THERAPEUTIC GENE-SWITCH CONSTRUCTS AND BIOREACTORS FOR THE EXPRESSION OF BIOTHERAPEUTIC MOLECULES, AND USES THEREOF

Background of the Invention

Field of the Invention

[0001] The present invention relates to methods and compositions for treating, ameliorating, or preventing a disease, disorder, or condition in a subject by introducing into the subject a therapeutic gene switch construct that controls expression of one or more therapeutic products. In a further embodiment, the present invention relates to methods and compositions for treating, ameliorating, or preventing a disease, disorder, or condition in a subject by introducing into the subject a "bioreactor," a therapeutic implant composed of a cell or cells that secrete a therapeutic protein. A bioreactor may be immuno-isolated by encapsulation or non-immunoisolated. In particular embodiments, the bioreactor comprises a therapeutic gene switch construct.

Background of the Invention

[0002] The concept of treating or preventing a disease in a subject through introduction of a polynucleotide encoding a therapeutic molecule, *e.g.*, a therapeutic polypeptide or therapeutic polynucleotide into cells of the subject, or introducing into the subject modified cells engineered to secrete the therapeutic molecule has been in existence for many years. Several difficulties in the practical aspects of the concept have hindered progress towards successful therapies. Direct introduction of genetic material into a subject to be treated presents difficulties such as: safety of delivery, obtaining sufficient expression levels of the therapeutic product for a sufficient period of time, limiting expression of the therapeutic product to desired cells, and maintaining the ability to modulate or pulse the expression of the therapeutic product, including the ability to turn off expression of the therapeutic product if it is no longer needed. Cell based therapies are subject to rejection via the subject's immune response, therefore immuno-isolation strategies such as cell encapsulation methods have been developed to increase the longevity of implanted cells and allow use of xenogeneic cells, *i.e.*, cells from a different

species. Current encapsulated and non-encapsulated cell therapies are engineered to secrete the therapeutic protein constitutively. Once implanted, protein secretion can not be regulated. To improve the safety and clinical application of direct or cell-mediated bioreactor therapeutic protein delivery it would be advantageous to be able to turn off the protein production or regulate the rate at which protein production occurs.

[0003] Thus, there is a need in the art for new therapeutic methods and compositions that provide these desired characteristics.

Summary of the Invention

[0004] The present invention relates to methods and compositions for treating, ameliorating, or preventing a disease, disorder, or condition in a subject.

[0005] In one embodiment, the present invention provides a method for treating, ameliorating, or preventing a disease, disorder, or condition in a subject, comprising:

[0006] (a) introducing into a subject (1) a first polynucleotide encoding a gene switch, where the gene switch comprises at least one transcription factor sequence encoding a ligand-dependent transcription factor through operable association with a therapeutic switch promoter, where the therapeutic switch promoter is constitutively active and (2) a second polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide in operable association with a factor-regulated promoter which is activated by said ligand-dependent transcription factor, where the first and second polynucleotides are introduced so as to permit their expression in the presence of ligand; and

[0007] (b) administering ligand to the subject to induce expression of the therapeutic polypeptide or therapeutic polynucleotide.

[0008] A further embodiment of the invention provides a method for expressing a therapeutic polypeptide or therapeutic polynucleotide in a subject, comprising:

(a) introducing into a subject (1) a first polynucleotide encoding a gene switch, where the gene switch comprises at least one transcription factor sequence encoding a ligand-dependent transcription factor through operable association with a therapeutic switch promoter, where the therapeutic switch promoter is activated under conditions associated with the disease, disorder, or condition to be treated, and (2) a second polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide in operable association with a factor-regulated

promoter which is activated by said ligand-dependent transcription factor, where the first and second polynucleotides are introduced so as to permit their expression in the subject under conditions associated with the disease, disorder, or condition; and

(b) administering ligand to the subject to induce expression of the therapeutic polypeptide or therapeutic polynucleotide.

[0009] A further embodiment of the invention provides a method for expressing a therapeutic polypeptide or therapeutic polynucleotide in a subject, comprising:

(a) introducing into a subject (1) a first polynucleotide encoding a gene switch, where the gene switch comprises at least one transcription factor sequence encoding a ligand-dependent transcription factor through operable association with a therapeutic switch promoter, where the therapeutic switch promoter is activated under conditions associated with a disease, disorder, or condition treatable by the therapeutic polypeptide or therapeutic polynucleotide, and (2) a second polynucleotide encoding the therapeutic polypeptide or therapeutic polynucleotide in operable association with a factor-regulated promoter which is activated by the ligand-dependent transcription factor, wherein said the and second polynucleotides are introduced so as to permit expression of the first polynucleotide under conditions associated with the disease, disorder, or condition; and

(b) administering ligand to the subject to induce expression of the therapeutic polypeptide or therapeutic polynucleotide.

[0010] In the methods described above, in one embodiment, the first polynucleotide encoding the therapeutic gene switch and the second polynucleotide encoding the therapeutic polypeptide or polynucleotide linked to a factor-regulated promoter are part of one larger polynucleotide, *e.g.*, a vector. In another embodiment, the first polynucleotide encoding the therapeutic gene switch and the second polynucleotide encoding the therapeutic polypeptide or polynucleotide linked to a factor-regulated promoter are separate polynucleotides which may be administered as a nucleic acid composition.

[0011] The invention further relates to therapeutic gene switch constructs that are useful in the disclosed methods.

[0012] The invention additionally relates to vectors comprising the therapeutic gene switch constructs of the invention.

[0013] The invention further provides a method for expressing a therapeutic polypeptide or therapeutic polynucleotide in one or more modified cells, comprising:

(a) introducing into a cell (1) a first polynucleotide encoding a gene switch, where the gene switch comprises at least one transcription factor sequence encoding a ligand-dependent transcription factor through operable association with a therapeutic switch promoter which is activated under conditions associated with a disease, disorder, or condition, and (2) a second polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide through operable association with a factor-regulated promoter which is activated by the ligand-dependent transcription factor, thereby producing a modified cell; and

(b) administering ligand to the modified cell to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0014] The invention further relates to modified cells comprising the therapeutic gene switch constructs of the invention.

[0015] The invention also relates to bioreactor devices comprising modified cells of the invention either non-encapsulated, or encapsulated in such a way to shield the cells from the subject's immune system. Such bioreactors may take the form, for example, of coated cells, micro-encapsulated cells, or macro-encapsulated cells.

[0016] The invention also relates to kits for carrying out the methods of the invention, comprising, *e.g.*, gene switch constructs, vectors, ligands, *etc.*

Brief Description of the Drawings

[0017] Figure 1 shows an embodiment of the therapeutic gene switch of the invention in which two transcription factor sequences encoding two separate portions of a ligand-dependent transcription factor complex are under the control of a single promoter. "AD" represents a transactivation domain; "HP" represents a heterodimerization partner domain. The AD and HP domains are expressed as a fusion protein termed a "coactivation protein" or "CAP." "DBD" represents a DNA binding domain; "LBD" represents a ligand binding domain. The DBD and LBD domains are expressed as a fusion protein termed a "ligand-dependent transcription factor," or "LTF."

"Transcriptional Linker" represents an IRES (Internal ribosomal entry site) or means of generating two separate protein products from a single open reading frame. "Therapeutic Product Sequence" represents a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide; "Therapeutic Product" represents a therapeutic polypeptide or therapeutic polynucleotide; and "TSP-1," represents either a constitutive therapeutic switch promoter, or a therapeutic switch promoter activated under conditions associated with a disease, disorder, or condition. CAP and LTF combine to form a ligand-dependent transcription factor complex (LDTFC) which in combination with ligand activates a factor-regulated promoter (FRP).

[0018] Figure 2 shows an embodiment of the therapeutic gene switch of the invention in which two transcription factor sequences (CAP and LTF) encoding two separate portions of a ligand-dependent transcription factor complex are under the control of different promoters. The terms AD, HP, CAP, DBD, LBD, LTF, "Therapeutic Product Sequence," "Therapeutic Product," TSP, LDTFC, and FRP are defined in the legend to Fig. 1. "TSP-1" and "TSP-2" represent two different therapeutic switch promoters, each of which is, independently, either a constitutive promoter or a promoter activated under conditions associated with a disease, disorder, or condition. In one embodiment TSP-1 is a constitutive promoter and TSP-2 is a promoter activated under conditions associated with a disease, disorder, or condition. CAP and LTF combine to form a LDTFC which in combination with ligand activates a FRP.

[0019] Figure 3 shows an embodiment of the therapeutic gene switch of the invention in which three transcription factor sequences (CAP, LTF-1, and LTF-2), which may combine to form two separate LDTFCs under the control of different promoters. The terms AD, HP, CAP, DBD, LBD, LTF, "Therapeutic Product Sequence," "Therapeutic Product," TSP, LDTFC, and FRP are defined in the legend to Fig. 1. DBD-A represents a first DNA binding domain which is fused with an LBD to form LTF-1, DBD-B represents a second DNA binding domain which is fused with an LBD to form LTF-2. "Therapeutic Product A" represents a first therapeutic polypeptide or therapeutic polynucleotide; "Therapeutic Product B" represents a second therapeutic polypeptide or therapeutic polynucleotide; and TSP-1, TSP-2, and TSP-3 represent three different therapeutic switch promoters, each of which is, independently, either a constitutive promoter or a promoter activated under conditions associated with a disease, disorder, or condition. In one

embodiment, TSP-1 is a constitutive therapeutic switch promoter and TSP-2, and TSP-3 are different therapeutic switch promoters, each of which is independently activated under conditions associated with a disease, disorder, or condition. CAP and LTF-1 combine to form LDTFC-1 which in combination with ligand activates FRP-1. CAP and LTF-2 combine to form LDTFC-2 which in combination with ligand activates FRP-2.

[0020] Figure 4 shows an embodiment of the therapeutic gene switch of the invention in which three transcription factor sequences encoding CAP and two separate LTF portions of a ligand-dependent transcription factor complex are under the control of different promoters. The terms AD, HP, CAP, DBD, LBD, LTF, "Therapeutic Product Sequence," "Therapeutic Product," TSP, LDTFC, and FRP are defined in the legend to Fig. 1. TSP-1, TSP-2, and TSP-3 represent three different therapeutic switch promoters, each of which is, independently, either a constitutive promoter or a promoter activated under conditions associated with a disease, disorder, or condition. In one embodiment, TSP-1 is a constitutive promoter and TSP-2 and TSP-3 are different promoters, each of which is independently activated under conditions associated with a disease, disorder, or condition. Either LTF-1 or LTF-2 may combine with CAP to form LDTFC-1 or LDTFC-2. Either LDTFC-1 or LDTFC-2, in combination with ligand, activates FRP.

[0021] Figure 5 is a diagram of a vector constructed under the scheme shown in Fig. 1, and engineered to express insulin growth factor-1 (IGF-1) under hypoxic conditions such as cardiac ischemia.

[0022] Figure 6 is a diagram of a vector constructed under the scheme shown in Fig. 2, and engineered to express basic fibroblast growth factor (bFGF) under hypoxic conditions such as cardiac ischemia.

[0023] Figure 7 is a diagram of a vector constructed under the scheme shown in Fig. 2, and engineered to express erythropoietin (EPO) under hypoxic conditions such as cardiac ischemia.

[0024] Figure 8 is is a diagram of a vector constructed under the scheme shown in Fig. 2, and engineered to express human B-type natriuretic peptide (BNP) under hypoxic conditions such as cardiac ischemia.

[0025] Figure 9 is is a diagram of a vector constructed under the scheme shown in Fig. 2, and engineered to express tissue plasminogen activator (tPA) under inflammatory conditions such as cardiac ischemia.

[0026] Figure 10 is is a diagram of a vector constructed under the scheme shown in Fig. 3, and engineered to express relaxin under inflammatory conditions and/or hepatocyte growth factor under hypoxic conditions, both conditions being associated with cardiac ischemia.

[0027] Figure 11 is is a diagram of a vector constructed under the scheme shown in Fig. 2, and engineered to express EPO under hypoxic conditions such as cardiac ischemia with expression being limited to cardiac myocytes.

[0028] Figure 12 is is a diagram of a vector constructed under the scheme shown in Fig. 4, and engineered to express IGF-1 under either inflammatory conditions or hypoxic conditions such as cardiac ischemia with expression being limited to cardiac myocytes.

[0029] Figure 13 is a diagram of a vector constructed under the scheme shown in Fig. 1, and engineered to express tumor necrosis factor binding protein 2 (Enbrel®) under inflammatory conditions such as rheumatoid arthritis.

[0030] Figure 14 is is a diagram of a vector constructed under the scheme shown in Fig. 4, and engineered to express tumor necrosis factor binding protein 2 (Enbrel®) either in reponse to TNF alpha expression or under inflammatory conditions, both conditions associated with rheumatoid arthritis.

[0031] Figure 15 is is a diagram of a vector constructed under the scheme shown in Fig. 3, and engineered to express tumor necrosis factor binding protein 2 (Enbrel®) under inflammatory conditions and/or EPO under HIF-driven hypoxic conditions, both conditions being associated with rheumatoid arthritis.

[0032] Figure 16 is a diagram of a vector constructed under the scheme shown in Fig. 1, and engineered to express human factor VIII:C constitutively.

[0033] Figure 17 is is a diagram of a vector constructed under the scheme shown in Fig. 2, and engineered to express human factor VIII:C under hypoxic conditions associated with hemophilia.

Detailed Description of the Invention

[0034] The invention relates to methods and compositions for using a gene switch to express a therapeutic polypeptide or therapeutic polynucleotide in a cell. The methods and compositions may be used *in vitro, ex vivo* or *in vivo*. The invention further relates to methods and compositions for using a gene switch controlling expression of a therapeutic

polypeptide or therapeutic polynucleotide for the treatment, amelioration, or prevention of diseases, disorders, or conditions in a subject. The methods of the invention can be carried out either *ex vivo* (by introducing the gene switch into isolated cells of a subject or non-autologous cells, and introducing the modified cells to the subject or into a different subject) or *in vivo* (by introducing the gene switch directly into cells of the subject). The methods of the invention involve the use of a gene switch in which expression of a ligand-dependent transcription factor is under the control of one or more therapeutic switch promoters. The methods also include, without limitation, applications of the gene switch technology in direct introduction into the subject to be treated, non-encapsulated and encapsulated cell therapies. The methods and compositions described herein provide a highly specific and tightly regulated therapeutic technique in which the level and timing of expression of a therapeutic product is controlled by administration of ligand to cells comprising the gene switch.

[0035] The following definitions are provided and should be helpful in understanding the scope and practice of the present invention.

[0036] The term "isolated" for the purposes of the present invention designates a biological material (cell, nucleic acid or protein) that has been removed from its original environment (the environment in which it is naturally present). For example, a polynucleotide present in the natural state in a plant or an animal is not isolated, however the same polynucleotide separated from the adjacent nucleic acids in which it is naturally present, is considered "isolated."

[0037] The term "purified," as applied to biological materials does not require the material to be present in a form exhibiting absolute purity, exclusive of the presence of other compounds. It is rather a relative definition.

[0038] "Nucleic acid," "nucleic acid molecule," "oligonucleotide," and "polynucleotide" are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to

the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA. A "nucleic acid composition" of the invention comprises one or more nucleic acids as described herein.

[0039] The term "fragment," as applied to polynucleotide sequences, refers to a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide sequence identical to the reference nucleic acid. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. Such fragments comprise, or alternatively consist of, oligonucleotides ranging in length from at least 6, 8, 9, 10, 12, 15, 18, 20, 21, 22, 23, 24, 25, 30, 39, 40, 42, 45, 48, 50, 51, 54, 57, 60, 63, 66, 70, 75, 78, 80, 90, 100, 105, 120, 135, 150, 200, 300, 500, 720, 900, 1000, 1500, 2000, 3000, 4000, 5000, or more consecutive nucleotides of a nucleic acid according to the invention.

[0040] As used herein, an "isolated nucleic acid fragment" refers to a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

[0041] A "gene" refers to a polynucleotide comprising nucleotides that encode a functional molecule, including functional molecules produced by transcription only (*e.g.*, a bioactive RNA species) or by transcription and translation (*e.g.*, a polypeptide). The term "gene" encompasses cDNA and genomic DNA nucleic acids. "Gene" also refers to a nucleic acid fragment that expresses a specific RNA, protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding

sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and/or coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. A chimeric gene may comprise coding sequences derived from different sources and/or regulatory sequences derived from different sources. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene or "heterologous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

[0042] "Heterologous DNA" refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. The heterologous DNA may include a gene foreign to the cell.

[0043] The term "genome" includes chromosomal as well as mitochondrial, chloroplast and viral DNA or RNA.

[0044] A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook *et al.* in *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization.

[0045] Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used, *e.g.*, 5X SSC, 0.1%

SDS, 0.25% milk, and no formamide; or 30% formamide, 5X SSC, 0.5% SDS. Moderate stringency hybridization conditions correspond to a higher T_m, *e.g.*, 40% formamide, with 5X or 6X SSC. High stringency hybridization conditions correspond to the highest T_m, *e.g.*, 50% formamide, 5X or 6X SSC.

[0046] Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The term "complementary" is used to describe the relationship between nucleotide bases that are capable of hybridizing to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine. Accordingly, the present invention also includes isolated nucleic acid fragments that are complementary to the complete sequences as disclosed or used herein as well as those substantially similar nucleic acid sequences.

[0047] In one embodiment of the invention, polynucleotides are detected by employing hybridization conditions comprising a hybridization step at T_m of 55°C, and utilizing conditions as set forth above. In other embodiments, the T_m is 60°C, 63°C, or 65°C.

[0048] Post-hybridization washes also determine stringency conditions. One set of conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 minutes (min), then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS is increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

[0049] The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (see Sambrook *et al.*, *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides,

the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook *et al.*, *supra*, 11.7-11.8).

[0050] In one embodiment of the invention, polynucleotides are detected by employing hybridization conditions comprising a hybridization step in less than 500 mM salt and at least 37°C, and a washing step in 2X SSPE at a temperature of at least 63°C. In another embodiment, the hybridization conditions comprise less than 200 mM salt and at least 37°C for the hybridization step. In a further embodiment, the hybridization conditions comprise 2X SSPE and 63°C for both the hybridization and washing steps.

[0051] In another embodiment, the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferably a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; *e.g.*, at least about 20 nucleotides; *e.g.*, at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

[0052] The term "probe" refers to a single-stranded nucleic acid molecule that can base pair with a complementary single stranded target nucleic acid to form a double-stranded molecule.

[0053] As used herein, the term "oligonucleotide" refers to a short nucleic acid that is hybridizable to a genomic DNA molecule, a cDNA molecule, a plasmid DNA or an mRNA molecule. Oligonucleotides can be labeled, *e.g.*, with ^{32}P-nucleotides or nucleotides to which a label, such as biotin, has been covalently conjugated. A labeled oligonucleotide can be used as a probe to detect the presence of a nucleic acid. Oligonucleotides (one or both of which may be labeled) can be used as PCR primers, either for cloning full length or a fragment of a nucleic acid, for DNA sequencing, or to detect the presence of a nucleic acid. An oligonucleotide can also be used to form a triple helix with a DNA molecule. Generally, oligonucleotides are prepared synthetically, preferably on a nucleic acid synthesizer. Accordingly, oligonucleotides can be prepared with non-naturally occurring phosphoester analog bonds, such as thioester bonds, *etc.*

[0054] A "primer" refers to an oligonucleotide that hybridizes to a target nucleic acid sequence to create a double stranded nucleic acid region that can serve as an initiation point for DNA synthesis under suitable conditions. Such primers may be used in a polymerase chain reaction or for DNA sequencing.

[0055] "Polymerase chain reaction" is abbreviated PCR and refers to an *in vitro* method for enzymatically amplifying specific nucleic acid sequences. PCR involves a repetitive series of temperature cycles with each cycle comprising three stages: denaturation of the template nucleic acid to separate the strands of the target molecule, annealing a single stranded PCR oligonucleotide primer to the template nucleic acid, and extension of the annealed primer(s) by DNA polymerase. PCR provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

[0056] "Reverse transcription-polymerase chain reaction" is abbreviated RT-PCR and refers to an *in vitro* method for enzymatically producing a target cDNA molecule or molecules from an RNA molecule or molecules, followed by enzymatic amplification of a specific nucleic acid sequence or sequences within the target cDNA molecule or molecules as described above. RT-PCR also provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

[0057] A DNA "coding sequence" refers to a double-stranded DNA sequence that encodes a polypeptide and can be transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of suitable regulatory sequences. "Suitable regulatory sequences" refers to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing sites, effector binding sites and stem-loop structures. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from mRNA, genomic DNA sequences, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0058] "Open reading frame" is abbreviated ORF and refers to a length of nucleic acid sequence, either DNA, cDNA or RNA, that comprises a translation start signal or

initiation codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

[0059] The term "head-to-head" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a head-to-head orientation when the 5' end of the coding strand of one polynucleotide is adjacent to the 5' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds away from the 5' end of the other polynucleotide. The term "head-to-head" may be abbreviated (5')-to-(5') and may also be indicated by the symbols (← →) or (3'←5'5'→3').

[0060] The term "tail-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a tail-to-tail orientation when the 3' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds toward the other polynucleotide. The term "tail-to-tail" may be abbreviated (3')-to-(3') and may also be indicated by the symbols (→ ←) or (5'→3'3'←5').

[0061] The term "head-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a head-to-tail orientation when the 5' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds in the same direction as that of the other polynucleotide. The term "head-to-tail" may be abbreviated (5')-to-(3') and may also be indicated by the symbols (→ →) or (5'→3'5'→3').

[0062] The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In particular, downstream nucleotide sequences generally relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0063] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence. In particular, upstream nucleotide sequences generally relate to sequences that are located on the 5' side of a coding sequence or starting point of

transcription. For example, most promoters are located upstream of the start site of transcription.

[0064] The terms "restriction endonuclease" and "restriction enzyme" are used interchangeably and refer to an enzyme that binds and cuts within a specific nucleotide sequence within double stranded DNA.

[0065] "Homologous recombination" refers to the insertion of a foreign DNA sequence into another DNA molecule, *e.g.*, insertion of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

[0066] Several methods known in the art may be used to propagate a polynucleotide according to the invention. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As described herein, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

[0067] A "vector" refers to any vehicle for the cloning of and/or transfer of a nucleic acid into a host cell. A vector may be a replicon to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "replicon" refers to any genetic element (*e.g.*, plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*, *i.e.*, capable of replication under its own control. The term "vector" includes both viral and nonviral vehicles for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors known in the art may be used to manipulate nucleic acids, incorporate response elements and promoters into genes, *etc.* Possible vectors include, for example, plasmids or modified viruses including, for example bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives, or the Bluescript vector. Another example of vectors that are useful in the present invention is the UltraVector™ Production System (Intrexon Corp., Blacksburg, VA) as described in WO 2007/038276, incorporated herein

by reference. For example, the insertion of the DNA fragments corresponding to response elements and promoters into a suitable vector can be accomplished by ligating the appropriate DNA fragments into a chosen vector that has complementary cohesive termini. Alternatively, the ends of the DNA molecules may be enzymatically modified or any site may be produced by ligating nucleotide sequences (linkers) into the DNA termini. Such vectors may be engineered to contain selectable marker genes that provide for the selection of cells that have incorporated the marker into the cellular genome. Such markers allow identification and/or selection of host cells that incorporate and express the proteins encoded by the marker.

[0068] Viral vectors, and particularly retroviral vectors, have been used in a wide variety of gene delivery applications in cells, as well as living animal subjects. Viral vectors that can be used include, but are not limited to, retrovirus, adeno-associated virus, pox, baculovirus, vaccinia, herpes simplex, Epstein-Barr, adenovirus, geminivirus, and caulimovirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers. In addition to a nucleic acid, a vector may also comprise one or more regulatory regions, and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (transfer to which tissues, duration of expression, *etc.*).

[0069] The term "plasmid" refers to an extra-chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

[0070] A "cloning vector" refers to a "replicon," which is a unit length of a nucleic acid, preferably DNA, that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. Cloning vectors may be capable of replication in one cell type and expression in another ("shuttle vector"). Cloning vectors may comprise one or more sequences that can be used for

selection of cells comprising the vector and/or one or more multiple cloning sites for insertion of sequences of interest.

[0071] The term "expression vector" refers to a vector, plasmid or vehicle designed to enable the expression of an inserted nucleic acid sequence following transformation into the host. The cloned gene, *i.e.*, the inserted nucleic acid sequence, is usually placed under the control of control elements such as a promoter, a minimal promoter, an enhancer, or the like. Initiation control regions or promoters, which are useful to drive expression of a nucleic acid in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving expression of these genes can be used in an expression vector, including but not limited to, viral promoters, bacterial promoters, animal promoters, mammalian promoters, synthetic promoters, constitutive promoters, tissue specific promoters, pathogenesis or disease related promoters, developmental specific promoters, inducible promoters, light regulated promoters; *CYC1, HIS3, GAL1, GAL4, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI,* alkaline phosphatase promoters (useful for expression in *Saccharomyces*); *AOX1* promoter (useful for expression in *Pichia*); β-lactamase, *lac, ara, tet, trp,* lP_L lP_R, *T7, tac, and trc* promoters (useful for expression in *Escherichia coli*); light regulated-, seed specific-, pollen specific-, ovary specific-, cauliflower mosaic virus 35S, CMV 35S minimal, cassava vein mosaic virus (CsVMV), chlorophyll a/b binding protein, ribulose 1,5-bisphosphate carboxylase, shoot-specific, root specific, chitinase, stress inducible, rice tungro bacilliform virus, plant super-promoter, potato leucine aminopeptidase, nitrate reductase, mannopine synthase, nopaline synthase, ubiquitin, zein protein, and anthocyanin promoters (useful for expression in plant cells); animal and mammalian promoters known in the art including, but are not limited to, the SV40 early (SV40e) promoter region, the promoter contained in the 3' long terminal repeat (LTR) of Rous sarcoma virus (RSV), the promoters of the E1A or major late promoter (MLP) genes of adenoviruses (Ad), the cytomegalovirus (CMV) early promoter, the herpes simplex virus (HSV) thymidine kinase (TK) promoter, a baculovirus IE1 promoter, an elongation factor 1 alpha (EF1) promoter, a phosphoglycerate kinase (PGK) promoter, a ubiquitin (Ubc) promoter, an albumin promoter, the regulatory sequences of the mouse metallothionein-L promoter and transcriptional control regions, the ubiquitous promoters (HPRT, vimentin, α-actin, tubulin and the like), the promoters of the intermediate filaments (desmin,

neurofilaments, keratin, GFAP, and the like), the promoters of therapeutic genes (of the MDR, CFTR or factor VIII type, and the like), pathogenesis or disease related-promoters, and promoters that exhibit tissue specificity and have been utilized in transgenic animals, such as the elastase I gene control region which is active in pancreatic acinar cells; insulin gene control region active in pancreatic beta cells, immunoglobulin gene control region active in lymphoid cells, mouse mammary tumor virus control region active in testicular, breast, lymphoid and mast cells; albumin gene, Apo AI and Apo AII control regions active in liver, alpha-fetoprotein gene control region active in liver, alpha 1-antitrypsin gene control region active in the liver, beta-globin gene control region active in myeloid cells, myelin basic protein gene control region active in oligodendrocyte cells in the brain, myosin light chain-2 gene control region active in skeletal muscle, and gonadotropic releasing hormone gene control region active in the hypothalamus, pyruvate kinase promoter, villin promoter, promoter of the fatty acid binding intestinal protein, promoter of the smooth muscle cell α-actin, and the like. In addition, these expression sequences may be modified by addition of enhancer or regulatory sequences and the like.

[0072] Vectors may be introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu *et al.*, *J. Biol. Chem. 267*:963 (1992); Wu *et al.*, *J. Biol. Chem. 263*:14621 (1988); and Hartmut *et al.*, Canadian Patent Application No. 2,012,311).

[0073] A polynucleotide according to the invention can also be introduced *in vivo* by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids *in vitro*. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome-mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA. 84*:7413 (1987); Mackey *et al.*, *Proc. Natl. Acad. Sci. USA 85*:8027 (1988); and Ulmer *et al.*, *Science 259*:1745 (1993)). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner *et al.*, *Science* 337:387 (1989)). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in WO95/18863, WO96/17823 and U.S. 5,459,127. The use of

lipofection to introduce exogenous genes into the specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly preferred in a tissue with cellular heterogeneity, such as pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting (Mackey *et al.* 1988, *supra*). Targeted peptides, *e.g.*, hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

[0074] Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptide (*e.g.*, WO95/21931), peptides derived from DNA binding proteins (*e.g.*, WO96/25508), or a cationic polymer (*e.g.*, WO95/21931).

[0075] It is also possible to introduce a vector *in vivo* as a naked DNA plasmid (see U.S. Patent Nos. 5,693,622, 5,589,466 and 5,580,859). Receptor-mediated DNA delivery approaches can also be used (Curiel *et al., Hum. Gene Ther. 3*:147 (1992); and Wu *et al., J. Biol. Chem.* 262:4429 (1987)).

[0076] The term "transfection" refers to the uptake of exogenous or heterologous RNA or DNA by a cell. A cell has been "transfected" by exogenous or heterologous RNA or DNA when such RNA or DNA has been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous RNA or DNA when the transfected RNA or DNA effects a phenotypic change. The transforming RNA or DNA can be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

[0077] "Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

[0078] In addition, the recombinant vector comprising a polynucleotide according to the invention may include one or more origins for replication in the cellular hosts in which their amplification or their expression is sought, markers or selectable markers.

[0079] The term "selectable marker" refers to an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, *i.e.*, resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like, wherein the effect is used to track the

inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like.

[0080] The term "reporter gene" refers to a nucleic acid encoding an identifying factor that is able to be identified based upon the reporter gene's effect, wherein the effect is used to track the inheritance of a nucleic acid of interest, to identify a cell or organism that has inherited the nucleic acid of interest, and/or to measure gene expression induction or transcription. Examples of reporter genes known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), β-galactosidase (LacZ), β-glucuronidase (Gus), and the like. Selectable marker genes may also be considered reporter genes.

[0081] "Promoter and "promoter sequence" are used interchangeably and refer to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters." Promoters that cause a gene to be expressed in a specific cell type are commonly referred to as "cell-specific promoters" or "tissue-specific promoters." Promoters that cause a gene to be expressed at a specific stage of development or cell differentiation are commonly referred to as "developmentally-specific promoters" or "cell differentiation-specific promoters." Promoters that are induced and cause a gene to be expressed following exposure or treatment of the cell with an agent, biological molecule, chemical, ligand, light, or the like that induces the promoter are commonly referred to as "inducible promoters" or "regulatable promoters." It is further recognized that since in

most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

[0082] The promoter sequence is typically bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

[0083] "Therapeutic switch promoter" ("TSP") refers to a promoter that controls expression of a gene switch component. Gene switches and their various components are described in detail elsewhere herein. In certain embodiments a TSP is constitutive, *i.e.*, continuously active. A consitutive TSP may be either constitutive-ubiquitous (*i.e.*, generally functions, without the need for additional factors or regulators, in any tissue or cell) or constitutive-tissue or cell specific (*i.e.*, generally functions, without the need for additional factors or regulators, in a specific tissue type or cell type). In certain embodiments a TSP of the invention is activated under conditions associated with a disease, disorder, or condition. In certain embodiments of the invention where two or more TSPs are involved the promoters may be a combination of constitutive and activatable promoters. As used herein, a "promoter activated under conditions associated with a disease, disorder, or condition" includes, without limitation, disease-specific promoters, promoters responsive to particular physiological, developmental, differentiation, or pathological conditions, promoters responsive to specific biological molecules, and promoters specific for a particular tissue or cell type associated with the disease, disorder, or condition, *e.g.* tumor tissue or malignant cells. TSPs can comprise the sequence of naturally occurring promoters, modified sequences derived from naturally occurring promoters, or synthetic sequences (*e.g.*, insertion of a response element into a minimal promoter sequence to alter the responsiveness of the promoter).

[0084] A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced (if the coding sequence contains introns) and translated into the protein encoded by the coding sequence.

[0085] "Transcriptional and translational control sequences" refer to DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

[0086] The term "response element" ("RE") refers to one or more cis-acting DNA elements which confer responsiveness on a promoter mediated through interaction with the DNA-binding domains of a transcription factor. This DNA element may be either palindromic (perfect or imperfect) in its sequence or composed of sequence motifs or half sites separated by a variable number of nucleotides. The half sites can be similar or identical and arranged as either direct or inverted repeats or as a single half site or multimers of adjacent half sites in tandem. The response element may comprise a minimal promoter isolated from different organisms depending upon the nature of the cell or organism into which the response element will be incorporated. The DNA binding domain of the transcription factor binds, in the presence or absence of a ligand, to the DNA sequence of a response element to initiate or suppress transcription of downstream gene(s) under the regulation of this response element. Examples of DNA sequences for response elements of the natural ecdysone receptor include: RRGG/TTCANTGAC/ACYY (SEQ ID NO: 1) (see Cherbas *et. al.*, *Genes Dev. 5*:120 (1991)); AGGTCA$N_{(n)}$AGGTCA, where $N_{(n)}$ can be one or more spacer nucleotides (SEQ ID NO: 2) (see D'Avino *et al.*, *Mol. Cell. Endocrinol. 113*:1 (1995)); and GGGTTGAATGAATTT (SEQ ID NO: 3) (see Antoniewski *et al.*, *Mol. Cell Biol. 14*:4465 (1994)).

[0087] "Factor-regulated promoter" ("FRP") refers to a promoter comprising at least one response element that is recognized by the DNA binding domain of a ligand-dependent transcription factor encoded by a gene switch of the invention.

[0088] The terms "operably linked," "operably associated," "through operable association," and the like refer to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (*i.e.*, that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

[0089] The term "expression" as used herein refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from a nucleic acid or polynucleotide. Expression may also refer to translation of mRNA into a protein or polypeptide.

[0090] The terms "cassette," "expression cassette" and "gene expression cassette" refer to a segment of DNA that can be inserted into a nucleic acid or polynucleotide at specific restriction sites or by homologous recombination. The segment of DNA comprises a polynucleotide that encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation. "Transformation cassette" refers to a specific vector comprising a polynucleotide that encodes a polypeptide of interest and having elements in addition to the polynucleotide that facilitate transformation of a particular host cell. Cassettes, expression cassettes, gene expression cassettes and transformation cassettes of the invention may also comprise elements that allow for enhanced expression of a polynucleotide encoding a polypeptide of interest in a host cell. These elements may include, but are not limited to: a promoter, a minimal promoter, an enhancer, a response element, a terminator sequence, a polyadenylation sequence, and the like.

[0091] For purposes of this invention, the term "gene switch" refers to the combination of a response element associated with a promoter, and a ligand-dependent transcription factor-based system which, in the presence of one or more ligands, modulates the expression of a gene into which the response element and promoter are incorporated.

[0092] The term "ecdysone receptor-based," with respect to a gene switch, refers to a gene switch comprising at least a functional part of a naturally occurring or synthetic ecdysone receptor ligand binding domain and which regulates gene expression in response to a ligand that binds to the ecdysone receptor ligand binding domain.

[0093] As used herein, the terms "bioreactor" or "bioreactor device" includes a cell or cells intended to secrete a therapeutic protein or therapeutic polynucleotide. In certain non-limiting embodiments, the bioreactor comprises modified cells as described elsewhere herein. In certain, but not all embodiments, bioreactor cells may be "immunoisolated." Bioreactor cells are considered "immunoisolated" from a subject when the cells are treated such that the cells, upon introduction or implantation into the subject, are protected from the subject's immune system. For example, immunoisolated

bioreactor cells may be contained within a barrier system which allows dissemination of said therapeutic protein or therapeutic polynucleotide, but which prevents direct contact of bioreactor cells with cells of the subject's immune system. Immunoisolated cells may be, for example, coated or encapsulated. Immunoisolation methods include but are not limited to conformal coating of cells, microencapsulation where cells are suspended in a biocompatible material and separated into spherical masses, or macroencapsulation, where the cells are enclosed in devices composed of natural or synthetic polymers that are used to enclose cells.

[0094] The terms "modulate" and "modulates" mean to induce, reduce or inhibit nucleic acid or gene expression, resulting in the respective induction, reduction or inhibition of protein or polypeptide production.

[0095] The polynucleotides or vectors according to the invention may further comprise at least one promoter suitable for driving expression of a gene in a modified cell.

[0096] Enhancers that may be used in embodiments of the invention include but are not limited to: an SV40 enhancer, a cytomegalovirus (CMV) enhancer, an elongation factor 1 (EF1) enhancer, yeast enhancers, viral gene enhancers, and the like.

[0097] A "3' reg" as defined herein, is an expression modulating element situated 3' to a coding region of a gene or transcript. Such elements include, without limitation: primary transcript-encoded Splicing elements, UTR from processed transcript, a polyadenylation signal or a DNA-encoded Transcription termination domain.

[0098] Termination control regions, *i.e.*, terminator or polyadenylation nucleotide sequences, may also be derived from various genes native to the preferred hosts. Optionally, a termination site may be unnecessary, however, it is most preferred if included. In a one embodiment of the invention, the termination control region may be comprised or be derived from a synthetic sequence, synthetic polyadenylation signal, an SV40 late polyadenylation signal, an SV40 polyadenylation signal, a bovine growth hormone (BGH) polyadenylation signal, viral terminator sequences, or the like.

[0100] The terms "3' non-coding sequences" or "3' untranslated region (UTR)" refer to DNA sequences located downstream (3') of a coding sequence and may comprise polyadenylation [poly(A)] recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The

polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor.

[0101] "Regulatory region" refers to a nucleic acid sequence that regulates the expression of a second nucleic acid sequence. A regulatory region may include sequences which are naturally responsible for expressing a particular nucleic acid (a homologous region) or may include sequences of a different origin that are responsible for expressing different proteins or even synthetic proteins (a heterologous region). In particular, the sequences can be sequences of prokaryotic, eukaryotic, or viral genes or derived sequences that stimulate or repress transcription of a gene in a specific or non-specific manner and in an inducible or non-inducible manner. Regulatory regions include origins of replication, RNA splice sites, promoters, enhancers, transcriptional termination sequences, and signal sequences which direct the polypeptide into the secretory pathways of the target cell.

[0102] A regulatory region from a "heterologous source" refers to a regulatory region that is not naturally associated with the expressed nucleic acid. Included among the heterologous regulatory regions are regulatory regions from a different species, regulatory regions from a different gene, hybrid regulatory sequences, and regulatory sequences which do not occur in nature, but which are designed by one having ordinary skill in the art.

[0103] "RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene. The complementarity of an antisense RNA may be with any part of the specific gene transcript, *i.e.*, at the 5' non-coding sequence, 3' non-coding sequence, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

[0104] "Polypeptide," "peptide" and "protein" are used interchangeably and refer to a polymeric compound comprised of covalently linked amino acid residues.

[0105] An "isolated polypeptide," "isolated peptide" or "isolated protein" refer to a polypeptide or protein that is substantially free of those compounds that are normally associated therewith in its natural state (*e.g.*, other proteins or polypeptides, nucleic acids, carbohydrates, lipids). "Isolated" is not meant to exclude artificial or synthetic mixtures with other compounds, or the presence of impurities which do not interfere with biological activity, and which may be present, for example, due to incomplete purification, addition of stabilizers, or compounding into a pharmaceutically acceptable preparation.

[0106] A "substitution mutant polypeptide" or a "substitution mutant" will be understood to mean a mutant polypeptide comprising a substitution of at least one wild-type or naturally occurring amino acid with a different amino acid relative to the wild-type or naturally occurring polypeptide. A substitution mutant polypeptide may comprise only one wild-type or naturally occurring amino acid substitution and may be referred to as a "point mutant" or a "single point mutant" polypeptide. Alternatively, a substitution mutant polypeptide may comprise a substitution of two or more wild-type or naturally occurring amino acids with two or more amino acids relative to the wild-type or naturally occurring polypeptide. According to the invention, a Group H nuclear receptor ligand binding domain polypeptide comprising a substitution mutation comprises a substitution of at least one wild-type or naturally occurring amino acid with a different amino acid relative to the wild-type or naturally occurring Group H nuclear receptor ligand binding domain polypeptide.

[0107] When the substitution mutant polypeptide comprises a substitution of two or more wild-type or naturally occurring amino acids, this substitution may comprise either an equivalent number of wild-type or naturally occurring amino acids deleted for the substitution, *i.e.*, 2 wild-type or naturally occurring amino acids replaced with 2 non-wild-type or non-naturally occurring amino acids, or a non-equivalent number of wild-type amino acids deleted for the substitution, *i.e.*, 2 wild-type amino acids replaced with 1 non-wild-type amino acid (a substitution+deletion mutation), or 2 wild-type amino acids replaced with 3 non-wild-type amino acids (a substitution+insertion mutation).

[0108] Substitution mutants may be described using an abbreviated nomenclature system to indicate the amino acid residue and number replaced within the reference polypeptide sequence and the new substituted amino acid residue. For example, a substitution mutant in which the twentieth (20^{th}) amino acid residue of a polypeptide is substituted may be abbreviated as "x20z", wherein "x" is the amino acid to be replaced, "20" is the amino acid residue position or number within the polypeptide, and "z" is the new substituted amino acid. Therefore, a substitution mutant abbreviated interchangeably as "E20A" or "Glu20Ala" indicates that the mutant comprises an alanine residue (commonly abbreviated in the art as "A" or "Ala") in place of the glutamic acid (commonly abbreviated in the art as "E" or "Glu") at position 20 of the polypeptide.

[0109] A substitution mutation may be made by any technique for mutagenesis known in the art, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson *et al., J. Biol. Chem. 253*:6551 (1978); Zoller *et al., DNA 3*:479 (1984); Oliphant *et al., Gene 44*:177 (1986); Hutchinson *et al., Proc. Natl. Acad. Sci. USA 83*:710 (1986)), use of TAB® linkers (Pharmacia), restriction endonuclease digestion/fragment deletion and substitution, PCR-mediated/oligonucleotide-directed mutagenesis, and the like. PCR-based techniques are preferred for site-directed mutagenesis (see Higuchi, 1989, "Using PCR to Engineer DNA", in *PCR Technology: Principles and Applications for DNA Amplification*, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70).

[0110] The term "fragment," as applied to a polypeptide, refers to a polypeptide whose amino acid sequence is shorter than that of the reference polypeptide and which comprises, over the entire portion with these reference polypeptides, an identical amino acid sequence. Such fragments may, where appropriate, be included in a larger polypeptide of which they are a part. Such fragments of a polypeptide according to the invention may have a length of at least 2, 3, 4, 5, 6, 8, 10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 30, 35, 40, 45, 50, 100, 200, 240, or 300 or more amino acids.

[0111] A "variant" of a polypeptide or protein refers to any analogue, fragment, derivative, or mutant which is derived from a polypeptide or protein and which retains at least one biological property of the polypeptide or protein. Different variants of the polypeptide or protein may exist in nature. These variants may be allelic variations characterized by differences in the nucleotide sequences of the structural gene coding for the protein, or may involve differential splicing or post-translational modification. The

skilled artisan can produce variants having single or multiple amino acid substitutions, deletions, additions, or replacements. These variants may include, *inter alia*: (a) variants in which one or more amino acid residues are substituted with conservative or non-conservative amino acids, (b) variants in which one or more amino acids are added to the polypeptide or protein, (c) variants in which one or more of the amino acids includes a substituent group, and (d) variants in which the polypeptide or protein is fused with another polypeptide such as serum albumin. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, *etc.*), chemical, and enzymatic techniques, are known to persons having ordinary skill in the art. In one embodiment, a variant polypeptide comprises at least about 14 amino acids.

[0112] The term "homology" refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known to the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s) and size determination of the digested fragments.

[0113] As used herein, the term "homologous" in all its grammatical forms and spelling variations refers to the relationship between proteins that possess a "common evolutionary origin," including proteins from superfamilies (*e.g.*, the immunoglobulin superfamily) and homologous proteins from different species (*e.g.*, myosin light chain, *etc.*) (Reeck *et al.*, *Cell 50*:667 (1987)). Such proteins (and their encoding genes) have sequence homology, as reflected by their high degree of sequence similarity. However, in common usage and in the present application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and not a common evolutionary origin.

[0114] Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of proteins that may or may not share a common evolutionary origin (see Reeck *et al.*, *Cell 50*:667 (1987)). In one embodiment, two DNA sequences are "substantially homologous"

or "substantially similar" when at least about 50% (*e.g.*, at least about 75%, 90%, or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art (see *e.g.*, Sambrook *et al.*, 1989, *supra*).

[0115] As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases do not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the present invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript. It is therefore understood that the invention encompasses more than the specific exemplary sequences. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

[0116] Moreover, the skilled artisan recognizes that substantially similar sequences encompassed by this invention are also defined by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS), with the sequences exemplified herein. Substantially similar nucleic acid fragments of the present invention are those nucleic acid fragments whose DNA sequences are at least about 70%, 80%, 90% or 95% identical to the DNA sequence of the nucleic acid fragments reported herein.

[0117] Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than about 40% of the amino acids are identical, or greater than 60% are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, *Version 7*, Madison, Wisconsin) pileup program.

[0118] The term "corresponding to" is used herein to refer to similar or homologous sequences, whether the exact position is identical or different from the molecule to which the similarity or homology is measured. A nucleic acid or amino acid sequence alignment may include spaces. Thus, the term "corresponding to" refers to the sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

[0119] A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul *et al., J. Mol. Biol. 215*:403 (1993)); available at ncbi.nlm.nih.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (*e.g.*, Southern hybridization) and isolation (*e.g., in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence.

[0120] The term "percent identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in: *Computational Molecular Biology* (Lesk, A. M., ed.) Oxford University Press, New York (1988); *Biocomputing: Informatics and Genome Projects* (Smith, D. W., ed.) Academic Press, New York (1993); *Computer Analysis of Sequence Data, Part I* (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey (1994); *Sequence Analysis in*

Molecular Biology (von Heinje, G., ed.) Academic Press (1987); and *Sequence Analysis Primer* (Gribskov, M. and Devereux, J., eds.) Stockton Press, New York (1991). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using sequence analysis software such as the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences may be performed using the Clustal method of alignment (Higgins *et al.*, *CABIOS. 5*:151 (1989)) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method may be selected: KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

[0121] The term "sequence analysis software" refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence analysis software includes, but is not limited to, the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI), BLASTP, BLASTN, BLASTX (Altschul *et al.*, *J. Mol. Biol. 215*:403 (1990)), and DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715 USA). Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the "default values" of the program referenced, unless otherwise specified. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

[0122] "Chemically synthesized," as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of DNA may be accomplished using well-established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons

favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

[0123] As used herein, two or more individually operable gene regulation systems are said to be "orthogonal" when; a) modulation of each of the given systems by its respective ligand, at a chosen concentration, results in a measurable change in the magnitude of expression of the gene of that system, and b) the change is statistically significantly different than the change in expression of all other systems simultaneously operable in the cell, tissue, or organism, regardless of the simultaneity or sequentially of the actual modulation. Preferably, modulation of each individually operable gene regulation system effects a change in gene expression at least 2-fold greater than all other operable systems in the cell, tissue, or organism, *e.g.*, at least 5-fold, 10-fold, 100-fold, or 500-fold greater. Ideally, modulation of each of the given systems by its respective ligand at a chosen concentration results in a measurable change in the magnitude of expression of the gene of that system and no measurable change in expression of all other systems operable in the cell, tissue, or organism. In such cases the multiple inducible gene regulation system is said to be "fully orthogonal." The present invention is useful to search for orthogonal ligands and orthogonal receptor-based gene expression systems such as those described in US 2002/0110861 A1, which is incorporated herein by reference in its entirety.

[0124] The term "exogenous gene" means a gene foreign to the subject, that is, a gene which is introduced into the subject through a transformation process, an unmutated version of an endogenous mutated gene or a mutated version of an endogenous unmutated gene. The method of transformation is not critical to this invention and may be any method suitable for the subject known to those in the art. Exogenous genes can be either natural or synthetic genes which are introduced into the subject in the form of DNA or RNA which may function through a DNA intermediate such as by reverse transcriptase. Such genes can be introduced into target cells, directly introduced into the subject, or indirectly introduced by the transfer of transformed cells into the subject.

[0125] The terms "therapeutic product" and "therapeutic molecule" as used herein refer to a therapeutic polypeptide ("TP", encoded by a "therapeutic proteins sequence" ("TPSQ")) or therapeutic polynucleotide which imparts a beneficial function to the subject to be treated. Therapeutic polypeptides may include, without limitation, peptides as small as

three amino acids in length, single- or multiple-chain proteins, and fusion proteins. Therapeutic polynucleotides may include, without limitation, antisense oligonucleotides, small interfering RNAs, ribozymes, and RNA external guide sequences. Non-limiting examples of therapeutic products are disclosed elsewhere herein. The therapeutic product may comprise a naturally occurring sequence, a synthetic sequence or a combination of natural and synthetic sequences.

[0126] The term "ligand-dependent transcription factor complex" or "LDTFC" refers to a transcription factor comprising one or more protein subunits, which complex can regulate gene expression driven by a "factor-regulated promoter" as defined herein. A model LDTFC is an "ecdysone receptor complex" generally refers to a heterodimeric protein complex having at least two members of the nuclear receptor family, ecdysone receptor ("EcR") and ultraspiracle ("USP") proteins (see Yao *et al.*, *Nature 366*:476 (1993)); Yao *et al.*, *Cell 71*:63 (1992)). A functional LDTFC such as an EcR complex may also include additional protein(s) such as immunophilins. Additional members of the nuclear receptor family of proteins, known as transcriptional factors (such as DHR38, *betaFTZ-1* or other insect homologs), may also be ligand dependent or independent partners for EcR and/or USP. A LDTFC such as an EcR complex can also be a heterodimer of EcR protein and the vertebrate homolog of ultraspiracle protein, retinoic acid-X-receptor ("RXR") protein or a chimera of USP and RXR. The terms "LDTFC" and "EcR complex" also encompass homodimer complexes of the EcR protein or USP, as well as single polypeptides or trimers, tetramer, and other multimers serving the same function.

[0127] A LDTFC such as an EcR complex can be activated by an active ecdysteroid or non-steroidal ligand bound to one of the proteins of the complex, inclusive of EcR, but not excluding other proteins of the complex. As used herein, the term "ligand," as applied to LDTFC-based gene switches *e.g.*, EcD complex based gene switches, describes small and soluble molecules having the capability of activating a gene switch to stimulate expression of a polypeptide encoded therein. Examples of ligands include, without limitation, an ecdysteroid, such as ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A, and the like, 9-cis-retinoic acid, synthetic analogs of retinoic acid, N,N'-diacylhydrazines such as those disclosed in U.S. Patent Nos. 6,013,836; 5,117,057; 5,530,028; and 5,378,726 and U.S. Published Application Nos. 2005/0209283 and 2006/0020146; oxadiazolines as described in U.S. Published Application No.

2004/0171651; dibenzoylalkyl cyanohydrazines such as those disclosed in European Application No. 461,809; N-alkyl-N,N'-diaroylhydrazines such as those disclosed in U.S. Patent No. 5,225,443; N-acyl-N-alkylcarbonylhydrazines such as those disclosed in European Application No. 234,994; N-aroyl-N-alkyl-N'-aroylhydrazines such as those described in U.S. Patent No. 4,985,461; amidoketones such as those described in U.S. Published Application No. 2004/0049037; each of which is incorporated herein by reference and other similar materials including 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, oxysterols, 22(R) hydroxycholesterol, 24(S) hydroxycholesterol, 25-epoxycholesterol, T0901317, 5-alpha-6-alpha-epoxycholesterol-3-sulfate (ECHS), 7-ketocholesterol-3-sulfate, famesol, bile acids, 1,1-biphosphonate esters, juvenile hormone III, and the like. Examples of diacylhydrazine ligands useful in the present invention include RG-115819 (3,5-Dimethyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N'-(2-methyl-3-methoxy-benzoyl)-hydrazide), RG-115932 ((*R*)-3,5-Dimethyl-benzoic acid N-(1-*tert*-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide), and RG-115830 (3,5-Dimethyl-benzoic acid N-(1-*tert*-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide). See, *e.g.*, U.S. Patent Appl. Serial No. 12/155,111, and PCT Appl. No. PCT/US2008/006757, both of which are incorporated herein by reference in their entireties.

[0128] A LDTFC such as an EcR complex includes proteins which are members of the nuclear receptor superfamily wherein all members are characterized by the presence of one or more polypeptide subunits comprising an amino-terminal transactivation domain ("AD," "TD," or "TA," used interchangeably herein), a DNA binding domain ("DBD"), and a ligand binding domain ("LBD"). The AD may be present as a fusion with a "heterodimerization partner" or "HP." A fusion protein comprising an AD and HP of the invention is referred to herein as a "coactivation protein" or "CAP." The DBD and LBD may be expressed as a fusion protein, referred to herein as a "ligand-inducible transcription factor ("LTF"). The fusion partners may be separated by a linker, *e.g.*, a hinge region. Some members of the LTF family may also have another transactivation domain on the carboxy-terminal side of the LBD. The DBD is characterized by the presence of two cysteine zinc fingers between which are two amino acid motifs, the P-box and the D-box, which confer specificity for ecdysone response elements. These

domains may be either native, modified, or chimeras of different domains of heterologous receptor proteins.

[0129] The DNA sequences making up the exogenous gene, the response element, and the LDTFC, *e.g.*, EcR complex, may be incorporated into archaebacteria, procaryotic cells such as *Escherichia coli*, *Bacillus subtilis*, or other enterobacteria, or eucaryotic cells such as plant or animal cells. However, because many of the proteins expressed by the gene are processed incorrectly in bacteria, eucaryotic cells are preferred. The cells may be in the form of single cells or multicellular organisms. The nucleotide sequences for the exogenous gene, the response element, and the receptor complex can also be incorporated as RNA molecules, preferably in the form of functional viral RNAs such as tobacco mosaic virus. Of the eucaryotic cells, vertebrate cells are preferred because they naturally lack the molecules which confer responses to the ligands of this invention for the EcR. As a result, they are "substantially insensitive" to the ligands of this invention. Thus, the ligands useful in this invention will have negligible physiological or other effects on transformed cells, or the whole organism. Therefore, cells can grow and express the desired product, substantially unaffected by the presence of the ligand itself.

[0130] The term "subject" means an intact insect, plant or animal. It is also anticipated that the ligands will work equally well when the subject is a fungus or yeast. When the subject is an intact animal, preferably the animal is a vertebrate, most preferably a mammal.

[0131] EcR ligands, when used with a LDTFC, *e.g.*, an EcR complex, which in turn is bound to the response element linked to an exogenous gene (*e.g.*, a reporter gene), provide the means for external temporal regulation of expression of the exogenous gene. The order in which the various components bind to each other, that is, ligand to receptor complex and receptor complex to response element, is not critical. Typically, modulation of expression of the exogenous gene is in response to the binding of a LDTFC, *e.g.*, an EcR complex, to a specific control, or regulatory, DNA element. The EcR protein, like other members of the nuclear receptor family, possesses at least three domains, an AD, a DBD, and a LBD. This receptor, like a subset of the nuclear receptor family, also possesses less well-defined regions responsible for heterodimerization properties (referred to herein as a "heterodimerization partner" or "HP"). Binding of the ligand to the ligand binding domain of a LTF, *e.g.*, an EcR protein, after heterodimerization with a CAP

including, *e.g.*, an AD and/or an HP, *e.g.*, a USP or RXR protein, enables the DNA binding domains of the heterodimeric proteins to bind to the response element in an activated form, thus resulting in expression or suppression of the exogenous gene. This mechanism does not exclude the potential for ligand binding to individual subunits, *e.g.*, LTF or CAP, *e.g.*, an EcR or USP, and the resulting formation of active homodimer complexes (*e.g.* EcR+EcR or USP+USP). In one embodiment, one or more of the receptor domains can be varied producing a chimeric gene switch. Typically, one or more of the three domains may be chosen from a source different than the source of the other domains so that the chimeric receptor is optimized in the chosen host cell or organism for transactivating activity, complementary binding of the ligand, and recognition of a specific response element. In addition, the response element itself can be modified or substituted with response elements for other DNA binding protein domains such as the GAL-4 protein from yeast (see Sadowski *et al.*, *Nature 335*:563 (1988) or LexA protein from *E. coli* (see Brent *et al.*, *Cell 43*:729 (1985)) to accommodate chimeric LDTFCs, *e.g.*, EcR complexes. Another advantage of chimeric systems is that they allow choice of a promoter used to drive the exogenous gene according to a desired end result. Such double control can be particularly important in areas of gene therapy, especially when cytotoxic proteins are produced, because both the timing of expression as well as the cells wherein expression occurs can be controlled. When exogenous genes, operatively linked to a suitable promoter, are introduced into the cells of the subject, expression of the exogenous genes is controlled by the presence of the ligand of this invention. Promoters may be constitutively or inducibly regulated or may be tissue-specific (that is, expressed only in a particular type of cell) or specific to certain developmental stages of the organism.

[0132] Numerous genomic and cDNA nucleic acid sequences coding for a variety of polypeptides, such as transcription factors and reporter genes, are well known in the art. Those skilled in the art have access to nucleic acid sequence information for virtually all known genes and can either obtain the nucleic acid molecule directly from a public depository, the institution that published the sequence, or employ routine methods to prepare the molecule.

[0133] For *in vivo* use, the ligands described herein may be taken up in pharmaceutically acceptable carriers, such as, for example, solutions, suspensions, tablets, capsules,

ointments, elixirs, and injectable compositions. Pharmaceutical compositions may contain from 0.01 % to 99% by weight of the ligand. Compositions may be either in single or multiple dose forms. The amount of ligand in any particular pharmaceutical composition will depend upon the effective dose, that is, the dose required to elicit the desired gene expression or suppression.

[0134] Suitable routes of administering the pharmaceutical preparations include oral, rectal, topical (including dermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) and by naso-gastric tube. It will be understood by those skilled in the art that the preferred route of administration will depend upon the condition being treated and may vary with factors such as the condition of the recipient.

[0135] One embodiment of the invention comprises methods for treating, ameliorating, or preventing a disease, disorder, or condition in a subject, comprising:

(a) introducing into cells of said subject (1) a polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor complex, operably linked to a therapeutic switch promoter, wherein the promoter is activated during said disease, disorder, or condition, and (2) a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide linked to a promoter which is activated by said ligand-dependent transcription factor complex; and

(b) administering ligand to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide;

wherein said therapeutic polypeptide or therapeutic polynucleotide is expressed at a level sufficient to treat, ameliorate, or prevent said disease, disorder, or condition.

[0136] One embodiment of the invention comprises methods for treating, ameliorating, or preventing a disease, disorder, or condition in a subject, comprising:

(a) introducing into a subject (1) a first polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor complex through operable association with a therapeutic switch promoter, and (2) a second polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide

operably associated with a factor-regulated promoter which is activated by said ligand-dependent transcription factor complex, wherein said first and second polynucleotides are introduced so as to permit expression of said ligand-dependent transcription factor complex; and

(b) administering ligand to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0137] One embodiment of the invention comprises methods for expressing a therapeutic polypeptide or therapeutic polynucleotide in a subject, comprising:

(a) introducing into a subject (1) a first polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor complex through operable association with a therapeutic switch promoter, and (2) a second polynucleotide encoding said therapeutic polypeptide or therapeutic polynucleotide operably associated with a factor-regulated promoter which is activated by said ligand-dependent transcription factor complex, wherein said first and second polynucleotides are introduced so as to permit expression of said ligand-dependent transcription factor complex; and

(b) administering ligand to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0138] In certain embodiments, the therapeutic switch promoter described in the methods is consititutive. In certain embodiments, the therapeutic switch promoter is activated under conditions associated with a disease, disorder, or condition, *e.g.*, the promoter is activated in response to a disease, in response to a particular physiological, developmental, differentiation, or pathological condition, and/or in response to one or more specific biological molecules; and/or the promoter is activated in particular tissue or cell types. In certain embodiments, the disease, disorder, or condition is responsive to the therapeutic polypeptide or polynucleotide. For example in certain non-limiting embodiments the therapeutic polynucleotide or polypeptide is useful to treat, prevent, ameliorate, reduce symptoms, prevent progression, or cure the disease, disorder or condition, but need not accomplish any one or all of these things. In certain embodiments, the first and second polynucleotides are introduced so as to permit expression of the ligand-dependent transcription factor complex under consitions

associated with a disease, disorder or condition. In one embodiment, the therapeutic methods are carried out such that the therapeutic polypeptide or therapeutic polynucleotide is expressed and disseminated through the subject at a level sufficient to treat, ameliorate, or prevent said disease, disorder, or condition. As used herein, "disseminated" means that the polypeptide is expressed and released from the modified cell sufficiently to have an effect or activity in the subject. Dissemination may be systemic, local or anything in between. For example, the therapeutic polypeptide or therapeutic polynucleotide might be systemically disseminated through the bloodstream or lymph system. Alternatively, the therapeutic polypeptide or therapeutic polynucleotide might be disseminated locally in a tissue or organ to be treated.

[0139] In one embodiment, the therapeutic methods are carried out by administering compositions of the invention, such as the first and second polynucleotides described above, directly to the subject to be treated, such that the polynucleotides are taken up, *in vivo*, by cells of the subject to be treated, and one or more therapeutic polypeptides or polynucleotides will be expressed by those cells under appropriate conditions, as described in detail elsewhere herein. Polynucleotides may be directly delivered to a subject to be treated by a variety of methods including, without limitation, viral vectors, *e.g.*, retroviral vectors, adeno-associated virus vectors, pox virus vectors, *e.g.*, vaccinia virus vectors, baculovirus vectors, herpes virus vectors, *e.g.*, herpes simplex vectors or Epstein-Barr virus vectors, adenovirus vectors, geminivirus vectors, or caulimovirus vectors; non-viral vectors such as plasmids, which may be delivered, for example complexed with liposomes, electrically charged lipids (cytofectins), biopolymers or as DNA-protein complexes.

[0140] In another embodiment, the therapeutic methods are carried out by introducing the compositions of the invention, such as the first and second polynucleotides described above, into the subject to be treated contained in one or more modified cells. Following administration of the modified cells the one or more therapeutic polypeptides or polynucleotides are expressed by the modified cells under appropriate conditions, as described in detail elsewhere herein. The term "modified cell" refers to a cell or cells into which at least a first and second polynucleotide as described above have been inserted. As such, "a modified cell" refers to the cell harboring the first and second polynucleotides, which may or may not be a cell from, or related to, the subject to be

treated. Such cells are included in the definition of "bioreactors" or "bioreactor devices" as described herein. As defined herein, however, a "bioreactor" or "bioreactor device" need not be not a modified cell, rather, a bioreactor or bioreactor device as defined herein is any cell or cells intended to secrete a therapeutic protein or therapeutic polynucleotide, whether or not the cell(s) are "modified cells."

[0141] In one embodiment, the therapeutic methods are carried out by introducing the compositions of the invention, such as the first and second polynucleotides described above, into cells that have been isolated from said subject, *i.e.*, autologous cells, to produce modified cells, and the modified cells are re-introduced into said subject.

[0142] Alternatively, modified cells may be prepared by introducing the compositions of the invention, such as the first and second polynucleotides described above, into cells which are not isolated from the subject, *i.e.*, they are non-autologous relative to the subject, to produce modified non-autologous (MNA) cells. Such MNA cells may be allogeneic relative to the subject to be treated, *i.e.*, they are derived from a genetically non-identical member of the same species as the subject. For example, in treating a human subject, the cells would be human cells, but not directly derived from the subject to be treated. Alternatively, MNA cells may be xenogeneic relative to the subject to be treated. *i.e.*, they are derived from a different species than the subject to be treated. For example, in treating a human subject the cells might be mouse cells, monkey cells, or pig cells.

[0143] MNA cells suitable for use in the present invention may be generated from any number of cells types, including, but not limited to immortalized cells, primary cells, and cells capable of terminal differentiation. Non-limiting examples of cells suitable for generating MNA modified cells for the present invention include C2C12 mouse myoblast cells, HEK293 human embryonic kidney cells, ARPE-19 cells, hMSC cells, pancreatic islet cells, MDCK cell, BHK cell, hybridoma cell CHO cell, an astrocyte derived cell, an oligodendrocyte derived cell, a myoblast derived cell, a parathyroid derived cell. In a specific embodiment where pancreatic islet cells are used to generate modified cells to treat a human subject, the pancreatic islet cells may be xenogeneic, *e.g.*, porcine islet cells, or allogeneic, *e.g.*, human islet cells derived from cadavers.

[0144] In one embodiment, the therapeutic methods are carried out *in vivo*.

[0145] In one embodiment, the polynucleotide encoding the gene switch and the polynucleotide encoding the therapeutic polypeptide or therapeutic polynucleotide linked to a promoter are part of one larger polynucleotide, *e.g.*, a vector. In another embodiment, the polynucleotide encoding the gene switch and the polynucleotide encoding the therapeutic polypeptide or therapeutic polynucleotide linked to a promoter are separate polynucleotides, which may be combined to form a "nucleic acid composition.".

[0146] In certain embodiments, a bioreactor of the invention comprises modified or non-modified cells surrounded by a barrier (*e.g.*, encapsulated) prior to being introduced into the subject. Such a bioreactor may be used with any subject instead of having to modify autologous cells from each individual. Cellular encapsulation methods have been used to immunoisolate cells while allowing, either selectively or unselectively, the release of desired biological materials. It may be desirable to provide encapsulation compositions and methods for making them, which are capable of providing improved structural characteristics and/or immune protection. Such compositions and methods may find use, where encapsulated cells can withstand mechanical, chemical or immune destruction within the subject to be treated, and would additionally provide for free permeability to nutrients, ions, oxygen, and other materials needed to both maintain the tissue and support normal metabolic functions, but impermeable to bacteria, lymphocytes, and large proteins of the type responsible for immunochemical reactions. Barriers suitable for use in the present invention allow dissemination of a therapeutic protein or therapeutic polynucleotide expressed by modified or non-modified cells contained within the barrier, but prevent direct contact of the cells with cells of the subject's immune system. The barrier may also function to prevent non-autologous or autologous modified or non-modified cells from escaping from the site of introduction, *e.g.*, rogue cells that might cause harm to the subject if allowed to circulate. In one embodiment the barrier is a selectively permeable barrier, *e.g.*, a barrier that is permeable to small molecules such as hormones and small peptides but impermeable to larger polypeptides such as antibodies. For example, the barrier may be impermeable to molecules with a molecular weight greater than about 100,000, about 50,000, about 25,000, about 10,000, about 5,000 or about 1,000 daltons.

[0147] Any number of barrier systems are suitable for use in the present invention. In one embodiment, for example, the barrier comprises a conformal coating which encases one or more cells. Typically a conformal coating is made of a polymer material, *e.g.*, polyethylene glycol or hydroxyethyl methacrylate-methyl methacrylate (HEMA-MMA). Conformal coatings typically enclose a small number of modified cells, *e.g.*, 1-10 cells, 1-20 cells, 1-30 cells 1-50 cells 1-70 cells or 1-90 cells. *See, e.g.*, Shoichet MS, Winn SR., *Adv Drug Delivery Rev. 42*:81-102 (2000), which is incorporated herein by reference in its entirety.

[0148] In other embodiments, a barrier system suitable for use in the present invention comprises a bioreactor, which comprises encapsulated cells. Two non-limiting encapsulation methods, microencapsulation and macroencapsulation, are known in the art. Typically, in microencapsulation, the cells are suspended in a biologically compatible encapsulation material which is then shaped into bead-like structures, whereas in macroencapsulation the device is generally manufactured prior to the addition of cells and can be composed of one or more synthetic membranes. As compared to conformal coatings, barrier systems comprising encapsulated cells tend to be more uniform in size, and tend to have uniform pore size allowing better control of protein dissemination. For encapsulation, living cells and other sensitive materials may be treated under sufficiently mild conditions allowing the cells or biomaterial to remain substantially unaffected by the encapsulation process, yet permitting the formation of a capsule of sufficient strength to exist over long periods of time.

[0149] Living cell(s) can be encapsulated and the resulting encapsulated cell(s) maintain long term *in vivo* activity by encapsulating the cells within a biocompatible semi-permeable membrane. One way to increase biocompatibility is to add an outer surface of biocompatible negatively-charged material. The term "biocompatible" as used herein refers collectively to both the intact capsule and its contents. Specifically, it refers to the capability of the implanted intact encapsulated cell to avoid detrimental effects of the body's various protective systems, such as immune system or foreign body fibrotic response, and remain functional for a significant period of time.

[0150] Bioreactors comprising encapsulated cells which are suitable for use in the present invention are especially useful for the administration of cells to an animal, wherein the immune response of the animal towards the cell is to be minimized. Cells which produce

antibodies, enzymes, and other bioactive materials can also be administered. The small size of the resulting encapsulated cells within the subject of the invention facilitate administration of the microcapsules by injection, implantation or transplantation into a subject.

[0151] Living cells can be encapsulated in a variety of gels, *e.g.*, alginate, to form implantable bead-like structures, *e.g.*, microbeads or microspheres to physically isolate the cells once implanted into a subject to be treated. To prevent entry of smaller molecular weight substances such as antibodies and complement (with a molecular weight of about 150 kDa) into these bead-like structures, they can be coated with a material such as poly-L-lysine, chitosan, or PAN-PVC, which provides an outer shell with a controlled pore size or they can be treated by *e.g.*, cross-linking, to control their internal porosity. Additional examples of useful materials include conventional biocompatible materials made up of natural or synthetic polymers or co-polymers, such as poly-L-lysine-alginate, collagen, gelatin, laminin, methyl methacrylate, hydroxyethyl methacrylate, MATRIGEL, VIRTOGEN, polyvinylalcohol, agarose, polyethylene glycol, hydrogels, polylactic acid, polyglycolic acid, poly(lactide-co-glycolide), polyhydroxybutyrate-polyhydroxyvalerate, copolymer, poly(lactide-co-caprolactone), polyesteramides, polyorthoesters, poly 13-hydroxybutyric acid, polyanhydrides, polyethylene terephthalate, polyetrafluoroethylene, pllyacrylates (including acrylic copolymers), polyvinylidenes, polyvinyl chloride copolymers, polyurethanes, polystyrenes, polyamides, cellulose acetates, cellulose nitrates, polysulfones (including polyether sulfones), polyphosphazenes, polyacrylonitriles, and poly(acrylonitrile/covinyl chloride).

[0152] One form of encapsulation is microencapsulation, which involves suspension of the cells in a liquid or gelatinous encapsulation material, which is then formed into a supporting particulate matrix, *e.g.*, a hydrogel matrix to form a bead-like structure, which serves as a core of an implantable device. The core maintains a proper cell distribution, provides strength, and enhances cell viability, longevity, and function. The core can also contribute to immunoisolation. It also protects the internal cells contained in the bead-like structures from direct cell-cell interactions that can elicit an undesirable immune response in the subject to be treated.

[0153] A barrier system may contain multiple layers, *e.g.*, where each layer serves a different purpose (*e.g.*, support, control of permeability). Barriers may comprise contrast agents or other properties that render the barrier imageable (*e.g.*, by x-ray, sonography, *etc.*) to ensure proper positioning of the implanted cells. Examples of barrier systems useful for cell implantation are described un U.S. Patent Nos. 7,226,978, RE39,542 (agarose), 6,960,351, 6,916,640, 6,911,227 (polyethylene glycol), 6,818,018, 6,808,705, 6,783,964, 6,762,959, 6,727,322, 6,610,668 (poly--14-N-acetylglucosamine (p-GlcNAc) polysaccharide), 6,558,665, RE38,027, 6,495,161, 6,368,612, 6,365,385, 6,337,008, 6,306,454 (polyalkylene), 6,303,355, 6,287,558 (gel super matrix), 6,281,015, 6,264,941, 6,258,870, 6,180,007, 6,126,936 (polyamine acid), 6,123,700, 6,083,523, 6,020,200, 5,916,790, 5,912,005, 5,908,623, 5,902,745, 5,858,746, 5,846,530 (polysaccaharides), 5,843,743, 5,837,747, 5,837,234, 5,834,274, 5,834,001, 5,801,033, 5,800,829, 5,800,828, 5,798,113, 5,788,988, 5,786,216, 5,773,286, 5,759,578, 5,700,848, 5,656,481, 5,653,975, 5,648,099, 5,550,178, 5,550,050, 4,806,355, 4,689,293, 4,680,174, 4,673,566, 4,409,331, 4,352,883, and U.S. Patent Application Publications 2006/0263405 (alginate/polymer) and 2004/0005302 (alignate-poly-L-lysine), each incorporated herein by references in its entirety.

[0154] In certain embodiments, a barrier system suitable for use in the present invention comprises microencapsulated cells. Microencapsulation generates approximately spherical and relatively uniform bead-like structures comprising encapsulated cells, where the bead-like structures are about 100-700 μm in diameter, *e.g.*, about 100, 200, 300, 400, 500, 600 or 700 μm in diameter. Microencapsulated cells of the invention may be produced using a variety of encapsulation materials as described above. In one embodiment, the encapsulation material comprises a hydrogel. In another embodiment the encapsulation material comprises a polymer. Suitable polymers include, without limitation, cellulose, *e.g.*, cellulose sulfate, and alginate. For example, one microcapsule of the invention comprises polyanionic alginate and a poly-cationic polymer to interact and form a physical permselective membrane barrier. An alternative method of microencapsulation comprises the formation of poly (L-lactide) acid (PLLA) or a poly-L-ornithine (PLO) alginate microspheres. *See, e.g.*, Darrabie, M.D. *et al. Biomaterials 26*:6846-6852 (2005) and Blasi, P. *et al. Int J Pharm. 324*:27-36 (2006). Alginate based microencapsulation materials may further contain ultra high viscosity (UHV) polymers,

which may also be biodegradable. *See, e.g.*, Zimmermann, U. *et al. Ann N Y Acad Sci. 944*:199-215 (2001).

[0155] Bioreactors of the present invention comprising microencapsulated cells typically comprise at least one up to about 1000 cells per "bead," *e.g.*, modified or non-modified cells intended to secrete a desired therapeutic polypeptide or polynucleotide as described herein. For example, a bioreactor of the invention which comprises microencapsulated cells may result in at least 50, at least 100, at least 200, at least 400, at least 500, at least 800 to about 1000 or more cells per "bead."

[0156] In certain embodiments, a bioreactor suitable for use in the present invention comprises cells enclosed in a macroencapsulation device. As compared to bioreactors comprising microencapsulated cells, bioreactors comprising macroencapsulation devices are typically larger and often non-spherical encapsulated cell entities, and may be composed of one or more synthetic membranes, *e.g.*, one, two, three, four, 8, 10, or more membranes, which may be the same composition of different compositions. As denoted by the name, macrocapsulated cell devices are of a size such that individual entities may be easily manipulated. For example, a typical macroencapsulation device may be an oblong shape, about 3mm, 4mm, 5mm, 6mm, 7mm, 8mm, 9mm, 10mm or more long and about 1 mm, 2 mm, 3 mm, 4 mm, or 5 mm or more in diameter. An exemplary but non-limiting macroencapsulation deviceof the invention is about 6 mm long and about 1 mm in diameter.

[0157] In certain embodiments a macroencapsulation device suitable for use in the present invention comprises two or more synthetic membranes, where the synthetic membranes have different pore sizes so as to regulate transit of therapeutic molecules through the device and their dissemination into the environment. In certain embodiments, a macroencapsulation device of the invention comprises a semi-permeable polymer outer membrane and an internal scaffold to support the cells. In non-limiting examples, the outer membrane comprises pores of about 15 nm to allow exchange of nutrients of therapeutic molecules. The internal scaffold may comprise any number of materials. In one non-limiting example the scaffold comprises poly (ethylene terephthalate) yarn (available from Neurotech (www.neurotechusa.com)).

[0158] In another non-limiting example, a macroencapsulaton device suitable for use in the invention comprises a polymeric membrane bilayer, where the bilayer comprises an

outer layer of 5μm poly(tetrafluoroethylene) (PTFE) membrane laminated onto an inner tighter pore 0.45 uM PTFE immunobarrier layer (available from Theracyte (www.theracyte.com)). Such a macroencapsulation device may further comprise a non-woven poly mesh layer exterior to said polymeric membrane bilayer. In yet another non-limiting example, a macroencapsulation device suitable for use in the invention is composed of polyethersulfone (PES) hollow fibers. *See, e.g.*, Li, Y., *et al. J. Membrane Sci. 245*:53-60 (2004).

[0159] Macroencapsulation devices suitable for use in the present invention may optionally have additional structures to allow convenient implantation into and retrieval from a subject to be treated. For example, a macroencapsulation device may comprise, without limitation, a suture clip, a loading port, a tether, or other structure for ease of use.

[0160] The interior space of macroencapsulation devices of the invention is typically suitable to comprise at least one up to about 10^5 cells, *e.g.*, modified or non-modified cells intended to secrete a desired therapeutic polypeptide or polynucleotide. For example, a macroencapsulation device of the invention may comprise at least 500, at least 1,000, at least 2,000, at least 4,000, at least 5,000, at least 8,000 to about 10,000 or more cells.

[0161] Some bioreactor devices, *e.g.*, encapsulated or coated modified or non-modified cells of the present invention intended to secrete a desired therapeutic polypeptide or polynucleotide, may further comprise protective cells, *e.g.*, within the barrier or capsule, where the protective cells are capable of providing protection to the modified or non-modified cells intended to secrete a desired therapeutic polypeptide or polynucleotide. Non-limiting examples of such protective cells include modified or non-modified sertoli cells and erythrocytes. Additionally, some bioreactor devices, *e.g.*, encapsulated or coated modified or non-modified cells of the present invention intended to secrete a desired therapeutic polypeptide or polynucleotide, may further comprise an outer coating capable of creating a more compatible or protective micro-environment. Exemplary, non-limiting micro-environments which may be created include an anti-inflammatory micro-environment and a pro-angiogenic micro-environment.

[0162] In still other embodiments, bioreactor devices of the present invention may include modified cells with a "safety-shutoff" mechanism. For example, modified cells contained in a bioreactor device may comprise a regulated suicide gene which encodes a

lethal polypeptide where the gene, upon activation, would induce destruction of the modified cell itself. For example, a modified cell might be programmed to die if it escapes from a barrier system, or if it undergoes oncogenic conversion. Non-limiting examples of lethal polypeptides suitable for use in the present invention are described in more detail below.

[0163] The subject on which the therapeutic methods are carried out may be any subject for which treatment or prevention is desired. For example, the subject may be one that is exhibiting one or more symptoms of a disease, disorder, or condition. The subject may also be one that is predisposed to a disease, disorder, or condition, *e.g.*, due to genetics, family history, or environmental exposure. The subject may be a member of the general public, *e.g.*, as part of a preventative immunization against a disease, disorder, or condition in a population.

[0164] The disease, disorder, or condition to be treated or prevented by the methods of the invention may be any disease, disorder, or condition for which one or more therapeutic switch promoters is available. Examples of diseases or disorders which may be treated or prevented by the methods of the invention include, without limitation, hyperproliferative diseases, disorders, or conditions (*e.g.*, cancer), cardiovascular diseases, disorders, or conditions, neural diseases, disorders, or conditions, autoimmune diseases, disorders, or conditions, bone diseases, disorders, or conditions, gastrointestinal diseases, disorders, or conditions, blood diseases, disorders, or conditions, metabolic diseases, disorders, or conditions, inflammatory diseases, disorders, or conditions, and infectious diseases, disorders, or conditions.

[0165] The therapeutic switch promoters of the invention may be any promoter that is useful for treating, ameliorating, or preventing a specific disease, disorder, or condition. Examples include, without limitation, promoters of genes that exhibit increased expression only during a specific disease, disorder, or condition and promoters of genes that exhibit increased expression under specific cell conditions (*e.g.*, proliferation, apoptosis, change in pH, oxidation state, oxygen level). In some embodiments where the gene switch comprises more than one transcription factor sequence, the specificity of the therapeutic methods can be increased by combining a disease- or condition- specific promoter with a tissue- or cell type-specific promoter to limit the tissues in which the

therapeutic product is expressed. Thus, tissue- or cell type-specific promoters are encompassed within the definition of therapeutic switch promoter.

[0166] As an example of disease-specific promoters, useful promoters for treating cancer include the promoters of oncogenes. Examples of classes of oncogenes include, but are not limited to, growth factors, growth factor receptors, protein kinases, programmed cell death regulators and transcription factors. Specific examples of oncogenes include, but are not limited to, sis, erb B, erb B-2, ras, abl, myc and bcl-2 and TERT. Examples of other cancer-related genes include tumor associated antigen genes and other genes that are overexpressed in neoplastic cells (*e.g.*, MAGE-1, carcinoembryonic antigen, tyrosinase, prostate specific antigen, prostate specific membrane antigen, p53, MUC-1, MUC-2, MUC-4, HER-2/neu, T/Tn, MART-1, gp100, GM2, Tn, sTn, and Thompson-Friedenreich antigen (TF)).

[0167] Examples of promoter sequences and other regulatory elements (*e.g.*, enhancers) that are known in the art and are useful as therapeutic switch promoters in the present invention are disclosed in the references listed in Tables 1 and 2, along with the disease/disorder (Table 1) or tissue specificity (Table 2) associated with each promoter. The promoter sequences disclosed in these references are herein incorporated by reference in their entirety.

Table 1

Promoter Sequence	Disease/Disorder	Patent/Published Application No.
Her-2/neu (ERBB2/c-erbB-2)	cancer	5,518,885
osteocalcin	calcified tumors	5,772,993
stromelysin-1	cancer	5,824,794
prostate specific antigen	prostate cancer	5,919,652
human sodium-iodide symporter	thyroid carcinoma	6,015,376
H19, IF-1, IGF-2	cancer	6,306,833
thymosin β15	breast, pancreatic, prostate cancer	6,489,463
T cell factor	cancer	6,608,037
cartilage-derived retinoic acid-sensitive protein	chondrosarcoma, mammary tumor	6,610,509

Promoter Sequence	Disease/Disorder	Patent/Published Application No.
insulin	pancreatic cancer	6,716,824
PEG-3	cancer	6,737,523
telomerase reverse transcriptase	cancer	6,777,203
melanoma differentiation associated gene-7	cancer	6,841,362
prostasin	cancer	6,864,093
telomerase catalytic subunit; cyclin-A	cancer	6,936,595
midkine; c-erbB-2	cancer	7,030,099
prostate-specific membrane antigen	prostate cancer	7,037,647
p51	cancer	7,038,028
telomerase RNA	cancer	7,084,267
prostatic acid phosphatase	prostate cancer	7,094,533
$PCA3_{dd3}$	prostate cancer	7,138,235
DF3/MUC1	cancer	7,247,297
hex II	cancer	2001/0011128
cyclooxygenase-2	cancer	2002/0107219
super PSA	prostate cancer	2003/0078224
skp2	cancer	2003/0109481
PRL-3	metastatic colon cancer	2004/0126785
CA125/M17S2	ovarian cancer	2004/0126824
IAI.3B	ovarian cancer	2005/0031591
CRG-L2	liver cancer	2005/0124068
TRPM4	prostate cancer	2006/0188990
RTVP	glioma	2006/0216731
TARP	prostate cancer, breast cancer	2007/0032439
telomere reverse transcriptase	cancer	2007/0059287
A4 amyloid protein	Alzheimer's disease	5,151,508
amyloid β-protein precursor	Alzheimer's disease	5,643,726
precursor of the Alzheimer's Disease A4 amyloid protein	Alzheimer's disease	5,853,985

Promoter Sequence	Disease/Disorder	Patent/Published Application No.
neuropeptide FF	CNS disorders	6,320,038
endoplasmic reticulum stress elements	stress	7,049,132
urocortin II	psychopathologies	7,087,385
tyrosine hydroxylase	neurological disorders	7,195,910
complement factor 3; serum amyloid A3	inflammation	5,851,822
tissue inhibitor of metalloproteinase-3 (TIMP-3)	rheumatism, cancer, autoimmune disease, inflammation	5,854,019
p75 tumor necrosis factor receptor	autoimmune disease	5,959,094
tumor necrosis factor-α	inflammation	6,537,784
peroxisome proliferator activated receptor/IIA-1 nonpancreatic secreted phospholipase A2	inflammation	6,870,044
SOCS-3	growth disorders, autoimmune disease, inflammation	2002/0174448
SR-BI	lipid disorders	5,965,790
Ob	obesity	5,698,389
site-1 protease	obesity, diabetes	7,045,294
TIGR	glaucoma	7,138,511
VL30	anoxia	5,681,706
excitatory amino acid transporter-2	nervous system ischemia	2004/0171108
MDTS9	renal failure	2006/0014931
LIM, pyrroline 5-carboxylate reductase, SIM2	prostate disorders	2006/0134688
Bax	apoptosis	5,744,310
fas	apoptosis	5,888,764
bbc3	apoptosis	7,202,024
PINK-1	PI-3 kinase/Akt pathway disorders	2006/0228776

Table 2

Promoter Sequence	Tissue Specificity	Patent/Published Application No.
troponin T	skeletal muscle	5,266,488
myoD	muscle	5,352,595
actin	muscle	5,374,544
smooth muscle 22α	arterial smooth muscle	5,837,534
utrophin	muscle	5,972,609
myostatin	muscle	6,284,882
smooth muscle myosin heavy chain	smooth muscle	6,780,610
cardiac ankyrin repeat protein	cardiac muscle	7,193,075
MLP	muscle	2002/0042057
smoothelin	smooth muscle	2003/0157494
MYBPC3	cardiomyocytes	2004/0175699
Tα1 α-tubulin	neurons	5,661,032
intercellular adhesion molecule-4 (ICAM-4)	neurons	5,753,502
γ-aminobutyric acid type A receptor β1 subunit	hippocampus	6,066,726
neuronal nicotinic acetylcholine receptor β2-subunit	neurons	6,177,242
presenilin-1	neurons	6,255,473
calcium-calmodulin-dependent kinase IIα	forebrain	6,509,190
$CRF_{2\alpha}$ receptor	brain	7,071,323
nerve growth factor	neurons	2003/ 159159
GLP-2 receptor	gut, brain	2002/0045173
type I transglutaminase	keratinocytes	5,643,746
K14	keratinocytes	6,596,515
stearoyl-CoA desaturase	skin	2002/0151018
megsin	renal cells	6,790,617
prolactin	pituitary	5,082,779
GDF-9	ovary, testes, hypothalamus, pituitary, placenta	7,227,013

Promoter Sequence	Tissue Specificity	Patent/Published Application No.
PSP94	prostate	2003/0110522
NRL; NGAL	mammary gland	5,773,290
long whey acidic protein	mammary gland	5,831,141
mammary associated amyloid A	mammary ductal epithelial cells	2005/0107315
endothelin-1	endothelial cells	5,288,846
serglycin	hematopoietic cells	5,340,739
platelet-endothelial cell adhesion molecule-1 (PECAM-1)	platelets, leukocytes, endothelial cells	5,668,012
Tie receptor tyrosine kinase	endothelial cells, bone marrow	5,877,020
KDR/flk-1	endothelial cells	5,888,765
endoglin	endothelial cells	6,103,527
CCR5	myeloid and lymphoid cells	6,383,746
CD11d	myeloid cells	6,881,834
platelet glycoprotein IIb	hematopoietic cells	6,884,616
preproendothelin-1	endothelial cells	7,067,649
interleukin-18 binding protein	mononuclear cells	2006/0239984
CD34	hematopoietic stem cells	5,556,954
Tec tyrosine kinase	hematopoietic stem cells, liver	6,225,459

[0168] Other genes that exhibit changes in expression levels during specific diseases or disorders and therefore may provide promoters that are useful in the present invention include, without limitation, the genes (along with the associated disease/disorder) listed in Table 3.

Table 3

Gene	Disease/Disorder	Patent/Published Application No.
MLH1, MSH2, MSH6, PMS1, APC	Colorectal cancer	7,148,016
LEF-1	Colon cancer	2002/0169300
F_2 receptor	Colon cancer	2002/0187502
TGF-β type II receptor	Colon cancer	2004/0038284
EYA4	Colon cancer	2005/0003463
PCA3	Prostate cancer	7,138,235
K2	Prostate cancer	6,303,361
PROST 03	Prostate cancer metastases	2002/0009455
PCAM-1	Prostate cancer	2002/0042062
PCADM-1	Prostate cancer	2003/0100033
$PCA3_{dd3}$	Prostate cancer	2003/0165850
PCAV	Prostate cancer	2006/0275747
PAcP	Androgen-insensitive prostate cancer	2006/0294615
SEQ ID NO: 1 of the patent 5,866,329, incorporated by reference herein	Liver cancer	5,866,329
SEQ ID NOS: 1, 3 of the U.S. patent application publication 2002/0115094, incorporated by reference herein	Hepatocellular cancer	2002/0115094
SEQ ID NO: 1 of the patent U.S. application publication 2005/0037372, incorporated by reference herein	Hepatocellular carcinoma	2005/0037372
ATB_0	Hepatocellular carcinoma	2006/0280725
SEQ ID NOS: 1, 3 of the U.S. patent application publication 2007/0042420, incorporated by reference herein	Liver cancer	2007/0042420
CSA-1	Chondrosarcoma	2001/0016649
SEQ ID NOS: 1-15 of the U.S. patent application publication 2001/0016651, incorporated by reference herein	Pancreatic cancer	2001/0016651

Gene	Disease/Disorder	Patent/Published Application No.
SEQ ID NOS: 1-15 of the U.S. patent application publication 2003/0212264, incorporated by reference herein	Pancreatic cancer	2003/0212264
SYG972	Breast cancer	2002/0055107
Urb-ctf	Breast cancer	2003/0143546
BCU399	Breast cancer	2003/0180728
TBX2	Breast cancer	2004/0029185
Cyr61	Breast cancer	2004/0086504
DIAPH3	Breast cancer	2005/0054826
SEQ ID NOS: 1-24 of the U.S. patent application publication 2007/0134669, incorporated by reference herein	Breast cancer	2007/0134669
Human aspartyl (asparaginyl) beta-hydroxylase	CNS cancer	2002/0102263
BEHAB	CNS cancer	2003/0068661
IL-8	Kaposi's Sarcoma	2003/0096781
SEQ ID NOS: 1-278 of the U.S. patent application publication 2002/0198362, incorporated by reference herein	Hematological cancers	2002/0198362
BLSA	B-cell cancer	2003/0147887
BP1	Leukemia	2003/0171273
DAP-kinase, HOXA9	Non-small cell lung cancer	2003/0224509
ARP	Clear cell renal carcinoma, inflammatory disorders	2004/0010119
Nbk	Renal cancer	2005/0053931
CD43	Ovarian cancer	2006/0216231
SEQ ID NOS: 1-84 of the U.S. patent application publication 2007/0054268, incorporated by reference herein	Ovarian cancer	2007/0054268
β7-hcG, β6-hCG, β6e-hCG, β5-hCG, β8-hcG, β3-hCG	Uterine tumors	2006/0292567

Gene	Disease/Disorder	Patent/Published Application No.
MTA1s	Hormone insensitive cancer	2006/0204957
Old-35, Old-64	Tumor proliferation	2003/0099660
LAGE-1	Cancer	6,794,131
CIF150/hTAF$_{II}$150	Cancer	6,174,679
P65 oncofetal protein	Cancer	5,773,215
Telomerase	Cancer	2002/0025518
CYP1B1	Cancer	2002/0052013
14-3-3σ	Cancer	2002/0102245
NES1	Cancer	2002/0106367
CAR-1	Cancer	2002/0119541
HMGI, MAG	Cancer	2002/0120120
ELL2	Cancer	2002/0132329
Ephrin B2	Cancer	2002/0136726
WAF1	Cancer	2002/0142442
CIF130	Cancer	2002/0143154
C35	Cancer	2002/0155447
BMP2	Cancer	2002/0159986
BUB3	Cancer	2002/0160403
Polymerase kappa	Cancer	2003/0017573
EAG1, EAG2	Cancer	2003/0040476
SEQ ID NOS: 18, 20, 22 of the U.S. patent application publication 2003/0044813, incorporated by reference herein	Cancer	2003/0044813
HMG I	Cancer	2003/0051260
HLTF	Cancer	2003/0082526
Barx2	Cancer	2003/0087243
SEQ ID NOS: 18, 20, 22, 32, 34, 36of the U.S. patent application publication 2003/0108920, incorporated by reference herein	Cancer	2003/0108920
Cables	Cancer	2003/0109443

Gene	Disease/Disorder	Patent/Published Application No.
Pp 32r1	Cancer	2003/0129631
BMP4	Cancer	2003/0134790
TS10q23.3	Cancer	2003/0139324
Nuclear spindle-associating protein	Cancer	2003/0157072
PFTAIRE	Cancer	2003/0166217
SEMA3B	Cancer	2003/0166557
MOGp	Cancer, multiple sclerosis, inflammatory disease	2003/0166898
Fortilin	Cancer	2003/0172388
SEQ ID NO: 1 of the U.S. patent application publication 2003/0215833, incorporated by reference herein	Cancer	2003/0215833
IGFBP-3	Cancer	2004/0005294
Polyhomeotic 2	Cancer	2004/0006210
PNQALRE	Cancer	2004/0077009
SEQ ID NOS: 1, 3 of the U.S. patent application publication 2004/0086916, incorporated by reference herein	Cancer	2004/0086916
SCN5A	Cancer	2004/0146877
miR15, miR16	Cancer	2004/0152112
Headpin	Cancer	2004/0180371
PAOh1/SMO	Cancer	2004/0229241
Hippo, Mst2	Cancer	2005/0053592
PSMA-like	Cancer, neurological disorders	2005/0064504
JAB1	Cancer	2005/0069918
NF-AT	Cancer	2005/0079496
P28ING5	Cancer	2005/0097626
MTG16	Cancer	2005/0107313
ErbB-2	Cancer	2005/0123538
HDAC9	Cancer	2005/0130146
GPBP	Cancer	2005/0130227

Gene	Disease/Disorder	Patent/Published Application No.
MG20	Cancer	2005/0153352
KLF6	Cancer	2005/0181374
ARTS1	Cancer	2005/0266443
Dock 3	Cancer	2006/0041111
Annexin 8	Cancer	2006/0052320
MH15	Cancer	2006/0068411
DELTA-N p73	Cancer	2006/0088825
RapR6	Cancer	2006/099676
StarD10	Cancer	2006/0148032
Ciz1	Cancer	2006/0155113
HLJ1	Cancer	2006/0194235
RapR7	Cancer	2006/0240021
A34	Cancer	2006/0292154
Sef	Cancer	2006/0293240
Killin	Cancer	2007/0072218
SGA-1M	Cancer	2007/0128593
TGFβ Type II receptor	Cancer	2002/0064786
GCA-associated genes	Giant cell arteritis	6,743,903
PRV-1	Polycythemia vera	6,686,153
SEQ ID NOS: 2, 4 of the U.S. patent 5,948,637, incorporated by reference herein	Ischemia	5,948,637
Vezf1	Vascular disorders	2002/0023277
MLP	Dilatative cardiomyopathy	2002/0042057
VEGI	Pathological angiogenesis	2002/0111325
PRO256	Cardiovascular disorders	2002/0123091
AOP2	Atherosclerosis	2002/0142417
Remodelin	Arterial restenosis, fibrosis	2002/0161211
Phosphodiesterase 4D	Stroke	2003/0054531
Prostaglandin receptor subtype EP3	Peripheral arterial occlusive disease	2003/0157599
CARP	Heart disorders	2004/0014706

Gene	Disease/Disorder	Patent/Published Application No.
HOP	Congenital heart disease	2004/0029158
SEQ ID NOS: 1-4 of the U.S. patent application publication 2004/0087784, incorporated by reference herein	Apoplexy	2004/0087784
PLTP	Atherosclerosis, vascular disease, hypercholesterolemia, Tangier's disease, familial HDL deficiency disease	2006/0252787
SEQ ID NOS: 1, 3-8, 15, 16 of the U.S. patent application publication 2007/0160996, incorporated by reference herein	Thrombosis	2007/0160996
UCP-2	Stroke	2002/0172958
FLJ11011	Fanconi's Anemia	2006/0070134
Codanin-1	Anemia	2006/0154331
SEQ ID NOS: 1, 6, 8 of the U.S. patent 5,763,591, incorporated by reference herein	Insulin-dependent diabetes mellitus	5,763,591
Resistin	Type II diabetes	2002/0161210
Archipelin	Diabetes	2003/0202976
SEQ ID NOS: 2, 7, 16, 27 of the U.S. patent application publication 2004/0053397, incorporated by reference herein	Diabetes, hyperlipidemia	2004/0053397
Neuronatin	Metabolic disorders	2004/0259777
Ncb5or	Diabetes	2005/0031605
7B2	Endocrine disorders	2005/0086709
PTHrP, PEX	Metabolic bone diseases	2005/0113303
KChIP1	Type II diabetes	2005/0196784
SLIT-3	Type II diabetes	2006/0141462
CX3CR1	Type II diabetes	2006/0160076
SMAP-2	Diabetes	2006/0210974

Gene	Disease/Disorder	Patent/Published Application No.
SEQ ID NOS: 2, 8, 12, 16, 22, 26, 28, 32 of the U.S. patent application publication 2006/0228706, incorporated by reference herein	Type II diabetes	2006/0228706
IC-RFX	Diabetes	2006/0264611
E2IG4	Diabetes, insulin resistance, obesity	2007/0036787
SEQ ID NOS: 2, 8, 10, 14, 18, 24, 26, 30, 34, 38, 44, 50, 54, 60, 62, 68, 74, 80, 86, 92, 98, 104, 110 of the U.S. patent application publication 2007/0122802, incorporated by reference herein	Diabetes	2007/0122802
UCP2	Body weight disorders	2002/0127600
Ob receptor	Body weight disorders	2002/0182676
Ob	Bodyweight disorders	2004/0214214
Dp1	Neurodegenerative disorders	2001/0021771
NRG-1	Schizophrenia	2002/0045577
Synapsin III	Schizophrenia	2002/0064811
NRG1AG1	Schizophrenia	2002/0094954
AL-2	Neuronal disorders	2002/0142444
Proline dehydrogenase	Bipolar disorder, major depressive disorder, schizophrenia, obsessive compulsive disorder	2002/0193581
MNR2	Chronic neurodegenerative disease	2002/0197678
ATM	Ataxia-telangiectasia	2004/0029198
Ho-1	Dementing diseases	2004/0033563
CON202	Schizophrenia	2004/0091928
Ataxin-1	Neurodegenerative disorders	2004/0177388
NR3B	Motor neuron disorders	2005/0153287
NIPA-1	Hereditary spastic paraplegia	2005/0164228

Gene	Disease/Disorder	Patent/Published Application No.
DEPP, adrenomedullin, csdA	Schizophrenia	2005/0227233
Inf-20	Neurodegenerative diseases	2006/0079675
EOPA	Brain development and degeneration disorders	2007/0031830
SERT	Autism	2007/0037194
FRP-1	Glaucoma	2002/0049177
Serum amyloid A	Glaucoma	2005/0153927
BMP2	Osteoporosis	2002/0072066
BMPR1A	Juvenile polyposis	2003/0072758
ACLP	Gastroschisis	2003/0084464
Resistin-like molecule β	Familial adenomatous polyposis, diabetes, insulin resistance, colon cancer, inflammatory bowel disorder	2003/0138826
Dlg5	Inflammatory bowel disease	2006/0100132
SEQ ID NOS: 1-82 of the U.S. patent application publication 2002/0119452, incorporated by reference herein	Osteoarthritis	2002/0119452
TRANCE	Immune system disorders	2003/0185820
Matrilin-3	Osteoarthritis	2003/0203380
Synoviolin	Rheumatoid arthritis	2004/0152871
SEQ ID NOS: 9, 35 of the U.S. patent application publication 2007/0028314, incorporated by reference herein	Osteoarthritis	2007/0028314
HIV LTR	HIV infection	5,627,023
SHIVA	HIV infection	2004/0197770
EBI 1, EBI 2, EBI 3	Epstein Barr virus infection	2002/0040133
NM23 family	Skin/intestinal disorders	2002/0034741

Gene	Disease/Disorder	Patent/Published Application No.
SEQ ID NO: 1 of the U.S. patent application publication 2002/0169127, incorporated by reference herein	Psoriasis	2002/0169127
Eps8	Skin disorders, wound healing	2003/0180302
Beta-10	Thyroid gland pathology	2002/0015981
SEQ ID NO: 2 of the U.S. patent application publication 2003/0207403, incorporated by reference herein	Thyroid conditions	2003/0207403
SEQ ID NO: 3 of the U.S. patent application publication 2007/0020275, incorporated by reference herein	Thyroid disorders	2007/0020275
Hair follicle growth factor	Alopecia	2003/0036174
Corneodesmosin	Alopecia	2003/0211065
GCR9	Asthma, lymphoma, leukemia	2003/0166150
SEQ ID NO: 1-71 of the U.S. patent application publication 2004/0002084, incorporated by reference herein	Asthma	2004/0002084
Bg	Chediak-Higashi syndrome	2002/0115144
SEQ ID NOS: 1-16 of the U.S. patent application publication 2002/0127555, incorporated by reference herein	Endometriosis	2002/0127555
FGF23	Hypophosphatemic disorders	2005/0156014
BBSR	Bardet-Biedl syndrome	2003/0152963
MIC-1	Fetal abnormalities, cancer, inflammatory disorders, miscarriage, premature birth	2004/0053325
MIA-2	Liver damage	2004/0076965
IL-17B	Cartilage degenerative disorders	2004/0171109

Gene	Disease/Disorder	Patent/Published Application No.
Formylglycine generating enzyme	Multiple sulfatase deficiency	2004/0229250
LPLA2	Pulmonary alveolar proteinosis	2006/0008455
CXCL1O	Respiratory illnesses	2006/0040329
SEQ ID NOS: 1, 2 of the U.S. patent application publication 2006/0140945, incorporated by reference herein	Nephropathy	2006/0140945
HFE2A	Iron metabolism disease	2007/0166711

[0169] Once a gene with an expression pattern that is modulated during a disease, disorder, or condition is identified, the promoter of the gene may be used in the gene switch of the invention. The sequence of many genes, including the promoter region, is known in the art and available in public databases, *e.g.*, GenBank. Thus, once an appropriate gene is identified, the promoter sequence can be readily identified and obtained. Another aspect of the present invention is directed towards identifying suitable genes whose promoter can be isolated and placed into a gene switch. The identity of the gene, therefore, may not be critical to specific embodiments of the present invention, provided the promoter can be isolated and used in subsequent settings or environments. The current invention thus includes the use of promoters from genes that are yet to be identified. Once suitable genes are identified, it is a matter of routine skill or experimentation to determine the genetic sequences needed for promoter function. Indeed, several commercial protocols exist to aid in the determination of the promoter region of genes of interest. By way of example, Ding *et al.* recently elucidated the promoter sequence of the novel *Sprouty4* gene (*Am. J. Physiol. Lung Cell. Mol. Physiol. 287*: L52 (2004), which is incorporated by reference) by progressively deleting the 5'-flanking sequence of the human *Sprouty4* gene. Briefly, once the transcription initiation site was determined, PCR fragments were generated using common PCR primers to clone segments of the 5'-flanking segment in a unidirectional manner. The generated segments were cloned into a luciferase reporter vector and luciferase activity was measured to determine the promoter region of the human *Sprouty4* gene.

[0170] Another example of a protocol for acquiring and validating gene promoters includes the following steps: (1) acquire diseased and non-diseased cell/tissue samples of similar/same tissue type; (2) isolate total RNA or mRNA from the samples; (3) perform differential microarray analysis of diseased and non-diseased RNA; (4) identify candidate disease-specific transcripts; (5) identify genomic sequences associated with the disease-specific transcripts; (6) acquire or synthesize DNA sequence upstream and downstream of the predicted transcription start site of the disease-specific transcript; (7) design and produce promoter reporter vectors using different lengths of DNA from step 6; and (8) test promoter reporter vectors in diseased and non-diseased cells/tissues, as well as in unrelated cells/tissues.

[0171] The source of the promoter that is inserted into the gene switch can be natural or synthetic, and the source of the promoter should not limit the scope of the invention described herein. In other words, the promoter may be directly cloned from cells, or the promoter may have been previously cloned from a different source, or the promoter may have been synthesized.

Gene Switch Systems

[0172] The gene switch may be any gene switch that regulates gene expression by addition or removal of a specific ligand. In one embodiment, the gene switch is one in which the level of gene expression is dependent on the level of ligand that is present. Examples of ligand-dependent transcription factor complexes that may be used in the gene switches of the invention include, without limitation, members of the nuclear receptor superfamily activated by their respective ligands (*e.g.*, glucocorticoid, estrogen, progestin, retinoid, ecdysone, and analogs and mimetics thereof) and rTTA activated by tetracycline. In one aspect of the invention, the gene switch is an EcR-based gene switch. Examples of such systems include, without limitation, the systems described in U.S. Patent Nos. 6,258,603, 7,045,315, U.S. Published Patent Application Nos. 2006/0014711, 2007/0161086, and International Published Application No. WO 01/70816. Examples of chimeric ecdysone receptor systems are described in U.S. Patent No. 7,091,038, U.S. Published Patent Application Nos. 2002/0110861, 2004/0033600, 2004/0096942, 2005/0266457, and 2006/0100416, and International Published Application Nos. WO

01/70816, WO 02/066612, WO 02/066613, WO 02/066614, WO 02/066615, WO 02/29075, and WO 2005/108617, each of which is incorporated by reference in its entirety. An example of a non-steroidal ecdysone agonist-regulated system is the RheoSwitch® Mammalian Inducible Expression System (New England Biolabs, Ipswich, MA). In another aspect of the invention, the gene switch is based on heterodimerization of FK506 binding protein (FKBP) with FKBP rapamycin associated protein (FRAP) and is regulated through rapamycin or its non-immunosuppressive analogs. Examples of such systems, include, without limitation, the ARGENT™ Transcriptional Technology (ARIAD Pharmaceuticals, Cambridge, MA) and the systems described in U.S. Patent Nos. 6,015,709, 6,117,680, 6,479,653, 6,187,757, and 6,649,595.

[0173] In one embodiment, the gene switch comprises a single transcription factor sequence encoding a ligand-dependent transcription factor complex under the control of a therapeutic switch promoter. The transcription factor sequence may encode a ligand-dependent transcription factor complex that is a naturally occurring or an artificial ligand-dependent transcription factor complex. An artificial transcription factor is one in which the natural sequence of the transcription factor has been altered, *e.g.*, by mutation of the sequence or by the combining of domains from different transcription factors. In one embodiment, the transcription factor comprises a Group H nuclear receptor ligand binding domain. In one embodiment, the Group H nuclear receptor ligand binding domain is from an ecdysone receptor, a ubiquitous receptor (UR), an orphan receptor 1 (OR-1), a steroid hormone nuclear receptor 1 (NER-1), a retinoid X receptor interacting protein–15 (RIP-15), a liver X receptor β (LXRβ), a steroid hormone receptor like protein (RLD-1), a liver X receptor (LXR), a liver X receptor α (LXRα), a farnesoid X receptor (FXR), a receptor interacting protein 14 (RIP-14), or a farnesol receptor (HRR-1). In another embodiment, the Group H nuclear receptor LBD is from an ecdysone receptor.

A. Ecdysone -based Gene Switch

[0174] The EcR and the other Group H nuclear receptors are members of the nuclear receptor superfamily wherein all members are generally characterized by the presence of an amino-terminal transactivation domain (AD, also referred to interchangeably as "TA" or "TD"), optionally fused to a heterodimerization partner (HP) to form a coactivation protein (CAP), a DNA binding domain (DBD), and a LBD fused to the DBD via a hinge region to form a ligand-dependent transcription factor (LTF). As used herein, the term

"DNA binding domain" comprises a minimal polypeptide sequence of a DNA binding protein, up to the entire length of a DNA binding protein, so long as the DNA binding domain functions to associate with a particular response element. Members of the nuclear receptor superfamily are also characterized by the presence of four or five domains: A/B, C, D, E, and in some members F (see US 4,981,784 and Evans, *Science 240*:889 (1988)). The "A/B" domain corresponds to the transactivation domain, "C" corresponds to the DNA binding domain, "D" corresponds to the hinge region, and "E" corresponds to the ligand binding domain. Some members of the family may also have another transactivation domain on the carboxy-terminal side of the LBD corresponding to "F".

[0175] The following polypeptide sequence was reported as a polypeptide sequence of Ecdysone receptor (Ecdysteroid receptor) (20-hydroxy-ecdysone receptor) (20E receptor) (EcRH) (Nuclear receptor subfamily 1 group H member 1) and has the accession number P34021 in Genbank.

[0176] Ecdysone receptor (878aa) from Drosophila melanogaster (Fruit fly) (SEQ ID NO:5)

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  1 mkrrwsnngg fmrlpeesss evtsssnglv lpsgvnmsps sldshdycdq dlwlcgnesg
 61 sfggsnghgl sqqqqsvitl amhgcsstlp aqttiiping nangnggstn gqyvpgatnl
121 galangmlng gfngmqqqiq nghglinstt pstpttplhl qqnlggaggg giggmgilhh
181 angtpnglig vvgggggvgl gvggggvggl gmqhtprsds vnsissgrdd lspssslngy
241 sanescdakk skkgpaprvq eelclvcgdr asgyhynalt cegckgffrr svtksavycc
301 kfgracemdm ymrrkcqecr lkkclavgmr pecvvpenqc amkrrekkaq kekdkmttsp
361 ssqhggngsl asgggqdfvk keildlmtce ppqhatipll pdeilakcqa rnipsltynq
421 laviykliwy qdgyeqpsee dlrrimsqpd enesqtdvsf rhiteitilt vqlivefakg
481 lpaftkipqe dqitllkacs sevmmlrmar rydhssdsif fannrsytrd sykmagmadn
541 iedllhfcrq mfsmkvdnve yalltaivif sdrpglekaq lveaiqsyyi dtlriyilnr
601 hcgdsmslvf yakllsilte lrtlgnqnae mcfslklknr klpkfleeiw dvhaippsvq
661 shlqitqeen erleraermr asvggaitag idcdsastsa aaaaaqhqpq pqpqpqpssl
721 tqndsqhqtq pqlqpqlppq lqgqlqpqlq pqlqtqlqpq iqpqpqllpv sapvpasvta
781 pgslsavsts seymggsaai gpitpattss itaavtasst tsavpmgngv gvgvgvggnv
841 smyanaqtam almgvalhsh qeqliggvav ksehstta
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[0177] The DBD is characterized by the presence of two cysteine zinc fingers between which are two amino acid motifs, the P-box and the D-box, which confer specificity for response elements. These domains may be either native, modified, or chimeras of different domains of heterologous receptor proteins. The EcR, like a subset of the nuclear receptor family, also possesses less well-defined regions responsible for heterodimerization properties. Because the domains of nuclear receptors are modular in nature, the LBD, DBD, and AD may be interchanged.

[0178] In another embodiment, the transcription factor comprises a AD, a DBD that recognizes a response element associated with the therapeutic protein or therapeutic polynucleotide whose expression is to be modulated; and a Group H nuclear receptor LBD. In certain embodiments, the Group H nuclear receptor LBD comprises a substitution mutation.

[0179] In another embodiment, the gene switch comprises a first transcription factor sequence, *e.g.*, a CAP, under the control of a first therapeutic switch promoter (TSP-1) and a second transcription factor sequence, *e.g.*, a LTF, under the control of a second therapeutic switch promoter (TSP-2), wherein the proteins encoded by said first transcription factor sequence and said second transcription factor sequence interact to form a protein complex (LDTFC), *i.e.*, a "dual switch"- or "two-hybrid"-based gene switch. The first and second TSPs may be the same or different. In this embodiment, the presence of two different TSPs in the gene switch that are required for therapeutic molecule expression enhances the specificity of the therapeutic method (see Figure 2). Figure 2 also demonstrates the ability to modify the therapeutic gene switch to treat any disease, disorder, or condition simply by inserting the appropriate TSPs.

[0180] In a further embodiment, both the first and the second transcription factor sequence, *e.g.*, a CAP or a LTF, are under the control of a single therapeutic switch promoter (*e.g.* TSP-1 in Figure 1). Activation of this promoter will generate both CAP and LTF with a single open reading frame. This can be achieved with the use of a transcriptional linker such as an IRES (internal ribosomal entry site). In this embodiment, both portions of the ligand-dependent transcription factor complex will be synthesized upon activation of TSP-1. TSP-1 can be a constitutive promoter or only activated under conditions associated with the disease, disorder, or condition.

[0181] In a further embodiment, one transcription factor sequence, *e.g.* a LTF, is under the control of a therapeutic switch promoter only activated under conditions associated with the disease, disorder, or condition (*e.g.*, TSP-2 or TSP-3 in Figure 4) and the other transcription factor sequence, *e.g.*, CAP, is under the control of a constitutive therapeutic switch promoter (*e.g.*, TSP-1 in Figure 4). In this embodiment, one portion of the ligand-dependent transcription factor complex will be constitutively present while the second portion will only be synthesized under conditions associated with the disease, disorder, or condition.

[0182] In another embodiment, one transcription factor sequence, *e.g.*, CAP, is under the control of a first TSP (*e.g.*, TSP-1 in Figure 3) and two or more different second transcription factor sequences, *e.g.*, LTF-1 and LTF-2 are under the control of different TSPs (*e.g.*, TSP-2 and TSP-3 in Figure 3). In this embodiment, each of the LTFs may have a different DBD that recognizes a different factor-regulated promoter sequence (*e.g.*, DBD-A binds to a response element associated with factor-regulated promoter-1 (FRP-1) and DBD-B binds to a response element associated with factor-regulated promoter-2 (FRP-2). Each of the factor-regulated promoters may be operably linked to a different therapeutic gene. In this manner, multiple treatments may be provided simultaneously.

[0183] In one embodiment, the first transcription factor sequence encodes a polypeptide comprising a AD, a DBD that recognizes a response element associated with the therapeutic product sequence whose expression is to be modulated; and a Group H nuclear receptor LBD, and the second transcription factor sequence encodes a transcription factor comprising a nuclear receptor LBD selected from the group consisting of a vertebrate retinoid X receptor (RXR), an invertebrate RXR, an ultraspiracle protein (USP), or a chimeric nuclear receptor comprising at least two different nuclear receptor ligand binding domain polypeptide fragments selected from the group consisting of a vertebrate RXR, an invertebrate RXR, and a USP (see WO 01/70816 A2 and US 2004/0096942 A1). The "partner" nuclear receptor ligand binding domain may further comprise a truncation mutation, a deletion mutation, a substitution mutation, or another modification.

[0184] In another embodiment, the gene switch comprises a first transcription factor sequence encoding a first polypeptide comprising a nuclear receptor LBD and a DBD that recognizes a response element associated with the therapeutic product sequence whose expression is to be modulated, and a second transcription factor sequence encoding a second polypeptide comprising an AD and a nuclear receptor LBD, wherein one of the nuclear receptor LBDs is a Group H nuclear receptor LBD. In a preferred embodiment, the first polypeptide is substantially free of an AD and the second polypeptide is substantially free of a DBD. For purposes of the invention, "substantially free" means that the protein in question does not contain a sufficient sequence of the domain in question to provide activation or binding activity.

[0185] In another aspect of the invention, the first transcription factor sequence encodes a protein comprising a heterodimerization partner and an AD (a "CAP") and the second transcription factor sequence encodes a protein comprising a DBD and a LBD (a "LTF").

[0186] When only one nuclear receptor LBD is a Group H LBD, the other nuclear receptor LBD may be from any other nuclear receptor that forms a dimer with the Group H LBD. For example, when the Group H nuclear receptor LBD is an EcR LBD, the other nuclear receptor LBD "partner" may be from an EcR, a vertebrate RXR, an invertebrate RXR, an ultraspiracle protein (USP), or a chimeric nuclear receptor comprising at least two different nuclear receptor LBD polypeptide fragments selected from the group consisting of a vertebrate RXR, an invertebrate RXR, and a USP (see WO 01/70816 A2, International Patent Application No. PCT/US02/05235 and US 2004/0096942 A1, incorporated herein by reference in their entirety). The "partner" nuclear receptor ligand binding domain may further comprise a truncation mutation, a deletion mutation, a substitution mutation, or another modification.

[0187] In one embodiment, the vertebrate RXR LBD is from a human *Homo sapiens*, mouse *Mus musculus*, rat *Rattus norvegicus*, chicken *Gallus gallus*, pig *Sus scrofa domestica*, frog *Xenopus laevis*, zebrafish *Danio rerio*, tunicate *Polyandrocarpa misakiensis*, or jellyfish *Tripedalia cysophora* RXR.

[0188] In one embodiment, the invertebrate RXR ligand binding domain is from a locust *Locusta migratoria* ultraspiracle polypeptide ("LmUSP"), an ixodid tick *Amblyomma americanum* RXR homolog 1 ("AmaRXR1"), an ixodid tick *Amblyomma americanum* RXR homolog 2 ("AmaRXR2"), a fiddler crab *Celuca pugilator* RXR homolog ("CpRXR"), a beetle *Tenebrio molitor* RXR homolog ("TmRXR"), a honeybee *Apis mellifera* RXR homolog ("AmRXR"), an aphid *Myzus persicae* RXR homolog ("MpRXR"), or a non-Dipteran/non-Lepidopteran RXR homolog.

[0189] In one embodiment, the chimeric RXR LBD comprises at least two polypeptide fragments selected from the group consisting of a vertebrate species RXR polypeptide fragment, an invertebrate species RXR polypeptide fragment, and a non-Dipteran/non-Lepidopteran invertebrate species RXR homolog polypeptide fragment. A chimeric RXR ligand binding domain for use in the present invention may comprise at least two different species RXR polypeptide fragments, or when the species is the same, the two or more

polypeptide fragments may be from two or more different isoforms of the species RXR polypeptide fragment.

[0190] In one embodiment, the chimeric RXR ligand binding domain comprises at least one vertebrate species RXR polypeptide fragment and one invertebrate species RXR polypeptide fragment.

[0191] In another embodiment, the chimeric RXR ligand binding domain comprises at least one vertebrate species RXR polypeptide fragment and one non-Dipteran/non-Lepidopteran invertebrate species RXR homolog polypeptide fragment.

[0192] The ligand, when combined with the LBD of the nuclear receptor(s), which in turn are bound to the response element of a FRP associated with a therapeutic product sequence, provides external temporal regulation of expression of the therapeutic product sequence. The binding mechanism or the order in which the various components of this invention bind to each other, that is, for example, ligand to LBD, DBD to response element, AD to promoter, *etc.*, is not critical.

[0193] In a specific example, binding of the ligand to the LBD of a Group H nuclear receptor and its nuclear receptor LBD partner enables expression of the therapeutic product sequence. This mechanism does not exclude the potential for ligand binding to the Group H nuclear receptor (GHNR) or its partner, and the resulting formation of active homodimer complexes (*e.g.* GHNR + GHNR or partner + partner). Preferably, one or more of the receptor domains is varied producing a hybrid gene switch. Typically, one or more of the three domains, DBD, LBD, and AD, may be chosen from a source different than the source of the other domains so that the hybrid genes and the resulting hybrid proteins are optimized in the chosen host cell or organism for transactivating activity, complementary binding of the ligand, and recognition of a specific response element. In addition, the response element itself can be modified or substituted with response elements for other DNA binding protein domains such as the GAL-4 protein from yeast (see Sadowski *et al.*, *Nature 335*:563 (1988)) or LexA protein from *Escherichia coli* (see Brent *et al.*, *Cell 43*:729 (1985)), or synthetic response elements specific for targeted interactions with proteins designed, modified, and selected for such specific interactions (see, for example, Kim *et al.*, *Proc. Natl. Acad. Sci. USA*, *94*:3616 (1997)) to accommodate hybrid receptors. Another advantage of two-hybrid systems is that they allow choice of a promoter used to drive the gene expression according to a desired end

result. Such double control may be particularly important in areas of gene therapy, especially when cytotoxic proteins are produced, because both the timing of expression as well as the cells wherein expression occurs may be controlled. When genes, operably linked to a suitable promoter, are introduced into the cells of the subject, expression of the exogenous genes is controlled by the presence of the system of this invention. Promoters may be constitutively or inducibly regulated or may be tissue-specific (that is, expressed only in a particular type of cells) or specific to certain developmental stages of the organism.

[0194] The DNA binding domain of the first hybrid protein binds, in the presence or absence of a ligand, to the DNA sequence of a response element to initiate or suppress transcription of downstream gene(s) under the regulation of this response element. .

[0195] The functional LDTFC, *e.g.*, an EcR complex, may also include additional protein(s) such as immunophilins. Additional members of the nuclear receptor family of proteins, known as transcriptional factors (such as DHR38 or *betaFTZ-1*), may also be ligand dependent or independent partners for EcR, USP, and/or RXR. Additionally, other cofactors may be required such as proteins generally known as coactivators (also termed adapters or mediators). These proteins do not bind sequence-specifically to DNA and are not involved in basal transcription. They may exert their effect on transcription activation through various mechanisms, including stimulation of DNA-binding of activators, by affecting chromatin structure, or by mediating activator-initiation complex interactions. Examples of such coactivators include RIP140, TIF1, RAP46/Bag-1, ARA70, SRC-1/NCoA-1, TIF2/GRIP/NCoA-2, ACTR/AIB1/RAC3/pCIP as well as the promiscuous coactivator C response element B binding protein, CBP/p300 (for review see Glass *et al.*, *Curr. Opin. Cell Biol.* 9:222 (1997)). Also, protein cofactors generally known as corepressors (also known as repressors, silencers, or silencing mediators) may be required to effectively inhibit transcriptional activation in the absence of ligand. These corepressors may interact with the unliganded EcR to silence the activity at the response element. Current evidence suggests that the binding of ligand changes the conformation of the receptor, which results in release of the corepressor and recruitment of the above described coactivators, thereby abolishing their silencing activity. Examples of corepressors include N-CoR and SMRT (for review, see Horwitz *et al.*, *Mol Endocrinol.* *10*:1167 (1996)). These cofactors may either be endogenous within the cell or organism,

or may be added exogenously as transgenes to be expressed in either a regulated or unregulated fashion.

B. Rapamycin based Gene Switch

[0196] The present invention further provides a gene switch system which utilizes FK506 binding protein as the ligand-dependent transcription factor complex and rapamycin as the ligand. In one embodiment, the construct encoding the gene switch comprises

(a) a first polynucleotide encoding a first chimeric protein which binds to rapamycin or an analog thereof and which comprises at least one *FK506*-binding protein (FKBP) domain and at least one protein domain heterologous thereto, wherein the FKBP domain comprises a peptide sequence selected from:

(1) a naturally occurring FKBP

(2) a variant of a naturally occurring FKBP in which up to 10 amino acid residues have been deleted, inserted, or replaced with substitute amino acids, and

(3) an FKBP encoded by a DNA sequence which selectively hybridizes to a DNA sequence encoding an FKBP of (1) or (2);

(b) a second polynucleotide encoding a second chimeric protein which forms a complex with both (a) rapamycin or a rapamycin analog and (b) the first chimeric protein, and which comprises at least one FKBP:rapamycin binding (FRB) domain and at least one protein domain heterologous thereto, wherein the FRB domain comprises a peptide sequence selected from:

(4) a naturally occurring FRB domain,

(5) a variant of a naturally occuring FRB domain in which up to 10 amino acid residues have been deleted, inserted, or replaced with substitute amino acids, and

(6) an FRB domain encoded by a DNA sequence which selectively hybridizes to a DNA sequence encoding an FRB of (4) or (5).

[0197] In this gene switch system, each of the first polynucleotide and the second polynucleotide are under the control of one or more therapeutic switch promoters as described elsewhere herein. Furthermore, in certain embodiments, at least one protein domain heterologous to the FKBP and/or FRB domains in the first and second chimeric protein may be one or more "action" or "effector" domains. Effector domains may be selected from a wide variety of protein domains including DNA binding domains, transcription activation domains, cellular localization domains and signaling domains (*i.e.*, domains which are capable upon clustering or multimerization, of triggering cell growth, proliferation, differentiation, apoptosis, gene transcription, etc.).

[0198] In certain embodiments, one fusion protein contains at least one DNA binding domain (*e.g.*, a GAL4 or ZFHD1 DNA-binding domain) and another fusion protein contains at least one transcription activation domain (*e.g.*, a VP16 or p65 transcription activation domain). Ligand-mediated association of the fusion proteins represents the formation of a transcription factor complex and leads to initiation of transcription of a target gene linked to a DNA sequence recognized by (*i.e.*, capable of binding with) the DNA-binding domain on one of the fusion proteins. Information regarding the gene expression system as well as the ligand is disclosed in U.S. Patent Nos. 6,187,757 B1, 6,649,595 B1, 6,509,152 B1, 6,479,653 B1, and 6,117,680 B1.

[0199] In other embodiments, the present invention provides a gene switch system which comprises polynucleotides encoding two fusion proteins which self-aggregate in the absence of a ligand, wherein (a) the first fusion protein comprises a conditional aggregation domain which binds to a selected ligand and a transcription activation domain, and (b) the second fusion protein comprising a conditional aggregation domain which binds to a selected ligand and a DNA binding domain, and (c) in the absence of ligand, the cells express a gene operably linked to regulatory DNA to which said DNA binding domain binds. Modified cells comprising the gene switch system are expanded in the presence of the ligand in an amount sufficient for repression of the gene. Ligand removal induces expression of the encoded protein that causes cell death. The nucleic acids encoding the two fusion proteins are under the control of at least one conditional promoter. The gene expression system utilizing conditional aggregation domains is disclosed in U.S. Publication No. 2002/0048792.

C. Procaryotic Repressor/ Operator based Gene Switch System

[0200] In one embodiment, the present invention provides gene switch system comprising (a) a first polynucleotide coding for a transactivator fusion protein comprising a prokaryotic tetracycline ("tet") repressor and a eucaryotic transcriptional activator protein domain; and (b) a second polynucleotide coding for a therapeutic protein or therapeutic polypeptide, wherein said second polynucleotide is operably linked to a minimal promoter and at least one tet operator sequence. The first polynucleotide coding for a transactivator fusion protein may comprise therapeutic switch promoter as described elsewhere herein. The expression of the lethal protein is up-regulated in the absence of tetracycline. (*see, e.g.*, Gossen *et al.* (1992) *Proc. Natl. Acad. Sci.* 89: 5547-5551; Gossen *et al.* (1993) *TIBS* 18 : 471-475; Furth *et al.* (1994) *Proc. Natl. Acad. Sci.* 91: 9302-9306; and Shockett *et al.* (1995) *Proc. Natl. Acad. Sci.* 92: 6522-6526). The TetO expression system is disclosed in U.S. Patent No. 5,464,758 B1.

[0201] In another embodiment, the gene switch system comprises the lactose ("Lac") repressor-operator systems from the bacterium *Escherichia coli*. The gene switch system of the present invention may also comprise (a) a first polynucleotide coding for a transactivator fusion protein comprising a prokaryotic lac I repressor and a eucaryotic transcriptional activator protein domain; and (b) a second polynucleotide coding for a therapeutic protein or therapeutic polypeptide, wherein said second polynucleotide is operably linked to a therapeutic switch promoter. In the Lac system, a lac operon is inactivated in the absence of lactose, or synthetic analogs such as isopropyl-b-D-thiogalactoside.

[0202] Additional gene switch systems include those described in the following: US 7,091,038; WO2004078924; EP1266015; US20010044151; US20020110861; US20020119521; US20040033600; US20040197861; US20040235097; US20060020146; US20040049437; US20040096942; US20050228016; US20050266457; US20060100416; WO2001/70816; WO2002/29075; WO2002/066612; WO2002/066613; WO2002/066614; WO2002/066615; WO2005/108617; US 6,258,603; US20050209283; US20050228016; US20060020146; EP0965644; US 7,304,162; US 7,304,161; MX234742; KR10-0563143; AU765306; AU2002-248500; and AU2002-306550.

D. Combination of the Gene Switch Systems

[0203] The present invention provides nucleic acid compositions, modified cells, and bioreactors comprising two or more gene switch systems comprising different ligand-dependent transcription factor complexes which are activated by an effective amount of one or more ligands, wherein the two or more gene switch systems comprise a first gene switch and a second gene switch, both of which selectively induce expression of one or more therapeutic polypeptides or therapeutic polynucleotides, upon binding to one or more ligands. Within the scope of the present invention are any numbers of and/or combinations of gene switch systems.

[0204] In one embodiment, the present invention provides a nucleic acid composition comprising:

(c) a first gene switch system which comprises:

i. a first gene expression cassette comprising a polynucleotide encoding a first hybrid polypeptide which comprises:

1. a transactivation domain, which activates a factor-regulated promoter operably associated with a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide; and

2. a heterodimer partner domain,

ii. a second gene expression cassette comprising a polynucleotide encoding a second hybrid polypeptide which comprises:

1. a DNA-binding domain, which recognizes a factor-regulated promoter operably associated with a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide; and

2. a ligand binding domain; and

iii. a third gene expression cassette comprising a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide comprising:

1. a factor-regulated promoter, which is activated by the transactivation domain of the second hybrid polypeptide; and,

2. a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide, and

b. a second gene expression system which comprises:

i. a first gene expression cassette comprising a polynucleotide encoding a first hybrid polypeptide which comprises:

1. a transactivation domain, which activates a factor-regulated promoter operably associated with a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide; and

2. a heterodimer partner domain,

ii. a second gene expression cassette comprising a polynucleotide encoding a second hybrid polypeptide which comprises:

1. a DNA-binding domain, which recognizes a factor-regulated promoter operably associated with a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide; and

2. a ligand binding domain; and

iii. a third gene expression cassette comprising a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide comprising:

1. a factor-regulated promoter, which is activated by the transactivation domain of the second hybrid polypeptide; and,

2. a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide.

[0205] The multiple inducible gene expression systems provide for expression of a given therapeutic polynucleotide or therapeutic polypeptide under conditions associated with different diseases, disorders or conditions, or expression of multiple therapeutic polypeptides or therapeutic polynucleotides either under the same conditions associated

with the same disease disorder or condition, or under different conditions associated with different diseases, disorders, or conditions.

[0206] In certain embodiments, the combination of two or more gene switch systems may be (1) a dual-switch ecdysone receptor based gene expression system and (2) a single-switch ecdysone receptor based gene switch. In other embodiments, the combination may be (1) an single- or dual-switch ecdysone receptor based gene switch and (2) a rapamycin based gene switch. Alternatively, the combination of gene switch systems may be two identical rapamycin based gene switch systems disclosed above. Any possible combinations of the gene switch systems are within the scope of the invention.

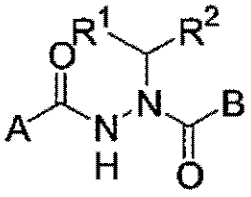
Ligands

[0207] The ligand for a ligand-dependent transcription factor complex of the invention binds to the protein complex comprising one or more of the ligand binding domain, the heterodimer partner domain, the DNA binding domain, and the transactivation domain. The choice of ligand to activate the ligand-dependent transcription factor complex depends on the type of the gene switch utilized.

[0208] For example, a ligand for the edysone receptor based gene switch may be selected from any suitable ligands. Both naturally occurring ecdysone or ecdyson analogs (*e.g.*, 20-hydroxyecdysone, muristerone A, ponasterone A, ponasterone B, ponasterone C, 26-iodoponasterone A, inokosterone or 26-mesylinokosterone) and non-steroid inducers may be used as a ligand for gene switch of the present invention. U.S. Patent No. 6,379,945 B1, describes an insect steroid receptor isolated from *Heliothis virescens* ("HEcR") which is capable of acting as a gene switch responsive to both steroid and certain non-steroidal inducers. Non-steroidal inducers have a distinct advantage over steroids, in this and many other systems which are responsive to both steroids and non-steroid inducers, for a number of reasons including, for example: lower manufacturing cost, metabolic stability, absence from insects, plants, or mammals, and environmental acceptability. U.S. Patent No. 6,379,945 B1 describes the utility of two dibenzoylhydrazines, 1,2-dibenzoyl-1-tert-butyl-hydrazine and tebufenozide (N-(4-ethylbenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butyl-hydrazine) as ligands for an ecdysone-based gene switch. Also included in the present invention as a ligand are other dibenzoylhydrazines, such as those disclosed in U.S. Pat. No. 5,117,057 B1. Use of tebufenozide as a chemical ligand for the ecdysone receptor from *Drosophila*

melanogaster is also disclosed in U.S. Patent No. 6,147,282. Additional, non-limiting examples of ecdysone ligands are 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, a 1,2-diacyl hydrazine, an N'-substituted-N,N'-disubstituted hydrazine, a dibenzoylalkyl cyanohydrazine, an N-substituted-N-alkyl-N,N-diaroyl hydrazine, an N-substituted-N-acyl-N-alkyl, carbonyl hydrazine or an N-aroyl-N'-alkyl-N'-aroyl hydrazine. (*See* U.S. Patent No. 6,723,531).

[0209] In one embodiment, the ligand for an ecdysone based gene switch system is a diacylhydrazine ligand or chiral diacylhydrazine ligand. The ligand used in the gene switch system may be compounds of Formula I



Formula I

wherein

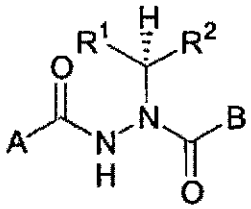
A is alkoxy, arylalkyloxy or aryloxy;

B is optionally substituted aryl or optionally substituted heteroaryl; and

R^1 and R^2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl;

or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

[0210] In another embodiment, the ligand may be enantiomerically enriched compounds of Formula II



Formula II

wherein

A is alkoxy, arylalkyloxy, aryloxy, arylalkyl, optionally substituted aryl or optionally substituted heteroaryl;

B is optionally substituted aryl or optionally substituted heteroaryl; and

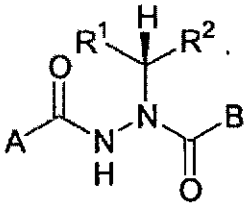
R^1 and R^2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl;

with the proviso that R^1 does not equal R^2;

wherein the absolute configuration at the asymmetric carbon atom bearing R^1 and R^2 is predominantly *S*;

or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

[0211] In certain embodiments, the ligand may be enantiomerically enriched compounds of Formula III



Formula III

wherein

A is alkoxy, arylalkyloxy, aryloxy, arylalkyl, optionally substituted aryl or optionally substituted heteroaryl;

B is optionally substituted aryl or optionally substituted heteroaryl; and

R^1 and R^2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl;

with the proviso that R^1 does not equal R^2;

wherein the absolute configuration at the asymmetric carbon atom bearing R^1 and R^2 is predominantly *R*;

or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

[0212] In one embodiment, a ligand may be *(R)*-3,5-dimethyl-benzoic acid N-(1-*tert*-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide having an enantiomeric excess of at least 95% or a pharmaceutically acceptable salt, hydrate, crystalline form or amorphous form thereof.

[0213] The diacylhydrazine ligands of Formula I and chiral diacylhydrazine ligands of Formula II or III, when used with an ecdysone-based gene switch system, provide the means for external temporal regulation of expression of a therapeutic polypeptide or therapeutic polynucleotide of the present invention.

[0214] The ligands used in the present invention may form salts. The term "salt(s)" as used herein denotes acidic and/or basic salts formed with inorganic and/or organic acids and bases. In addition, when a compound of Formula I, II or III contains both a basic moiety and an acidic moiety, zwitterions ("inner salts") may be formed and are included within the term "salt(s)" as used herein. Pharmaceutically acceptable (*i.e.*, non-toxic, physiologically acceptable) salts are used, although other salts are also useful, *e.g.*, in isolation or purification steps which may be employed during preparation. Salts of the compounds of Formula I, II or III may be formed, for example, by reacting a compound

with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

[0215] The ligands which contain a basic moiety may form salts with a variety of organic and inorganic acids. Exemplary acid addition salts include acetates (such as those formed with acetic acid or trihaloacetic acid, for example, trifluoroacetic acid), adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides (formed with hydrochloric acid), hydrobromides (formed with hydrogen bromide), hydroiodides, 2-hydroxyethanesulfonates, lactates, maleates (formed with maleic acid), methanesulfonates (formed with methanesulfonic acid), 2-naphthalenesulfonates, nicotinates, nitrates, oxalates, pectinates, persulfates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates (such as those formed with sulfuric acid), sulfonates (such as those mentioned herein), tartrates, thiocyanates, toluenesulfonates such as tosylates, undecanoates, and the like.

[0216] The ligands which contain an acidic moiety may form salts with a variety of organic and inorganic bases. Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as benzathines, dicyclohexylamines, hydrabamines (formed with N,N-bis(dehydroabietyl)ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glucamides, t-butyl amines, and salts with amino acids such as arginine, lysine and the like.

[0217] Non-limiting examples of the ligands for the inducible gene expression system utilizing the FK506 binding domain are FK506, Cyclosporin A, or Rapamycin. FK506, rapamycin, and their analogs are disclosed in U.S. Patent Nos. 6,649,595 B2 and 6,187,757. *See also* U.S. Patent Nos. 7,276,498 and 7,273,874.

[0218] The ligands described herein may be administered alone or as part of a pharmaceutical composition comprising a pharmaceutically acceptable carrier. In one embodiment, the pharmacetical compoistion are in the form of solutions, suspensions, tablets, capsules, ointments, elixirs, or injectable compositions.

Pharmaceutical Compositions

[0219] Pharmaceutically acceptable carriers include fillers such as saccharides, for example lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added such as the above-mentioned starches and also carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. In one embodiment, dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated saccharide solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropylmethyl-cellulose phthalate, are used. Dye stuffs or pigments may be added to the tablets or dragee coatings, for example, for identification or in order to characterize combinations of active compound doses.

[0220] Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules or nanoparticles which may optionally be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In one embodiment, the is dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin, optionally with stabilizers.

[0221] Fattty oils may comprise mono-, di- or triglycerides. Mono-, di- and triglycerides include those that are derived from C_6, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20} and C_{22} acids. Exemplary diglycerides include, in particular, diolein, dipalmitolein, and mixed caprylin-caprin diglycerides. Triglycerides include vegetable oils, fish oils, animal fats, hydrogenated vegetable oils, partially hydrogenated vegetable oils, synthetic

triglycerides, modified triglycerides, fractionated triglycerides, medium and long-chain triglycerides, structured triglycerides, and mixtures thereof. Exemplary triglycerides include: almond oil; babassu oil; borage oil; blackcurrant seed oil; canola oil; castor oil; coconut oil; corn oil; cottonseed oil; evening primrose oil; grapeseed oil; groundnut oil; mustard seed oil; olive oil; palm oil; palm kernel oil; peanut oil; rapeseed oil; safflower oil; sesame oil; shark liver oil; soybean oil; sunflower oil; hydrogenated castor oil; hydrogenated coconut oil; hydrogenated palm oil; hydrogenated soybean oil; hydrogenated vegetable oil; hydrogenated cottonseed and castor oil; partially hydrogenated soybean oil; partially soy and cottonseed oil; glyceryl tricaproate; glyceryl tricaprylate; glyceryl tricaprate; glyceryl triundecanoate; glyceryl trilaurate; glyceryl trioleate; glyceryl trilinoleate; glyceryl trilinolenate; glyceryl tricaprylate/caprate; glyceryl tricaprylate/caprate/laurate; glyceryl tricaprylate/caprate/linoleate; and glyceryl tricaprylate/caprate/stearate.

[0222] In one embodiment, the triglyceride is the medium chain triglyceride available under the trade name LABRAFAC CC. Other triglycerides include neutral oils, *e.g.*, neutral plant oils, in particular fractionated coconut oils such as known and commercially available under the trade name MIGLYOL, including the products: MIGLYOL 810; MIGLYOL 812; MIGLYOL 818; and CAPTEX 355. Other triglycerides are caprylic-capric acid triglycerides such as known and commercially available under the trade name MYRITOL, including the product MYRITOL 813. Further triglycerides of this class are CAPMUL MCT, CAPTEX 200, CAPTEX 300, CAPTEX 800, NEOBEE M5 and MAZOL 1400.

[0223] Pharmaceutical compositions comprising triglycerides may further comprise lipophilic and/or hydrophilic surfactants which may form clear solutions upon dissolution with an aqueous solvent. One such surfactant is tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS). Examples of such compositions are described in U.S. Pat. 6,267,985.

[0224] In another embodiment, the pharmaceutically acceptable carrier comprises LABRASOL (Gattefosse SA), which is PEG-8 caprylic/capric glycerides. In another embodiment, the pharmaceutically acceptable carrier comprises PL90G, vitamin E TPGS, and Miglyol 812N.

[0225] Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of one or more of the ligands with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the ligand with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

[0226] Suitable formulations for parenteral administration include aqueous solutions of the ligand in water-soluble form, for example, water-soluble salts and alkaline solutions. In addition, suspensions of the ligand as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides or polyethylene glycol-400. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers.

[0227] The topical compositions may be formulated as oils, creams, lotions, ointments and the like by choice of appropriate carriers. Suitable carriers include vegetable or mineral oils, white petrolatum (white soft paraffin), branched chain fats or oils, animal fats and high molecular weight alcohol (greater than C_{12}). Emulsifiers, stabilizers, humectants and antioxidants may also be included as well as agents imparting color or fragrance, if desired. Additionally, transdermal penetration enhancers can be employed in these topical formulations. Examples of such enhancers can be found in U.S. Pat. Nos. 3,989,816 and 4,444,762.

[0228] Creams may be formulated from a mixture of mineral oil, self-emulsifying beeswax and water in which ligand, dissolved in a small amount of an oil such as almond oil, is admixed. A typical example of such a cream is one which includes about 40 parts water, about 20 parts beeswax, about 40 parts mineral oil and about 1 part almond oil.

[0229] Ointments may be formulated by mixing a suspension of the ligand in a vegetable oil such as almond oil with warm soft paraffin and allowing the mixture to cool. A typical example of such an ointment is one which includes about 30% almond oil and about 70% white soft paraffin by weight.

[0230] Lotions may be conveniently prepared by preparing a suspension of the ligand in a suitable high molecular weight alcohol such as propylene glycol or polyethylene glycol.

[0231] Examples of antioxidants which may be added to the pharmaceutical compositions include BHA and BHT.

[0232] In one embodiment, the pharmaceutical composition comprises 30 mg ligand per mL LABRASOL in a solid gelatin capsule. In another embodiment, the capsule contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 mg ligand.

[0233] Pharmaceutical compositions may contain from 0.01 % to 99% by weight of the ligand. Compositions may be either in single or multiple dose forms. The amount of ligand in any particular pharmaceutical composition will depend upon the effective dose, that is, the dose required to elicit the desired gene expression or suppression. In one embodiment, 0.1 to 7.5 mg/kg is administered to the subject. In another embodiment, 0.1 to 3 mg/kg is administered to the subject. In another embodiment, 0.1 to 3 mg/kg is administered.

[0234] Suitable routes of administering the pharmaceutical compositions include oral, rectal, topical (including dermal, buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal, intra-tumoral and epidural) and by naso-gastric tube. It will be understood by those skilled in the art that the route of administration will depend upon the condition being treated and may vary with factors such as the condition of the recipient. The pharmaceutical compositions may be administered one or more times daily.

Therapeutic Molecules

[0235] The therapeutic molecule, *e.g.*, the polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide may be any sequence that encodes a polypeptide or polynucleotide that is useful for the treatment, amelioration, or prevention of a disease, disorder, or condition. Therapeutic polypeptides may be any polypeptide known to be effective for treating, ameliorating, or preventing a disease, disorder, or condition. Examples of classes of therapeutic polypeptides that may be used in the invention include, without limitation, cytokines, chemokines, hormones, antibodies, engineered immunoglobulin-like molecules, single chain antibodies, fusion proteins, enzymes, immune co-stimulatory molecules, immunomodulatory molecules,

transdominant negative mutants of target proteins, toxins, conditional toxins, antigens, tumor suppressor proteins, growth factors, membrane proteins, vasoactive proteins and peptides, anti-viral proteins or variants thereof. Therapeutic polynucleotides include, without limitation, antisense sequences, small interfering RNAs, ribozymes, and RNA external guide sequences. Therapeutic polynucleotides may be targeted to any transcript associated with a particular disease, disorder, or condition and for which it is desired to decrease or eliminate expression. Numerous genes exhibiting elevated expression during a disease, disorder, or condition are known in the art, including the genes listed in Tables 1-3 above.

[0236] The polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide is operably linked to or operably associated with a factor-regulated promoter comprising at least one response element that is recognized by the DBD of the ligand-dependent transcription factor complex encoded by the gene switch. In one embodiment, the promoter comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of the response element. Promoters comprising the desired response elements may be naturally occurring promoters or artificial promoters created using techniques that are well known in the art, *e.g.*, one or more response elements operably linked to a minimal promoter.

[0237] Specific therapeutic polypeptides which may be expressed using a therapeutic gene-switch include, but are not limited to antibodies, including monoclonal antibodies, minimal antibodies, fusion proteins, endogenous protein mimetics, enzymes, hormones, cytokines, chemokines, growth factors, and fragments, variants or derivatives of any such polypeptides. Non-limiting representative therapeutic molecules are described below. All references to these molecules, including patent publications, scientific literature, and polynucleotide and polypeptide sequence accession numbers, are hereby incorporated by reference in their entireties.

Monoclonal Antibodies

[0238] Therapeutic gene-switch constructs of the present invention may be used to express therapeutic monoclonal antibodies, or fragments, variants or analogs thereof (collectively "monoclonal antibodies"). Such monoclonal antibodies are useful for treatment of diseases and disorders including, without limitation, cancer, autoimmune diseases (*e.g.*, MS, Crohn's disease, rheumatoid arthritis), cancer, infectious diseases, inflammatory diseases, allergies, heart disases, and transplantation rejection. Antibodies

for use in the present invention include any known therapeutic monoclonal antibodies including, but not limited to those listed below, monoclonal antibodies which bind to the same epitope or target as any known monoclonal antibodies. Monoclonal antibody constructs suitable for expression via therapeutic gene switch constructs include multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), and fragments comprising either a VL or VH domain. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019. Immunoglobulin or antibody molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains.

[0239] In certain embodiments, the present invention includes therapeutic gene switch constructs which encode monoclonal antibodies against antigens including, but not limited to CTLA4, CD25, HER-2/neu (ErbB2), CD20, TNFα, EGFR, and VEGF.

[0240] Anti-CTLA4 antibodies employable in the present invention, and methods of producing them, are described in the International Application No. PCT/US99/30895, published on June 29, 2000 as WO 00/37504 (*e.g.*, ticilimumab, also known as 11.2.1 and CP-675,206), European Patent Appl. No. EP 1262193 A1 , published April 12, 2002, U.S. Patent Application No. 09/472,087, now issued as U.S. Patent No. 6,682,736, U.S. Patent Application No. 09/948,939, now published as U.S. Pat. App. Pub. No. 2002/0086014 (*e.g.*, ipilimumab, also known as 10D1 and MDX-010, Medarex, Princeton, NJ),

[0241] Anti-CD25 antibodies employable in the present invention include, without limitation, Daclizumab. See, *e.g.*, US Patent No. 5,530,101. Daclizumab (brand name: Zenapax®, marketed by Roche) is a humanized IgG1 monoclonal antibody directed against CD25 (IL-2 receptor). Functioning as an IL-2 receptor antagonist, it binds with high affinity to the Tac subunit of the high-affinity IL-2 receptor complex. Daclizumab is

indicated for the prophylaxis of acute organ rejection in renal transplant patients when used in combination with cyclosporine and corticosteroids.

[0242] Anti-HER-2/neu (ErbB2) antibodies employable in the invention include, without limitation, Trastuzumab. See, *e.g.*, U.S. Patent No. 5,677,171. Trastuzumab (brand name: Herceptin®, marketed by Genentech) is a humanized, monoclonal antibody targeted against the extracellular domain of the c-erbB2/HER2/neu protein, a transmembrane receptor protein (structurally related to the Epidermal Growth Factor receptor) which is overexpressed in certain types of breast cancer. As a mediator of antibody-dependent cellular cytotoxicity, trastuzumab is preferentially toxic to HER2-expressing cancer cells.

[0243] Anti-CD20 antibodies employable in the invention include, without limitation rituximab (see, *e.g.*, U.S. Patent No. 5,736,137). Rituximab (brand name: Rituxan®, marketed by Biogen Idec and Genentech) is a chimeric (murine/human) monoclonal IgG1κ antibody. Rituximab was initially designed and licensed for treatment of non-Hodgkin's lymphoma, and more recently has been licensced for treatment of anti-TNF refractory rheumatoid arthritis.

[0244] Anti-TNFα antibodies employable in the invention include, without limitation, Adalimumab (see, *e.g.*, U.S. Patent No. 7,223,394), and Infliximab (see, *e.g.*, U.S. Patent No. 7138118). Adalimumab, (brand name: Humira®, marketed by Abbott) is a recombinant human IgG1κ monoclonal antibody which binds specifically to TNFα, thereby blocking interaction of TNFα with the p55 and p75 surface TNF receptors. Adalimumab is licensed for use in rheumatoid arthritis, and juvenile idiopathic arthritis. Additional indications for adalimumab include Crohn's disease, plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis. Infliximab (brand name: Remicade, marketed by Centocor) is a recombinant chimeric IgG1κ monoclonal antibody which binds specifically to TNFα, thereby blocking interaction of TNFα with the p55 and p75 surface TNF receptors. Infliximab is licensed for use in Crohn's disease. Additional indications include rheumatoid arthritis, psoriatic arthritis, severe chronic plaque psoriasis, and ankylosing spondylitis.

[0245] Anti EGFR (Epidermal Growth Factor Receptor) antibodies employable in the invention include, without limitation, Cetuximab (see, *e.g.*, U.S. Patent No. 6,217,866). Cetuximab (brand name: Erbitux®, marketed by Imclone and Bristol-Meyers Squibb

(North America) and by Merck KGaA (other areas) wis a chimeric monoclonal antibody which binds specifically to EGFR. Cetuximab is indicated for metastatic colorectal cancer; and head and neck cancer.

[0246] Anti-VEGF antibodies employable in the invention include, without limitation, Bevacizumab (see, *e.g.*, U.S. Patent No. 6,383,486). Bevacizumab (brand name: Avastin®, marketed by Genentech) is a human monoclonal antibody that inhibits the function of vascular endothelial growth factor (VEGF), thus inhibiting tumor neoangiogenesis. Bevacizumab is indicated for treatment in combination with other anti-cancer chemotherapeutics for the first- and second-line treatment of patients with metastatic colorectal cancer and first-line treatment of patients with recurrent or metastatic non-squamous non-small cell lung cancer (NSCLC).

Fusion Proteins

[0247] Therapeutic gene-switch constructs of the present invention may be used to express therapeutic fusion proteins, such as a chimeric TNFα binding protein 2. Tumor necrosis factor binding protein 2 (Enbrel) is produced from the membrane form by proteolytic processing. Enbrel is a recombinant fusion protein consisting of two soluble TNF receptors joined by the Fc fragment of a human IgG1 molecule. It binds to TNF-alpha and blocks TNF-alpha interaction with its receptor. Enbrel is used to treat moderate to severe rheumatoid arthritis. The amino acid sequence coding for Enbrel is available from public database as accession number P20333.

[0248] The polynucleotide sequences of Enbrel are available from public databases as accession numbers DD292498 and DD 292499, sequences of which are incorporated by reference herein.

Enzymes

[0249] Therapeutic gene-switch constructs of the present invention may be used to express therapeutic enzymes, including tissue plasminogen activator. Plasminogen activator, tissue type isoform 3 preproprotein (tPA) is a secreted serine protease which converts the proenzyme plasminogen to plasmin, a fibrinolytic enzyme. This enzyme plays a role in cell migration and tissue remodeling. The amino acid sequences coding for tPA are available from public databases as accession numbers NP_127509 and NP_000921 (both human), sequences of which are incorporated by reference herein.

[0250] The polynucleotide sequences coding for tPA are available from public databases as accession numbers NM_033011 and NM_000930 (both human), sequences of which are incorporated by reference herein.

Endogenous protein mimetics

[0251] Therapeutic gene-switch constructs of the present invention may be used to express therapeutic mimetics of endogenous proteins, such as the following.

[0252] Alphanate (Coagulation factor III), along with calcium and phospholipid, acts as a cofactor for factor IXa when it converts factor X to the activated form of factor Xa. Alphanate is purified Factor VIII (also know as Antihemophilic factor) and von Willebrand factor. Alphanate is approved for the prevention and control of bleeding in patients with Factor VIII deficiency due to hemophilia A or acquired Factor VIII deficiency. The amino acid sequences coding for factor VIII are available from public databases as accession numbers AAA52485 (human); and AAA37385 (mouse), sequences of which are incorporated by reference herein.

[0253] The polynucleotide sequences coding for factor VIII are available from public databases as accession numbers M14113 (human); and L05573 (mouse), sequences of which are incorporated by reference herein.

[0254] Aralast (Alpha-1 proteinase inhibitor) amino acid sequences are available from public databases as accession numbers AAB59375 (human alpha 1-antitrypsin); AAC28869 (mouse alpha-1 protease inhibitor); and AAA40788 (rat alpha-1-antitrypsin), sequences of which are incorporated by reference herein.

[0255] The polynucleotide sequences coding for alpha-1 proteinase inhibitor are available from public databases as accession numbers K01396 (human) ; M75721 (mouse); and M32247 (rat), sequences of which are incorporated by reference herein.

[0256] Nesiritide (Natrecor®) is a recombinant form of human B-type natriuretic peptide (hBNP) that has been approved for the intravenous treatment of patients with acute decompensated congestive heart failure (CHF) who have dyspnea at rest or with minimal activity. The amino acid sequence coding for Brain natriuretic peptide is available from public database as accession number NP_002512, sequence of which is incorporated by reference herein.

[0257] The polynucleotide sequence coding for brain natriuretic peptide is available from public database as accession number NM_002521, sequence of which is incorporated by reference herein.

[0258] The amino acid sequence coding for human insulin is available from public database as accession number AAH05255, sequence of which is incorporated by reference herein.

[0259] The polynucleotide sequence coding for human insulin is available from public database as accession number BC005255, sequence of which is incorporated by reference herein.

[0260] Granulocyte/macrophage colony-stimulating factor (GM-CSF) is a cytokine that functions as a white blood cell growth factor, stimulates stems cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes. The amino acid sequences coding for granulocyte/macrophage colony-stimulating factor (GM-CSF) are available from public databases as accession numbers AAA52122 (human); NP_034099 (mouse); NP_001032749 (rat Csf2ra); and NP_598239 (Csf2rb), sequences of which are incorporated by reference herein.

[0261] The polynucleotide sequences coding for GM-CSF are available from public databases as accession numbers M11734 (human); NM_009969 (mouse); NM_001037660 (rat Csf2ra); and NM_133555 (rat Csf2rb), sequences of which are incorporated by reference herein.

[0262] The amino acid sequences coding for erythropoietin are available from public databases as accession numbers AAH93628 (human); AAI19266 (mouse); and BAA01593 (rat), sequences of which are incorporated by reference herein.

[0263] The polynucleotide sequences coding for erythropoietin are available from public databases as accession numbers BC093628 (human); BC119265 (mouse); and D10763 (rat), sequences of which are incorporated by reference herein.

[0264] The amino acid sequences coding for growth hormone are available from public databases as accession numbers AAA98618 (human); NP_032143 (mouse); and NP_001030020 (rat), sequences of which are incorporated by reference herein.

[0265] The polynucleotide sequences coding for growth hormone are available from public databases as accession numbers M13438 (human); NM_008117 (mouse); and NM_001034848 (rat), sequences of which are incorporated by reference herein.

Recombinant Protein

[0266] Therapeutic gene-switch constructs of the present invention may be used to express therapeutic recombinant proteins, such as the botulinum toxin. The botulinum toxin inhibits neurotransmitter acetylcholine release at nerve terminals, and is available under the name BOTOX for the treatment of strabismus and blepharospasm associated with dystonia and cervical dystonia. BOTOX is also used for the treatment of hemifacial spasm and a number of other neurological disorders characterized by abnormal muscle contraction. The amino acid sequence coding for botulinum neurotoxin type A precursor (BoNT/A) (Bontoxilysin-A) (BOTOX) are available from public databases as accession numbers P10845.

Treatment of Cardiovascular Diseases

[0267] The present invention is further directed to a method of treating, ameliorating, or preventing cardiovascular disease, comprising administering to a subject in need of such treatment a therapeutic gene product which ameliorates, prevents, or treats cardiovascular related diseases under control of the switch proteins referenced earlier. Such treatment may be delivered directly to the subject to be treated, or via a bioreactor containing encapsulated or non-encapsulated non-modified or genetically modified cells which secrete one or more therapeutic proteins or therapeutic polypeptides as described elsewhere herein. According to this embodiment, the cell will express one or more therapeutic gene products effective in treating cardiovascular disease when transplanted into a subject, *e.g.*, into an infarct zone of a cardiovascular disease patient. Examples of such therapeutic gene products are described in more detail below. In certain embodiments, a genetically modified cell of the present invention expresses the one or more therapeutic gene products constitutively, *i.e.*, one or more heterologous therapeutic gene products are expressed in the cell continuously. Alternatively, expression of one, two, three, or more heterologous therapeutic gene products expressed by the cell is controlled by a therapeutic gene switch. In certain aspects, bioreactors for treatment, amelioration, or prevention of cardiovascular disease comprise encapsulated cells, *e.g.*, the cells are encapsulated in an alginate-based formulation. Examples and methods of cell encapsulation, to provide, *e.g.*, a physical or immunological barrier from the subject being treated, are described in detail elsewhere herein.

[0268] The invention further provides a nucleic acid composition comprising one or more polynucleotides which express therapeutic gene products, *e.g.*, therapeutic polypeptides and/or therapeutic polynucleotides, useful for the treatment, amelioration, or prevention of cardiovascular disease through operable association with a promoter. In certain embodiments a promoter controlling expression of a therapeutic gene product is activated by a ligand-dependent transcription factor complex, where at least a portion of the transcription factor is expressed via operable linkage to one or more therapeutic switch promotesr, where the activity of the therapeutic switch promoters is constitutive and/or is modulated under conditions associated with a tissue type or associated with a disease, disorder, or condition. In embodiments relating to the treatment, amelioration, or prevention of cardiovascular disease, a therapeutic switch promoter could be, for example, a heart-specific promoter, or a promoter which is activated during conditions such as congestive heart failure, ischemic heart disease, hypertensive heart disease, coronary artery disease, peripheral vascular disease and ischemic cardiac events, *e.g.*, myocardial infarction, heart attack, heart failure, arrhythmia, myocardial rupture, pericarditis, and cardiogenic shock. Exemplary promoters are presented in Tables 1 - 3. Additional promoters are described elsewhere herein, for example in Examples 1 - 8. Additional promoters can also be easily identified via methods described herein.

[0269] Examples of therapeutic switch promoters useful for regulated gene switch expression in cardiac cells or under conditions related to cardiac diseases, disorders, or conditions include, without limitation: the S100A6 promoter, which is tissue-specific for cardiac myocytes (Tsoporis *et al., J. Biol. Chem.* (2008) (Epub ahead of print; PMID: 18753141)); Atrial Naturetic Factor (ANF) promoter, Alpha-myosin heavy chain promoter, c-fos promoter, BNP promoter, or alpha actins promoter, all of which are tissue-specific for cardiomyocytes (Nelson *et al., J. Mol. Cell. Cardiol.* 39(3):479 (2005)); Erythropoietin promoter, which is activated in myocardium under ischemic conditions (Su *et al., Proc. Natl. Acad. Sci. U. S. A.* 99(14):9480 (2002)); AlphaB-Crystallin (CRYAB) promoter including, for example, a BRG1-response element, which is tissue-specific for vertebrate eye lens (Duncan B. and Zhao K. *DNA Cell. Biol.* 26(10):745 (2007)); AlphaB-Crystallin (CRYAB) promoter with cis-acting regulatory elements, *e.g.*, alpha BE-1, alpha BE-2, alpha BE-3, and MRF, which is tissue-specific for skeletal muscle (Gopal-Srivastava *et al., J. Mol. Cell Biol.* 15(12):7081 (1995));

NCX1 promoter, which is tissue-specific for cardiomyocytes (Xu *et al.*, *J. Biol. Chem.* 281(45):34430 (2006)); Beta myosin heavy chain promoter, which is tissue-specific for cardiomyocytes (Nelson *et al.*, *J. Mol. Cell Cardiol.* 39(3):479 (2005), Ross *et al.*, *Development.* 122(6):1799 (1996), and Lee *et al.*, *Mol. Cell. Biol.* 14(2):1220 (1994)), Myosin light chain-2 ventricular promoter including an HF-1a/HF-1b/MEF-2 combinatorial element (Ross *et al.*, *Development.* 122(6):1799 (1996)) or an HF-1a/HF-1b element and an HF-3 regulatory element, (Lee *et al.*, *Mol. Cell. Biol.* 14(2):1220 (1994)), which is tissue-specific for cardiac ventricles; Myosin light chain promoters, *e.g.*, MLC1F and MLC3F, which are differentially activated during skeletal muscle development (Kelly *et al.*, *J. Cell. Biol.* 129(2):383 (1995)); Myosin light chain 2v (MLC-2v) promoter, which is tissue-specific for cardiac muscles (Su *et al.*, *Proc. Natl. Acad. Sci. U S A.* 101(46):16280 (2004)); and Cardiac troponin I (TnIc) promoter, which is tissue-specific and developmental stage-specific in cardiac muscles (Bhavsar *et al.*, *J. Mol. Cell Cardiol.* 32(1):95 (2000)).

[0270] The invention further provides one or more vectors comprising the aforementioned nucleic acid composition, and one or more genetically modified cells comprising such vectors. Such cells may be allogeneic, autologous, or xenogeneic relative to the subject to be treated. The invention further provides one or more encapsulation methodologies for the treatment, amelioration, or prevention of cardiovascular disease, comprising the aforementioned modified cells, where the cells have been treated in such a way as to be protected from a subject's immune system upon introduction into the subject. Such treatments include, without limitation, provision of a conformal coating, microencapsulation, or macroencapsulation.

[0271] Cardiovascular diseases include, but are not limited to congestive heart failure, ischemic heart disease, hypertensive heart disease, coronary artery disease, peripheral vascular disease and ischemic cardiac events, *e.g.*, myocardial infarction, heart attack, heart failure, arrhythmia, myocardial rupture, pericarditis, and cardiogenic shock. Causes of such events include, without limitation, thrombosis, embolism, atherosclerosis, and stenosis. Populations predisposed include, without limitation, smokers, persons with diabetes, hypertension, or dyslipidemia.

[0272] Suitable therapeutic molecules for the treatment, amelioration, or prevention of cardiovascular disease include, without limitation, pro-angiogenic factors, cardioprotective factors, and cardioregenerative factors.

[0273] The therapeutic molecules useful for the present invention to prevent, treat, or ameliorate cardiovascular diseases include, without limitation, the atrial natriuretic factor (ANF), carperitide, brain Natriuretic factor (BNP), nesiritide, relaxin, vascular endothelial growth factor (VEGF165), hepatocyte growth factor (HGF), Angiopoietin-1 (Ang-1), basic fibroblast growth factor (bFGF), fibroblast growth factor 4 (FGF-4), insulin-like growth factor 1 (IGF-1), hypoxia-inducible factor1-alpha (HIF1-alpha), erythropoietin, tissue plasminogen activator (tPA), growth hormone, Stromal-Derived Factor-1 (SDF-1), sarco-endoplasmic reticulum Ca2+-ATPase (SERCA2a), adenylycyclase type VI (AC6), S100A1, parvalbumin, phosphatase inhibitor 2, and phosphatase inhibitor 1. These molecules are known to exert the effects on cardiac tissues through various mechanisms, *e.g.*, hemodynamics, angiogenesis, cardiac regeneration, anti-fibrosis, and/or cardiac repair. These therapeutic molecules may provide multiple therapeutic actions and may be used in combination with each other or other molecules that are known in public.

[0274] In one embodiment, pro-angiogenic gene therapy clinical trials for the treatment, amelioration, or prevention of cardiovascular disease are currently being performed using therapeutic proteins useful for promoting neo-vascularization. These include, without limitation, pro-angiogenic factors such as VEGF, HGF, bFGF, Ang-1, FGF-4, IGF-1, and HIF1-alpha as well as fragments, variants and derivatives thereof. Identification of suitable molecules for promoting neo-vascularization are well within the capabilities of a person of ordinary skill in the art Such pro-angiogenic factors stimulate neo-angiogenesis to supply oxygen and nutrients within the infarct zone. This will limit infarct zone expansion and sustain any cardiac progenitors that migrate into the infarct. .

[0275] Indeed, pro-angionic factor VEGF165 is known to induce neovascularization (Benest *et al.*, *Microcirculation*. 13(6):423 (2006); Riley *et al.*, *Biomaterials*. 27(35):5935 (2006); Shyu *et al.*, *Life Sci.* 73(5):563 (2003); Arsic *et al.*, *Mol Ther.* 7(4):450 (2003); Ye *et al.*, *J. Heart Lung Transplant.* 24(9):1393 (2005); Lubiatowski *et al.*, *Plast. Reconstr. Surg.* 110(1):149 (2002) (Erratum in: *Plast. Reconstr. Surg.* 111(3):1380 (2003)); Kim *et al.*, *Ann. Thorac. Surg.* 83(2):640 (2007) (Comment in: *Ann. Thorac. Surg.* 83(2):646 (2007)); Thurston G., *J. Anat.* 200(6):575 (2002); Ryu *et al.*, *Mol. Ther.*

13(4):705 (2006); Chae *et al., Arterioscler. Thromb. Vasc. Biol.* 20(12):2573 (2000); and Chen *et al., Acta. Pharmacol. Sin.* 28(4):493 (2007)). The shortcomings of early clinical trials in therapeutic neovascularization have been partly attributed to the single administration of high doses of growth factor. *See* Zacchigna *et al., Hum. Gene Ther.* 18(6):515 (2007) and Yla-Herttuala *et al., J Am Coll Cardiol.* 49(10):1015 (2007) (Comment in: *J Am Coll Cardiol.* 50(2):186 (2007)). Since then, preclinical data on VEGF expression and release has suggested that prolonged exposure results in the formation of stable vessels, whereas short-term delivery merely produces leaky vessels that regress easily. High local concentrations caused, for example, by VEGF-A-producing myoblasts results in leaky and abnormal vessels, whereas moderate amounts of the growth factor initiated the growth of healthy vessels. *See* Arsic *et al., Mol Ther.* 7(4):450 (2003); Benest *et al., Microcirculation.* 13(6):423 (2006); Yamauchi *et al., J Gene Med.* 5(11):994 (2003); Jiang *et al., Acta Cardiol.* 61(2):145 (2006); Ozawa *et al., J Clin Invest.* 113(4):516 (2004). Additionally, the combination of VEGF (initiation of angiogenesis) and Ang-1 (maturation of vessels) has been shown to result in more stable vascular growth. *See* Thurston G., *J Anat.* 200(6):575 (2002); Jiang *et al., Acta Cardiol.* 61(2):145 (2006); Benest *et al., Microcirculation.* 13(6):423 (2006); Zhou *et al., Gene Ther.* 12(3):196 (2005) (Erratum in: *Gene Ther.* 12(6):552 (2005); Liu *et al., Scand Cardiovasc* J. 41(2):95 (2007); Shyu *et al., Life Sci.* 73(5):563 (2003); Yamauchi *et al., J Gene Med.* 5(11):994 (2003); Arsic *et al., Mol Ther.* 7(4):450 (2003); Ye *et al., J Heart Lung Transplant.* 24(9):1393 (2005); Ye *et al., Eur J Heart Fail.* 9(1):15 (2007); Lubiatowski *et al., Plast Reconstr Surg.* 110(1):149 (2002) (Erratum in: *Plast Reconstr Surg.* 111(3):1380 (2003)); Ryu *et al., Mol Ther.* 13(4):705 (2006); Chen *et al., Eur J Pharmacol.* 568(1-3):222 (2007); Chae *et al., Arterioscler Thromb Vasc Biol.* 20(12):2573 (2000); and Chen *et al., Acta Pharmacol Sin.* 28(4):493 (2007).

[0276] Therefore, pro-angiogenic factor VEGF165 is known to prevent, treat or ameliorate various cardiovascular disease (Yamauchi *et al., J Gene Med.* 5(11):994 (2003) and Xu *et al., Cytotherapy.* 6(3):204 (2004) (Comment in: *Cytotherapy.* 7(1):74 (2005))) including, without limitation, myocardial infarction (Zhou *et al., Gene Ther.* 12(3):196 (2005) (Erratum in: *Gene Ther.* 12(6):552 (2005); Ye *et al., Circulation.* 116(11 Suppl):I113 (2007); Liu *et al., Scand Cardiovasc J.* 41(2):95 (2007); Ye *et al., Eur. J. Heart Fail.* 7(6):945 (2005); Zhang *et al., Cell Transplant.* 14(10):787 (2005);

Bonaros *et al.*, *Interact. Cardiovasc. Thorac. Surg.* 7(2):249 (2008); Shyu *et al.*, *J. Biomed. Sci.* 13(1):47 (2006); Ventura *et al.*, *J Biol Chem.* 282(19):14243 (2007); Sugimoto *et al.*, *Jpn. J. Thorac. Cardiovasc. Surg.* 51(5):192 (2003); Yau *et al.*, *Ann. Thorac. Surg.* 83(3):1110 (2007) (Comment in: *Ann Thorac Surg.* 83(3):1119 (2007)); Rong *et al.*, *Chin. Med. J. (Engl).* 121(4):347 (2008); Yang *et al.*, *Cardiology.* 107(1):17 (2007); Wang *et al.*, *J. Mol. Cell Cardiol.* 40(5):736 (2006); Chen *et al.*, *Eur J Clin Invest.* 35(11):677 (2005); Suzuki *et al.*, *Circulation.* 104(12 Suppl 1):I207 (2001); Ye *et al.*, *Ann. Acad. Med. Singapore.* 32(5 Suppl):S21 (2003); and Haider *et al.*, *J Mol Med.* 82(8):539 (2004) (Comment in: *J Mol Med.* 82(8):485 (2004))) or ischemia or reperfusion injury (Becker *et al.*, *Int J Cardiol.* 113(3):348 (2006); Gao *et al.*, *Can. J. Cardiol.* 23(11):891 (2007); Ye *et al. Eur J Heart Fail.* 9(1):15 (2007); Chen *et al.*, *Eur. J. Pharmacol.* 568(1-3):222 (2007); and Jiang *et al.*, *Acta Cardiol.* 61(2):145 (2006)).

[0277] Furthermore, another pro-angiogenic factor HGF (human nucleotide sequence accession No.: M29145, human amino acid sequence accession No.: NP_000592.3), which provides multipotent actions, are useful for the present invention. . HGF, mediated by c-Met receptor, provides a pro-angiogenic effect through mitogenic activity on endothelial cells, a cardioprotective anti-apoptotic effect on cardiomyocytes, an anti-fibrotic effect through suppression of TGF-beta1 signaling, and is a type I collagen regenerative factor through mobilization of CD117(+)/c-Met(+) stem cells into ischemic myocardium. *See* Li *et al.*, *Chin Med J (Engl)* 121(4):336 (2008);Guo *et al.*, *Arch. Med. Res.* 39(2):179 (2008); Ventura *et al.*, *J. Biol. Chem.* 282(19):14243 (2007); Yang *et al.*, *Gene Ther.* 13(22):1564 (2006); Tambara *et al.*, *Circulation.* 112(9 Suppl):I129 (2005); Zhang *et al.*, *Tissue Eng. Part* A. 14(6):1025 (2008); and Sakaguchi *et al.*, *Ann. Thorac. Surg.* 79(5):1627 (2005).

[0278] Similarly, bFGF (amino acid sequence accession no. NP_001997) has been shown to have the added effect of cardioprotection by promoting angiogenesis, neovascularization, and tissue regeneration. (Doi *et al.*, *Heart Vessels.* 22(2):104 (2007); Fujita *et al.*, *J.* Surg. *Res.* 126(1):27 (2005); Fujita *et al.*, *Wound Repair Regen.* 15(1):58 (2007); Hosaka *et al.*, *Circulation.* 110(21):3322 (2004); Iwakura *et al.*, *Heart Vessels.* 18(2):93 (2003); Lai *et al.*, *Tissue Eng.* 12(9):2499 (2006); Nakajima *et al.*, *J. Artif. Organs.* 7(2):58 (2004); Perets *et al.*, *J. Biomed. Mater. Res. A.* 65(4):489 (2003); Pike *et al.*, *Biomaterials.* 27(30):5242 (2006); Sakakibara *et al.*, *J Thorac Cardiovasc Surg.*

124(1):50 (2002); Sakakibara *et al., Eur J Cardiothorac Surg.* 24(1):105 (2003); Shao *et al., Circ J.* 70(4):471 (2006); Tabata Y. and Ikada Y., *Biomaterials.* 20(22):2169 (1999); Yamamoto *et al., Artif. Organs.* 27(2):181 (2003); Yamamoto *et al., Jpn. Circ. J.* 65(5):439 (2001); Yang *et al., Ophthalmic Res.* 32(1):19 (2000); and Zhu *et al., Chin. Med. Sci. J.* 15(4):210 (2000)) In certain embodiments, bFGF may be used to prevent, treat, or ameliorate osteoarthritis. *See* Inoue *et al., Arthritis Rheum.* 54(1):264 (2006);

[0279] IGF-1 (human amino acid sequence accession No.: NP_001104753.1) is also known to exert multipotent function of protecting cardiomyocytes from apoptosis and enhancing neovascularization (Su *et al., Am J Physiol Heart Circ Physiol.* 284(4):H1429 (2003); Chao *et al., J. Gene Med.* (4):277 (2003); Rabinovsky E.D. and Draghia-Akli R., *Mol Ther.* 9(1):46 (2004); and Barton *et al., Circulation.* 112(9 Suppl):I46 (2005)) and may be used in the present invention.

[0280] In addition, FGF-4 may be used as a therapeutic molecule to prevent, treat, or ameliorate chronic ischemic heart disease by inducing myocardial angio-/arteriogenesis. (Kapur N.K. and Rade J.J., *Trends Cardiovasc. Med.* 18(4):133 (2008); Henry *et al.*, J. *Am. Coll. Cardiol.* 50(11):1038 (2007); Grines *et al.; Am. J. Cardiol.* 92(9B):24N (2003); (no author listed) *BioDrugs.* 16(1):75 (2002)).

[0281] Furthermore, HIF1-alpha gene therapy, *e.g.*, HIF1-alpha (aa 1-390)/VP16 (aa 413-490), is known to treat, prevent, or ameliorate ischemic disease by enhancing BNP gene expression (Rajagopalan *et al., Circulation.* 115(10):1234 (2007) (Comment in: *Circulation.* 115(10):1180 (2007)); Wilhide M.E. and Jones W.K., *Mol Pharmacol.* 69(6):1773 (2006) (Comment on: *Mol Pharmacol.* 69(6):1953 (2006)); Luo *et al., Mol Pharmacol.* 69(6):1953 (2006) (Comment in: *Mol. Pharmacol.* 69(6):1773 (2006)) or improve angiogenesis in myocardial infarction (Shyu *et al., Cardiovasc Res.* 54(3):576 (2002); Vincent *et al., Circulation.* 102(18):2255 (2000)).

[0282] In certain embodiments, cardioprotective factors for the treatment, amelioration, or prevention of cardiovascular diseases are provided, either alone, or in combination with angiogenic factors and/or cardioregenerative factors. Cardioprotective molecules provide anti-fibrotic, anti-apoptotic signal to resident cardiomyocytes, limiting infact zone size and supplying survival signals to migrating stem cells. In certain embodiments, the cardioprotective factor is erythropoietin alfa (EPO) (human amino acid accession no. CAA26095.1), *e.g.*, human erythropoietin alfa or EPOGEN®, manufactured by Amgen.

Erythropoietin has been shown to have cardioprotective, angiogenic and neuroprotective effects (Ben-Dor *et al.*, *Cardiovasc Drugs Ther.* 21(5):339 (2007); Lin *et al.*, *Circ J.* 71(1):132 (2007); Prunier *et al.*, *Am J Physiol Heart Circ Physiol.* 292(1):H522 (2007)).

[0283] Other cardioprotective hormones demonstrated to be protective against experimental myocardial ischemia-reperfusion injury include, without limitation, adrenomedullin, bradykinin, relaxin, ANF, also known as atrial natriuretic peptide (ANP, human nucleotide sequence accession No.: NM_006172, human amino acid sequence accession No.: NP_006163), BNP, also known as B-type natriuretic peptide or GC-B (human amino acid sequence accession No.:NP_002512.1; human nucleotide sequence accession No.: M25296), C-type natriuretic peptide (CNP), carperitide, tissue plasminogen activator (tPA) and urocortins. Many have also been shown to reduce fibrosis or mediate hemodynamics. Nesiritide (brand name Natrecor®, marketed by Scios), a recombinant form of human B-type natriuretic peptide, ANF, and Carperitide are used in the treatment, amelioration, or prevention of acute decompensated heart failure, and may also be used in the present invention (Burnett J.C. Jr., *J. Cardiol.* 48(5):235 (2006)).

[0284] Relaxin (human amino acid accession no. NP_604390.1), known for its effects on the female reproductive system, is also a potent vasodilator of the systemic and coronary circulation by a mechanism of action involving nitric oxide, and influences cardiac beating rate. Relaxin is also known as a cardiovascular drug that may prevent, treat, or ameliorate ischemic heart disease (acute and chronic myocardial infarction), cardiac fibrosis, and obliterative peripheral arterial disease and restore cardiac function in cell transplantation. (Nistri *et al.*, *Pharmacol. Res.* 57(1):43 (2008); Samuel *et al.*, *Adv. Exp. Med. Biol.* 612:88 (2007); Du XJ., J. *Cell Mol. Med.* 11(5):1101 (2007); Formigli *et al.*, *J. Cell Mol. Med.* 11(5):1087 (2007); Bathgate *et al.*, *Mol. Cell Endocrinol.* 280(1-2):30 (2008); Nistri *et al.*, *Cardiovasc. Hematol.* Agents Med. Chem. 5(2):101 (2007); Moore *et al.*, *Endocrinology.* 148(4):1582 (2007); Lekgabe *et al.*, *Endocrinology.* 147(12):5575 (2006); Samuel *et al.*, *Pharmacol. Ther.* 112(2):529 (2006); Zhang *et al.*, *Peptides.* 26(9):1632 (2005); Perna *et al.*, *Ann. N.Y. Acad. Sci.* 1041:431 (2005); Perna *et al.*, *FASEB J.* 19(11):1525 (2005); Samuel *et al.*, *Endocrinology.* 145(9):4125 (2004); Masini *et al.*, *Br J Pharmacol.* 137(3):337 (2002); Ndisang *et al.*, *Inflamm. Res.* 50 Suppl.

2:S122-3 (2001); Dschietzig *et al.*, *FASEB. J.* 15(12):2187 (2001); Bani *et al.*, *Am J Pathol.* 152(5):1367 (1998); and Masini *et al.*, *Inflamm. Res.* 45 Suppl 1:S27 (1996))

[0285] In certain embodiments, therapeutic proteins of the invention useful for the treatment, amelioration, or prevention of cardiovascular diseases have multiple therapeutic benefits. For example, in the early phase after myocardial infarction, elevated (SDF-1, human nucleotide sequence accession No.: U16752, human amino acid sequence accession No.: NP_954637) levels have been reported in the infarct zone. This provides the required stimulus for mobilization of stem cells from BM niches to the damaged site as part of a natural repair process. SDF-1 recruits bone marrow haematopoietic stem cells (primarily $CD31^+$, $C\text{-}kit^+$ and $CD34^+$ cells) to the infarcted heart resulting in both neoangiogenic and cardioprotective activities. Furthermore, SDF-1 activates the cell-survival factor protein kinase B (PKB/Akt) via the G protein-coupled receptor CXCR4 regenerative factors. *See also* U.S. Patent Appl. Publ. No. 20060111290 A1; Elmadbouh *et al.*, *J Mol Cell Cardiol.* 42(4):792 (2007); Bonaros *et al.*, *Interact Cardiovasc Thorac Surg.* 7(2):249 (2008); Zhang *et al.*, *J Mol Cell Cardiol.* 44(2):281 (2008); Ma *et al.*, *Basic Res Cardiol.* 100(3):217 (2005); and Zhang *et al.*, *Tissue Eng.* 13(8):2063 (2007).

[0286] In addition, tPA (human amino acid accession no. 28274638), *e.g.*, human tissue Plasminogen Activator or Retavase®, manufactured by PDL BioPharma, Inc. is known to prevent, treat, or ameliorate post cardiac transplant complications by inhibiting graft atherosclerosis (Scholl *et al.*, *J Heart Lung Transplant.* 20(3):322 (2001); Dunn *et al.*, *Circulation.* 93(7):1439 (1996) (Comment in: *Circulation.* 93(7):1319 (1996)); and Gong *et al.*, *Gene Ther.* 14(21):1537 (2007)). Furthermore, the growth hormone is also known to prevent, treat, or ameliorate cardiovascular disease and may be used in the present invention (Isgaard J. and Bergh C.H., *BioDrugs.* 12(4):245 (1999); Fazio *et al.*, *J. Clin. Endocrinol. Metab.* 92(11):4218 (2007); Climent *et al.*, *Curr Med Chem.* 14(13):1399 (2007); Perez-Berbel *et al.*, *Int J Cardiol.* 124(3):393 (2008) (Comment on: *Int J Cardiol.* 110(3):313 (2006)); and Le Corvoisier *et al.*, *J Clin Endocrinol Metab.* 92(1):180 (2007)) by promoting angiogenesis and attenuate apoptosis (Rong *et al.*, *Chin Med J (Engl).* 121(4):347 (2008)).

[0287] In other embodiments, the therapeutic molecules that restore cardiac function are included in the present invention. Cardiac repair molecules include, but are not limited to, SERCA2a, AC6, S100A1, parvalbumin, phosphatase inhibitor 2 and phosphatase

inhibitor 1. For example, SERCA2a is known to improve cardiac contractility *in vivo* and *in vitro* and cardiac function in heart failure (Asahi *et al.*, *Proc Natl Acad Sci U S A.* 101(25):9199 (2004); Cavagna *et al.*, *J Physiol.* 528 Pt 1:53 (2000); Chaudhri *et al.*, *Mol Cell Biochem.* 251(1-2):103 (2003); Davia *et al.*, *J Mol Cell Cardiol.* 33(5):1005 (2001); del Monte *et al.*, *Circulation.* 100(23):2308 (1999) (Comment in: *Circulation.* 100(23):2303 (1999); del Monte *et al.*, *Circulation.* 104(12):1424 (2001); Hajjar *et al.*, *Circ Res.* 81(2):145 (1997) (Comment in: *Circ Res.* 88(4):373 (2001) and *Circulation.* 101(7):790 (2000)); Kawase *et al.*, *J Am Coll Cardiol.* 51(11):1112 (2008); Maier *et al.*, *Cardiovasc Res.* 67(4):636 (2005) (Comment in: *Cardiovasc Res.* 67(4):581 (2005); Meyer M. and Dillmann W.H., *Cardiovasc Res.* 37(2):360 (1998); Miyamoto *et al.*, *Proc Natl Acad Sci U S A.* 97(2):793 (2000); Muller *et al.*, *Cardiovasc Res.* 59(2):380 (2003); Sakata *et al.*, *J Mol Cell Cardiol.* 42(4):852 (2007); Sakata *et al.*, *Am J Physiol Heart Circ Physiol.* 292(2):H1204 (2007); Schmidt *et al.*, *Circulation.* 101(7):790 (2000) (Comment in: *Circulation.* 101(7):738 (2000), *Circ Res.* 81(2):145 (1997), *Circ Res.* 83(9):889 (1998), and *Circulation.* 95(2):423 (1997)) Suarez *et al.*, *Am J Physiol Heart Circ Physiol.* 287(5):H2164 (2004); Terracciano *et al.*, *Cell Calcium.* 31(6):299 (2002); Trost *et al.*, *Diabetes.* 51(4):1166 (2002); and Vetter *et al.*, *FASEB J.* 16(12):1657 (2002))

[0288] Furthermore, AC6 is known to restore affinity of SERCA2a to calcium and maximum velocity of cardiac calcium uptake by sarcoplasmic reticulum in cardiomyopathy (Gao *et al.*, *Proc Natl Acad Sci U S A.* 95(3):1038 (1998); Roth *et al.*, *Circulation.* 99(24):3099 (1999); Lai *et al.*, *Circulation.* 102(19):2396 (2000); Roth *et al.*, *Circulation.* 105(16):1989 (2002) (Comment in: *Circulation.* 105(16):1876 (2002)); Gao *et al.*, *Cardiovasc Res.* 56(2):197 (2002) (Comment in: *Cardiovasc Res.* 56(2):181 (2002)); Roth *et al.*, *Basic Res Cardiol.* 98(6):380 (2003); Roth *et al.*, *Am J Physiol Heart Circ Physiol.* 287(1):H172 (2004); Gao *et al.*, *J Biol Chem.* 279(37):38797 (2004); Tang *et al.*, *Am J Physiol Heart Circ Physiol.* 287(5):H1906 (2004); Lai *et al.*, *Circulation.* 110(3):330 (2004) (Comment in: Circulation. 110(3):242 (2004); Roth *et al.*, *Hum Gene Ther.* 15(10):989 (2004); Timofeyev *et al.*, *J Mol Cell Cardiol.* 41(1):170 (2006) (Comment in: *J Mol Cell Cardiol.* 41(3):424 (2006); Takahashi *et al.*, *Circulation.* 114(5):388 (2006) (Erratum in: *Circulation.* 114(11):e497 (2006); Comment in: *Circulation.* 114(5):365 (2006); Sastry *et al.*, *J Am Coll Cardiol.* 48(3):559 (2006); Rebolledo *et al.*, Hum Gene Ther. 17(10):1043 (2006); Hammond H.K., *Ann N Y Acad*

Sci. 1080:426 (2006); Phan *et al.*, *Trends Cardiovasc Med.* 17(7):215 (2007); Tang *et al.*, *Circulation.* 117(1):61 (2008); and Lai *et al.*, *J Am Coll Cardiol.* 51(15):1490 (2008)).

[0289] In certain embodiments, the Ca2+-binding protein S100A1 may restore cardiac function and therefore be used in the present invention. S100A1 is known to increase myocardial contraction *in vivo* and reduce propensity toward heart failure after myocardial infarction. (Most *et al.*, *J Clin Invest.* 114(11):1550 (2004); Most *et al.*, *Circulation.* 114(12):1258 (2006); Pleger *et al.*, *Mol Ther.* 12(6):1120 (2005); Pleger *et al.*, *Eur J Med* Res. 11(10):418 (2006); Remppis *et al.*, *J Gene Med.* 6(4):387 (2004); Most *et al.*, *Am J Physiol Regul Integr Comp Physiol.* 293(2):R568 (2007); Remppis *et al.*, *Basic Res Cardiol.* 97 Suppl 1:I56 (2002); Pleger *et al.*, *Circulation.* 115(19):2506 (2007); and Most *et al.*, *J Biol Chem.* 278(36):33809 (2003)). Other non-limiting examples of the therapeutic molecules that improve or restore cardiac function are: paralbumin (Hirsch *et al.*, *Am J Physiol Heart Circ Physiol.* 286(6):H2314 (2004); Michele *et al.*, *Mol Ther.* 10(2):399 (2004); and Sakata *et al.*, *J Mol Cell Cardiol.* 42(4):852 (2007)), phosphatase inhibitor 2 (Yamada *et al.*, *FASEB J.* 20(8):1197 (2006); Gupta *et al.*, *Mol Cell Biochem.* 269(1-2):49 (2005); and Kirchhefer *et al.*, *Cardiovasc* Res. 68(1):98 (2005)) and phosphatase inhibitor 1 (Gupta *et al.*, *Mol Cell Biochem.* 269(1-2):49 (2005) and Gupta *et al.*, *Am J Physiol Heart Circ Physiol.* 285(6):H2373 (2003))

[0290] Additional therapeutic molecules that may be useful for the present invention to prevent, treat, or ameliorate a disease or disorder include, but are not limited to, monoclonal antibodies (*e.g.*, HERCEPTIN®-HC, HERCEPTIN®-LC, TICILIMUMAB®-HC, TICILIMUMAB®-LC, ZENAPAX®-HC, ZENAPAX®-LC, HUMIRA®-HC, HUMIRA®-LC, RITUXAN®-HC, RITUXAN®-LC, IPILIMUMAB®-HC, IPILIMUMAB®-LC, AVASTIN®-HC, AVASTIN®-LC, Erbitux®-HC, and ERBITUX®-LC), recombinant enzymes (*e.g.*, RETAVASE®, ACTRAPID®-A chain, ACTRAPID®-B chain, NEULASTA®, pre-pro insulin, EPOGEN®, and NORDITROPIN®), fusion protein (*e.g.*, ENBREL®), or any purified proteins (*e.g.*, ALPHANATE® and ARALAST®). In addition, identification of suitable therapeutic molecules for preventing, treating, or ameliorating a particular disease or disorder is well within the capabilities of a person of ordinary in the art.

Vectors and Host Cells

[0291] To introduce the polynucleotides into the cells, a vector can be used. The vector may be, for example, a plasmid vector or a single-or double-stranded RNA or DNA viral vector. Such vectors may be introduced into cells by well-known techniques for introducing DNA and RNA into cells. Viral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing viral competent cells.

[0292] Thus, at a minimum, the vectors must include the polynucleotides of the invention. Other components of the vector may include, but are not limited to, selectable markers, chromatin modification domains, additional promoters driving expression of other polypeptides that may also be present on the vector (*e.g.*, a lethal polypeptide), genomic integration sites, recombination sites, and molecular insertion pivots. The vectors may comprise any number of these additional elements, either within or not within the polynucleotides, such that the vector can be tailored to the specific goals of the therapeutic methods desired.

[0293] In one embodiment of the present invention, the vectors that are introduced into the cells further comprise a "selectable marker gene" which, when expressed, indicates that the therapeutic gene switch construct of the present invention has been integrated into the genome of the modified cell. In this manner, the selector gene can be a positive marker for the genome integration. While not critical to the methods of the present invention, the presence of a selectable marker gene allows the practitioner to select for a population of live cells where the vector construct has been integrated into the genome of the cells. Thus, certain embodiments of the present invention comprise selecting cells where the vector has successfully been integrated. As used herein, the term "select" or variations thereof, when used in conjunction with cells, is intended to mean standard, well-known methods for choosing cells with a specific genetic make-up or phenotype. Typical methods include, but are not limited to, culturing cells in the presence of antibiotics, such as G418, neomycin and ampicillin. Other examples of selectable marker genes include, but are not limited to, genes that confer resistance to dihydrofolate reductase, hygromycin, or mycophenolic acid. Other methods of selection include, but are not limited to, a selectable marker gene that allows for the use of thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase or adenine phosphoribosyltransferase as

selection agents. Cells comprising a vector construct comprising an antibiotic resistance gene or genes would then be capable of tolerating the antibiotic in culture. Likewise, cells not comprising a vector construct comprising an antibiotic resistance gene or genes would not be capable of tolerating the antibiotic in culture.

[0294] As used herein, a "chromatin modification domain" (CMD) refers to nucleotide sequences that interact with a variety of proteins associated with maintaining and/or altering chromatin structure, such as, but not limited to, DNA insulators. *See* Ciavatta *et al., Proc. Nat'l Acad. Sci. U.S.A.*, 103:9958 (2006), which is incorporated by reference herein. Examples of CMDs include, but are not limited to, the chicken β-globulin insulator and the chicken hypersensitive site 4 (cHS4). The use of different CMD sequences between one or more gene programs (*i.e.*, a promoter, coding sequence, and 3' regulatory region), for example, can facilitate the use of the differential CMD DNA sequences as "mini homology arms" in combination with various microorganism or *in vitro* recombineering technologies to "swap" gene programs between existing multigenic and monogenic shuttle vectors. Other examples of chromatin modification domains are known in the art or can be readily identified.

[0295] Particular vectors for use with the present invention are expression vectors that code for polypeptides or polynucleotides. Generally, such vectors comprise *cis*-acting control regions effective for expression in a modified cell, operatively linked to the polynucleotide to be expressed. Appropriate *trans*-acting factors are supplied by the modified cell, supplied by a complementing vector or supplied by the vector itself upon introduction into the cell.

[0296] A great variety of expression vectors can be used to express polypeptides or polynucleotides. Such vectors include chromosomal, episomal and virus-derived vectors, *e.g.*, vectors derived from bacterial plasmids, from bacteriophage, from yeast episomes, from yeast chromosomal elements, from viruses such as adeno-associated viruses, lentiviruses, baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. All may be used for expression in accordance with this aspect of the present invention. Generally, any vector suitable to maintain,

propagate or express polynucleotides or polypeptides in a cell may be used for expression in this regard.

[0297] The polynucleotide sequence in the expression vector is operatively linked to appropriate expression control sequence(s) including, for instance, a promoter to direct mRNA transcription. Representatives of additional promoters include, but are not limited to, constitutive promoters and tissue specific or inducible promoters. Examples of constitutive eukaryotic promoters include, but are not limited to, the promoter of the mouse metallothionein I gene (Hamer *et al., J. Mol. Appl. Gen. 1*:273 (1982)); the TK promoter of Herpes virus (McKnight, *Cell 31*:355 (1982)); the SV40 early promoter (Benoist *et al.*, *Nature 290*:304 (1981)); and the vaccinia virus promoter. All of the above listed references are incorporated by reference herein. Additional examples of the promoters that could be used to drive expression of a protein or polynucleotide include, but are not limited to, tissue-specific promoters and other endogenous promoters for specific proteins, such as the albumin promoter (hepatocytes), a proinsulin promoter (pancreatic beta cells) and the like. In general, expression constructs will contain sites for transcription, initiation and termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs may include a translation initiating AUG at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

[0298] In addition, the constructs may contain control regions that regulate, as well as engender expression. Generally, such regions will operate by controlling transcription, such as repressor binding sites and enhancers, among others.

[0299] Examples of eukaryotic vectors include, but are not limited to, pW-LNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Amersham Pharmacia Biotech; and pCMVDsRed2-express, pIRES2-DsRed2, pDsRed2-Mito, and pCMV-EGFP available from Clontech. Many other vectors are well-known and commercially available.

[0300] Particularly useful vectors, which comprise molecular insertion pivots for rapid insertion and removal of elements of gene programs, are described in United States Published Patent Application No. 2004/0185556, United States Patent Application No. 11/233,246 and International Published Application Nos. WO 2005/040336 and WO

2005/116231, all of which are incorporated by reference. An example of such vectors is the UltraVector™ Production System (Intrexon Corp., Blacksburg, VA), as described in WO 2007/038276, incorporated herein by reference. As used herein, a "gene program" is a combination of genetic elements comprising a promoter (P), an expression sequence (E) and a 3' regulatory sequence (3), such that "PE3" is a gene program. The elements within the gene program can be easily swapped between molecular pivots that flank each of the elements of the gene program. A molecular pivot, as used herein, is defined as a polynucleotide comprising at least two non-variable rare or uncommon restriction sites arranged in a linear fashion. In one embodiment, the molecular pivot comprises at least three non-variable rare or uncommon restriction sites arranged in a linear fashion. Typically any one molecular pivot would not include a rare or uncommon restriction site of any other molecular pivot within the same gene program. Cognate sequences of greater than 6 nucleotides upon which a given restriction enzyme acts are referred to as "rare" restriction sites. There are, however, restriction sites of 6 bp that occur more infrequently than would be statistically predicted, and these sites and the endonucleases that cleave them are referred to as "uncommon" restriction sites. Examples of either rare or uncommon restriction enzymes include, but are not limited to, AsiS I, Pac I, Sbf I, Fse I, Asc I, Mlu I, SnaB I, Not I, Sal I, Swa I, Rsr II, BSiW I, Sfo I, Sgr AI, AflIII, Pvu I, Ngo MIV, Ase I, Flp I, Pme I, Sda I, Sgf I, Srf I, and Sse8781 I.

[0301] The vector may also comprise restriction sites for a second class of restriction enzymes called homing endonuclease (HE) enzymes. HE enzymes have large, asymmetric restriction sites (12-40 base pairs), and their restriction sites are infrequent in nature. For example, the HE known as I-SceI has an 18 bp restriction site (5'TAGGGATAACAGGGTAAT3' (SEQ ID NO:4)), predicted to occur only once in every $7x10^{10}$ base pairs of random sequence. This rate of occurrence is equivalent to only one site in a genome that is 20 times the size of a mammalian genome. The rare nature of HE sites greatly increases the likelihood that a genetic engineer can cut a gene program without disrupting the integrity of the gene program if HE sites were included in appropriate locations in a cloning vector plasmid.

[0302] Selection of appropriate vectors and promoters for expression in a host cell is a well-known procedure, and the requisite techniques for vector construction and

introduction into the host cell, as well as its expression in the host cell are routine skills in the art.

[0303] The introduction of the polynucleotides into the cells can be a transient transfection, stable transfection, or can be a locus-specific insertion of the vector. Transient and stable transfection of the vectors into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, Basic Methods in Molecular Biology (1986); Keown *et al.*, 1990, Methods Enzymol. 185: 527-37; Sambrook *et al.*, 2001, Molecular Cloning, A Laboratory Manual, Third Edition, Cold Spring Harbor Laboratory Press, N.Y., which are hereby incorporated by reference. These stable transfection methods result in random insertion of the vector into the genome of the cell. Further, the copy number and orientation of the vectors are also, generally speaking, random.

[0304] In another embodiment, locus-specific insertion may be carried out by recombinase-site specific gene insertion. Briefly, bacterial recombinase enzymes, such as, but not limited to, PhiC31 integrase can act on "pseudo" recombination sites within the human genome. These pseudo recombination sites can be targets for locus-specific insertion using the recombinases. Recombinase-site specific gene insertion is described in Thyagarajan *et al., Mol. Cell Biol.* 21:3926 (2001), which is hereby incorporated by reference. Other examples of recombinases and their respective sites that may be used for recombinase-site specific gene insertion include, but are not limited to, serine recombinases such as R4 and TP901-1 and recombinases described in WO 2006/083253, incorporated herein by reference.

[0305] In order to stably integrate the one or more gene expression systems in the genome of a modified cell, any known methods of integration may be used for the purpose of this invention. In one embodiment, locus-specific insertion may be carried out by recombinase-site specific gene insertion. Briefly, bacterial recombinase enzymes, such as, but not limited to, PhiC31 integrase may act on "pseudo" recombination sites within the human genome. See US publication No. 2004/0003420 A1; Groth *et al., Proc. Natl. Acad. Science*, 97, 5995-6000 (2000). These pseudo recombination sites may be targets for locus-specific insertion using the recombinases. Recombinase-site specific

gene insertion is described in Thyagarajan, B. *et al., Mol. Cell Biol.* 21(12):3926-34 (2001).

[0306] In certain embodiments, the first inducible gene expression system further comprises an integrase, which will stably integrate the first gene switch system into pseudo-sites within the genome of the targeted cells. A second gene switch system may also comprise an integrase, which will integrate the second gene switch system into the pseudo-sites within the genome of the targeted cells. The first gene switch system may further comprise an integrase acceptor site, which may allow integration of the second inducible gene switch system in the pre-positioned acceptor site within the genome of the targeted cells.

[0307] The following polypeptide sequence was reported as a polypeptide sequence encoding the *Streptomyces* phase PhiC31 integrase polypeptide sequence and has the accession number NP_047974 in Genbank.

[0308] *Streptomyces* phage phiC31 integrase (605 aa) (SEQ ID NO:6)

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  1 mdtyagaydr qsrerenssa aspatqrsan edkaadlqre verdggrfrf vghfseapgt
 61 safgtaerpe ferilnecra grlnmiivyd vsrfsrlkvm daipivsell algvtivstq
121 egvfrqgnvm dlihlimrld ashkesslks akildtknlq relggyvggk apygfelvse
181 tkeitrngrm vnvvinklah sttpltgpfe fepdvirwww reikthkhlp fkpgsqaaih
241 pgsitglckr mdadavptrg etigkktass awdpatvmri lrdpriagfa aeviykkkpd
301 gtpttkiegy riqrdpitlr pveldcgpii epaewyelqa wldgrgrgkg lsrgqailsa
361 mdklycecga vmtskrgees ikdsyrcrrr kvvdpsapgq hegtcnvsma aldkfvaeri
421 fnkirhaegd eetlallwea arrfgkltea peksgeranl vaeradalna leelyedraa
481 gaydgpvgrk hfrkqqaalt lrqqgaeerl aeleaaeapk lpldqwfped adadptgpks
541 wwgrasvddk rvfvglfvdk ivvtksttgr gqgtpiekra sitwakpptd ddeddaqdgt
601 edvaa
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[0309] Other examples of recombinases and their respective sites that may be used for recombinase-site specific gene insertion include, but are not limited to, serine recombinases such as R4 and TP901-1. Site-specific recombinases (SSRs), such as the bacteriophage P1-derived Cre recombinase recognize specific DNA sequences ("recognition sites," "recognition sequences," or "integrase acceptor site") and catalyze recombination between two recognition sites. Cre recombinase, for example, recognizes the 34 base pair (bp) loxP motif (Austin *et al., Cell* 25,729-736 (1981)). If the two sites are located on the same DNA molecule in the same orientation, the intervening DNA sequence is excised by the recombinase from the parental molecule as a closed circle, leaving one recognition site on each of the reaction products. If the two sites are in inverted orientation, the recognition-site flanked region is inverted through recombinase

mediated recombination. Alternatively, if the two recognition sites are located on different molecules, recombinase-mediated recombination will lead to integration of a circular molecule or translocation between two linear molecules.

[0310] In addition to Cre, a few recombinases have been shown to exhibit some activity in mammalian cells. The best characterized examples are the yeast derived FLP and Kw recombinases, which exhibit optimal activity at 30°C and are unstable at 37°C (Buchholz *et al., Nature Biotech.*, 16,657-662 (1998); Ringrose *et al., Eur. J. Biochem.*, 248,903-912). Other recombinases that show some activity in mammalian cells include a mutant integrase of phage lambda, the integrases of phage HK022, mutant gamma delta-resolvase and beta- recombinase (Lorbach *et al., J. Mol. Biol.*, 296, 1175-81 (2000); Kolot *et al., Moi. Biol. Rep.* 26,207-213 (1999); Schwikardi *et al., FEBS Lett.* , 471,147-150 (2000); Diaz *et al., J. Biol. Chem.*, 274, 6634-6640 (1999)). Moreover, an improved version of the phiC31 integrase has been developed. This modified C31-Int (C31-Int (CNLS)) carries a C-terminal nuclear localization signal (NLS) and displays a recombination efficiency in mammalian cells that is significantly enhanced over the wild type form and is comparable to that of Cre recombinase (EP 1205490; US Publication No. 2004/0003420 A1). This makes the C31-Int a valuable tool for mammalian genome modification.

[0311] In one embodiment of the invention, the vector is inserted into a bio-neutral site in the genome. A bio-neutral site is a site in the genome where insertion of the polynucleotides interferes very little, if any, with the normal function of the cell. Bio-neutral sites may be analyzed using available bioinformatics. Many bio-neutral sites are known in the art, *e.g.*, the ROSA-equivalent locus. Other bio-neutral sites may be identified using routine techniques well known in the art. Characterization of the genomic insertion site(s) is performed using methods known in the art. To control the location, copy number and/or orientation of the polynucleotides when introducing the vector into the cells, methods of locus-specific insertion may be used. Methods of locus-specific insertion are well-known in the art and include, but are not limited to, homologous recombination and recombinase-mediated genome insertion. Of course, if locus-specific insertion methods are to be used in the methods of the present invention, the vectors may comprise elements that aid in this locus-specific insertion, such as, but not limited to, homologous recombination. For example, the vectors may comprise one,

two, three, four or more genomic integration sites (GISs). As used herein, a "genomic integration site" is defined as a portion of the vector sequence which nucleotide sequence is identical or nearly identical to portions of the genome within the cells that allows for insertion of the vector in the genome. In particular, the vector may comprise two genomic insertion sites that flank at least the polynucleotides. Of course, the GISs may flank additional elements, or even all elements present on the vector.

[0312] In a further embodiment, the vector may comprise a chemo-resistance gene, *e.g.*, the multidrug resistance gene mdr1, dihydrofolate reductase, or O^6-alkylguanine-DNA alkyltransferase. The chemo-resistance gene may be under the control of a constitutive (*e.g.*, CMV) or inducible (*e.g.*, RheoSwitch®) promoter. In this embodiment, if it is desired to treat a disease in a subject while maintaining the modified cells within the subject, a clinician may apply a chemotherapeutic agent to destroy diseased cells while the modified cells would be protected from the agent due to expression of a suitable chemo-resistance gene and may continue to be used for treatment, amelioration, or prevention of a disease, disorder, or condition. By placing the chemo-resistance gene under an inducible promoter, the unnecessary expression of the chemo-resistance gene can be avoided, yet it will still be available in case continued treatment is needed. If the modified cells themselves become diseased, they could still be destroyed by inducing expression of a lethal polypeptide as described below.

[0313] The methods of the invention are carried out by introducing the polynucleotides encoding the gene switch and the therapeutic polypeptide or therapeutic polynucleotide into cells of a subject. Any method known for introducing a polynucleotide into a cell known in the art, such as those described above, can be used.

[0314] When the polynucleotides are to be introduced into cells *ex vivo*, the cells may be obtained from a subject by any technique known in the art, including, but not limited to, biopsies, scrapings, and surgical tissue removal. The isolated cells may be cultured for a sufficient amount of time to allow the polynucleotides to be introduced into the cells, *e.g.*, 2, 4, 6, 8, 10, 12, 18, 24, 36, 48, hours or more. Methods for culturing primary cells for short periods of time are well known in the art. For example, cells may be cultured in plates (*e.g.*, in microwell plates) either attached or in suspension.

[0315] For *ex vivo* therapeutic methods, cells are isolated from a subject and cultured under conditions suitable for introducing the polynucleotides into the cells. Once the

polynucleotides have been introduced into the cells, the cells are incubated for a sufficient period of time to allow the ligand-dependent transcription factor complex to be expressed, *e.g.*, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 18, or 24 hours or more. At some point after the introduction of the polynucleotides into the cells (either before or after significant levels of the ligand-dependent transcription factor complex is expressed), the cells are introduced back into the subject. Reintroduction may be carried out by any method known in the art, *e.g.*, intravenous infusion or direct injection into a tissue or cavity. In one embodiment, the presence of the polynucleotides in the cells is determined prior to introducing the cells back into the subject. In another embodiment, cells containing the polynucleotides are selected (*e.g.*, based on the presence of a selectable marker in the polynucleotides) and only those cells containing the polynucleotides are reintroduced into the subject. After the cells are reintroduced to the subject, ligand is administered to the subject to induce expression of the therapeutic polypeptide or therapeutic polynucleotide. In an alternative embodiment, the ligand may be added to the cells even before the cells are reintroduced to the subject such that the therapeutic polypeptide or therapeutic polynucleotide is expressed prior to reintroduction of the cells. The ligand may be administered by any suitable method, either systemically (*e.g.*, orally, intravenously) or locally (*e.g.*, intraperitoneally, intrathecally, intraventricularly, direct injection into the tissue or organ where the cells were reintroduced). The optimal timing of ligand administration can be determined for each type of cell and disease, disorder, or condition using only routine techniques.

[0316] In a different embodiment, the *ex vivo* therapeutic methods may be carried out using non-autologous cells, *e.g.*, cells that are allogeneic or xenogeneic to the subject, instead of autologous cells from the subject. The polynucleotides may be introduced into the non-autologous cells ex vivo to produce modified cells and the modified cells may then be introduced into the subject. The non-autologous cells may be any cells that are viable after transplantation into a subject, including, without limitation, stem cells (such as embryonic stem cells or hematopoietic stem cells) and fibroblasts.

[0317] The *in vivo* therapeutic methods of the invention involve direct *in vivo* introduction of the polynucleotides into the cells of the subject. The polynucleotides may be introduced into the subject systemically or locally (*e.g.*, at the site of the disease, disorder, or condition). Once the polynucleotides have been introduced to the subject, the

ligand may be administered to induce expression of the therapeutic polypeptide or therapeutic polynucleotide. The ligand may be administered by any suitable method, either systemically (*e.g.*, orally, intravenously) or locally (*e.g.*, intraperitoneally, intrathecally, intraventricularly, direct injection into the tissue or organ where the disease, disorder, or condition is occurring). The optimal timing of ligand administration can be determined for each type of cell and disease, disorder, or condition using only routine techniques.

[0318] In one embodiment, the ligand may be administered to the subject continuously or intermittently, and the pattern of ligand administration may be altered as necessary depending on the status of the disease, disorder, or condition. The level of expression of the therapeutic polypeptide or therapeutic polynucleotide can be modulated both by the schedule of ligand administration and the amount of ligand that is administered, permitting careful control of the therapeutic treatment.

[0319] The therapeutic methods of the invention may also be coupled with diagnostic technologies in order to improve treatment outcomes in various approaches tht are known in the art as pharmacodiagnostics or theranostics. For example, administration of the ligand may be coordinated with monitoring of the status or progression of the disease, disorder, or condition. In one embodiment, the polynucleotides of the invention are introduced into a cell together with one or more polynucleotides designed to diagnose or monitor a disease, disorder, or condition. In another embodiment, the diagnostic polynucleotides are present on the same vector comprising the polynucleotides of the invention. In this embodiment, the therapeutic treatment and the diagnostic test for monitoring effectiveness of the treatment are administered together in one unit, ensuring that all cells that receive the treatment also receive the diagnostic test. In one embodiment, the diagnostic polynucleotides comprise a diagnostic switch promoter (*i.e.*, a promoter whose activity is modulated during a disease, disorder, or condition) operably linked to a reporter gene, and monitoring of the status of the disease, disorder, or condition involves detecting the level of expression of the reporter gene.

[0320] In another theranostic embodiment of the invention, the level of expression of a therapeutic polypeptide or therapeutic polynucleotide is monitored through detecting the level of expression of a reporter gene, wherein the level of expression of the reporter directly correlates with the level of expression of the therapeutic polypeptide or

therapeutic polynucleotide. For example, the level of expression of a therapeutic protein such as interleukin-12 may be monitored non-invasively in various tissues through a bioneutral reporter such as the human type 2 somatostatin receptor, which may be imaged with a radiolabeled somatostatin analog (*see, e.g.*, Zinn *et al., J. Nucl. Med 41*:887-895 (2000)). The reporter may be linked to the same promoter as the therapeutic polypeptide or polynucleotide, or may be placed under a different promoter that is modulated by the therapeutic polypeptide or polynucleotide.

[0321] An additional embodiment of the invention relates to methods for expressing a therapeutic polypeptide or therapeutic polynucleotide in a subject, comprising:

(a) introducing into cells of said subject (1) a polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor complex, operably linked to a therapeutic switch promoter, wherein the promoter is activated during said disease, disorder, or condition, and (2) a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide linked to a promoter which is activated by said ligand-dependent transcription factor complex, to produce modified cells; and

(b) administering ligand to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0322] In one embodiment, the methods for expressing a therapeutic polypeptide or therapeutic polynucleotide in a subject may be carried out using laboratory animals (*e.g.*, mice, rats, cats, dogs, monkeys) or farm animals (*e.g.*, pigs, sheep, cows). For example, methods of expressing therapeutic products in animals may be carried out for research purposes or for the large scale production of therapeutic polypeptides or therapeutic polynucleotides.

[0323] A further embodiment of the invention relates to methods for expressing a therapeutic polypeptide or therapeutic polynucleotide in a cell, comprising:

(a) introducing into said cell (1) a polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor complex, operably linked to a therapeutic switch promoter, wherein the promoter is activated during said disease, disorder, or condition, and (2) a polynucleotide

encoding a therapeutic polypeptide or therapeutic polynucleotide linked to a promoter which is activated by said ligand-dependent transcription factor complex, to produce a modified cell; and

(b) administering ligand to said modified cell to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0324] Another embodiment of the invention is a method for expressing a therapeutic polypeptide or therapeutic polynucleotide in one or more modified cells, comprising:

(a) introducing into a cell (1) a first polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor complex through operable association with a therapeutic switch promoter, wherein said therapeutic switch promoter is activated under conditions associated with a disease, disorder, or condition, and (2) a second polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide operably associated with a factor-regulated promoter which is activated by said ligand-dependent transcription factor complex, thereby producing a modified cell; and

(b) administering ligand to said modified cell to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0325] In one embodiment, the methods for expressing a therapeutic polypeptide or therapeutic polynucleotide in a cell may be carried out *in vitro*, *e.g.*, in cells in culture. For example, *in vitro* methods of therapeutic product expression may be carried out for research use or for the large scale production of therapeutic polypeptides or therapeutic polynucleotides.

[0326] In any embodiments described herein, the polynucleotides or vector comprising the polynucleotides may comprise a sequence encoding a lethal polypeptide that can be turned on to express a product that will kill a cell containing the polynucleotides or vector. Lethal polypeptide expression can be used to eliminate the modified cells from a subject, either because treatment is no longer needed or because of a problem with the modified cells (*e.g.*, hyperproliferation or toxicity). A lethal polypeptide, as used herein, is a polypeptide that, when expressed, is lethal to the cell that expresses the polypeptide, either because the polypeptide itself is lethal or the polypeptide produces a compound that

is lethal. As used herein, a lethal polypeptide includes polypeptides that induce cell death in any fashion, including but not limited to, necrosis, apoptosis and cytotoxicity. Examples of lethal polypeptides include, but are not limited to, apoptosis inducing tumor suppressor genes such as, but not limited to, p53, Rb and BRCA-1, toxins such as diphtheria toxin (DTA), shigella neurotoxin, botulism toxin, tetanus toxin, cholera toxin, CSE-V2 and other variants of scorpion protein toxins to name a few, suicide genes such as cytosine deaminase and thymidine kinase, and cytotoxic genes, *e.g.*, tumor necrosis factor, interferon-alpha. The present invention is not limited by the identity of the lethal polypeptide, provided that the polypeptide is capable of being lethal to the cell in which it is expressed. If the modified cells are short-lived cells (*e.g.*, cells with a limited lifespan (*e.g.*, about 10 days or less, such as dendritic cells), it may not be necessary to include a lethal polypeptide in the polynucleotides or vector as the cells will be naturally removed over a short period of time.

[0327] For each of the methods described above, in one embodiment, the polynucleotide encoding the gene switch and the polynucleotide encoding the therapeutic polypeptide or therapeutic polynucleotide linked to a promoter are part of one larger polynucleotide, *e.g.*, a vector. In another embodiment, the polynucleotide encoding the gene switch and the polynucleotide encoding the therapeutic polypeptide or therapeutic polynucleotide linked to a promoter are separate polynucleotides, which may be combined to form a "nucleic acid composition."

[0328] In one aspect, the invention relates to polynucleotides that may be used in the methods of the invention. In one embodiment, the polynucleotide encodes a gene switch, the gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor complex, operably linked to a therapeutic switch promoter, wherein the activity of the promoter is modulated during said disease, disorder, or condition. In another embodiment, the polynucleotide further encodes a therapeutic polypeptide or therapeutic polynucleotide linked to a factor-regulated promoter which is activated by said ligand-dependent transcription factor complex. In one embodiment, the gene switch is an EcR-based gene switch. In another embodiment, the gene switch comprises a first transcription factor sequence under the control of a first therapeutic switch promoter and a second transcription factor sequence under the control of a second therapeutic switch

promoter, wherein the proteins encoded by said first transcription factor sequence and said second transcription factor sequence interact to form a protein complex which functions as a ligand-dependent transcription factor complex. In one embodiment, the first therapeutic switch promoter and the second therapeutic switch promoter are different. In another embodiment, the first therapeutic switch promoter and the second therapeutic switch promoter are the same. In another embodiment, the first transcription factor sequence encodes a protein comprising a heterodimer partner and a transactivation domain and the second transcription factor sequence encodes a protein comprising a DNA binding domain and a ligand-binding domain. In a further embodiment, the polynucleotide also encodes a lethal polypeptide operably linked to an inducible promoter.

[0329] Another aspect of the invention relates to vectors comprising any of the polynucleotides described above. In one embodiment, the vector is a plasmid vector or a viral vector.

[0330] In another aspect, the invention provides kits that may be used in conjunction with methods of the invention. Kits according to this aspect of the invention may comprise one or more containers, which may contain one or more components selected from the group consisting of one or more nucleic acid molecules, restriction enzymes and one or more cells comprising such nucleic acid molecules. Kits of the invention may further comprise one or more containers containing supporting cells suitable for supporting the cells of the invention in culture, one or more containers containing cell culture media suitable for culturing cells of the invention, one or more containers containing antibiotics suitable for use in culturing cells of the invention, one or more containers containing buffers, one or more containers containing transfection reagents, one or more containers containing substrates for enzymatic reactions, and/or one or more ligands for gene switch activation.

[0331] Kits of the invention may contain a wide variety of nucleic acid molecules that can be used with the invention. Examples of nucleic acid molecules that can be supplied in kits of the invention include those that contain promoters, sequences encoding gene switches, enhancers, repressors, selection markers, transcription signals, translation signals, primer hybridization sites (*e.g.*, for sequencing or PCR), recombination sites, restriction sites and polylinkers, sites that suppress the termination of translation in the

presence of a suppressor tRNA, suppressor tRNA coding sequences, sequences that encode domains and/or regions, origins of replication, telomeres, centromeres, and the like. In one embodiment, kits may comprise a polynucleotide comprising a gene switch without any therapeutic switch promoters, the polynucleotide being suitable for insertion of any therapeutic switch promoters of interest. Nucleic acid molecules of the invention may comprise any one or more of these features in addition to polynucleotides as described above.

[0332] Kits of the invention may comprise containers containing one or more recombination proteins. Suitable recombination proteins include, but are not limited to, Cre, Int, IHF, Xis, Flp, Fis, Hin, Gin, Cin, Tn3 resolvase, ΦC31, TndX, XerC, and XerD. Other suitable recombination sites and proteins are those associated with the GATEWAY™ Cloning Technology available from Invitrogen Corp., Carlsbad, CA, and described in the product literature of the GATEWAY™ Cloning Technology (version E, September 22, 2003), the entire disclosures of which are incorporated herein by reference.

[0333] Kits of the invention can also be supplied with primers. These primers will generally be designed to anneal to molecules having specific nucleotide sequences. For example, these primers can be designed for use in PCR to amplify a particular nucleic acid molecule. Sequencing primers may also be supplied with the kit.

[0334] One or more buffers (*e.g.*, one, two, three, four, five, eight, ten, fifteen) may be supplied in kits of the invention. These buffers may be supplied at working concentrations or may be supplied in concentrated form and then diluted to the working concentrations. These buffers will often contain salt, metal ions, co-factors, metal ion chelating agents, *etc.* for the enhancement of activities or the stabilization of either the buffer itself or molecules in the buffer. Further, these buffers may be supplied in dried or aqueous forms. When buffers are supplied in a dried form, they will generally be dissolved in water prior to use.

[0335] Kits of the invention may contain virtually any combination of the components set out above or described elsewhere herein. As one skilled in the art would recognize, the components supplied with kits of the invention will vary with the intended use for the kits. Thus, kits may be designed to perform various functions set out in this application and the components of such kits will vary accordingly.

EXAMPLES

[0336] The examples which follow further illustrate the invention, but should not be construed to limit the scope of the invention in any way. The practice of the present invention, including the following examples will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, and recombinant DNA, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning: A Laboratory Manual (3-Volume Set), J. Sambrook, D. W. Russell, Cold Spring Harbor Laboratory Press (2001); Genes VIII, B. Lewin, Prentice Hall (2003); PCR Primer, C.W. Dieffenbach and G.S. Dveksler, CSHL Press (2003); DNA Cloning, D. N. Glover ed., Volumes I and II (1985); Oligonucleotide Synthesis: Methods and Applications (Methods in Molecular Biology), P. Herdewijn (Ed.), Humana Press (2004); Culture of Animal Cells: A Manual of Basic Technique, 4th edition, R. I. Freshney, Wiley-Liss (2000); Oligonucleotide Synthesis, M. J. Gait (Ed.), (1984); Mullis *et.al* U.S. Pat. No: 4,683,195; Nucleic Acid Hybridization, B. D. Hames & S. J. Higgins eds. (1984); Nucleic Acid Hybridization, M. L. M. Anderson, Springer (1999); Animal Cell Culture and Technology, 2nd edition, M. Butler, BIOS Scientific Publishers (2004); Immobilized Cells and Enzymes: A Practical Approach (Practical Approach Series), J. Woodward, IRL Press (1992); Transcription And Translation, B. D. Hames & S. J. Higgins (Eds.) (1984); Culture Of Animal Cells, R. I. Freshney, Alan R. Liss, Inc., (1987); A Practical Guide To Molecular Cloning, 3rd edition, B. Perbal, John Wiley & Sons Inc. (1988); Gene Transfer Vectors For Mammalian Cells, J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory (1987); Methods In Enzymology, Vols. 154 and 155, Wu *et.al* (Eds.); Immunochemical Methods In Cell And Molecular Biology, Mayer and Walker, (Eds.), Academic Press, London (1987); and in Ausubel *et.al*, Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Maryland (1989).

Example 1

[0337] This example describes a gene therapy vector useful for the treatment of ischemic heart disease through the promotion of angiogenesis. Insulin like growth factor 1 is a hormone that may be useful in the treatment of ischemic heart disease. (IGF-1, GenBank Accession No.: NP_001104753.1, SEQ ID NO:20). Use of IGF-1 in preclinical models is

associated with improved cardiac function, anti-apoptosis, neo-vascularization and cardiac muscle regeneration (reviewed in Santini, M.P., *et al. Novartis Found Symp. 274*:228-38 (2006); discussion 239-43, 272-6; and Saetrum Opgaard, O., and Wang, P.H. *Growth Horm IGF Res. 15*:89-94 (2005)). For this purpose, an example of inducible IGF-1 expression, in response to ischemia and/or inflammation is given. An inducible expression system for the expression if IGF-1 upon administration of ligand, under hypoxic conditions which occur in ischemic tissue is shown in Fig. 5.

SEQ ID NO:20:

MGKISSLPTQLFKCCFCDFLKVKMHTMSSSHLFYLALCLLTFTSSATAGPETLCGAELVD ALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLEMYCAPLKPAKSAR SVRAQRHTDMPKTQKYQPPSTNKNTKSQRRKGSTFEERK

[0338] The complete nucleotide sequence of the construct shown in Fig. 5 is presented as SEQ ID NO:7. The nucleotide coordinates for salient elements of the construct are shown in Table 4.

TABLE 4

Label	**Direction**	**Length**	**Start**	**End**
3'Reg(HSVTKpA)	reverse	259	197	455
Neo	reverse	795	462	1256
SV40 early promoter	reverse	278	1446	1723
3'Reg(SV40pA)	reverse	221	1830	2050
LTF[Gal4(DBD):EcR(LBD)]	reverse	1467	2057	3523
TL (cMyc ires)	reverse	408	3536	3943
CAP[VP16(AD):RXR(HP)]	reverse	975	3950	4924
TSP-1 (Cardiac hypoxia-inducible)	reverse	578	4958	5535
FRP[6xGalRE:Minimal Promoter]	forward	189	5946	6134
Insulin like growth factor (IGF-1) Coding Region	forward	477	6348	6824
3'Reg(hGH PolyA)	forward	627	6897	7523

Replication Origin	reverse	589	7957	8545
AmpR	reverse	858	8920	9777
bla Promoter	reverse	39	9811	9849

[0339] The vector shown in Fig. 5 is modeled according to the gene switch system shown in Fig. 1. Under this system, both the CAP subunit, and the LTF subunit of the ligand-dependent transcription factor complex (LDTFC) are expressed through operable association with a single therapeutic switch promoter (TSP-1) via use of an internal ribosome entry site (IRES). The promoter utilized in this system is a UV-conformed synthetic hypoxia-inducible promoter.

[0340] The coding region for the therapeutic product, IGF-1, is operably associated with a factor-regulated promoter (FRP) which is activated upon contact with the LDTFC in the presence of ligand.

[0341] The construct shown in Fig. 5 is inserted into a suitable vector system, for example, a viral vector, for delivery to a subject in need of treatment for ischemic heart disease.

[0342] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of gene therapy vectors are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and the transcription factor may be expressed under the appropriate physiological conditions. The LTF encoded by the vector will be expressed under hypoxic conditions associated with, *e.g.*, cardiac ischemia. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of IGF-1 under control of the factor-regulated promoter. IGF-1 expression in turn promotes targeted angiogenesis in the ischemic tissue.

Example 2

[0343] This example describes a bioreactor/cell therapy vector useful for the treatment of ischemic cardiovascular disease through the promotion of angiogenesis and cardioprotection. The vector, shown in Fig. 6, will confer expression of human basic

fibroblast growth factor (bFGF, GenBank Accession No.: NP_001997, SEQ ID NO:21) upon administration of ligand, under hypoxic conditions which occur in ischemic tissue.

SEQ ID NO:21:

MVGVGGGDVEDVTPRPGGCQISGRGARGCNGIPGAAAWEAALPRRRPRRHPSVNPRSR
AAGSPRTRGRRTEERPSGSRLGDRGRGRALPGGRLGGRGRGRAPERVGGRGRGRGTAA
PRAAPAARGSRPGPAGTMAAGSITTLPALPEDGGSGAFPPGHFKDPKRLYCKNGGFFLRI
HPDGRVDGVREKSDPHIKLQLQAEERGVVSIKGVCANRYLAMKEDGRLLASKCVTDEC
FFFERLESNNYNTYRSRKYTSWYVALKRTGQYKLGSKTGPGQKAILFLPMSAKS

[0344] The complete nucleotide sequence of the construct shown in Figure 6 is presented as SEQ ID NO:8. The nucleotide coordinates for salient elements of the construct are shown in Table 5.

Table 5

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
SV40 early promoter	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992
TSP-1 (constitutive)	reverse	571	3026	3596
3'Reg(SV40pA)	reverse	221	3719	3939
LTF[Gal4(DBD):EcR(LBD)]	reverse	1467	3946	5412
TSP-2(hypoxia-inducible)	reverse	870	5446	6315
FRP[6x GalRE:Minimal Promoter]	forward	189	6648	6836
TSPq(bFGF)	forward	867	7050	7916

3'Reg(hGH PolyA)	forward	627	7989	8615
Replication Origin	reverse	589	9049	9637
AmpR	reverse	858	9882	10739
bla Promoter	reverse	39	10773	10811

[0345] The vector shown in Fig. 6 is modeled according to the gene switch system shown in Fig. 2. Under this system, the CAP subunit of the LDTFC is expressed through operable association with a first, constitutive therapeutic switch promoter, TSP-1, and the LTF subunit of the LDTFC is expressed through operable association with a second, inducible therapeutic switch promoter (TSP-2). The promoter used in this construct is the hypoxia-inducible control promoter-1.

[0346] The coding region for the therapeutic product, bFGF, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0347] The construct shown in Fig. 6 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, *e.g.*, the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for ischemic heart disease.

[0348] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of cell-based therapies are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and the LTF encoded by the vector will be expressed under hypoxic conditions associated with, *e.g.*, cardiac ischemia. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of bFGF under control of the

FRP . bFGF expression in turn promotes targeted angiogenesis and/or cardioprotection in the ischemic tissue.

Example 3

[0349] This example describes a bioreactor/cell therapy vector useful for the treatment of ischemic cardiovascular disease through the promotion of cardioprotection. The vector, shown in Fig. 7, will confer expression of human erythropoietin (EPO, GenBank Accession No.: CAA26095.1, SEQ ID NO:22) upon administration of ligand, under hypoxic conditions which occur in ischemic tissue. Erythropoietin has been shown to function in cardioprotection and anti-remodeling, in response to ischemia.

SEQ ID NO:22:

MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLQRYLLEAKEAENITTGCAEHC
SLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEP
LQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLR
GKLKLYTGEACRTGDR

[0350] The complete nucleotide sequence of the construct shown in Fig. 7 is presented as SEQ ID NO:9. The nucleotide coordinates for salient elements of the construct are shown in Table 6.

Table 6

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
SV40 early promoter	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992
TSP-1(constitutive)	reverse	571	3026	3596
3'Reg(SV40pA)	reverse	221	3719	3939
LTF[Gal4(DBD):EcR(LBD)]	reverse	1467	3946	5412
TSP2(Hypoxia-inducible)	reverse	870	5446	6315

FRP(6x GalRE:minimal promoter)	forward	189	6648	6836
TSPQ(Epo)	forward	582	7050	7631
3'Reg(hGH PolyA)	forward	627	7704	8330
Replication Origin	reverse	589	8764	9352
AmpR	reverse	858	9597	10454
bla Promoter	reverse	39	10488	10526

[0351] The vector shown in Fig. 7 is modeled according to the gene switch system shown in Fig. 2. Under this system, the CAP subunit of the LDTFC is expressed through operable association with a first, constitutive therapeutic switch promoter (TSP-1), and the LTF subunit of the LDTFC is expressed through operable association with a second, inducible therapeutic switch promoter (TSP-2). The inducible therapeutic switch promoter used in this vector is the hypoxia-inducible control promoter-1.

[0352] The coding region for the therapeutic product, EPO, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0353] The construct shown in Fig. 7 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, *e.g.*, the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for ischemic heart disease.

[0354] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of cell-based therapies are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and LTF encoded by the vector will be expressed under hypoxic conditions associated with, *e.g.*, cardiac ischemia. Ligand will be administered to the subject to be treated which will

combine with the expressed LDTFC to drive expression of EPO under control of the FRP. EPO expression in turn promotes targeted cardioprotection in the ischemic tissue.

Example 4

[0355] This example describes a bioreactor/cell therapy vector useful for the treatment of ischemic cardiovascular disease through the promotion of antiogenesis and hemodynamics. The vector, shown in Fig. 8, will confer expression of human brain natriuretic factor (BNP, GenBank Accession No.: NP_002512, SEQ ID NO:23) upon administration of ligand, under hypoxic conditions which occur in ischemic tissue. BNP, as well as other natriuretic peptides, such as relaxin, ANF, CNP and adrenomodulin, has been shown to function in cardioprotection, vasodilation and anti-remodeling, in the heart. For this purpose, an example of inducible expression of BNP, in response to ischemia is given.

SEQ ID NO:23:

MDPQTAPSRALLLLLFLHLAFLGGRSHPLGSPGSASDLETSGLQEQRNHLQGKLSELQVE QTSLEPLQESPRPTGVWKSREVATEGIRGHRKMVLYTLRAPRSPKMVQGSGCFGRKMD RISSSSGLGCKVLRRH

[0356] The complete nucleotide sequence of the construct shown in Fig. 8 is presented as SEQ ID NO:10. The nucleotide coordinates for salient elements of the construct are shown in Table 7.

Table 7

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
SV40 early promoter	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992
TSP-1(constitutive)	reverse	571	3026	3596
3'Reg(SV40pA)	reverse	221	3719	3939

LTF[GAL4(DBD):EcR(LBD)]	reverse	1467	3946	5412
TSP-2(Hypoxia-inducible)	reverse	870	5446	6315
FRP(6x GalRE:Minimal Promoter)	forward	189	6648	6836
TSPQ(BNP)	forward	405	7050	7454
3'Reg(hGH PolyA)	forward	627	7527	8153
Replication Origin	reverse	589	8587	9175
AmpR	reverse	858	9420	10277
bla Promoter	reverse	39	10311	10349

[0357] The vector shown in Fig. 8 is modeled according to the gene switch system shown in Fig. 2. Under this system, the CAP subunit of the LDTFC is expressed through operable association with a first, constitutive therapeutic switch promoter (TSP-1), and the LTF subunit of the LDTFC is expressed through operable association with a second, inducible therapeutic switch promoter (TSP-2). The inducible TSP-2 used in this vector is the hypoxia-inducible control promoter-1.

[0358] The coding region for the therapeutic product, BNP, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0359] The construct shown in Fig. 8 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, *e.g.*, the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for ischemic heart disease.

[0360] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of cell-based therapies are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and the

LTF encoded by the vector will be expressed under hypoxic conditions associated with, *e.g.*, cardiac ischemia. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of BNP under control of the FRP. BNP expression in turn promotes targeted cardioprotection, vasodilation and anti-remodeling in the ischemic tissue.

Example 5

[0361] This example describes a bioreactor/cell therapy vector useful for the treatment of ischemic cardiovascular disease through the breakdown of fibrin deposition in the heart. The vector, shown in Fig. 9, will confer expression of human tissue plasminogen activator (tPA, GenBank Accession No.: AAO34406, SEQ ID NO:24) upon administration of ligand, under inflammatory conditions which occur in ischemic tissue. Tissue plasminogen activator is a serine protease that catalyzes the conversion of plasminogen to the activated enzyme plasmin, that degrades fibrin. The use of recombinant tPA has been proven effective as a thrombolytic, for the breakdown of fibrin clots, in diseases such as pulmonary embolism, myocardial infarction and stroke. In addition to clot formation, excess fibrin deposition in the heart and vasculature is associated with insulin resistant diabetes, atherosclerosis and myocardial infarction in response to inflammation. For this purpose, an example of inducible expression of tPA, in response to ischemia is given.

SEQ ID NO:24:

MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARSYQVICRDEKTQMIYQQHQSWLR
PVLRSNRVEYCWCNSGRAQCHSVPVKSCSEPRCFNGGTCQQALYFSDFVCQCPEGFAG
KCCEIDTRATCYEDQGISYRGTWSTAESGAECTNWNSSALAQKPYSGRRPDAIRLGLGN
HNYCRNPDRDSKPWCYVFKAGKYSSEFCSTPACSEGNSDCYFGNGSAYRGTHSLTESGA
SCLPWNSMILIGNVYTAQNPSAQALGLGKHNYCRNPDGDAKPWCHVLKNRRLTWEYC
DVPSCSTCGLRQYSQPQFRIKGGLFADIASHPWQAAIFAKHRRSPGERFLCGGILISSCWIL
SAAHCFQERFPPHHLTVILGRTYRVVPGEEEQKFEVEKYIVHKEFDDDTYDNDIALLQLK
SDSSRCAQESSVVRTVCLPPADLQLPDWTECELSGYGKHEALSPFYSERLKEAHVRLYPS
SRCTSQHLLNRTVTDNMLCAGDTRSGGPQANLHDACQGDSGGPLVCLNDGRMTLVGII
SWGLGCGQKDVPGVYTKVTNYLDWIRDNMRP

[0362] The complete nucleotide sequence of the construct shown in Fig. 9 is presented as SEQ ID NO:11. The nucleotide coordinates for salient elements of the construct are shown in Table 8.

Table 8

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
SV40 early promoter	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992
TSP-1 (constitutive)	reverse	571	3026	3596
3'Reg(SV40pA)	reverse	221	3719	3939
LTF[GAL4(DBD):EcR(LBD)]	reverse	1467	3946	5412
TSP-2 (hypoxia inducible)	reverse	770	5446	6215
FRP[6xGalRE:Minimal Promoter]	forward	189	6548	6736
TPSQ (Reteplase, tPA)	forward	1689	6950	8638
3'Reg(hGH PolyA)	forward	627	8711	9337
Replication Origin	reverse	589	9771	10359
AmpR	reverse	858	10604	11461
bla Promoter	reverse	39	11495	11533

[0363] The vector shown in Fig. 9 is modeled according to the gene switch system shown in Fig. 2. Under this system, the CAP subunit of the LDTFC is expressed through operable association with a first, constitutive therapeutic switch promoter (TSP-1), and the LTF subunit of the LDTFC is expressed through operable association with a second,

inducible therapeutic switch promoter (TSP-2). The inducible therapeutic switch promoter used in this vector is the human Plexin D1 promoter.

[0364] The coding region for the therapeutic product, tPA, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0365] The construct shown in Fig. 9 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, *e.g.*, the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for ischemic heart disease.

[0366] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of cell-based therapies are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and the LTF encoded by the vector will be expressed in the event of an inflammatory response associated with, *e.g.*, cardiac ischemia. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of tPA under control of the FRP. tPA expression in turn promotes targeted break-up of fibrin deposition in the ischemic tissue.

Example 6

[0367] This example describes a bioreactor/cell therapy vector useful for the treatment of ischemic cardiovascular disease through the promotion of cardioprotection, antiogenesis and hemodynamics. The vector, shown in Fig. 10, will confer expression of two therapeutic polypeptides, human relaxin (GenBank Accession No.: NP_604390.1, SEQ ID NO:25) and human hepatocyte growth factor (HGF, GenBank Accession No.: NP_000592.3, SEQ ID NO:26) upon administration of ligand, under inflammatory conditions and/or hypoxia, respectively, both of which occur in ischemic tissue. Relaxin

is a potent vasodilator of the systemic and coronary circulation by a mechanism of action involving nitric oxide, and influences cardiac beating rate. HGF provides a pro-angiogenic effect, a cardioprotective anti-apoptotic effect, an anti-fibrotic effect, and is a type I collagen regenerative factor in ischemic myocardium. For this purpose, an example of separately controlled inducible expression of relaxin and HGF in response to ischemia is given.

SEQ ID NO:25:

MPRLFFFHLLGVCLLLNQFSRAVADSWMEEVIKLCGRELVRAQIAICGMSTWSKRSLSQ
EDAPQTPRPVAEIVPSFINKDTETINMMSEFVANLPQELKLTLSEMQPALPQLQQHVPVL
KDSSLLFEEFKKLIRNRQSEAADSSPSELKYLGLDTHSRKKRQLYSALANKCCHVGCTKR
SLARFC

SEQ ID NO:26:

MWVTKLLPALLLQHVLLHLLLLPIAIPYAEGQRKRRNTIHEFKKSAKTTLIKIDPALKIKT
KKVNTADQCANRCTRNKGLPFTCKAFVFDKARKQCLWFPFNSMSSGVKKEFGHEFDLY
ENKDYIRNCIIGKGRSYKGTVSITKSGIKCQPWSSMIPHEHSFLPSSYRGKDLQENYCRNP
RGEEGGPWCFTSNPEVRYEVCDIPQCSEVECMTCNGESYRGLMDHTESGKICQRWDHQ
TPHRHKFLPERYPDKGFDDNYCRNPDGQPRPWCYTLDPHTRWEYCAIKTCADNTMNDT
DVPLETTECIQGQGEGYRGTVNTIWNGIPCQRWDSQYPHEHDMTPENFKCKDLRENYC
RNPDGSESPWCFTTDPNIRVGYCSQIPNCDMSHGQDCYRGNGKNYMGNLSQTRSGLTCS
MWDKNMEDLHRHIFWEPDASKLNENYCRNPDDDAHGPWCYTGNPLIPWDYCPISRCE
GDTTPTIVNLDHPVISCAKTKQLRVVNGIPTRTNIGWMVSLRYRNKHICGGSLIKESWVL
TARQCFPSRDLKDYEAWLGIHDVHGRGDEKCKQVLNVSQLVYGPEGSDLVLMKLARP
AVLDDFVSTIDLPNYGCTIPEKTSCSVYGWGYTGLINYDGLLRVAHLYIMGNEKCSQHH
RGKVTLNESEICAGAEKIGSGPCEGDYGGPLVCEQHKMRMVLGVIVPGRGCAIPNRPGIF
VRVAYYAKWIHKIILTYKVPQS

[0368] The complete nucleotide sequence of the construct shown in Fig. 10 is presented as SEQ ID NO:13. The nucleotide coordinates for salient elements of the construct are shown in Table 9.

Table 9

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	197	455
NEO	reverse	804	462	1265
SV40e Promoter	reverse	280	1385	1664
3'Reg(Synthetic PolyA)	reverse	49	1782	1830
CAP[VP16(AD):RxR(HP)]	reverse	975	1837	2811
TSP-1(ubiquitous)	reverse	571	2845	3415
3'Reg(SV40pA)	reverse	221	3538	3758
LTF-1[EcR(LBD):Gal4(DBD)]	reverse	1467	3765	5231
TSP-2(inflammatory inducible)	reverse	770	5265	6034
FRP-1[6x GalRE:Minimal Promoter]	forward	189	6405	6593
TPSQ-1(Relaxin)	forward	558	6807	7364
3'Reg(hGH PolyA)	forward	627	7437	8063
FRP-2[8X LexA RE:Minimal Promoter]	forward	216	8437	8652
TPSQ-2(HGF)	forward	2187	8866	11052
3'Reg(SV40 early pA)	forward	135	11112	11246
TSP-3(Hypoxia-inducible)	forward	870	11478	12347
LTF-2[LexA(DBD) CfEcR-DEF(LBD)]	forward	1629	12381	14009
EcR-LBD (DEF)	forward	1014	12996	14009
3'Reg(Human S100 CABP)	forward	765	14016	14780
Replication Origin	reverse	589	15114	15702
AmpR	reverse	858	16038	16895
bla Promoter	reverse	39	16929	16967

[0369] The vector shown in Fig. 10 is modeled according to the gene switch system shown in Fig. 3. Under this system, the CAP subunit of the LDTFC is expressed through operable association with a first, constitutive therapeutic switch promoter (TSP-1), a first LTF (LTF-1) subunit is expressed through operable association with a second, inducible therapeutic switch promoter (TSP-2), and a second LTF (LTF-2) subunit is expressed through operable association with a third, inducible therapeutic switch promoter (TSP-3). TSP-2 in this vector is the human Plexin D1 promoter and TSP-3 in this vector is the hypoxia-inducible control promoter 1.

[0370] The coding region for the first therapeutic product, relaxin, is operably associated with a first FRP (FRP-1) having response elements which recognize the first DNA binding domain DBD-A of LTF-1, and the coding region for the second therapeutic product, HGF, is operably associated with a second FRP (FRP-2) having response elements which recognize the second DNA binding domain (DBD-B) of LTF-2. Both FRPs are activated upon contact with the respective LDTFC in the presence of ligand.

[0371] The construct shown in Fig. 10 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, *e.g.*, the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for ischemic heart disease.

[0372] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of cell-based therapies are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and the LTF-1 and/or LTF-2 will be expressed in the event of an inflammatory response and/or hypoxia associated with, *e.g.*, cardiac ischemia. One or more ligands will be administered to the subject to be treated which will combine with the expressed LDTFC(s) to drive expression of relaxin and/or HGF under control of FRPs.

Example 7

[0373] This example describes a bioreactor/cell therapy vector useful for the treatment of ischemic cardiovascular disease through the promotion of cardiac repair and cardioprotection. The vector, shown in Fig. 11, will confer expression of human EPO (see Example 3) upon administration of ligand, under hypoxic conditions which occur in ischemic tissue. EPO has been shown to function in cardioprotection and anti-remodeling, in response to ischemia. In this example, EPO expression is specifically limited to cardiac tissue.

[0374] The vector shown in Fig. 11 is modeled according to the gene switch system shown in Fig. 2. Under this system, the CAP subunit is expressed through operable association with a promoter which is specific for cardiac tissue (Nxc1 cardiomyocyte-specific promoter), and the LTF subunit is expressed through operable association with the hypoxia-inducible control promoter-1.

[0375] The coding region for the therapeutic product, EPO, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0376] The complete nucleotide sequence of the construct shown in Figure 11 is presented as SEQ ID NO:12. The nucleotide coordinates for salient elements of the construct are shown in Table 10.

Table 10

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
SV40 early promoter	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992
TSP-1(Cardiac Specific)	reverse	240	3026	3265
3'Reg(SV40pA)	reverse	221	3388	3608
LTF[Gal4(DBD):EcR(LBD)]	reverse	1467	3615	5081

TSP-2(ischemic,inducible)	reverse	870	5115	5984
FRP[6x GalRE:Minimal Promoter]	forward	189	6317	6505
TPSQ (Epo)	forward	582	6719	7300
3'Reg(hGH PolyA)	forward	627	7373	7999
Replication Origin	reverse	589	8433	9021
AmpR	reverse	858	9266	10123
bla Promoter	reverse	39	10157	10195

[0377] The construct shown in Fig. 11 is inserted into a suitable vector system, for example, a viral vector, for delivery to a subject in need of treatment for ischemic heart disease.

[0378] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of gene therapy vectors are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and the LDTFC may be expressed under the appropriate physiological conditions. The LDTFC encoded by the vector will be expressed specifically in cardiomyocytes under hypoxic conditions associated with, *e.g.*, cardiac ischemia. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of EPO under control of the FRP. EPO expression in turn promotes targeted cardioprotection in the ischemic tissue.

Example 8

[0379] This example describes a gene therapy vector useful for the treatment of ischemic heart disease through the promotion of cardiac repair and angiogenesis. The vector, shown in Fig. 12, will confer expression of human IGF-1 (see Example 1) upon administration of ligand, under either hypoxic conditions in an inflammatory response, both of which may occur in ischemic tissue. For this purpose, an example of inducible IGF-1 expression, in response to hypoxia and/or an inflammatory response is given. In this example, inducible expression if IGF-1 is specifically limited to cardiomyocytes.

[0380] The vector shown in Fig. 12 is modeled according to the gene switch system shown in Fig. 4. Under this system, the CAP subunit is expressed through operable association with a promoter which is specific for cardiac tissue (Nxc1 cardiomyocyte-specific promoter), and LTF subunits (LTF-1 and LTF-2) of the LDTFC are expressed through either of two inducible TSPs, the first through operable association with the hypoxia-inducible control promoter-1, and the second through operable association with the human plexin D1 promoter.

[0381] The coding region for the therapeutic product, IGF-1, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0382] The complete nucleotide sequence of the construct shown in Figure 12 is presented as SEQ ID NO:14. The nucleotide coordinates for salient elements of the construct are shown in Table 11.

Table 11

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
SV40 early promoter	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992
Transactivation Domain	reverse	261	2732	2992
TSP-1(cardiac-specific)	reverse	240	3026	3265
3'Reg(SV40pA)	reverse	221	3388	3608
LTF-1[Gal4(DBD):EcR(LBD)]	reverse	1467	3615	5081
TSP-2(Hypoxia-inducible)	reverse	870	5115	5984
FRP[6xGalRE:Minimal promoter]	forward	189	6317	6505
Minimal promoter	forward	60	6446	6505
TPSQ (IGF-1)	forward	477	6719	7195
3'Reg(hGH PolyA)	forward	627	7268	7894

TSP-3(Inflammatory inducible)	forward	770	8040	8809
LTF-2[Gal42(DBD):EcR(LBD)]	forward	1467	8843	10309
CfEcR-LBD(2)	forward	1014	9296	10309
3'Reg(Human S100 Calcium Binding Protein pA)	forward	765	10316	11080
Replication Origin	reverse	589	11414	12002
AmpR	reverse	858	12247	13104
bla Promoter	reverse	39	13138	13176

[0383] The construct shown in Fig. 12 is inserted into a suitable vector system, for example, a viral vector, for delivery to a subject in need of treatment for ischemic heart disease.

[0384] The vector may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to cardiac tissue, *e.g.*, via angioplasty. Methods for systemic and/or local administration of gene therapy vectors are well known in the art. Upon delivery the vector will be taken up by cells, *e.g.*, cardiac cells, and the LDTFC may be expressed under the appropriate physiological conditions. The LDTFC encoded by the vector will be expressed specifically in cardiomyocytes under hypoxic conditions, and/or in the event of an inflammatory response, associated with, *e.g.*, cardiac ischemia. Ligand will be administered to the subject to be treated which will combine with the expressed transcription factor to drive expression of IGF-1 under control of the FRP. IGF-1 expression in turn promotes targeted angiogenesis in the ischemic tissue.

Example 9

[0385] This example describes a bioreactor/cell therapy vector useful for the treatment of rheumatoid arthritis, active ankylosing spondylitis or plaque psoriasis or for inhibition of structural damage by the active arthritis ("RA or related diseases"). Conventional treatment of RA and related diseases includes traditional Disease Modifying Anti-Rheumatic Drugs (DMARDs) as well as biologic DMARDs such as etanercept, infliximab, and adalimumab. For example, etanercept (Enbrel®), manufactured by

Amgen, is a fusion protein that contains two extracellular domains of human TNF-alpha receptor 2 fused to a Fc portion by a hinge peptide. *See* U.S. Patent No. 7,276,477 (incorporated herein by reference in its entirety). Etanercept should be administered s.c. once or twice a week. Use of the etanercept gene switch system utilizing inflammation or cytokine response promoters may therefore increase convenience and safety by limiting any production of etanercept in the absence of TNF activation.

[0386] The complete nucleotide sequence of the construct shown in Fig. 13 is presented as SEQ ID NO: 16. The nucleotide coordinates for salient elements of the construct are shown in Table 12.

Table 12

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	197	455
Neo	reverse	795	462	1256
SV40 early promoter	reverse	278	1446	1723
3'Reg(SV40pA)	reverse	221	1830	2050
LTF[GAL4(DBD):EcR(LBD)]	reverse	1467	2057	3523
TL(cMyc ires)	reverse	408	3536	3943
CAP[VP16(AD):RxR(HP)]	reverse	975	3950	4924
TSP-1(TNF-responsive inflammatory inducible)	reverse	800	4958	5757
FRP[6x GalRE:Minimal Promoter]	forward	189	6168	6356
TPSQ(entanercept)	forward	1407	6570	7976
3'Reg(hGH PolyA)	forward	627	8049	8675
Replication Origin	reverse	589	9109	9697
AmpR	reverse	858	10072	10929
bla Promoter	reverse	39	10963	11001

[0387] The vector shown in Fig. 13 is modeled according to the gene switch system shown in Fig. 1. Under this system, both the CAP subunit and the LTF subunit of the LDTFC are expressed through operable association with a single TSP-1 via use of an internal ribosome entry site (IRES). The promoter utilized in this system is a vascular cell adhesion molecule (VCAM1) promoter, which is activated by TNF-alpha. Another example of the TNF-alpha regulated promoters that may be used for the invention is human pentraxin 3(PTX3) promoter, which is responsive to TNF-alpha or Interleukin (IL)-1 beta. *See* Basile *et al., J. Biol. Chem.* 272(13): 8172 (1997).

[0388] The coding region for the therapeutic product, etanercept, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0389] The construct shown in Fig. 13 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, e.g., the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for RA.

[0390] The cells may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to joints. Systemic and/or local administration of gene therapy cells are well known in the art. Upon delivery of the cells, the LDTFC may be expressed under the appropriate physiological conditions. The LDTFC encoded by the vector will be expressed in the presence of TNF-alpha associated with, *e.g.*, RA. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of etanercept under control of the TNF-alpha regulated promoter. Etanercept expression in turn captures TNF-alpha and reduces the TNF-alpha concentration in the tissues.

Example 10

[0391] This example describes a bioreactor/cell therapy vector useful for the treatment of RA and related disease through reducing the TNF-alpha level. The vector shown in Fig. 14 will confer expression of etanercept upon administration of ligand, under either the presence of TNF-alpha and/or severe inflammation, both of which may occur in RA or related diseases. For this purpose, an example of inducible etanercept expression, in response to the presence of TNF-alpha and/or an inflammatory response is given.

[0392] The vector shown in Fig. 14 is modeled according to the gene switch system shown in Fig. 4. Under this system, the CAP subunit is expressed through operable association with a constitutive promoter (TSP-1), and a LTF subunit of a LDTFC is expressed by either of two inducible transcription cassettes, the first (LTF-1) through operable association with human plasminogen activator inhibitor type-2 (PAI2) promoter (TSP-2), and the second (LTF-2) through operable association with the human serum amyloid A1 (SAA1) promoter (TSP-3). The human PAI2 promoter is activated in the presence of TNF- alpha. *See* Mahony *et al. Eur. J. Biochem.* 263(3) (1999) and Matsuo *et al., Biochem. J.* 405: 605 (2007). The SAA1 promoter is upregulated not directly by proinflammatory cytokines such as TNF-alpha, but by other acute inflammatory signals such as glucocorticoid. *See* Kumon *et al., Scandinavian J. Immunol.* 56: 504 (2002).

[0393] The coding region for the therapeutic product, etanercept, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0394] The complete nucleotide sequence of the construct shown in Fig. 14 is presented as SEQ ID NO: 15. The nucleotide coordinates for salient elements of the construct are shown in Table 13.

Table 13

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
SV40 early promoter	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992

Transactivation Domain	reverse	261	2732	2992
TSP-1(constitutive)	reverse	571	3026	3596
3'Reg(SV40pA)	reverse	221	3719	3939
LTF-1[Gal4(DBD):EcR(LBD)]	reverse	1467	3946	5412
TSP-2(TNF-responsive Inflammatory inducible)	reverse	252	5446	5697
FRP[6x GalRE:Minimal Promoter]	forward	189	6030	6218
TPSQ(etanercept)	forward	1407	6432	7838
3'Reg(hGH PolyA)	forward	627	7911	8537
TSP-3(Inflammatory inducible)	forward	253	8683	8935
LTF-2(Gal4(DBD):CfEcR(LBD))	forward	1467	8969	10435
EcRLBD(2)	forward	1014	9422	10435
3'Reg(Human S100 CABP)	forward	765	10442	11206
Replication Origin	reverse	589	11540	12128
AmpR	reverse	858	12373	13230
bla Promoter	reverse	39	13264	13302

[0395] The construct shown in Fig. 14 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, e.g., the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for RA.

[0396] The cells may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to joints. Methods for systemic and/or local

administration of gene therapy cells are well known in the art. Upon delivery of the cells, the LDTFC may be expressed under the appropriate physiological conditions. The LDTFC encoded by the vector will be expressed specifically in the administered cells under the presence of TNF-alpha and/or severe inflammation. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of etanercept under control of the FRP. Etanercept expression in turn captures TNF-alpha and reduces the TNF-alpha concentration in the tissues.

Example 11

[0397] This example describes a bioreactor/cell therapy vector useful for the treatment of RA. The vector will confer expression of two therapeutic polypeptides, etanercept and human erythropoietin (EPO) upon administration of ligand, under the presence of TNF-alpha and/or inflammatory conditions, respectively, both of which occur in RA patients. EPO induces erythrogenesis in anemic RA patients. *See* Mercuriali *et al. Transfusion* 34(6): 501 (2003). For this purpose, an example of separately controlled inducible expression of etanercept and EPO in response to RA and anemia, respectively, is given.

[0398] The complete nucleotide sequence of the construct shown in Fig. 15 is presented as SEQ ID NO: 17. The nucleotide coordinates for salient elements of the construct are shown in Table 14.

Table 14

Label	**Direction**	**Length**	**Start**	**End**
3'Reg(HSVTKpA)	reverse	259	197	455
NEO	reverse	804	462	1265
SV40e Promoter	reverse	280	1385	1664
3'Reg(Synthetic PolyA)	reverse	49	1782	1830
CAP[VP16(AD):RxR(HP)]	reverse	975	1837	2811
TSP-1(constitutive)	reverse	571	2845	3415
3'Reg(SV40pA)	reverse	221	3538	3758
LTF-1[Gal4(DBD):EcR(LBD)]	reverse	1467	3765	5231

TSP-2(Inflammatory-inducible)	reverse	253	5265	5517
FRP-1[6x GalRE:Minimal promoter]	forward	189	5888	6076
TPSQ-1(etanercept)	forward	1407	6290	7696
3'Reg(hGH PolyA)	forward	627	7769	8395
FRP-2[8X LexA:Minimal Promoter]	forward	216	8769	8984
TPSQ-2(Epo)	forward	582	9198	9779
3'Reg(SV40 early pA)	forward	135	9839	9973
TSP-3(Hypoxia-inducible)	forward	870	10205	11074
LTF2[LexA(DBD):CfEcR-DEF(LBD)]	forward	1629	11108	12736
3'Reg(Human S100 Calcium Binding Protein pA)	forward	765	12743	13507
Replication Origin	reverse	589	13841	14429
AmpR	reverse	858	14765	15622
bla Promoter	reverse	39	15656	15694

[0399] The vector shown in Fig. 15 is modeled according to the gene switch system shown in Fig. 3. Under this system, the CAP subunit of the LDTFC is expressed through operable association with a first, constitutive TSP-1, a first LTF subunit of a LDTFC (LTF-1) is expressed through operable association with a second, inducible TSP-2, and a second LTF subunit of a LDTFC (LTF-2) is expressed through operable association with a third, inducible TSP-3. The second inducible TSP-2 used in this vector is the human serum amyloid A1 (SAA1) promoter and the third inducible TSP-3 used in this vector is the hypoxia-inducible control promoter 1.

[0400] The coding region for the first therapeutic product, etanercept, is operably associated with a first FPR-1 having response elements which recognize the first DNA binding domain (DBD-A) associated with LTF-1 , and the coding region for the second therapeutic product, EPO, is operably associated with a second FPR-2 having response elements which recognize the second DNA binding domain (DBD-B) associated with

LTF-2. Both factor- regulated promoters are activated upon contact with the respective LDTFC in the presence of ligand.

[0401] The construct shown in Fig. 15 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, e.g., the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for RA.

[0402] The cells may be delivered to a subject systemically, for example, via intravenous infusion, or may be delivered directly to joints. Methods for systemic and/or local administration of gene therapy cells are well known in the art. Upon delivery of the cells, the LDTFC(s) may be expressed under the appropriate condition. One or more ligands will be administered to the subject to be treated which will combine with the expressed LDTFC(s) to drive expression of etanercept and/or EPO under control of FRP-1 or FRP-2. Etanercept expression in turn captures TNF-alpha and reduces the TNF-alpha concentration in the tissues, and EPO expression induces erythrogenesis and improves anemia.

Example 12

[0403] This example describes a bioreactor/cell therapy vector useful for the treatment of hemophilia. Hemophilia is caused by lack of either Factor VIII or Factor IX. Deficiency of Factor VIII is called hemophilia A, and deficiency of Factor IX is called hemophilia B. Hemophilia A or B may be treated by administering recombinantly produced Factor VIII or IX, respectively. *See* Garcia-Martin *et al., J. Gene Med.* 4(2): 215 (2002). For example, recombinantly produced Factor VIII that may be used in the present invention includes, without limitation, full length Factor VIII such as RECOMBINATE® (markted by Baxter), BIOCLATE® (marketed by Aventis), KOGENATE® (marketed by Bayer), HELIXATE® (marketed by Aventis), or ADVATE® (marketed by Baxter), B-domain deleted

Factor VIII such as REFACTO® and XYNTHA® (both marked by Genetics Institute and Wyeth), or Factor VIII and von Willebrand Factor complex such as ALPHANATE® (marketed by Grifols Biologicals, Inc.). For this purpose, an example of inducible ALPHANATE® expression for a bioreactor/cell therapy in response to administration of ligand is shown in Fig. 16. Use of bioreactor/cell therapy improves problems of stability and continuous infusion. *See* Pipe S.W., J. *Thromb. Haemost.* 3(8): 1692 (2005).

[0404] The complete nucleotide sequence of the construct shown in Fig. 16 is presented as SEQ ID NO: 18. The nucleotide coordinates for salient elements of the construct are shown in Table 15.

Table 15

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	318	576
Neo	reverse	795	583	1377
3'Reg(SV40 early promoter)	reverse	278	1567	1844
3'Reg(Synthetic PolyA)	reverse	49	1963	2011
CAP[VP16(AD):RxR(HP)]	reverse	975	2018	2992
TSP-1(constitutive)	reverse	571	3026	3596
3'Reg(SV40pA)	reverse	221	3719	3939
LTF[Gal4(DBD):EcR(LBD)]	reverse	1467	3946	5412
TSP-2(constitutive)	reverse	1417	5446	6862
FRP[6xGalRE:Minimal promoter]	forward	189	7195	7383
TSPQ(Hu Factor VIII)	forward	7002	7597	14598
3'Reg(hGH PolyA)	forward	627	14671	15297
Replication Origin	reverse	589	15731	16319
AmpR	reverse	858	16564	17421
bla Promoter	reverse	39	17455	17493

[0405] The vector shown in Fig. 16 is modeled according to the gene switch system shown in Fig. 2. Under this system, the CAP subunit is expressed through operable association with a first, constitutive promoter (TSP-1), and the LTF subunit of the LDTFC is expressed through operable association with a second, constitutive promoter (TSP-2). The promoter utilized for the first constitutive promoter is UbC (short) promoter, and the promoter utilized for the second constitutive promoter is UbB (short) promoter.

[0406] The coding region for the therapeutic product, ALPHANATE®, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0407] The construct shown in Fig. 16 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, e.g., the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for hemophilia.

[0408] The cells may be delivered to a subject systemically, for example, via intravenous infusion. Systemic and/or local administration of gene therapy cells are well known in the art. Upon delivery the cells, the LDTFC may be expressed constitutively. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of ALPHANATE® under control of the factor regulated promoter. ALPHANATE® expression in turn treats the symptoms of hemophilia.

Example 13

[0409] This example describes a bioreactor/cell therapy vector useful for the treatment of hemophilia. The vector shown in Fig. 17 is modeled according to the gene switch system shown in Fig. 1. Under this system, both CAP subunit, and the LTF subunit of the LDTFC are expressed through operable association with a single constitutive promoter

(TSP-1) via use of an internal ribosome entry site (IRES). The constitutive promoter is UbC (short) promoter.

[0410] The coding region for the therapeutic product, ALPHANATE®, is operably associated with a FRP which is activated upon contact with the LDTFC in the presence of ligand.

[0411] The complete nucleotide sequence of the construct shown in Fig. 17 is presented as SEQ ID NO: 19. The nucleotide coordinates for salient elements of the construct are shown in Table 16.

Table 16 (MOD 8361)

Label	Direction	Length	Start	End
3'Reg(HSVTKpA)	reverse	259	197	455
Neo	reverse	795	462	1256
SV40 early promoter	reverse	278	1446	1723
3'Reg(SV40pA)	reverse	221	1830	2050
LTF[Gal4(DBD):EcR(LBD)]	reverse	1467	2057	3523
TL(cMyc ires)	reverse	408	3536	3943
CAP[VP16(AD):RxR(HP)]	reverse	975	3950	4924
TSP-1(constitutive)	reverse	571	4958	5528
FRP[6x GalRE:Minimal Promoter]	forward	189	5939	6127
TSPQ(Human Factor VIII)	forward	7002	6341	13342
3'Reg(hGH PolyA)	forward	627	13415	14041
Replication Origin	reverse	589	14475	15063
AmpR	reverse	858	15438	16295
bla Promoter	reverse	39	16329	16367

[0412] The construct shown in Fig. 17 may be prepared in a vector suitable for introduction into cells prior to introduction into the subject to be treated. The cells may

be autologous cells removed from the subject to be treated or non-autologous allogeneic or xenogeneic cells, either primary cells or cell-lines maintained in culture. The vector is introduced into the cells via any standard method, *e.g.*, transfection, transduction, lipofection, or electroporation, to produce modified cells. Following introduction of the vector, the modified cells may optionally be treated to produce a barrier system, e.g., the cells may be coated or encapsulated so as to provide immunoisolation. The modified cells will then be formulated as a bioreactor for administration to a subject in need of treatment for hemophilia.

[0413] The cells may be delivered to a subject systemically, for example, via intravenous infusion. Systemic and/or local administration of gene therapy cells are well known in the art. Upon delivery the cells, the LDTFC may be expressed constitutively. Ligand will be administered to the subject to be treated which will combine with the expressed LDTFC to drive expression of ALPHANATE® under control of the factor regulated promoter. ALPHANATE® expression in turn treats the symptoms of hemophilia.

[0414] Additional embodiments of the invention include the following:

[0415] E1. A method for treating, ameliorating, or preventing a disease or disorder in a subject, comprising:

(a) introducing into cells of said subject (1) a polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor, operably linked to a therapeutic switch promoter, wherein the promoter is activated during said disease or disorder, and (2) a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide linked to a promoter which is activated by said ligand-dependent transcription factor, to produce modified cells; and

(b) administering ligand to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide;

[0416] wherein said therapeutic polypeptide or therapeutic polynucleotide is expressed at a level sufficient to treat, ameliorate, or prevent said disease or disorder.

[0417] E2. A method for expressing a therapeutic polypeptide or therapeutic polynucleotide in a subject, comprising:

(a) introducing into cells of said subject (1) a polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor, operably linked to a therapeutic switch promoter, wherein the promoter is activated during said disease or disorder, and (2) a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide linked to a promoter which is activated by said ligand-dependent transcription factor, to produce modified cells; and

(b) administering ligand to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0418] E3. The method of E1 or E2, wherein said polynucleotides are introduced into cells that have been isolated from said subject to produce modified cells, and the modified cells are re-introduced into said subject.

[0419] E4. The method of E1 or E2, wherein said method is carried out *in vivo*.

[0420] E5. The method of E1 or E2, wherein said gene switch is an ecdysone receptor (EcR)-based gene switch.

[0421] E6. The method of E5, wherein said ligand binds to the EcR ligand binding domain.

[0422] E7. The method of E6, wherein said ligand is a diacylhydrazine.

[0423] E8. The method of E7, wherein said ligand is selected from the group consisting of RG-115819, RG-115932, and RG-115830.

[0424] E9. The method of E6, wherein said ligand is an amidoketone or oxadiazoline.

[0425] E10. The method of E1 or E2, wherein said gene switch comprises a first transcription factor sequence under the control of a first therapeutic switch promoter and a second transcription factor sequence under the control of a second therapeutic switch promoter, wherein the proteins encoded by said first transcription factor sequence and said second transcription factor sequence interact to form a protein complex which functions as a ligand-dependent transcription factor.

[0426] E11. The method of E10, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are different.

[0427] E12. The method of E10, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are the same.

[0428] E13. The method of E10, wherein said first transcription factor sequence encodes a protein comprising a heterodimer partner and a transactivation domain.

[0429] E14. The method of E10, wherein said second transcription factor sequence encodes a protein comprising a DNA binding domain and a ligand-binding domain.

[0430] E15. The method of E1 or E2, wherein one of said polynucleotides further encodes a lethal polypeptide operably linked to an inducible promoter.

[0431] E16. A method for expressing a therapeutic polypeptide or therapeutic polynucleotide in a cell, comprising:

(a) introducing into said cell (1) a polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor, operably linked to a therapeutic switch promoter, wherein the promoter is activated during said disease or disorder, and (2) a polynucleotide encoding a therapeutic polypeptide or therapeutic polynucleotide linked to a promoter which is activated by said ligand-dependent transcription factor, to produce a modified cell; and

(b) administering ligand to said modified cell to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

[0432] E17. The method of E16, wherein said method is carried out *in vitro*.

[0433] E18. The method of E16, wherein said method is carried out *ex vivo* in a cell that has been isolated from a subject.

[0434] E19. The method of E16, wherein said method is carried out *in vivo*.

[0435] E20. The method of E16, wherein said gene switch is an EcR-based gene switch.

[0436] E21. The method of E20, wherein said ligand binds to the EcR ligand binding domain.

[0437] E22. The method of E21, wherein said ligand is a diacylhydrazine.

[0438] E23. The method of E22, wherein said ligand is selected from the group consisting of RG-115819, RG-115932, and RG-115830.

[0439] E24. The method of E21, wherein said ligand is an amidoketone or oxadiazoline.

[0440] E25. The method of E16, wherein said gene switch comprises a first transcription factor sequence under the control of a first therapeutic switch promoter and a second transcription factor sequence under the control of a second therapeutic switch promoter, wherein the proteins encoded by said first transcription factor sequence and said second transcription factor sequence interact to form a protein complex which functions as a ligand-dependent transcription factor.

[0441] E26. The method of E25, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are different.

[0442] E27. The method of E25, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are the same.

[0443] E28. The method of E25, wherein said first transcription factor sequence encodes a protein comprising a heterodimer partner and a transactivation domain.

[0444] E29. The method of E25, wherein said second transcription factor sequence encodes a protein comprising a DNA binding domain and a ligand-binding domain.

[0445] E30. The method of E16, wherein one of said polynucleotides further encodes a lethal polypeptide operably linked to an inducible promoter.

[0446] E31. A polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes a ligand-dependent transcription factor, operably linked to a therapeutic switch promoter, wherein the activity of the promoter is modulated during said disease or disorder.

[0447] E32. The polynucleotide of E31, further encoding a reporter gene linked to a promoter which is activated by said ligand-dependent transcription factor.

[0448] E33. The polynucleotide of E31, wherein said gene switch is an EcR-based gene switch.

[0449] E34. The polynucleotide of E31, wherein said gene switch comprises a first transcription factor sequence under the control of a first therapeutic switch promoter and a second transcription factor sequence under the control of a second therapeutic switch promoter, wherein the proteins encoded by said first transcription factor sequence and said second transcription factor sequence interact to form a protein complex which functions as a ligand-dependent transcription factor.

[0450] E35. The polynucleotide of E34, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are different.

[0451] E36. The polynucleotide of E34, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are the same.

[0452] E37. The polynucleotide of E34, wherein said first transcription factor sequence encodes a protein comprising a heterodimer partner and a transactivation domain.

[0453] E38. The polynucleotide of E34, wherein said second transcription factor sequence encodes a protein comprising a DNA binding domain and a ligand-binding domain.

[0454] E39. The polynucleotide of E31, wherein said polynucleotide further encodes a lethal polypeptide operably linked to an inducible promoter.

[0455] E40. A vector comprising the polynucleotide of E31.

[0456] E41. The vector of E40, which is a plasmid vector.

[0457] E42. The vector of E40, which is a viral vector.

[0458] E43. A kit comprising the polynucleotide of E31.

[0459] E44. A kit comprising the vector of E42.

[0460] The present invention further relates to instructions for performing one or more methods of the invention. Such instructions can instruct a user of conditions suitable for performing methods of the invention. Instructions of the invention can be in a tangible form, for example, written instructions (*e.g.*, typed on paper), or can be in an intangible form, for example, accessible *via* a computer disk or over the internet.

[0461] It will be recognized that a full text of instructions for performing a method of the invention or, where the instructions are included with a kit, for using the kit, need not be provided. One example of a situation in which a kit of the invention, for example, would not contain such full length instructions is where the provided directions inform a user of the kits where to obtain instructions for practicing methods for which the kit can be used. Thus, instructions for performing methods of the invention can be obtained from internet web pages, separately sold or distributed manuals or other product literature, *etc.* The invention thus includes kits that direct a kit user to one or more locations where instructions not directly packaged and/or distributed with the kits can be found. Such instructions can be in any form including, but not limited to, electronic or printed forms.

[0462] Having now fully described the invention, it will be understood by those of ordinary skill in the art that the same can be performed within a wide and equivalent range of conditions, formulations and other parameters without affecting the scope of the invention or any embodiment thereof. All patents, patent applications and publications cited herein are fully incorporated by reference herein in their entirety.

WHAT IS CLAIMED IS:

1. Use of

(1) a first polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes components of a ligand-dependent transcription factor complex (LDTFC) through operable association with a therapeutic switch promoter that is activated under conditions associated with a disease, disorder, or condition,

with the proviso that said promoter is not a stress-inducible promoter, and

(2) a second polynucleotide encoding a therapeutic polypeptide useful in treating said disease, disorder or condition operably associated with a factor-regulated promoter which is activated by said ligand-dependent transcription factor complex,

wherein said first and second polynucleotides are introduced so as to permit expression of said ligand-dependent transcription factor complex,

in a method for treating, ameliorating, or preventing a disease, disorder, or condition in a subject;

wherein said ligand is administered to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

2. Use of

(1) a first polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes components of a ligand-dependent transcription factor complex through operable association with a therapeutic switch promoter that is activated under conditions associated with a disease, disorder, or condition,

with the proviso that said promoter is not a stress-inducible promoter, and

(2) a second polynucleotide encoding said therapeutic polypeptide useful in treating said disease, disorder or condition operably associated with a factor-regulated promoter which is activated by said ligand-dependent transcription factor complex,

wherein said first and second polynucleotides are introduced so as to permit expression of said ligand-dependent transcription factor complex,

in a method for expressing a therapeutic polypeptide or therapeutic polynucleotide in a subject,

wherein said ligand is administered to said subject to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

3. The use of claim 1 or claim 2, wherein said therapeutic switch promoter is constitutive.

4. The use of claim 1 or claim 2, wherein said therapeutic polypeptide or therapeutic polynucleotide is expressed and disseminated at a level sufficient to treat, ameliorate, or prevent said disease, disorder, or condition.

5. The use of claim 1 or claim 2, wherein said gene switch is an ecdysone receptor (EcR)-based gene switch.

6. The use of claim 5, wherein said ligand binds to the EcR ligand binding domain.

7. The use of claim 6, wherein said ligand is a diacylhydrazine.

8. The use of claim 7, wherein said ligand is selected from the group consisting of RG-115819, RG-115932, and RG-115830.

9. The use of claim 6, wherein said ligand is an amidoketone or oxadiazoline.

10. The use of claim 1 or claim 2, wherein said gene switch comprises a first transcription factor sequence under the control of a first therapeutic switch promoter and a second transcription factor sequence under the control of a second therapeutic switch promoter, wherein the proteins encoded by said first transcription factor sequence and said second transcription factor sequence interact to form a protein complex which functions as a ligand-dependent transcription factor complex.

11. The use of claim 10, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are different.

12. The use of claim 10, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are the same.

13. The use of claim 10, wherein said first transcription factor sequence encodes a protein comprising a heterodimer partner and a transactivation domain.

14. The use of claim 10, wherein said second transcription factor sequence encodes a protein comprising a DNA binding domain and a ligand-binding domain.

15. The use of claim 1 or claim 2, wherein said first and second polynucleotides are introduced into said subject in one or more modified cells.

16. The use of claim 15, wherein said modified cells are prepared by introducing said first and second polynucleotides into cells that have been isolated from said subject to produce modified autologous cells, which are then re-introduced into said subject.

17. The use of claim 15, wherein said modified cells are modified non-autologous (MNA) cells into which said first and second polynucleotides have been incorporated.

18. The use of claim 17, wherein said MNA cells are allogeneic to said subject.

19. The use of claim 17, wherein said MNA cells are xenogeneic to said subject.

20. The use of claim 17, wherein said MNA cells are generated from a cell type selected from the group consisting of immortalized cells and primary cells capable of terminal differentiation.

21. The use of claim 20, wherein said MNA cells are generated from a cell type selected from the group consisting of C2C12 mouse myoblast cells, HEK293 human embryonic kidney cells, ARPE-19 cells, hMSC cells, pancreatic islet cells, MDCK cell, a CHO cell, an astrocyte derived cell, an oligodendrocyte derived cell, and a myoblast derived cell.

22. The use of claim 21, wherein said pancreatic islet cells are porcine islet cells.

23. The use of claim 21, wherein said pancreatic islet cells are human islet cells derived from cadavers.

24. The use of claim 15, wherein said modified cells have been treated such that said cells, upon introduction into said subject, are protected from the subject's immune system.

25. The use of claim 24, wherein said modified cells are contained within a barrier system which allows dissemination of said therapeutic protein or therapeutic polynucleotide, but which prevents direct contact of said modified cells with cells of the subject's immune system.

26. The use of claim 25, wherein said barrier system comprises modified cells with a conformal coating.

27. The use of claim 26, wherein said conformal coating comprises a polymer.

28. The use of claim 27, wherein said polymer is selected from the group consisting of polyethylene glycol, and hydroxyethyl methacrylate-methyl methacrylate (HEMA-MMA).

29. The use of claim 25, wherein said barrier system comprises encapsulated modified cells.

30. The use of claim 29, wherein said modified cells are contained in a macroencapsulation device.

31. The use of claim 30, wherein said macroencapsulation device comprises one or more synthetic membranes.

32. The use of claim 31, wherein said macroencapsulation device comprises two or more synthetic membranes, said synthetic membranes comprising different pore sizes so as to regulate mass transit through said macroencapsulation device.

33. The use of claim 30, wherein said macroencapsulation device comprises a semi-permeable polymer outer membrane and an internal scaffold which supports cells.

34. The use of claim 33, wherein said macroencapsulation device is about 6 mm long and about 1 mm in diameter.

35. The use of claim 33, wherein said outer membrane comprises pores of about 15 nm.

36. The use of claim 33, wherein said internal scaffold comprises poly (ethylene terephthalate) yarn.

37. The use of claim 30, wherein the interior space of said macroencapsulation device comprises at least one to about 10^5 modified cells.

38. The use of claim 30, wherein said macroencapsulation device comprises a polymeric membrane bilayer, said bilayer comprising an outer layer of 5μm PTFE membrane laminated onto an inner tighter pore 0.45 μM PFTE immunobarrier layer.

39. The use of claim 38, wherein said macroencapsulation device further comprises a non-woven poly mesh layer exterior to said polymeric membrane bilayer.

40. The use of claim 30, wherein said macroencapsulation device comprises a PES hollow fiber device.

41. The use of claim 30, wherein said macroencapsulation device further comprises a suture clip.

42. The use of claim 30, wherein said macroencapsulation device is implantable and retrievable.

43. The use of claim 29, wherein said modified cells are microencapsulated.

44. The use of claim 43, wherein said modified cells are suspended in a biologically compatible encapsulation material which is then shaped into bead-like structures.

45. The use of claim 44, wherein said encapsulation material comprises a hydrogel.

46. The use of claim 44, wherein said encapsulation material comprises a polymer selected from the group consisting of cellulose, and alginate.

47. The use of claim 46, wherein said polymer comprises polyanionic alginate and a poly-cationic polymer to interact and form a physical permselective membrane barrier.

48. The use of claim 44, wherein said bead-like structures each comprise at least one to about 10^3 modified cells.

49. The use of claim 44, wherein said bead-like structures comprise poly (L-lactide) acid (PLLA) alginate microspheres.

50. The use of claim 46, wherein said bead-like structures further comprise a poly-L-ornithine (PLO) alginate coating.

51. The use of claim 46, wherein said encapsulation material comprises an alginate based ultra high viscosity (UHV)-biopolymer.

52. The use of claim 51, wherein said alginate based UHV-polymer is biodegradable.

53. The use of claim 44, wherein said bead-like structures are approximately spherical and are about 500μm in diameter.

54. The use of claim 25, wherein said barrier system further comprises protective cells capable of providing protection to said modified cells, wherein said protective cells are selected from the group consisting of sertoli cells and erythrocytes.

55. The use of claim 25, wherein said barrier system further comprises an outer coating capable of creating a protective micro-environment.

56. The use of claim 55, wherein said protective micro-environment is anti-inflammatory.

57. The use of claim 55, wherein said protective micro-environment is pro-angiogenic.

58. The use of claim 15, wherein said modified cells further comprise a regulated suicide gene which, upon activation, will induce destruction of said modified cells

59. The use of claim 58, wherein said suicide gene is activated in the event of escape from a barrier system or oncogenic conversion.

60. A method for expressing a therapeutic polypeptide or therapeutic polynucleotide in one or more modified cells, comprising:

(a) introducing into a cell (1) a first polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes components of a ligand-dependent transcription factor complex through operable association with a therapeutic switch promoter that is activated under conditions associated with a disease, disorder or condition, with the proviso that said promoter is not a stress-inducible promoter, and (2) a second polynucleotide encoding a therapeutic polypeptide useful in treating said disease, disorder or condition operably associated with a factor-regulated promoter which is activated by said ligand-dependent transcription factor complex, thereby producing a modified cell; and

(b) administering ligand to said modified cell to induce expression of said therapeutic polypeptide or therapeutic polynucleotide.

61. The method of claim 60, wherein said therapeutic switch promoter is constitutive.

62. The method of claim 60, wherein said method is carried out *in vitro*.

63. The method of claim 60, wherein said method is carried out *ex vivo* in a cell that has been isolated from a subject.

64. A nucleic acid composition comprising a first polynucleotide encoding a gene switch, said gene switch comprising at least one transcription factor sequence, wherein said at least one transcription factor sequence encodes components of a ligand-dependent transcription factor complex through operable association to a therapeutic switch promoter that is activated under conditions associated with a disease, disorder or condition, with the proviso that said promoter is not a stress-inducible promoter.

65. The nucleic acid composition of claim 64, wherein said therapeutic switch promoter is constitutive.

66. The nucleic acid composition of claim 64, further comprising a second polynucleotide encoding a polypeptide useful in treating said disease, disorder, or condition through operable association with a promoter which is activated by said ligand-dependent transcription factor complex.

67. The nucleic acid composition of claim 66, wherein said polypeptide or polynucleotide associated with a disease, disorder, or condition comprises a therapeutic polypeptide or a therapeutic polynucleotide capable of treating, ameliorating or preventing a disease, disorder, or condition.

68. The nucleic acid composition of any one of claims 64-67, wherein said gene switch is an EcR-based gene switch.

69. The nucleic acid composition of claim 68, wherein said ligand binds to the EcR ligand binding domain.

70. The nucleic acid composition of claim 69, wherein said ligand is a diacylhydrazine.

71. The nucleic acid composition of claim 70, wherein said ligand is selected from the group consisting of RG-115819, RG-115932, and RG-115830.

72. The nucleic acid composition of claim 69, wherein said ligand is an amidoketone or oxadiazoline.

73. The nucleic acid composition of claim any one of claims 64-67, wherein said gene switch comprises a first transcription factor sequence under the control of a first therapeutic switch promoter and a second transcription factor sequence under the control of a second therapeutic switch promoter, wherein the proteins encoded by said first transcription factor sequence and said second transcription factor sequence interact to form a protein complex which functions as a ligand-dependent transcription factor complex.

74. The nucleic acid composition of claim 73 wherein said first therapeutic switch promoter and said second therapeutic switch promoter are different.

75. The nucleic acid composition of claim 73, wherein said first therapeutic switch promoter and said second therapeutic switch promoter are the same.

76. The nucleic acid composition of claim 73, wherein said first transcription factor sequence encodes a protein comprising a heterodimer partner and a transactivation domain.

77. The nucleic acid composition of claim 73, wherein said second transcription factor sequence encodes a protein comprising a DNA binding domain and a ligand-binding domain.

78. The nucleic acid composition of claim 66, further comprising a third polynucleotide encoding a lethal polypeptide operably linked to an inducible promoter.

79. A vector comprising the nucleic acid composition of claim 66.

80. The vector of claim 79, which is a plasmid vector.

81. The vector of claim 79, which is a viral vector.

82. A kit comprising the nucleic acid composition of any one of claim 66.

83. A kit comprising the vector of claim 79.

84. A method of producing a modified cell, comprising introducing into a cell the nucleic acid composition of any one of claims 66-78.

85. A method of producing a modified cell, comprising introducing into a cell the vector of claim 79.

86. A modified cell comprising the nucleic acid composition of any one of claims 66-78.

87. A modified cell comprising the vector of claim 79.

88. The modified cell of claim 86 which is a modified autologous cell derived from cells isolated from a subject in need of said polypeptide or polynucleotide, wherein said modified cell is suitable for re-introduction into said subject.

89. The modified cell of claim 86 which is derived from a cell type selected from the group consisting of immortalized cells and primary cells capable of terminal differentiation.

90. The modified cell of claim 89 which is derived from a cell type selected from the group consisting of C2C12 mouse myoblast cells, HEK293 human embryonic kidney cells, ARPE-19 cells, hMSC cells, pancreatic islet cells, MDCK cell, a CHO cell, an astrocyte derived cell, an oligodendrocyte derived cell, and a myoblast derived cell.

91. The modified cell of claim 90, wherein said pancreatic islet cell is a porcine islet cell.

92. The modified cell of claim 90, wherein said pancreatic islet cell is a human islet cell derived from cadavers.

93. The modified cell of claim 86, further comprising a regulated suicide gene which, upon activation, will induce destruction of said modified cell

94. The modified cell of claim 93, wherein said suicide gene is activated in the event of escape from a barrier system or oncogenic conversion.

95. A bioreactor device comprising one or more modified cells as recited in any one of claims 86-94.

96. The bioreactor device of claim 95, wherein said modified cells have been treated such that said cells, upon introduction into a subject, are protected from the subject's immune system.

97. The bioreactor device of claim 95, wherein said modified cells are contained within a barrier system which allows dissemination of said polypeptide or polynucleotide, but which prevents direct contact of said modified cells with cells of the subject's immune system.

98. The bioreactor device of claim 97, wherein said barrier system comprises modified cells with a conformal coating.

99. The bioreactor device of claim 98, wherein said conformal coating comprises a polymer.

100. The bioreactor device of claim 99, wherein said polymer is selected from the group consisting of polyethylene glycol, and hydroxyethyl methacrylate-methyl methacrylate (HEMA-MMA).

101. The bioreactor device of claim 97, wherein said barrier system comprises encapsulated modified cells.

102. The bioreactor device of claim 101, wherein said modified cells are contained in a macroencapsulation device.

103. The bioreactor device of claim 102, wherein said macroencapsulation device comprises one or more synthetic membranes.

104. The bioreactor device of claim 103, wherein said macroencapsulation device comprises two or more synthetic membranes, said synthetic membranes comprising different pore sizes so as to regulate mass transit through said macroencapsulation device.

105. The bioreactor device of claim 102, wherein said macroencapsulation device comprises a semi-permeable polymer outer membrane and an internal scaffold which supports cells.

106. The bioreactor device of claim 105, wherein said macroencapsulation device is about 6 mm long and about 1 mm in diameter.

107. The bioreactor device of claim 105, wherein said outer membrane comprises pores of about 15 nm.

108. The bioreactor device of claim 105, wherein said internal scaffold comprises poly (ethylene terephthalate) yarn.

109. The bioreactor device of claim 102, wherein the interior space of said macroencapsulation device comprises at least one to about 10^5 modified cells.

110. The bioreactor device of claim 102, wherein said macroencapsulation device comprises a polymeric membrane bilayer, said bilayer comprising an outer layer of 5μm PTFE membrane laminated onto an inner tighter pore 0.45 μM PFTE immunobarrier layer.

111. The bioreactor device of claim 110, wherein said macroencapsulation device further comprises a non-woven poly mesh layer exterior to said polymeric membrane bilayer.

112. The bioreactor device of claim 102, wherein said macroencapsulation device comprises a PES hollow fiber device.

113. The bioreactor device of claim 102, wherein said macroencapsulation device further comprises a suture clip.

114. The bioreactor device of claim 102, wherein said macroencapsulation device is implantable and retrievable.

115. The bioreactor device of claim 101, wherein said modified cells are microencapsulated.

116. The bioreactor device of claim 115, wherein said modified cells are suspended in a biologically compatible encapsulation material which is then shaped into bead-like structures.

117. The bioreactor device of claim 116, wherein said encapsulation material comprises a hydrogel.

118. The bioreactor device of claim 116, wherein said encapsulation material comprises a polymer selected from the group consisting of cellulose, and alginate.

119. The bioreactor device of claim 118, wherein said polymer comprises polyanionic alginate and a poly-cationic polymer to interact and form a physical permselective membrane barrier.

120. The bioreactor device of claim 116, wherein said bead-like structures each comprise at least one to about 10^3 modified cells.

121. The bioreactor device of claim 116, wherein said bead-like structures comprise poly (L-lactide) acid (PLLA) alginate microspheres.

122. The bioreactor device of claim 116, wherein said bead-like structures further comprise a poly-L-ornithine (PLO) alginate coating.

123. The bioreactor device of claim 118, wherein said encapsulation material comprises an alginate based ultra high viscosity (UHV)-biopolymer.

124. The bioreactor device of claim 123, wherein said alginate based UHV-polymer is biodegradable.

125. The bioreactor device of claim 116, wherein said bead-like structures are approximately spherical and are about 500μm in diameter.

126. The bioreactor device of claim 97, wherein said barrier system further comprises protective cells within said capsule capable of providing protection to said modified cells, wherein said protective cells are selected from the group consisting of sertoli cells and erythrocytes.

127. The bioreactor device of claim 97, wherein said barrier system further comprises an outer coating capable of creating a protective micro-environment.

128. The bioreactor device of claim 127, wherein said protective micro-environment is anti-inflammatory.

129. The bioreactor device of claim 127, wherein said protective micro-environment is pro-angiogenic.

130. Any one of claims 64-78 for use in the treatment of a disease, disorder, or condition.

131. Any one of claims 79-81 for use in the treatment of a disease, disorder, or condition.

132. Any one of claims 86-94 for use in the treatment of a disease, disorder, or condition.

133. Any one of claims 95-129 for use in the treatment of a disease, disorder, or condition.

134. Any one of claims 64-78 for use in the preparation of a medicament for the treatment of a disease, disorder, or condition selected from the group consisting of a hyperproliferative disease, disorder, or condition, a cardiovascular disease, disorder, or condition, a neural disease, disorder, or condition, an autoimmune disease, disorder, or condition, a bone disease, disorder, or condition, a gastrointestinal disease, disorder, or condition, a blood disease, disorder, or condition, a metabolic disease, disorder, or condition, an inflammatory disease, disorder, or condition, and an infectious disease, disorder, or condition.

135. Any one of claims 79-81 for use in the preparation of a medicament for the treatment of a disease, disorder, or condition selected from the group consisting of a

hyperproliferative disease, disorder, or condition, a cardiovascular disease, disorder, or condition, a neural disease, disorder, or condition, an autoimmune disease, disorder, or condition, a bone disease, disorder, or condition, a gastrointestinal disease, disorder, or condition, a blood disease, disorder, or condition, a metabolic disease, disorder, or condition, an inflammatory disease, disorder, or condition, and an infectious disease, disorder, or condition.

136. Any one of claims 86-94 for use in the preparation of a medicament for the treatment of a disease, disorder, or condition selected from the group consisting of a hyperproliferative disease, disorder, or condition, a cardiovascular disease, disorder, or condition, a neural disease, disorder, or condition, an autoimmune disease, disorder, or condition, a bone disease, disorder, or condition, a gastrointestinal disease, disorder, or condition, a blood disease, disorder, or condition, a metabolic disease, disorder, or condition, an inflammatory disease, disorder, or condition, and an infectious disease, disorder, or condition.

137. Any one of claims 95-129 for use in the preparation of a medicament for the treatment of a disease, disorder, or condition selected from the group consisting of a hyperproliferative disease, disorder, or condition, a cardiovascular disease, disorder, or condition, a neural disease, disorder, or condition, an autoimmune disease, disorder, or condition, a bone disease, disorder, or condition, a gastrointestinal disease, disorder, or condition, a blood disease, disorder, or condition, a metabolic disease, disorder, or condition, an inflammatory disease, disorder, or condition, and an infectious disease, disorder, or condition.

SEQUENCE LISTING

<110> MERENICK, Bethany Lynn
BEECH, Robert P.
REED, Thomas D.
TRETIAKOVA, Anna P.
PETERSON, Richard E.

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<210> 20
<211> 158
<212> PRT
<213> Homo sapiens

<220>
<223> Human Insulin like growth factor (IGF-1)

<400> 20

Met Gly Lys Ile Ser Ser Leu Pro Thr Gln Leu Phe Lys Cys Cys Phe
1 5 10 15

```
Cys Asp Phe Leu Lys Val Lys Met His Thr Met Ser Ser Ser His Leu
            20                  25                  30

Phe Tyr Leu Ala Leu Cys Leu Leu Thr Phe Thr Ser Ser Ala Thr Ala
        35                  40                  45

Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
    50                  55                  60

Val Cys Gly Asp Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr Gly
65                  70                  75                  80

Ser Ser Ser Arg Arg Ala Pro Gln Thr Gly Ile Val Asp Glu Cys Cys
                85                  90                  95

Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro Leu
            100                 105                 110

Lys Pro Ala Lys Ser Ala Arg Ser Val Arg Ala Gln Arg His Thr Asp
        115                 120                 125

Met Pro Lys Thr Gln Lys Tyr Gln Pro Pro Ser Thr Asn Lys Asn Thr
    130                 135                 140

Lys Ser Gln Arg Arg Lys Gly Ser Thr Phe Glu Glu Arg Lys
145                 150                 155
```

<210> 21
<211> 288
<212> PRT
<213> Homo sapiens

<220>
<223> Human basic fibroblast growth factor (bFGF)

<400> 21

```
Met Val Gly Val Gly Gly Gly Asp Val Glu Asp Val Thr Pro Arg Pro
1               5                   10                  15

Gly Gly Cys Gln Ile Ser Gly Arg Gly Ala Arg Gly Cys Asn Gly Ile
            20                  25                  30

Pro Gly Ala Ala Ala Trp Glu Ala Ala Leu Pro Arg Arg Arg Pro Arg
        35                  40                  45

Arg His Pro Ser Val Asn Pro Arg Ser Arg Ala Ala Gly Ser Pro Arg
```

```
    50                  55                  60

Thr Arg Gly Arg Arg Thr Glu Glu Arg Pro Ser Gly Ser Arg Leu Gly
65                  70                  75                  80

Asp Arg Gly Arg Gly Arg Ala Leu Pro Gly Gly Arg Leu Gly Gly Arg
                85                  90                  95

Gly Arg Gly Arg Ala Pro Glu Arg Val Gly Gly Arg Gly Arg Gly Arg
            100                 105                 110

Gly Thr Ala Ala Pro Arg Ala Ala Pro Ala Ala Arg Gly Ser Arg Pro
        115                 120                 125

Gly Pro Ala Gly Thr Met Ala Ala Gly Ser Ile Thr Thr Leu Pro Ala
    130                 135                 140

Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro Gly His Phe Lys
145                 150                 155                 160

Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly Phe Phe Leu Arg Ile
                165                 170                 175

His Pro Asp Gly Arg Val Asp Gly Val Arg Glu Lys Ser Asp Pro His
            180                 185                 190

Ile Lys Leu Gln Leu Gln Ala Glu Glu Arg Gly Val Val Ser Ile Lys
        195                 200                 205

Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg Leu
    210                 215                 220

Leu Ala Ser Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu Arg Leu
225                 230                 235                 240

Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp
                245                 250                 255

Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr
            260                 265                 270

Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
        275                 280                 285
```

<210> 22
<211> 193

<212> PRT
<213> Homo sapiens

<220>
<223> Human erythropoietin

<400> 22

Met Gly Val His Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu
1 5 10 15

Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu
20 25 30

Ile Cys Asp Ser Arg Val Leu Gln Arg Tyr Leu Leu Glu Ala Lys Glu
35 40 45

Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu
50 55 60

Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg
65 70 75 80

Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu
85 90 95

Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser
100 105 110

Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala Val Ser Gly
115 120 125

Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Lys Glu
130 135 140

Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
145 150 155 160

Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser Asn Phe Leu
165 170 175

Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly Asp
180 185 190

Arg

<210> 23

<211> 134
<212> PRT
<213> Homo sapiens

<220>
<223> Human BNP

<400> 23

Met Asp Pro Gln Thr Ala Pro Ser Arg Ala Leu Leu Leu Leu Leu Phe
1 5 10 15

Leu His Leu Ala Phe Leu Gly Gly Arg Ser His Pro Leu Gly Ser Pro
20 25 30

Gly Ser Ala Ser Asp Leu Glu Thr Ser Gly Leu Gln Glu Gln Arg Asn
35 40 45

His Leu Gln Gly Lys Leu Ser Glu Leu Gln Val Glu Gln Thr Ser Leu
50 55 60

Glu Pro Leu Gln Glu Ser Pro Arg Pro Thr Gly Val Trp Lys Ser Arg
65 70 75 80

Glu Val Ala Thr Glu Gly Ile Arg Gly His Arg Lys Met Val Leu Tyr
85 90 95

Thr Leu Arg Ala Pro Arg Ser Pro Lys Met Val Gln Gly Ser Gly Cys
100 105 110

Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys
115 120 125

Lys Val Leu Arg Arg His
130

<210> 24
<211> 562
<212> PRT
<213> Homo sapiens

<220>
<223> Human tPA

<400> 24

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
1 5 10 15

Ala Val Phe Val Ser Pro Ser Gln Glu Ile His Ala Arg Phe Arg Arg
20 25 30

```
Gly Ala Arg Ser Tyr Gln Val Ile Cys Arg Asp Glu Lys Thr Gln Met
        35                  40                  45

Ile Tyr Gln Gln His Gln Ser Trp Leu Arg Pro Val Leu Arg Ser Asn
    50                  55                  60

Arg Val Glu Tyr Cys Trp Cys Asn Ser Gly Arg Ala Gln Cys His Ser
65                  70                  75                  80

Val Pro Val Lys Ser Cys Ser Glu Pro Arg Cys Phe Asn Gly Gly Thr
                85                  90                  95

Cys Gln Gln Ala Leu Tyr Phe Ser Asp Phe Val Cys Gln Cys Pro Glu
            100                 105                 110

Gly Phe Ala Gly Lys Cys Cys Glu Ile Asp Thr Arg Ala Thr Cys Tyr
        115                 120                 125

Glu Asp Gln Gly Ile Ser Tyr Arg Gly Thr Trp Ser Thr Ala Glu Ser
    130                 135                 140

Gly Ala Glu Cys Thr Asn Trp Asn Ser Ser Ala Leu Ala Gln Lys Pro
145                 150                 155                 160

Tyr Ser Gly Arg Arg Pro Asp Ala Ile Arg Leu Gly Leu Gly Asn His
                165                 170                 175

Asn Tyr Cys Arg Asn Pro Asp Arg Asp Ser Lys Pro Trp Cys Tyr Val
            180                 185                 190

Phe Lys Ala Gly Lys Tyr Ser Ser Glu Phe Cys Ser Thr Pro Ala Cys
        195                 200                 205

Ser Glu Gly Asn Ser Asp Cys Tyr Phe Gly Asn Gly Ser Ala Tyr Arg
    210                 215                 220

Gly Thr His Ser Leu Thr Glu Ser Gly Ala Ser Cys Leu Pro Trp Asn
225                 230                 235                 240

Ser Met Ile Leu Ile Gly Asn Val Tyr Thr Ala Gln Asn Pro Ser Ala
                245                 250                 255

Gln Ala Leu Gly Leu Gly Lys His Asn Tyr Cys Arg Asn Pro Asp Gly
            260                 265                 270
```

```
Asp Ala Lys Pro Trp Cys His Val Leu Lys Asn Arg Arg Leu Thr Trp
        275                 280                 285

Glu Tyr Cys Asp Val Pro Ser Cys Ser Thr Cys Gly Leu Arg Gln Tyr
    290                 295                 300

Ser Gln Pro Gln Phe Arg Ile Lys Gly Gly Leu Phe Ala Asp Ile Ala
305                 310                 315                 320

Ser His Pro Trp Gln Ala Ala Ile Phe Ala Lys His Arg Arg Ser Pro
                325                 330                 335

Gly Glu Arg Phe Leu Cys Gly Gly Ile Leu Ile Ser Ser Cys Trp Ile
            340                 345                 350

Leu Ser Ala Ala His Cys Phe Gln Glu Arg Phe Pro Pro His His Leu
        355                 360                 365

Thr Val Ile Leu Gly Arg Thr Tyr Arg Val Val Pro Gly Glu Glu Glu
    370                 375                 380

Gln Lys Phe Glu Val Glu Lys Tyr Ile Val His Lys Glu Phe Asp Asp
385                 390                 395                 400

Asp Thr Tyr Asp Asn Asp Ile Ala Leu Leu Gln Leu Lys Ser Asp Ser
                405                 410                 415

Ser Arg Cys Ala Gln Glu Ser Ser Val Val Arg Thr Val Cys Leu Pro
            420                 425                 430

Pro Ala Asp Leu Gln Leu Pro Asp Trp Thr Glu Cys Glu Leu Ser Gly
        435                 440                 445

Tyr Gly Lys His Glu Ala Leu Ser Pro Phe Tyr Ser Glu Arg Leu Lys
    450                 455                 460

Glu Ala His Val Arg Leu Tyr Pro Ser Ser Arg Cys Thr Ser Gln His
465                 470                 475                 480

Leu Leu Asn Arg Thr Val Thr Asp Asn Met Leu Cys Ala Gly Asp Thr
                485                 490                 495

Arg Ser Gly Gly Pro Gln Ala Asn Leu His Asp Ala Cys Gln Gly Asp
            500                 505                 510
```

```
Ser Gly Gly Pro Leu Val Cys Leu Asn Asp Gly Arg Met Thr Leu Val
        515                 520                 525

Gly Ile Ile Ser Trp Gly Leu Gly Cys Gly Gln Lys Asp Val Pro Gly
    530                 535                 540

Val Tyr Thr Lys Val Thr Asn Tyr Leu Asp Trp Ile Arg Asp Asn Met
545                 550                 555                 560

Arg Pro
```

<210> 25
<211> 185
<212> PRT
<213> Homo sapiens

<220>
<223> Human relaxin

<400> 25

```
Met Pro Arg Leu Phe Phe Phe His Leu Leu Gly Val Cys Leu Leu Leu
1               5                   10                  15

Asn Gln Phe Ser Arg Ala Val Ala Asp Ser Trp Met Glu Glu Val Ile
            20                  25                  30

Lys Leu Cys Gly Arg Glu Leu Val Arg Ala Gln Ile Ala Ile Cys Gly
        35                  40                  45

Met Ser Thr Trp Ser Lys Arg Ser Leu Ser Gln Glu Asp Ala Pro Gln
    50                  55                  60

Thr Pro Arg Pro Val Ala Glu Ile Val Pro Ser Phe Ile Asn Lys Asp
65                  70                  75                  80

Thr Glu Thr Ile Asn Met Met Ser Glu Phe Val Ala Asn Leu Pro Gln
                85                  90                  95

Glu Leu Lys Leu Thr Leu Ser Glu Met Gln Pro Ala Leu Pro Gln Leu
            100                 105                 110

Gln Gln His Val Pro Val Leu Lys Asp Ser Ser Leu Leu Phe Glu Glu
        115                 120                 125

Phe Lys Lys Leu Ile Arg Asn Arg Gln Ser Glu Ala Ala Asp Ser Ser
    130                 135                 140
```

```
Pro Ser Glu Leu Lys Tyr Leu Gly Leu Asp Thr His Ser Arg Lys Lys
145                 150                 155                 160

Arg Gln Leu Tyr Ser Ala Leu Ala Asn Lys Cys Cys His Val Gly Cys
                165                 170                 175

Thr Lys Arg Ser Leu Ala Arg Phe Cys
            180                 185
```

<210> 26
<211> 728
<212> PRT
<213> Homo sapiens

<220>
<223> Human hepatocyte growth factor (HGF)

<400> 26

```
Met Trp Val Thr Lys Leu Leu Pro Ala Leu Leu Leu Gln His Val Leu
1               5                   10                  15

Leu His Leu Leu Leu Leu Pro Ile Ala Ile Pro Tyr Ala Glu Gly Gln
            20                  25                  30

Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser Ala Lys Thr
        35                  40                  45

Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys Thr Lys Lys Val
    50                  55                  60

Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr Arg Asn Lys Gly Leu
65                  70                  75                  80

Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys Ala Arg Lys Gln Cys
                85                  90                  95

Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly Val Lys Lys Glu Phe
            100                 105                 110

Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp Tyr Ile Arg Asn Cys
        115                 120                 125

Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr Val Ser Ile Thr Lys
    130                 135                 140

Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met Ile Pro His Glu His
145                 150                 155                 160
```

```
Ser Phe Leu Pro Ser Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr
                165                 170                 175

Cys Arg Asn Pro Arg Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser
            180                 185                 190

Asn Pro Glu Val Arg Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu
        195                 200                 205

Val Glu Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp
    210                 215                 220

His Thr Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro
225                 230                 235                 240

His Arg His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp
                245                 250                 255

Asp Asn Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr
            260                 265                 270

Thr Leu Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys
        275                 280                 285

Ala Asp Asn Thr Met Asn Asp Thr Asp Val Pro Leu Glu Thr Thr Glu
    290                 295                 300

Cys Ile Gln Gly Gln Gly Glu Gly Tyr Arg Gly Thr Val Asn Thr Ile
305                 310                 315                 320

Trp Asn Gly Ile Pro Cys Gln Arg Trp Asp Ser Gln Tyr Pro His Glu
                325                 330                 335

His Asp Met Thr Pro Glu Asn Phe Lys Cys Lys Asp Leu Arg Glu Asn
            340                 345                 350

Tyr Cys Arg Asn Pro Asp Gly Ser Glu Ser Pro Trp Cys Phe Thr Thr
        355                 360                 365

Asp Pro Asn Ile Arg Val Gly Tyr Cys Ser Gln Ile Pro Asn Cys Asp
    370                 375                 380

Met Ser His Gly Gln Asp Cys Tyr Arg Gly Asn Gly Lys Asn Tyr Met
385                 390                 395                 400
```

```
Gly Asn Leu Ser Gln Thr Arg Ser Gly Leu Thr Cys Ser Met Trp Asp
                405                 410                 415

Lys Asn Met Glu Asp Leu His Arg His Ile Phe Trp Glu Pro Asp Ala
            420                 425                 430

Ser Lys Leu Asn Glu Asn Tyr Cys Arg Asn Pro Asp Asp Asp Ala His
        435                 440                 445

Gly Pro Trp Cys Tyr Thr Gly Asn Pro Leu Ile Pro Trp Asp Tyr Cys
    450                 455                 460

Pro Ile Ser Arg Cys Glu Gly Asp Thr Thr Pro Thr Ile Val Asn Leu
465                 470                 475                 480

Asp His Pro Val Ile Ser Cys Ala Lys Thr Lys Gln Leu Arg Val Val
                485                 490                 495

Asn Gly Ile Pro Thr Arg Thr Asn Ile Gly Trp Met Val Ser Leu Arg
            500                 505                 510

Tyr Arg Asn Lys His Ile Cys Gly Gly Ser Leu Ile Lys Glu Ser Trp
        515                 520                 525

Val Leu Thr Ala Arg Gln Cys Phe Pro Ser Arg Asp Leu Lys Asp Tyr
    530                 535                 540

Glu Ala Trp Leu Gly Ile His Asp Val His Gly Arg Gly Asp Glu Lys
545                 550                 555                 560

Cys Lys Gln Val Leu Asn Val Ser Gln Leu Val Tyr Gly Pro Glu Gly
                565                 570                 575

Ser Asp Leu Val Leu Met Lys Leu Ala Arg Pro Ala Val Leu Asp Asp
            580                 585                 590

Phe Val Ser Thr Ile Asp Leu Pro Asn Tyr Gly Cys Thr Ile Pro Glu
        595                 600                 605

Lys Thr Ser Cys Ser Val Tyr Gly Trp Gly Tyr Thr Gly Leu Ile Asn
    610                 615                 620

Tyr Asp Gly Leu Leu Arg Val Ala His Leu Tyr Ile Met Gly Asn Glu
625                 630                 635                 640
```

```
Lys Cys Ser Gln His His Arg Gly Lys Val Thr Leu Asn Glu Ser Glu
                645                 650                 655

Ile Cys Ala Gly Ala Glu Lys Ile Gly Ser Gly Pro Cys Glu Gly Asp
            660                 665                 670

Tyr Gly Gly Pro Leu Val Cys Glu Gln His Lys Met Arg Met Val Leu
        675                 680                 685

Gly Val Ile Val Pro Gly Arg Gly Cys Ala Ile Pro Asn Arg Pro Gly
    690                 695                 700

Ile Phe Val Arg Val Ala Tyr Tyr Ala Lys Trp Ile His Lys Ile Ile
705                 710                 715                 720

Leu Thr Tyr Lys Val Pro Gln Ser
                725
```