to 9 metric tons. For comparison, in 2002, total mAb-based therapy production was only 916 kg while sales were around $ 5.2 billion. Clearly, a production platform that can substantially reduce both COGs and capitalization costs, and at the same time hold out the promise of treating substantially more afflicted individuals would be of tremendous benefit to the healthcare system in general.

[0007] Like Xolair and other anti-IgE mAbs, the naturally occurring receptor FcεRIα, binds IgE with high affinity. The human FcεRIα is a tetrameric complex located on the surface of mast cells and basophils, eosinophils, platelets and megakaryocytes. In these cell types, the receptor is comprised of an α chain, containing the ligand binding site, a β chain and a dimer of inter-molecularly, disulphide linked, γ chains, linked by inter-molecular disulphide bonds which are critically important for receptor accumulation. β chains are absent from this complex on the surface of both Langerhans cells and activated monocytes. The cytoplasmic tail and transmembrane domain of FcεRIα are not required for ligand binding and the extracellular domain of FcεRIα (ecFcεRIα) does not require glycosylation for ligand binding.

[0008] Recent success in the expression of recombinant proteins from both the nuclear and chloroplast genomes in the green alga *Chlamydomonas reinhardtii* serves to highlight the potential of algae for the production of recombinant proteins. The chloroplast organelle of algae and plants also is a particularly favorable site for recombinant protein production. Due to the presence of chaperones and foldases required for correct assembly of complex eukaryotic proteins imported from the cytosol, the chloroplast is well-suited for production of mammalian proteins that are both soluble and correctly folded. Algal-based production systems also offer tremendous advantages over traditional, as well as many alternative, expression platforms when it comes to speed, scalability, COGs and perhaps most importantly capital costs. However, despite demonstration of the feasibility of expressing human antibodies containing Fc domains in the chloroplasts of microalgae, no certainty was afforded that algae and their chloroplast organelles were amenable to expression and accumulation of chimeric or non-antibody molecules containing mammalian Fc domains.

SUMMARY ~~OF THE INVENTION~~

[0009] ~~The present invention d~~Discloseds herein is a method of using plastid (chloroplast) organelle expression to generate fusion proteins that incorporate immunoglobulin-derived Fc domains suitable for conferring improved serum stability to therapeutic proteins. In addition, the method provides for efficient isolation and purification of such fusion proteins from complex extracts. These fused molecules may modulate the interaction between proteins containing stereoselective

binding domains and specific ligands. ~~The present invention a~~Also discloseds are nucleic acid constructs encoding such fusion proteins and the use of these fusion proteins in the treatment of various disorders, including inflammatory, metabolic and proliferative disorders. By incorporating an Fc domain capable of interacting with the neonatal FcRn receptor, it is possible to extend the pharmacokinetics of therapeutic proteins, thus minimizing adverse reactions caused by high doses, decreasing the frequency of injection, maximizing trancytosis to specific tissue sites, and decreasing production costs by reducing the dosage required to gain a useful pharmacological effect. In addition, expression of Fc-fusion proteins in the chloroplast results in the absence of asparagine-linked glycosylation on the Fc domain, thus prevents unwanted or undesired activation of Fc-mediated effector functions.

[0009a] In one aspect, there is provided a method for producing a soluble and active bifunctional fusion protein comprising, (a) transforming an algal chloroplast with one or more expression constructs, said one or more expression constructs comprising in operable linkage, nucleic acid signal sequence elements for homologous recombination and expression of said bifunctional fusion protein in said algal chloroplast, a first polynucleotide sequence encoding a fragment crystallizable (Fc) region or fragment thereof, and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine, or an enzyme, and (b) expressing said first and said second polynucleotide sequences to produce a soluble and active bifunctional fusion protein in said algalchloroplast, wherein said Fc region or fragment thereof is aglycosylated.

[0009b] In another aspect, there is provided a nucleic acid construct comprising in operable linkage: nucleic acid signaling elements for homologous recombination and expression of a soluble and active bifunctional fusion protein in an algal chloroplast; a first polynucleotide sequence encoding a fragment crystallizable (Fc) region or fragment thereof; and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine, or an enzyme, wherein said first and second polynucleotide sequences are expressed as a soluble and active bifunctional fusion protein in said algal chloroplast, and wherein said Fc region or fragment thereof is aglycosylated.

[0009c] In another aspect, there is provided an algal cell or a progeny thereof comprising the construct as defined above stably integrated into the chloroplast of said algal cell.

[00010] In one embodiment, a nucleic acid construct is disclosed including, in operable linkage, nucleic acid signaling elements for homologous recombination and expression of the fusion protein in a plant or algae plastid and a first polynucleotide sequence encoding a polypeptide including an Fc region and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine, or an enzyme, such as a metabolic enzyme whose deficiency results in a clinical disease state, where the first and second polynucleotide sequences are expressed as a fusion protein.

[00011] In one embodimentaspect, the first polynucleotide encodes a non-plastid, non-plant, eukaryotic polypeptide. In another embodimentaspect, the first polynucleotide encodes an aglycosylated Fc domain, that mediates binding to the FcRN receptor and Protein A or Protein G, but not to Fc receptors that mediate opsonization, cell lysis, and degranulation of mast cells, basophils and eosinophils.

[00012] In another embodimentaspect, the extracellular domain (ECD) of a receptor includes, but is not limited to, a cytokine receptor ECD, immunoglobulin receptor ECD, a T-cell receptor ECD, a cluster of differentiation antigen receptor ECD, growth factor receptor ECD, tissue factor receptor ECD, blood factor receptor ECD. In another embodimentaspect, the enzyme is DNAse I, a lipase, a protease, an amylase, uricase, asparaginase, collagenase, streptokinase, urokinase, tissue plasminogen activator, thrombin, lactoferrin, lysozyme, adenosine deaminase, N-acetylgalactosamine-4-sulfatase, alpha galactosidase, beta galactosidase, alpha glucocerebrosidase, beta glucocerebrosidase, alpha glucosidase, or iduronidase.

[00013] In one embodimentaspect, the growth factors include, but are not limited to, TGF-β, G-CSF, GM-CSF, IGF1, NGF, BDNF, NT3, PDGF, EPO, TPO, myostatin, GDF9, bFGF, EGF, and HGF.

[00014] In another aspect, the receptor ECD is IL-17 receptor ECD, TNFα receptor ECD, high affinity IgE receptor ECD, low affinity IgE receptor ECD, α chain high affinity IL-4 receptor ECD, IL-5 receptor ECD, IL-13 receptor ECD, IL-2 receptor ECD, cytotoxic T-lymphocyte-

associated antigen 4 (CTLA-4), receptor activator of nuclear factor κβ (RANK), and tissue factor.

[00015] In one embodimentaspeet, the cytokines include, but are not limited to, IL-1a, IL-1b, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-14, IL-15, IL-17, INF-α, INF-β, INF-γ, MIP-1a, MIP-1b, TGF-β, RANTES, MCP-1, MCP-2, MCP-3, MCP-4, and PF-4.

[00016] In another embodimentaspeet, the surface glycoproteins include, but are not limited to, integrins, immunoglobulin superfamily members, selectins, and cadherins. In a related embodimentaspeet, the glycoprotein is the extracellular Ig-like domain of human myelin oligodendrocyte glycoprotein (MOG).

[00017] In one embodiment, a plant cell or algae cell or progeny thereof is disclosed which contains a construct, where the construct includes, in operable linkage, nucleic acid signaling elements for homologous recombination and expression of the Fc-fusion protein in a plant or algae plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine or an enzyme, where the first and second polynucleotide sequences are expressed as a fusion protein.

[00018] In another embodiment, a plant cell or algae cell plastid is disclosed which contains a construct which includes, in operable linkage, nucleic acid signaling elements for homologous recombination and expression of the Fc-fusion protein in a plant or algae plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine or an enzyme, where the first and second polynucleotide sequences are expressed as a fusion protein.

[00019] In one embodimentaspeet, the plant cell, algae cell or progeny contains the first and second polynucleotides that are stably integrated into the plastid of the cell. In another embodimentaspeet, a vector includes such a construct.

[00020] In one embodiment, a method of producing an Fc-fusion protein is disclosed, including contacting a plastid with one or more expression constructs, where the expression constructs include, in operably linkage, a nucleic acid signal element for homologous recombination and expression of the fusion protein in the plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, cytokine or an enzyme, wherein the first and second polynucleotide sequences are expressed as a fusion protein,

allowing the construct to integrate into the genome of the plastid, and expressing the fusion protein encoded by the construct.

[00021] In another embodiment, a method of producing an aglycosylated Fc-fusion protein is disclosed, including contacting a plastid with one or more expression constructs, where the expression constructs include, in operably linkage, a nucleic acid signal element for homologous recombination and expression of the fusion protein in the plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, cytokine or an enzyme, wherein the first and second polynucleotide sequences are expressed as a fusion protein, allowing the construct to integrate into the genome of the plastid, and expressing the fusion protein encoded by the construct.

[00022] In one embodimentaspect, the plastid is in a plant cell or algae cell or progeny thereof.

[00023] In a related embodimentaspect, the method further includes isolating the expressed protein from the plastid.

[00024] In another embodimentaspect, the isolation method comprises capture of the Fc domain on Protein A or Protein G.

[00025] In one embodimentaspect, an Fc-fusion protein is functional in vivo, and may include, but is not limited to, an ecFcεRIα-Fc fusion protein.

[00026] In one embodimentaspect, an Fc-fusion protein is functional in vivo, and may include, but is not limited to, an IGF1- Fc fusion protein.

[00027] In another embodiment, a plastid is disclosed which includes a nucleic acid expression construct, where the construct includes, in operable linkage, nucleic acid signaling elements for homologous recombination and expression of the fusion protein in a plant or algae plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine or an enzyme, where the first and second polynucleotide sequences are expressed as a fusion protein. In a related embodimentaspect, the plastid is a chloroplast.

[00028] In one embodiment, microalgae, macroalgae or progeny thereof, contain a plastid, where the plastid includes a nucleic acid expression construct, where the construct includes, in operable linkage, nucleic acid signaling elements for homologous recombination and expression of the fusion protein in a plant or algae plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine or an enzyme, where the first and second polynucleotide sequences are expressed as a fusion protein.

[00029] In one embodimentaspeet, the algae is *Chlamydomonas reinhardtii.*

[00030] In another embodimentaspeet, the microalgae is Dunaliella, Ankistrodesmus, Botryococcus, Chlorella, Haematococcus, Scenedesmus, Volvox or Porphyridium.

[00031] In another embodiment, an isolated fusion protein is disclosed which is generated by the steps including contacting a plastid with one or more expression constructs, where the expression constructs include, in operably linkage, a nucleic acid signal element for homologous recombination and expression of the fusion protein in the plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine or an enzyme, wherein the first and second polynucleotide sequences are expressed as a fusion protein, allowing the construct to integrate into the genome of the plastid, and expressing the fusion protein encoded by the construct.

[00032] In one embodiment, a method of treating a eukaryotic cell is disclosed including contacting the eukaryotic cell with a fusion protein isolated from a plant cell or algae cell or a plant cell or algae cell plastid which contains a construct which includes, in operable linkage, nucleic acid signaling elements for homologous recombination and expression of the fusion protein in a plant or algae plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine or an enzyme, where the first and second polynucleotide sequences are expressed as a fusion protein.

[00033] In another embodiment, a method of modulating receptor mediated signaling and/or immunologic response is disclosed including treating animal or human cells with a therapeutically effective dose of the fusion protein which is generated by the steps including contacting a plastid with one or more expression constructs, where the expression constructs include, in operably linkage, a nucleic acid signal element for homologous recombination and expression of the fusion protein in the plastid and a first polynucleotide sequence encoding an Fc domain and a second polynucleotide sequence encoding an extracellular domain (ECD) of a receptor or surface glycoprotein, a growth factor, a cytokine or an enzyme, wherein the first and second polynucleotide sequences are ex pressed as a fusion protein, allowing the construct to integrate into the genome of the plastid, and expressing the fusion protein encoded by the construct.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] Figure 1 shows a stained gel analysis of chloroplast expressed Fc-fusion protein after capture by Protein A affinity chromatography.