

A LIGAND OF THE PROTEIN "BEACON"

FIELD OF THE INVENTION

5 The present invention relates generally to a ligand for a protein associated with modulating obesity, diabetes and metabolic energy levels in animals and humans and to genetic sequences encoding the ligand. More particularly, the present invention is directed to a ligand of the protein "beacon" and its homologues. The identification of the ligand molecule permits the development of a range of therapeutic and diagnostic protocols for obesity, diabetes and energy
10 imbalance.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by author in this specification are collected
15 at the end of the description.

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical, veterinary and allied human and animal health fields. This is particularly the case in the investigation of the genetic bases involved in the etiology of certain
20 disease conditions. One particularly significant condition from the stand point of morbidity and mortality is obesity and its association with non-insulin-dependent diabetes mellitus (NIDDM) and cardiovascular disease.

Obesity is defined as a pathological excess of body fat and is the result of an imbalance between
25 energy intake and energy expenditure for a sustained period of time. Obesity is the most common metabolic disease found in affluent societies. The prevalence of obesity in these nations is alarmingly high, ranging from 10% to upwards of 50% in some subpopulations (Bouchard, 1994). Of particular concern is the fact that the prevalence of obesity appears to be rising consistently in affluent societies and is now increasing rapidly in less prosperous nations
30 as they become more affluent and/or adopt cultural practices from the more affluent countries (Zimmet, 1992).

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In Australia, for example, studies using the definition of obesity of BMI>30 have found prevalence rates for obesity of 8.2-9.3% in men and 9.1-11.1% in women (Risk Factor Prevalence Study Management Committee, 1990; Waters and Bennett, 1995). The prevalence rates for obesity are increasing in Australia, as they are in many affluent societies. Bennett and Magnus (1994) found that the mean weight of Australian females aged 20-69 increased by 3.1 kg (from 61.7 to 64.8 kg) from 1980 to 1989, while the corresponding increase in males was 1.8 kg (from 77.0 to 78.8 kg). No change in height was observed during this period. Accordingly, the crude prevalence rates of obesity increased from 8.0 to 13.2% in females and from 9.3 to 11.5% in males (Bennett and Magnus, 1994). All of the above changes were statistically significant ($p<0.05$).

The high and increasing prevalence of obesity has significant health implications. Obesity has been identified as a key risk indicator of preventable morbidity and mortality due to disease such as NIDDM and cardiovascular disease (National Health and Medical Research Council, 1996). The annual costs of obesity in Australia, for example, associated with these and other disease conditions have been conservatively estimated at AU\$810 million (National Health and Medical Research Council, 1996).

A genetic basis for the etiology of obesity is indicated *inter alia* from studies in twins, adoption studies and population-based analyses which suggest that genetic effects account for 25-80% of the variation in body weight in the general population (Bouchard 1994; Kopelman *et al*, 1994; Ravussin, 1995). It is considered that genes determine the possible range of body weight in an individual and then the environment influences the point within this range where the individual is located at any given time (Bouchard, 1994).

Obesity is a complex and heterogeneous disorder and of considerable relevance to society. However, despite numerous studies into genes thought to be involved in the pathogenesis of obesity, there have been surprisingly few significant findings in this area. In addition, genome-wide scans in various population groups have not produced definitive evidence of the chromosomal regions having a major effect on obesity.

The hypothalamus has long been recognized as a key brain area in the regulation of energy intake. Early studies led to the dual-centre hypothesis which proposed that two opposing centres in the hypothalamus were responsible for the initiation and termination of eating, the lateral hypothalamus (LHA; "hunger centre") and ventromedial hypothalamus (VMH; "satiety centre"; Stellar, 1954). The dual-centre hypothesis has been repeatedly modified to accommodate the increasing information about the roles played by various other brain regions, neurotransmitter systems, and hormonal and neural signals originating in the gut on the regulation of food intake. In addition to the LHA and VMH, the paraventricular nucleus (PVN) is now considered to have an important integrative function in the control of energy intake.

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A large number of neurotransmitters have been investigated as possible hypothalamic regulators of feeding behaviour including neuropeptide Y (NPY), glucagon-like peptide 1 (GLP-1), melanin-concentrating hormone (MCH), serotonin, cholecystokinin and galanin. Some of these neurotransmitters stimulate food intake, some act in an anorexigenic manner and some have diverse effects on energy intake depending on the site of administration. For example, gamma-aminobutyric acid (GABA) inhibits food intake when injected into the LHA, but stimulates eating when injected into the VMH or PVN (Leibowitz, 1985). Feeding behaviour is thought to be greatly influenced by the interaction of stimulatory and inhibitory signals in the hypothalamus.

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In work leading up to the present invention, the inventors made a significant break through in determining a genetic basis of obesity by identifying a genetic sequence referred to as "*beacon*" which is differentially expressed in lean and obese animals. This genetic sequence is associated with energy balance and is also involved in modulating obesity and diabetes. See International Patent Application No. PCT/AU98/00902 filed on 30 October, 1998 in the names of International Diabetes Institute and Deakin University. This International application is incorporated herein by reference. The inventors now propose the presence of a ligand capable of interacting with the protein, beacon. The interaction between beacon and its ligand is proposed to be a factor in obesity, diabetes and energy imbalance. The identification of a beacon-interacting ligand provides the means for developing a range of therapeutic and diagnostic agents for conditions such as obesity and diabetes.

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SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of
5 a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier, i.e. <400>1, <400>2, etc. A sequence listing is provided at the end of the description.

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Accordingly, one aspect of the present invention provides a ligand of a protein or a derivative, homologue, analogue or mimetic of said protein which protein is produced in larger amounts in hypothalamus tissue of obese animals compared to lean animals.

15 Another aspect of the present invention provides a ligand or a derivative, homologue, analogue or mimetic which ligand is capable of interacting with a protein which is produced in a larger amount of hypothalamus tissue of obese animals compared to lean animals and which is encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>4 or a nucleotide sequence having at least about 50% similarity thereto or a nucleotide sequence capable of
20 hybridizing <400>1 or <400>4 under low stringency conditions.

Yet another aspect of the present invention is directed to a ligand capable of interacting with a protein which comprises the amino acid sequence substantially as set forth in <400>2 or <400>5 or an amino acid sequence having at least 50% similarity thereto and wherein said protein is
25 produced in larger amounts in hyperthalamus tissue of obese animals compared to lean animals.

In still another aspect of the present invention, the nucleotide sequence substantially as set forth in <400>6 and/or <400>7 or a nucleotide sequence having at least about 50% similarity to one or both of <400>6 or <400>7 after optimal alignment or a nucleotide sequence capable of
30 hybridizing to one or both of <400>6 or <400>7 under low stringency conditions.

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In still yet another aspect of the present invention, the ligand comprises a nucleotide sequence substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at least about 50% similarity to a nucleotide sequence in Figure 2 or Figure 3 after optimal alignment or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3
5 under low stringency conditions.

Another aspect of the present invention contemplates a method of identifying a ligand of the protein beacon or its derivatives, said method comprising introducing a first genetic construct in a yeast strain, said genetic construct comprising a nucleotide sequence encoding all or part
10 of beacon fused to a nucleotide sequence encoding one of a DNA binding (DB) domain or an activation domain (AD) and introducing a second genetic construct into said yeast comprising a cDNA, said second genetic construct comprising elements of a cDNA library fused to a nucleotide sequence encoding the other of a DB domain or AD domain and selecting yeast cells which comprise both genetic constructs and in which a reporter gene has been subjected to two-
15 hybrid dependent transcription.

Yet another aspect of the present invention contemplates a method for modulating expression of *beacon* ligand in a mammal, said method comprising contacting the *beacon* ligand gene with an effective amount of a modulator of *beacon* ligand expression for a time and under conditions
20 sufficient to up-regulate or down-regulate or otherwise modulate expression of *beacon* ligand.

Still another aspect of the present invention contemplates a method of modulating activity of beacon in a mammal, said method comprising administering to said mammal a modulating effective amount of a soluble *beacon* ligand or a derivative thereof for a time and under
25 conditions sufficient to increase or decrease beacon activity.

Still yet another aspect of the present invention contemplates, in one embodiment, a composition comprising a soluble form of *beacon* ligand or a modulator of *beacon* ligand expression and one or more pharmaceutically acceptable carriers and/or diluents.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a representation showing the nucleotide sequence of both strands of a differentially expressed band in hypothalamus tissue of lean and obese *Psammomys obesus* corresponding to
5 *beacon*. The amino acids encoded by each codon are shown above in single letter code and the numbering refers to the amino acid position from the start codon.

Figure 1B is a representation of a nucleotide and corresponding amino acid sequence of the short form of *beacon*. Note that amino acid 15 may be His or Arg and the corresponding codon
10 may be CGC or CAC, respectively.

Figure 2 is a representation of the partial nucleotide sequence of pPC86 clone 31 which encodes a *beacon* ligand.

15 **Figure 3** is a representation of multiple sequence alignments of pPC86 clone 31, which encodes a *beacon* ligand, with CLK4M, STYMA and CLK1H.

Figure 4 is a diagrammatic representation of the yeast two-hybrid screening protocol.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of a ligand for the product of a gene associated *inter alia* with regulation of energy balance, obesity and diabetes. The preferred
5 gene is referred to as "beacon" and was identified following differential screening of hypothalamic mRNA between lean and obese animals (see International Patent Application No. PCT/AU98/00902).

The term "ligand" means a peptide, polypeptide or protein which binds, forms a close
10 interaction to or which otherwise associates with a protein involved in energy imbalance, obesity and diabetes. Examples of ligands contemplated by the present invention include cell bound receptors, soluble receptors, intracellular ligands, extracellular ligands and partners in a complex comprising the protein involved in energy imbalance, obesity and diabetes. A single ligand may be involved in interaction with the protein or a complex of two or more ligands may be required
15 to form a complex with the subject protein. The term "ligand" also includes binding or interacting partners, cell bound receptors and soluble receptors.

The terms "lean" and "obese" are used in their most general sense but should be considered relative to the standard criteria for determining obesity. Generally, for human subjects the
20 definition of obesity is BMI>30 (Risk Factor Prevalence, 1990; Waters and Bennett, 1995).

Conveniently, an animal model may be employed to study the effects of obese and lean animals. In particular, the present invention is exemplified using the *Psammomys obesus* (the Israeli sand rat) animal model of dietary-induced obesity and NIDDM. In its natural desert habitat, an active
25 lifestyle and saltbush diet ensure that they remain lean and normoglycemic (Shafir and Gutman, 1993). However, in a laboratory setting on a diet of *ad libitum* chow (on which many other animal species remain healthy), a range of pathophysiological responses are seen (Barnett *et al.*, 1994a, b; Barnett *et al.*, 1995). By the age of 16 weeks, more than half of the animals become obese and approximately one-third develop NIDDM. Only hyperphagic animals go on to
30 develop hyperglycemia, highlighting the importance of excessive energy intake in the pathophysiology of obesity and NIDDM in *Psammomys obesus* (Collier *et al.*, 1997a; Walder

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et al., 1997a). Other phenotypes found include hyperinsulinemia, dyslipidemia and impaired glucose tolerance (Collier *et al.*, 1997a, b). *Psammomys obesus* exhibit a range of bodyweight and blood glucose and insulin levels which forms a continuous curve that closely resembles the patterns found in human populations, including the inverted U-shaped relationship between
5 blood glucose and insulin levels known as "Starling's curve of the pancreas" (Barnett *et al.*, 1994a; DeFronzo, 1988). It is the heterogeneity of the phenotypic response of *Psammomys obesus* which make it an ideal model to study the etiology and pathophysiology of obesity and NIDDM.

10 A preferred aspect of the present invention is directed to a ligand capable of interacting with "beacon", the product of the gene "beacon". The nucleotide sequence of *beacon* is set forth in <400>1 and <400>4. The amino acid sequence of *beacon* is set forth in <400>2 and <400>5.

Accordingly, another aspect of the present invention provides a ligand or a derivative,
15 homologue, analogue or mimetic which ligand is capable of interacting with a protein which is produced in a larger amount of hypothalamus tissue of obese animals compared to lean animals and which is encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>4 or a nucleotide sequence having at least about 50% similarity thereto or a nucleotide sequence capable of hybridizing <400>1 or <400>4 under low stringency conditions.

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According, another aspect of the present invention is directed to a ligand capable of interacting with a protein which comprises the amino acid sequence substantially as set forth in <400>2 or <400>5 or an amino acid sequence having at least 50% similarity thereto and wherein said protein is produced in larger amounts in hyperthalamus tissue of obese animals compared to
25 lean animals.

Reference herein to similarity is generally at a level of comparison of at least 15 consecutive or substantially consecutive nucleotides or at least 5 consecutive or substantially consecutive amino acid residues. Generally, similarity or identity is determined after optimal alignment of
30 the sequences.

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The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity.

10 Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", "percentage of sequence similarity", "percentage of sequence identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units, inclusive of
15 nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window"
20 to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a
25 comparison window may be conducted by computerised implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST
30 family of programs as for example disclosed by Altschul *et al.* (1997). A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.* (1998).

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The terms "sequence similarity" and "sequence identity" as used herein refers to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences
5 over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g. A, T, C, G, I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e. the window size),
10 and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, California, USA) using standard defaults as used in the reference manual accompanying the software. Similar comments apply in relation
15 to sequence similarity.

Reference herein to a low stringency includes and encompasses from at least about 0 to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization, and at least about 1 M to at least about 2 M salt for washing conditions.
20 Generally, low stringency is at from about 25-30°C to about 42°C. The temperature may be altered and higher temperatures used to replace formamide and/or to give alternative stringency conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and
25 at least about 0.5 M to at least about 0.9 M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41 (G+C)\%$ (Marmur and Doty, 1962). However, the T_m of a duplex
30 DNA decreases by 1°C with every increase of 1% in the number of mismatch base pairs (Bonner and Laskey, 1974). Formamide is optional in these hybridization conditions. Accordingly,

particularly preferred levels of stringency are defined as follows: low stringency is 6 x SSC buffer, 0.1% w/v SDS at 25-42°C; a moderate stringency is 2 x SSC buffer, 0.1% w/v SDS at a temperature in the range 20°C to 65°C; high stringency is 0.1 x SSC buffer, 0.1% w/v SDS at a temperature of at least 65°C.

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The nucleotide sequence or amino acid sequence of the beacon ligand of the present invention may correspond to exactly the same sequence of the naturally occurring ligand or its gene (or corresponding cDNA) or may carry one or more nucleotide or amino acid substitutions, additions and/or deletions.

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Any number of approaches may be employed to identify the ligand.

In one particularly useful method, a yeast two-hybrid system is employed. The yeast two-hybrid system is an *in vivo* genetic technique that can be utilized for the identification of protein:protein interactions. The essence of the two-hybrid system is that interaction between two proteins (X and Y) can be identified by reconstituting active transcription factor dimers. In yeast, these dimers are formed between two fusion proteins, one of which contains a DNA binding (DB) domain fused to the first protein of interest X and the other, an activation domain (AD) fused to a second protein Y. Interaction between DB-X and AD-Y forms a functional transcription factor that activates chromosomally integrated-reporter genes driven by promoters containing the relevant DB binding sites. When a selectable marker such as *HIS3* is used as a reporter gene, two-hybrid dependent transcription activation can be monitored by growth on plates lacking histidine. This technique can, therefore, be applied to test whether two known proteins interact or to detect an unknown protein, encoded by a cDNA library, that interacts with a protein of interest.

Accordingly, another aspect of the present invention contemplates a method of identifying a ligand of the protein beacon or its derivatives, said method comprising introducing a first genetic construct in a yeast strain, said genetic construct comprising a nucleotide sequence encoding all or part of beacon fused to a nucleotide sequence encoding one of a DNA binding (DB) domain or an activation domain (AD) and introducing a second genetic construct into said

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yeast comprising a cDNA, said second genetic construct comprising elements of a cDNA library fused to a nucleotide sequence encoding the other of a DB domain or AD domain and selecting yeast cells which comprise both genetic constructs and in which a reporter gene has been subjected to two-hybrid dependent transcription.

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According to this embodiment, if the cDNA from the cDNA library encodes a binding partner for beacon, then a dimer forms and the DB and AD domains permit transcription of the reporter gene.

10 In one embodiment, the yeast reporter gene is *HIS3* although any other reporter gene may be employed. Preferably, the reporter gene provides a selectable marker.

In a particularly preferred embodiment, the ligand comprises the nucleotide sequence substantially as set forth in <400>6 and/or <400>7 or a nucleotide sequence having at least
15 about 50% similarity to one or both of <400>6 or <400>7 after optimal alignment or a nucleotide sequence capable of hybridizing to one or both of <400>6 or <400>7 under low stringency conditions.

In another particularly preferred embodiment, the ligand comprises a nucleotide sequence
20 substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at least about 50% similarity to a nucleotide sequence in Figure 2 or Figure 3 after optimal alignment or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3 under low stringency conditions.

25 For convenience, the ligand capable of interaction with beacon is referred to as "beacon ligand". The corresponding genetic sequence encoding beacon ligand is referred to herein as "*beacon* ligand". Reference herein to *beacon* ligand includes, where appropriate, reference to the genomic gene or cDNA as well as any naturally occurring or induced derivatives. Apart from the substitutions, deletions and/or additions to the nucleotide sequence, the present invention
30 further encompasses mutants, fragments, parts and portions of the nucleotide sequence corresponding to the *beacon* ligand. The *beacon* gene itself may encode a "short" form or "long"

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form of beacon. Both the long and short forms of beacon are biologically active, and suppression of the activity of either or both forms is included in this invention. The short form of beacon can be readily synthesized *in vitro*, while the long form can be produced using expression vectors. A beacon ligand may be any protein including a heat shock protein and/or
5 a cdc-like protein. In a preferred embodiment, the beacon ligand is a cdc-like kinase with strong homology to mouse clk4.

A homologue of beacon ligand or *beacon* ligand is considered to be a ligand from another animal species. The *beacon* ligand gene is exemplified herein from *Psammomys obesus*
10 hypothalamus. The invention extends, however, to the homologous gene, as determined by nucleotide sequence and/or function, from humans, primates, livestock animals (e.g. cows, sheep, pigs, horses, donkeys), laboratory test animals (e.g. mice, guinea pigs, hamsters, rabbits), companion animals (e.g. cats, dogs) and captured wild animals (e.g. rodents, foxes, deer, kangaroos).

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Apart from the yeast two-hybrid method, the ligand of the present invention and, in particular, beacon ligand, may also be identifiable by a number of other means. In one method, beacon or a ligand binding portion thereof is labelled with a reporter molecule and used to screen cells, cell lysate and biological fluid (including blood, serum, lymph fluid) for binding to ligand. For
20 cloning of *beacon* ligand, a cDNA library is conveniently prepared and expressed in a suitable cell such as CHO cells and the presence of beacon ligand is then determined by, for example, beacon or a ligand binding portion thereof labelled with a reporter molecule.

The identification of cell types having a beacon ligand is readily determined by incubated cells
25 with beacon, with or without neuropeptide Y (NPY) or leptin and screening for an effect. Generally, the effect is the expression of select genes or screening for signal transduction or screening for phenotypic changes. Another useful technique involves the yeast two-hybrid system. This is particularly useful where the ligand is intracellular and not expressed on the cell surface.

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The present invention provides the *beacon* ligand gene as well as a peptide, polypeptide or

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protein encoded thereby. The nucleic acid molecule of this aspect of the present invention and in particular *beacon* ligand gene and its derivatives and homologues may be in isolated or purified form and/or may be ligated to a vector such as an expression vector. Expression may be in a eukaryotic cell line (e.g. mammalian, insect or yeast cells) or in microbial cells (e.g. *E. coli*) or both.

The derivatives of the *beacon* ligand nucleic acid molecule of the present invention include oligonucleotides, PCR primers, antisense molecules, molecules suitable for use in co-suppression and fusion nucleic acid molecules. Ribozymes and DNA enzymes are also contemplated by the present invention directed to *beacon* ligand or its mRNA.

Reference herein to a *beacon* ligand includes reference to isolated or purified naturally occurring *beacon* ligand molecules as well as any derivatives, homologues, analogues and mimetics thereof. Derivatives includes parts, fragments and portions of a *beacon* partner as well as single and multiple amino acid substitutions, deletions and/or additions to the *beacon* partner.

Other derivatives of a *beacon* ligand include chemical analogues. Analogues of a *beacon* ligand contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic

condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

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Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-
10 chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides.

15 Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

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Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of
25 amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 1.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5 α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
10 aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15 D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20 D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
25 D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30 D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib

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	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
5	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
10	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
15	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptyl glycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
20	D- α -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncp
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
25	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
30	D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu

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N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
5 N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
10 γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L- α -methylalanine	Mala
L- α -methylarginine	Marg	L- α -methylasparagine	Masn
15 L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
L- α -methylhistidine	Mhis	L- α -methylhomophenylalanine	Mhphe
L- α -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
20 L- α -methylleucine	Mleu	L- α -methyllysine	Mlys
L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
25 L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmbc		
30 ethylamino)cyclopropane			

Crosslinkers can be used, for example, to stabilize 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-
5 reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α -methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and
10 the N or C terminus.

All such modifications may also be useful in stabilizing the beacon partner molecule for use in *in vivo* administration protocols or for diagnostic purposes.

15 The identification of a beacon ligand permits the generation of a range of therapeutic molecules capable of modulating expression of *beacon* or *beacon* ligand or modulating the activity of beacon or beacon ligand. Modulators contemplated by the present invention includes agonists and antagonists of *beacon* ligand expression. Antagonists of *beacon* ligand expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules
20 which increase promoter activity or which interfere with negative regulatory mechanisms. Antagonists of beacon ligand include antibodies and inhibitor peptide fragments. All such molecules may first need to be modified to enable such molecules to penetrate cell membranes. Alternatively, viral agents may be employed to introduce genetic elements to modulate expression of a *beacon* ligand. Insofar as beacon acts in association with other genes such as the
25 *ob* gene which encodes leptin, the therapeutic molecules of the present invention may target both the *beacon* ligand and *ob* ligand genes or their translation products.

The present invention contemplates, therefore, a method for modulating expression of *beacon* ligand in a mammal, said method comprising contacting the *beacon* ligand gene with an
30 effective amount of a modulator of *beacon* ligand expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *beacon* ligand.

- 20 -

For example, a nucleic acid molecule encoding *beacon* ligand or a derivative or homologue thereof may be introduced into a cell to enhance the ability of that cell to produce *beacon* ligand. Conversely, *beacon* ligand antisense sequences such as oligonucleotides may be introduced to decrease the availability of *beacon* ligand molecules.

5

Another aspect of the present invention contemplates a method of modulating activity of *beacon* in a mammal, said method comprising administering to said mammal a modulating effective amount of a soluble *beacon* ligand or a derivative thereof for a time and under conditions sufficient to increase or decrease *beacon* activity. The derivative of *beacon* ligand may be a
10 proteinaceous molecule or a chemical entity such as a product identified from a natural product library or chemical library.

One convenient means of screening for antagonists of *beacon* ligand when in the form of a receptor is to incubate a cell carrying a *beacon* ligand in the form of a receptor with *beacon* with
15 or without a potential antagonist and screening for a differential effect when the antagonist is applied. Again, the effect may be gene expression, signal transduction and/or phenotypic changes.

Modulating levels of *beacon* ligand expression or *beacon* ligand activity is important in the
20 treatment of a range of conditions such as obesity, anorexia, energy imbalance, diabetes, metabolic syndrome, dyslipidemia, hypertension and insulin resistance. It may also be useful in the agricultural industry to assist in the generation of leaner animals, or where required, more obese animals. Accordingly, the mammal contemplated by the present invention includes but is not limited to humans, primates, livestock animals (e.g. pigs, sheep, cows, horses, donkeys),
25 laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), companion animals (e.g. dogs, cats) and captured wild animals (e.g. foxes, kangaroos, deer). A particularly preferred host is a human, primate or livestock animal.

Accordingly, the present invention contemplates in one embodiment a composition comprising
30 a soluble form of *beacon* ligand or a modulator of *beacon* ligand expression and one or more pharmaceutically acceptable carriers and/or diluents. The compositions may also comprise leptin

or modulators of leptin activity or *ob* expression.

For brevity, all such components of such a composition are referred to as "active components".

- 5 The compositions of active components in a form suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as
10 bacteria and fungi.

The carrier can be a solvent or other medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

15

- The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about
20 by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

- Sterile injectable solutions are prepared by incorporating the active components in the required amount in the appropriate solvent with optionally other ingredients, as required, followed by
25 sterilization by, for example, filter sterilization, irradiation or other convenient means. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

30

When the active molecules are suitably protected they may be orally administered, for example,

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with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μ g and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active

ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active component may be compounded for convenient and effective administration in sufficient amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. A unit dosage form can, for example, contain the principal active component in amounts ranging from 0.5 μ g to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In general terms, effective amounts of active ingredient will range from 0.01 ng/kg/body weight to above 10,000 mg/kg/body weight. Alternative amounts range from 0.1 ng/kg/body weight to above 1000 mg/kg/body weight. Active ingredients may be administered per minute, hour, day, week, month or year depending on the condition being treated. The route of administration may vary and includes intravenous, intraperitoneal, sub-cutaneous, intramuscular, intranasal, *via* suppository, *via* infusion, *via* drip, orally or *via* other convenient means. Compositions may be formulated in a variety of ways and reference may be conveniently made to Remington Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Pennsylvania, USA.

30

The pharmaceutical composition may also comprise genetic molecules such as a vector capable

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of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating *beacon* ligand expression or beacon ligand activity. The vector may, for example, be a viral vector.

- 5 Still another aspect of the present invention is directed to antibodies to beacon ligand and its derivatives and homologues. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to beacon or may be specifically raised to beacon or derivatives or homologues thereof. In the case of the latter, beacon ligand or its derivatives or homologues may first need to be associated with a carrier molecule. The antibodies and/or
- 10 recombinant beacon ligand or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

For example, beacon ligand and its derivatives can be used to screen for naturally occurring beacon or antibodies to beacon ligand which may occur in certain autoimmune diseases or

15 where cell death is occurring. These may occur, for example in some autoimmune diseases. Alternatively, specific antibodies can be used to screen for beacon partner. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA.

Antibodies to beacon partner of the present invention may be monoclonal or polyclonal and may

20 be selected from naturally occurring antibodies to the beacon or may be specifically raised to the beacon or its derivatives. In the case of the latter, the beacon protein may need first to be associated with a carrier molecule. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include

25 fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also be used as a diagnostic tool or as a means for purifying beacon ligand.

For example, specific antibodies can be used to screen for beacon partner proteins. The latter

30 would be important, for example, as a means for screening for levels of beacon partner in a cell extract or other biological fluid or purifying beacon made by recombinant means from culture

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supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal
5 or fragments of antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of beacon partner.

10 Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of beacon partner or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any
15 of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the
20 ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art (see, for example, Douillard and Hoffman, 1981; Kohler and Milstein, 1975, Kohler and Milstein, 1976).

25

Another aspect of the present invention contemplates a method for detecting beacon or a derivative or homologue thereof in a biological sample from a subject said method comprising contacting said biological sample with a beacon ligand or a derivative or homologue thereof for a time and under conditions sufficient for a complex to form, and then detecting said complex.

30

The presence of the complex is indicative of the presence of beacon. This assay may be

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quantitated or semi-quantitated to determine a propensity to develop obesity or other conditions or to monitor a therapeutic regimen.

Conveniently, beacon partner is immobilized to a solid support and a biological sample brought
5 into contact with the immobilized molecule.

The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable
10 for conducting an immunoassay. The binding processes to immobilize *beacon* partner are well-known in the art and generally consist of cross-linking, covalently binding or physically adsorbing the molecule to the solid support. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes or overnight if more convenient) and under suitable conditions (e.g. from room temperature to
15 about 37°C) to allow binding of beacon to its receptor. Following the incubation period, solid phase is washed and an antibody added directed to beacon. The presence of antibody binding is indicative of beacon being immobilized to its receptor.

Generally, either the beacon partner or an antibody to beacon is labelled with a receptor
20 molecule.

In an alternative embodiment, beacon itself or a ligand binding portion thereof is labelled with a reported molecule and used to screen for beacon ligand.

25 By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

30

The present invention also contemplates genetic assays such as involving PCR analysis to detect

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beacon ligand or its derivatives.

The present invention further extends to a clk ligand which is independent of beacon or beacon-clk interaction. The clk ligand is useful for a range of applications such as acting as an
5 antagonist for clk interaction with other ligands. The clk ligand of this aspect of the present invention, for example, is the treatment of diabetes and/or other conditions associated with clk. This aspect of the present invention further contemplates nucleic acid molecules encoding the clk ligand as well as compositions comprising the clk ligand such as pharmaceutical compositions.

10

The present invention is further described by reference to the following non-limiting Figures and Examples.

A summary of sequence identifiers used throughout the subject specification is provided in
15 Table 2.

TABLE 2

SEQUENCE IDENTIFIER	DESCRIPTION
<400>1	Nucleotide sequence for <i>beacon</i>
<400>2	Amino acid sequence for <i>beacon</i>
<400>3	Complementary sequence for <400>1
<400>4	Nucleotide sequence for human <i>beacon</i>
<400>5	Amino acid sequence for short form of <i>beacon</i>
<400>6	Partial nucleotide sequence of pPC86 clone 31 <i>beacon</i> ligand
<400>7	Partial nucleotide sequence of pPC86 clone 31 <i>beacon</i> ligand

A summary of the single and three letter abbreviations for amino acid residues used in the present specification is provided in Table 3.

TABLE 3

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

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EXAMPLE 1***Animals***

A *Psammomys obesus* colony is maintained at Deakin University, Geelong, Victoria, Australia, with the breeding pairs fed *ad libitum* a diet of lucerne and chow. Experimental animals were weaned at four weeks of age and given a diet of standard laboratory chow from which 12% of energy was derived from fat, 63% from carbohydrate and 25% from protein (Barastoc, Pakenham, Australia). Animals were housed individually in a temperature controlled room ($22 \pm 1^\circ\text{C}$) with a 12-12-hour light-dark cycle. The animals used in the study were aged 16-20 weeks.

EXAMPLE 2***Beacon ligand in cell lines***

This example provides means for identifying a beacon ligand (e.g. beacon receptor) in cells lines which also helps in the study of signal transduction mechanisms. Examples of suitable cell lines include 3T2, GT1-7, HepG2 and primary cultures from Israeli Sand Rats. Cells are treated with beacon with and without neuropeptide Y(NPY) or leptin and an effect observed. Generally, approximately 3-5 genes are selected for changes in expression. Analysis is generally conducted in macro-or micro-assays. Once a cell is identified carrying a beacon receptor, a cDNA library is prepared and *beacon* ligand identified. Signal transduction studies may also be conducted (e.g. Ca^{2+} , cAMP, kinases, phosphatases).

EXAMPLE 3***Intracellular beacon ligand***

In cases where a cell does not secrete beacon or synthesize it as a cellular receptor, the yeast two-hybrid system is useful for identifying beacon ligand as a binding partner of beacon.

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EXAMPLE 4***Fusion protein fishing******Yeast Two-hybrid System***

5 (using the ProQuest Two-hybrid System available from Life Technologies)

The steps employed are as follows:

- 10 ● Clone the beacon gene in frame with the *GAL4* activation domain of the yeast vector pPC86. Libraries available include: expression human (brain and foetal brain), mouse (embryo 8.5 or 10 days, liver, brain and lymph node), *C. elegans*, HeLa cell and rat (liver and brain). A custom library (*Pssamomys obesus*) is also created.
- 15 ● Transform the yeast strain MaV203 with the pDBLeu-beacon plasmid construct.
- 20 ● Test for self activation of the pDBLeu-beacon fusion protein and determine the concentration of 3-Amino-1,2,4-Triazole (3AT) required to titrate basal HIS3 expression levels. HIS3 encodes imidazole glycerol phosphate dehydratase, an enzyme involved in histidine biosynthesis. This enzyme is specifically inhibited in a dose-dependent manner by 3AT. To maximize sensitivity of the HIS3 reporter gene, strain MaV203 expresses a basal level of HIS3. By determining the threshold of resistance to 3AT and including that concentration of 3AT in plates lacking histidine, even slight increases in HIS3 reporter is detected, enhancing the likelihood of detecting even weak protein:protein interactions.
- 25 ● Transform MaV203 cells containing the pDBLeu-beacon construct with pC86 library using antibiotic resistance (ampicillin and kanamycin) to select for cells that contain both plasmids and induce the HIS3 reporter gene.
- 30 ● Purify cells containing candidate interacting proteins then patch isolated colonies onto a masterplate.

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- Replica plate from the master plate onto selective plates to determine whether the three reporter genes are induced.
- For cells inducing the reporter genes confirm that DB-beacon and AD-fusion protein (from the cDNA library) interact when AD-fusion protein is retested with fresh DB-beacon plasmid by either a retransformation assay or a version of plasmid shuffling.
- Protein:protein interactions detected are confirmed by biological assays such as DNA sequencing of the cDNA clone to determine if the interacting protein has been previously identified. This fusion protein is also expressed in *E. coli* and co-precipitation experiments using antibodies raised against beacon or monoclonal antibodies raised against GAL4-AD domains (protein tags derived from the expression vectors). Other methods are employed to confirm that these proteins interact such as by surface receptor panning and fusion protein fishing.

15

A full length beacon sequence is cloned into the bacterial expression plasmid pGEX (Pharmacia Biotech). pGEX vectors allow for inducible, high level intracellular expression of genes as fusions with *Schistoma japonicum* glutathione S-transferase (GST). Induced bacterial cultures expressing pGEX-beacon are lysed by sonication in 50mM Tris-HCl (pH 7.4) containing 1% w/v Triton X-100, 1% v/v Tween 20, 2 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride, and 10 µg/ml aprotinin. Affinity resins for the isolation of beacon binding proteins are prepared by immobilizing GST-beacon onto glutathione-Sepharose 4B beads (Pharmacia). Similar resins are prepared using GST alone to act as a control for the specificity of interaction. Affinity resins are incubated in the presence of brain lysates (or other tissue lysates of interest) and after extensive washing, proteins bound to resins are released either by boiling in SDS sample buffer or by elution with Tris-HCl (pH 7.4) containing 0.5% v/v Triton X-100. Binding proteins are then separated by SDS-PAGE and visualized by silver staining or Coomassie Blue staining. Regions of the gel containing beacon binding proteins are excized and the gel slices digested and purified by anion-exchange and reverse phase HPLC prior to amino acid sequencing.

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EXAMPLE 5***Yeast two-hybrid screening***

The full length Beacon gene was cloned into the expression vector pDBLeu, which encodes the
5 *GAL 4* DNA binding domain. To screen and identify potential proteins that interact with the
73aa Beacon gene product, a commercial human brain cDNA library was purchased. This cDNA
expression library was constructed in the two-hybrid activation domain vector pPC86.
Following vector construction, pDBLeu - Beacon (pDB73Be) was introduced into the yeast
strain MaV203 by transformation. MaV203 cells containing pDB73Be were then used to
10 introduce, by transformation, the pPC86 cDNA library. Candidate positive clones were
identified by growth on media that selects for cells containing both plasmids as well as
induction of the *HIS3* reporter gene. Greater than 10^6 transformants were screened for
interaction with 73aa Beacon. Of this figure, 28 clones were identified as preliminary positives
as a result of induction of the *HIS3* reporter gene.

15

Clones containing candidate interacting proteins were purified and re-tested for induction of
three independent reporter genes. Of the 28 clones examined, three clones (clones 12, 16 and
31) were identified as containing potential interacting proteins due induction of all three reporter
genes. To further ascertain the authenticity of interaction with 73aa Beacon, plasmid DNA from
20 each clone was selectively isolated and re-introduced into MaV203. The re-transformation assay
confirmed these clones as containing potential positive interactors with 73aa Beacon.
Specifically, clones 12 and 16 were both identified as containing potential weak interacting
proteins with 73aa Beacon. In contrast, clone 31 was shown to contain a strong interacting
protein. The results are shown in Table 4. Plasmid DNA from each clone has been selectively
25 isolated and partial sequences for the unknown cDNA's determined.

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TABLE 4
Reporter gene expression

Clone	-HIS	-URA	X-gal	Result
Clone 12	+	+	White	+ve weak interacting proteins
Clone 16	+	+	White	+ve weak interacting proteins
Clone 31	+++	+++	Blue	+ve strong interacting proteins

Of the three clones that exhibited positive interaction with 73aa Beacon, clones 12 and 16 revealed overlapping partial cDNA sequences that were found to be 100% homologous to that of the human heat shock protein 2 (HSPB2) in the regions examined. HSPB2 belongs to the small heat shock protein (HSP20) family and has been shown to bind and activate the myotonic dystrophy protein kinase. *In vivo*, heat shock proteins have been shown to interact with large numbers of different proteins, consequently interaction of HSBP2 with 73aa Beacon may prove to be biologically irrelevant in the context of Beacon action.

Partial cDNA sequence data for clone 31 (<400>7) was found to be highly homologous to that for the gene encoding mouse cdc2/CDC28-like protein kinase 4 in the region examined (Table 5). This kinase is considered to be closely related to the yeast cdc2/CDC28 kinases that have been shown to regulate the cell cycle. In a particularly preferred embodiment, the present invention is predicted on the beacon ligand being clone 31/pPC86, which has strong homology to mouse clk4.

TABLE 5
Sequence data summary

	Clone	Sequence
5	Clone 12/pPC86 cDNA	Partial sequence - 100% homologous to HSPB2
	Clone 16/pPC86 cDNA	Partial sequence - 100% homologous to HSPB2
	Clone 31/pPC86 cDNA	Partial sequence shows strong homology to mouse cdc2/CDC28-like protein kinase 4

10 Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or
15 features.

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CLAIMS

1. A ligand of a protein or a derivative, homologue, analogue or mimetic of said protein which protein is produced in larger amounts in hypothalamus tissue of obese animals compared to lean animals.
2. A ligand according to Claim 1 wherein the protein is encoded by a nucleotide sequence substantially as set forth in <400>1 or <400>4, a nucleotide sequence having at least 50% similarity after optimal alignment to <400>1 or <400>4 or a nucleotide sequence which is capable of hybridizing to <400>1 or <400>4 under low stringency conditions.
3. A ligand according to Claim 1 or 2 wherein the protein comprises an amino acid sequence substantially as set forth in <400>2 or <400>5 or an amino acid sequence having at least about 50% similarity after optimal alignment to <400>2 or <400>5.
4. A ligand according to any one of Claims 1 to 3 wherein protein is encoded by the nucleotide sequence substantially as set forth in <400>6 or <400>7 or a nucleotide sequence having at least 50% similarity after optimal alignment to <400>6 or <400>7 or a nucleotide sequence capable of hybridizing to <400>6 or <400>7 under low stringency conditions.
5. A ligand according to any one of Claims 1 to 3 wherein the ligand is encoded by the nucleotide sequence substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at least about 50% similarity after optimal alignment to a nucleotide sequence in Figure 2 or Figure 3 or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3 under low stringency conditions.
6. A ligand according to Claim 1 wherein the ligand is a heat shock protein or a cdc-like protein.
7. A ligand according to Claim 6 wherein the ligand is a cdc-like kinase.



8. A method of identifying a ligand of the protein beacon or its derivatives, said method comprising introducing a first genetic construct in a yeast strain, said genetic construct comprising a nucleotide sequence encoding all or part of beacon fused to a nucleotide sequence encoding one of a DNA binding (DB) domain or an activation domain (AD) and introducing a second genetic construct into said yeast comprising a cDNA, said second genetic construct comprising elements of a cDNA library fused to a nucleotide sequence encoding the other of a DB domain or AD domain and selecting yeast cells which comprise both genetic constructs and in which a reporter gene has been subjected to two-hybrid dependent transcription.
9. A method according to Claim 8 wherein the ligand is encoded by the nucleotide sequence substantially as set forth in <400>6 or <400>7 or a nucleotide sequence having at least 50% similarity after optimal alignment to <400>6 or <400>7 or a nucleotide sequence capable of hybridizing to <400>6 or <400>7 under low stringency conditions.
10. A method according to Claim 8 wherein the ligand is encoded by the nucleotide sequence substantially as set forth in Figure 2 or Figure 3 or a nucleotide sequence having at least about 50% similarity after optimal alignment to a nucleotide sequence in Figure 2 or Figure 3 or a nucleotide sequence capable of hybridizing to a nucleotide sequence in Figure 2 or Figure 3 under low stringency conditions.
11. A ligand according to Claim 8 wherein the ligand is a heat shock protein or a cdc-like protein.
12. A ligand according to Claim 11 wherein the ligand is a cdc-like kinase.
13. A ligand identified by the method of Claim 8.
14. A method for modulating expression of *beacon* ligand in a mammal, said mammal comprising contacting the *beacon* ligand gene with an effective amount of a modulator of *beacon* ligand expression for a time and under conditions or otherwise modulate expression of *beacon* ligand.



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15. A method of modulating activity of beacon in a mammal, said method comprising administering to said mammal a modulating effective amount of a soluble *beacon* ligand or a derivative thereof for a time and under conditions sufficient to increase or decrease beacon activity.
16. A composition comprising a soluble form of beacon ligand or a modulator of *beacon* ligand expression and one or more pharmaceutically acceptable carriers and/or diluents.
17. A composition according to Claim 16 further comprising a leptin, a modulator of leptin activity or a modulator of *ob* expression.