METHODS AND REAGENTS FOR IDENTIFYING INSULIN RESPONSE MODULATORS AND THERAPEUTIC USES THEREOF

Methods of identifying insulin response modulators are provided. In particular, methods that feature identifying modulators of insulin-responsive aminopeptidase (IRAP) and insulin-degrading enzyme (IDE), or activities associated therewith, are provided. Therapeutic methods utilizing compounds identified according to the methods of the invention are also provided.
METHODS AND REAGENTS FOR IDENTIFYING INSULIN RESPONSE MODULATORS AND THERAPEUTIC USES THEREFOR

Related Applications

This application claims the benefit of prior-filed provisional patent application Serial No. 60/290,494, filed May 11, 2001, entitled “METHODS AND REAGENTS FOR IDENTIFYING INSULIN RESPONSE MODULATORS AND THERAPEUTIC USES THEREFOR.” The entire content of the above-referenced application is incorporated herein by this reference.

Background of the Invention

The regulation of blood glucose levels by insulin is achieved mainly by increased glucose transport exclusively into adipose and skeletal muscle tissue; De Fronzo et al. (1981) Diabetes 30:1000-1007 and James et al. (1985) Am. J. Physiol. 248:E567-E574.


It is believed that GLUT4 recycles in cells as a constituent of tissue-specific secretory-like microsomal structures, known as "GLUT4-containing vesicles". In addition to GLUT 4, these vesicles have also been determined to include phosphatidylinositol 4-kinase, Del Vecchio and Pilch (1991) J. Biol. Chem. 266:13278-13283; vesicle-associated membrane proteins ("VAMPS"), Cain et al. (1992) J. Biol. Chem. 267:11681-11684; secretory component-associated membrane proteins ("SCAMPS"), Thoidis et al. (1993) J. Biol. Chem. 268:11691-11696; and Laurie et al.

Given the important role of IRAP in regulating insulin-responsive translocation of the glucose transporter, GLUT4, there exists a need for understanding in greater detail the mechanism by which IRAP regulates GLUT4 translocation, for identifying proteins that interact with IRAP and, in particular, for identifying modulators of such interactions for use in regulating a variety insulin-sensitive cellular responses.

Summary of the Invention

The present invention is based, at least in part, on the identification of a heretofore unrecognized biological activity of a protein known in the art as insulin degrading enzyme ("IDE"). In particular, the present invention is based on the discovery that IDE interacts with insulin-responsive aminopeptidase ("IRAP"), an important component of GLUT4-containing vesicles. IDE was identified as an IRAP interacting partner (or IRAP binding protein) by affinity purification of IDE on a matrix comprising amino acid residues 1-109 of IRAP. Binding of IDE to IRAP is competitively inhibited by a bioactive fragment comprising residues 1-109 of IRAP.
The present inventors are the first to identify a novel interaction between IDE and IRAP. In particular, the present inventors have demonstrated that IDE specifically binds IRAP, a critical and insulin-regulatable component of GLUT4 vesicles. Importantly, this IDE:IRAP interaction was identified in differentiated adipocytes, known to be a critical insulin responsive cell type. Based at least in part on the discovery that IRAP and IDE, two important insulin response regulators, interact, the present invention features methods of identifying insulin response modulators, in particular, methods that involve IDE and IRAP polypeptide reagents and/or cells that overexpress IDE and/or IRAP. In particular, the methods (e.g., cell-free and/or cell-based methods) feature determining the ability of a test compound to effect the interaction of IDE, or a bioactive fragment thereof, with IRAP or a bioactive fragment thereof, or to effect an activity associated with such an interaction, the ability to effect such an interaction being determinative of the compound’s ability to modulate insulin responsiveness. Reagents, for example, polypeptide reagents and cellular reagents, fusion proteins, antibodies and the like are also featured. Other aspect of the present invention feature modulators identified by the methods described herein as well as therapeutic methods for modulating insulin responsiveness using such modulators.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

**Brief Description of the Drawings**

*Figure 1A-B* schematically depicts the amino acid sequence of human and rat IRAP. The N-terminal 109 amino acid fragment used to isolate IDE as an IRAP binding protein is indicated by bold underlining and the corresponding IDE-binding fragment in human IRAP is indicated by underlining. The amino acid sequences of human and rat IRAP are set forth as SEQ ID NO:1 and SEQ ID NO:2, respectively.

*Figure 2* depicts the amino acid sequence of human IDE. The amino acid sequence of human IDE is set forth as SEQ ID NO:3.

**Detailed Description of the Invention**

The present invention is based, at least in part, on the discovery of a previously unrecognized activity of insulin degrading enzyme (IDE). In particular, the present invention is based on the discovery of an interaction between IDE and IRAP (“insulin-responsive aminopeptidase”), an important component of insulin-responsive GLUT4-containing vesicles. IDE is a 110-kDa neutral metalloendopeptidase with sensitivity towards thiol reagents (for a recent review see Duckworth et al. (1998) *Endocrine Rev.*

Numerous experiments suggest that IDE is the principal enzyme controlling insulin degradation in various cells. First, insulin fragments generated \textit{in vitro} by the purified endopeptidase are identical to those isolated from intact liver and hepatocytes (Hamel et al. (1988) J. Biol. Chem. 263:6703-6708; and Duckworth et al. (1988) J. Biol. Chem. 263:1826-1833, respectively). Furthermore, inhibitors of IDE prevent insulin degradation in different cells (Kayalar and Wong (1989) J. Biol. Chem. 264:8928-8934; and Gehm and Rosner (1991) Endocrinol. 128:1603-1610), and the hormone can be cross-linked \textit{in vivo} to the peptidase (Hari et al. (1987) Endocrinol. 120:829-831). Moreover, monoclonal antibodies specific to IDE block insulin degradation in HepG2 cells (Shii et al. (1986) Proc. Natl. Acad. Sci. USA 83:4147-4151). Finally, overexpression of the endopeptidase in COS cells led to a several-fold increase in the intracellular rate of insulin degradation (Kuo et al. (1991) Mol. Endocrinol. 5:1467-1476).

For many years insulin degradation has been viewed as a passive process and not considered to be involved in regulation of metabolism. It is now clear that insulin removal and degradation are regulated processes and that abnormalities in insulin clearance are integral to diseases such as obesity and type 2 diabetes, with several studies suggesting that the increase in circulating insulin in obesity and type 2 diabetes is due, at least in part, to reduced hepatic clearance (Hossein et al. (2000) Hum. Mol. Genet. 9:2149-2158). Syndrome X, the genetic abnormality resulting in insulin resistance, hyperinsulinemia, dyslipidemia, hypertension, and other abnormalities, may include primary alterations in insulin clearance and degradation. Hypertension has also been independently associated with altered insulin clearance (Mondon et al. (1989) Am J. Physiol. 257:E491-E498).

Recently, there have been suggestions that insulin interaction with and degradation by IDE may play a more direct role in generating insulin effects. IDE complexes with, and regulates, certain cytosolic organelles, specifically proteasomes,
androgen and glucocorticoid receptors, and possibly peroxisomes. IDE increases proteasome and steroid hormone receptor activity, and this activation is reversed by insulin. This raises the possibility of a direct intracellular modulation of IDE that could modulate protein and fat metabolism. The recent findings would place intracellular IDE action into the insulin signal transduction pathway for mediating the intermediate effects of insulin on fat and protein turnover.

Accordingly, the present invention features methods of identifying insulin response modulators. In one embodiment, the invention features contacting an assay composition with a test compound, the assay composition including IDE and IRAP (or bioactive fragments of the respective proteins) with a test compound and assaying for modulation of IDE:IRAP binding or alternatively, for an activity associated with IDE:IRAP binding. Preferred activities include molecular activities of IDE and/or IRAP (e.g., an aminopeptidase activity or insulin degradative activity). In another embodiment, the invention features contacting a cell that expresses or overexpresses IDE and/or IRAP (or bioactive fragments of the respective proteins) with a test compound and assaying for modulation of IDE:IRAP binding or alternatively, for an activity associated with IDE:IRAP binding. Preferred activities include cellular activities of IDE and/or IRAP (e.g., IRAP translocation, extracellular aminopeptidase activity, regulation of intracellular GLUT4 trafficking, regulation of intracellular retention of GLUT4, modulation of cellular protein degradation, modulation of fat metabolism, modulation of insulin clearance, modulation of proteosome activation, modulation of ubiquitination and/or peroxisome targeting activity).

Compounds identified by the methods of the present invention are also featured (e.g., positive and negative modulators) as are pharmaceutical compositions that include such modulators.

The invention further features a method for identifying an IRAP:IDE modulator, the method involving contacting a composition comprising IRAP and IDE with a test compound and determining the ability of the test compound to enhance binding of the IRAP to the IDE, such that the modulator is identified. In yet another embodiment, the invention features a method for identifying an IRAP:IDE modulator, the method involving contacting a composition comprising IRAP or bioactive fragment thereof and IDE or bioactive fragment thereof with a test compound and determining the ability of the test compound to inhibit binding of the IRAP or bioactive fragment thereof to the IDE or bioactive fragment thereof, such that the modulator is identified.

Therapeutic methods and/or methods of effecting desired responses in an individual are also featured. In one embodiment, the invention features a method of modulating insulin responsiveness in a subject that involves administering to the subject
an insulin response modulator identified according to one of the screening assays
described herein, such that insulin responsiveness is modulated. In another embodiment,
the invention features a method of regulating blood glucose levels in a subject that
involves administering to the subject an insulin response modulator identified according
to one of the screening assays described herein, such that insulin responsiveness is
modulated. In yet another embodiment, the invention features a method of restoring
insulin sensitivity in a subject comprising administering to the subject an insulin
response modulator identified according to one of the screening assays described herein,
such that insulin sensitivity is restored.

Also featured are anti-IDE antibodies as well as pharmaceutical compositions of
such antibodies.

Various aspects of the invention are described in further detail in the following
subsections:

I. Screening Assays:

IA. Cell Free Assays

In one embodiment, an assay of the present invention is a cell-free assay in
which a composition comprising assay reagents (e.g., an IDE polypeptide, IRAP
polypeptide or biologically active portions thereof), is contacted with a test compound
and the ability of the test compound to modulate binding of the IDE polypeptide to the
IRAP polypeptide (or bioactive fragments thereof) is determined. Binding of IDE or
IRAP (or bioactive fragments thereof) can be accomplished, for example, by coupling
the polypeptide or fragment with a radioisotope or enzymatic label such that binding
of polypeptide reagents can be determined by detecting the labeled compound or
polypeptide in a complex. For example, test compounds or polypeptides can be labeled
with $^{125}$I, $^{35}$S, $^{14}$C, or $^{3}$H, either directly or indirectly, and the radioisotope detected
by direct counting of radioemmission or by scintillation counting. Alternatively,
polypeptides can be enzymatically labeled with, for example, horseradish peroxidase,
alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of
conversion of an appropriate substrate to product.

Determination of binding of reagents can also be accomplished using a
technology such as real-time Biomolecular Interaction Analysis (BIA). Sjolander, S.
Opin. Struct. Biol. 5:699-705. As used herein, "BIA" is a technology for studying
biospecific interactions in real time, without labeling any of the interactants (e.g.,
Changes in the optical phenomenon of surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules. In a preferred embodiment, the assay includes contacting IRAP polypeptide or biologically active portion thereof with an IRAP target molecule, e.g., IDE or a bioactive fragment thereof to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the IRAP polypeptide, wherein determining the ability of the test compound to interact with the IRAP polypeptide comprises determining the ability of the test compound to preferentially bind to IRAP or the bioactive portion thereof as compared to the IRAP target molecule (e.g., IDE). In another embodiment, the assay includes contacting the IDE polypeptide or biologically active portion thereof with an IDE target molecule, e.g., IRAP or a bioactive fragment thereof to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to modulate binding between the IDE polypeptide and the IRAP polypeptide.

In another embodiment, the assay is a cell-free assay in which a composition comprising an IRAP polypeptide and an IDE polypeptide (or bioactive portions thereof) is contacted with a test compound and the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the IRAP polypeptide or IDE polypeptide (or biologically active portions thereof) is determined.

Determining the ability of the test compound to modulate the activity of an IRAP or an IDE polypeptide can be accomplished, for example, by determining the ability of the IDE polypeptide to modulate the activity of a downstream IDE binding partner or target molecule (e.g., IRAP) by one of the methods described herein for cell-based assays. For example, the catalytic/ enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described (e.g., the aminopeptidase activity of IRAP).

In yet another embodiment, the cell-free assay involves contacting an IDE polypeptide or biologically active portion thereof with an IDE target molecule which binds the IDE polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound (e.g., IRAP) to preferentially modulate the activity of an IDE binding partner or target molecule, as compared to the IDE.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either IDE or IRAP to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. The ability of a test compound to modulate IDE polypeptide activity, IRAP polypeptide activity, or interaction of an IDE polypeptide
with an IRAP polypeptide in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/IDE fusion proteins or glutathione-S-transferase/IRAP fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed IRAP polypeptide or IDE polypeptide, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of IDE binding or activity or IRAP binding or activity determined using standard techniques.

Additional exemplary IRAP and/or IDE fusion proteins include, but are not limited to, chitin binding domain (CBD) fusion proteins, hemagglutinin epitope tagged (HA)-fusion proteins, His fusion proteins (e.g., His tagged proteins), FLAG tagged fusion proteins, AU1 tagged proteins, and the like.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either an IDE polypeptide or an IDE target IRAP polypeptide can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated IDE polypeptide or IRAP polypeptide can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with IDE polypeptide or IRAP polypeptide but which do not interfere with binding of the IDE polypeptide to IRAP polypeptide can be derivatized to the wells of the plate, and unbound IRAP or IDE polypeptide trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the IDE polypeptide or IRAP polypeptide, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the IDE polypeptide or IRAP polypeptide.
In yet another aspect of the invention, the IDE or IRAP polypeptides can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Biotechniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with IDE or IRAP ("binding proteins" or "target molecules") and are involved in IDE or IRAP activity. Such target molecules are also likely to be involved in the regulation of cellular activities modulated by the IDE polypeptides or IRAP polypeptides.

At least one exemplary two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a first polypeptide (the "bait" polypeptide, e.g., IRAP or IDE) is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact, in vivo, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the bait polypeptide.

Another exemplary two-hybrid system, referred to in the art as the CytoTrap™ system, is based in the modular nature of molecules of the Ras signal transduction cascade. Briefly, the assay features a fusion protein comprising the "bait" protein and Son-of-Severless (SOS) and the cDNAs for unidentified proteins (the "prey") in a vector that encodes myristylated target proteins. Expression of an appropriate bait-prey combination results in translocation of SOS to the cell membrane where it activates Ras.

Cytoplasmic reconstitution of the Ras signaling pathway allows identification of proteins that interact with the bait protein of interest, for example, an IRAP or IDE protein. Additional mammalian two hybrid systems are also known in the art and can be utilized to identify IRAP or IDE interacting proteins. Moreover, at least one of the above-described assays can be utilized to identify IRAP-interacting domains or regions of the IDE protein or alternative, to identify IDE-interacting domain or regions of the IRAP protein.
IB. Cell Based Assays

In one embodiment, an assay is a cell-based assay in which a cell capable of expressing a IDE polypeptide, or biologically active portion thereof, is contacted with a test compound and the ability of the test compound to modulate the expression of the IDE polypeptide, or biologically active portion thereof, determined. In another embodiment, an assay is a cell-based assay in which a cell which expresses an IDE polypeptide or IRAP polypeptide (or biologically active portions thereof) is contacted with a test compound and the ability of the test compound to modulate the activity of the IDE polypeptide or IRAP polypeptide (or biologically active portions thereof) determined. The cell, for example, can be of mammalian origin or a yeast cell. The polypeptides, for example, can be expressed heterologously or native to the cell. Determining the ability of the test compound to modulate the activity of an IDE or IRAP polypeptide (or biologically active portions thereof) can be accomplished by assaying for any of the activities of an IDE or IRAP polypeptide described herein. Determining the ability of the test compound to modulate the activity of an IDE polypeptide or IRAP polypeptide (or biologically active portions thereof) can also be accomplished by assaying for the activity of an IDE target molecule. In one embodiment, determining the ability of the test compound to modulate the activity of an IDE polypeptide, or biologically active portion thereof, is accomplished by assaying for the ability to bind IRAP or a bioactive portion thereof. In another embodiment, determining the ability of the test compound to modulate the activity of an IDE polypeptide, or biologically active portion thereof, is accomplished by assaying for the activity of IRAP (e.g., by assaying for aminopeptidase activity). In a preferred embodiment, the cell overexpresses the IDE polypeptide, or biologically active portion thereof, and optionally, overexpresses IRAP, or biologically active portion thereof. In another preferred embodiment, the cell expresses IRAP, or biologically active portion thereof. In yet another preferred example, the cell is contacted with a compound which stimulates an IDE-associated activity or IRAP-associated activity (e.g., insulin) and the ability of a test compound to modulate the IDE-associated activity is determined.

As used herein, the term “bioactive” fragment includes any portion (e.g., a segment of contiguous amino acids) of an IDE or IRAP protein sufficient to exhibit or exert at least one IDE- or IRAP-associated activity including, for example, the ability to bind to IRAP or IDE, respectively. In one embodiment, the bioactive peptide is derived from the amino acid sequence of IRAP. In another embodiment, the bioactive peptide corresponds to the N-terminal cytoplasmic domain, also referred to herein as the cytoplasmic interacting domain (i.e., amino acids 1-109 of IRAP) or a smaller bioactive fragment thereof (e.g., about amino acids 50-85, preferably about amino acids 55-82 of
IRAP). In yet another embodiment, the bioactive peptide corresponds to a trafficking motif of IRAP, *i.e.*, a motif which signals intracellular trafficking from a first to a second cellular location (*e.g.*, membrane location). In another embodiment, the bioactive peptide is derived from IDE and can include, for example, amino acid residues sufficient to effect enzymatic activity.

According to the cell-based assays of the present invention, determining the ability of the test compound to modulate the activity of the IRAP polypeptide or biologically active portion thereof, can be determined by assaying for any of the native activities of an IRAP polypeptide described herein, for example, assaying for GLUT4 translocation, IRAP translocation, IRAP and/or GLUT4 sorting, retention of IRAP and/or GLUT4, intracellular trafficking of IRAP and/or GLUT4-containing vesicles, subcellular fractionation or glucose uptake. IRAP trafficking, for example, can be monitored by labeling cells with biotin (*i.e.*, cell surface biotinylation) followed by detection of labeled IRAP in intracellular fractions, indicating trafficking. Moreover, the activity of IRAP, can be determined by assaying for an indirect activity which is coincident to the activity of IRAP. For example, the effect of the test compound on the ability of an IRAP-expressing cell to uptake glucose in an insulin-dependent manner can be assayed in the presence of the test compound. Furthermore, determining the ability of the test compound to modulate the activity of the IRAP and/or IDE polypeptide or biologically active portion thereof, can be determined by assaying for an activity which is not native to the IDE or IRAP polypeptide, but for which the cell has been recombinantly engineered. For example, the cell can be engineered to express a target molecule which is a recombinant protein comprising a bioactive portion of IRAP operatively linked to a non-IRAP polypeptide. In an exemplary embodiment, the cytoplasmic domain of IRAP is operatively linked to the transmembrane and extracellular domains of, for example, the transferrin receptor, and the effect of the test compound on the ability of the chimeric protein to traffic intracellularly, determined. (Johson *et al.* (1998) *J. Biol. Chem.* 273:17968-17977 provide an example of the making of such a chimera.) It is also intended that in preferred embodiments, the cell-based assays of the present invention comprise a final step of identifying the compound as a modulator of IDE activity or IRAP activity.
II. Assay reagents

IIA. Test Compounds

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including:

5 biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) Anticancer Drug Des. 12:145).


In a preferred embodiment, the library is a natural product library.

IIB. Antibodies, Bioactive Fragments and Fusion Proteins

Another aspect of the invention features biologically active portions (i.e., bioactive fragments) of IDE or IRAP, including polypeptide fragments suitable for use as immunogens to raise anti-IDE antibodies or IRAP antibodies or to make IDE or IRAP fusion proteins. In one embodiment, IDE or IRAP immunogens or bioactive fragments can be generated from IDE or IRAP isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, IDE or IRAP immunogens or bioactive fragments are produced by recombinant DNA techniques. Alternative to recombinant expression, an IDE or IRAP immunogen or bioactive fragment can be synthesized chemically using standard peptide synthesis techniques.
An immunogen, bioactive fragment or fusion protein, as used herein is preferably "isolated" or "purified". The terms "isolated" and "purified" are used interchangeably herein. "Isolated" or "purified" means that the immunogen, bioactive fragment or fusion protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the polypeptide is derived, substantially free of other protein fragments, for example, non-desired fragments in a digestion mixture, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations in which the polypeptide is separated from other components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of polypeptide having less than about 30% (by dry weight) of non-IDE or non-IRAP polypeptide (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-IDE or non-IRAP polypeptide, still more preferably less than about 10% of non-IDE or non-IRAP polypeptide, and most preferably less than about 5% non-IDE or non-IRAP polypeptide.

When the immunogen, bioactive portion or fusion protein is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the polypeptide preparation. When the immunogen, bioactive fragment or fusion protein is produced by, for example, chemical or enzymatic processing from isolated or purified IDE or IRAP protein, the preparation is preferably free of enzyme reaction components or chemical reaction components and is free of non-desired IDE or IRAP fragments, i.e., the desired polypeptide represents at least 75% (by dry weight) of the preparation, preferably at least 80%, more preferably at least 85%, and even more preferably at least 90%, 95%, 99% or more or the preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of polypeptide in which the polypeptide is separated from chemical precursors or other chemicals which are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations having less than about 30% (by dry weight) of chemical precursors or reagents, more preferably less than about 20% chemical precursors or reagents, still more preferably less than about 10% chemical precursors or reagents, and most preferably less than about 5% chemical precursors or reagents.

Bioactive fragments of IDE or IRAP include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the IDE protein or the IRAP protein, respectively, which include less amino acids than the
full length protein, and exhibit at least one biological activity of the full-length protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the full-length protein. A biologically active portion of an IDE or IRAP polypeptide can be a polypeptide which is, for example, 10, 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more amino acids in length. For example, in one embodiment, a bioactive portion of an IRAP protein comprises at least a N-terminal or cytoplasmic interacting domain, as defined herein, or a smaller bioactive portion of the N-terminal interacting domain. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native IDE or IRAP protein. Mutants of IRAP and/or IDE can also be utilized as assay reagents, for example, mutants having reduced, enhanced or otherwise altered biological properties identified according to one of the activity assays described herein.

To determine the percent identity of two amino acid sequences (or of two nucleotide or amino acid sequences), the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the first sequence or second sequence for optimal alignment). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same residue as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100), optionally penalizing the score for the number of gaps introduced and/or length of gaps introduced.

The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In one embodiment, the alignment generated over a certain portion of the sequence aligned having sufficient identity but not over portions having low degree of identity (i.e., a local alignment). A preferred, non-limiting example of a local alignment algorithm utilized for the comparison of sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-68, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-77. Such an algorithm is incorporated into the BLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST alignments can be generated and percent identity calculated using BLAST protein searches (e.g., the XBLAST program) using IDE, IRAP or a portion thereof as a query, score = 50, wordlength = 3.
In another embodiment, the alignment is optimized by introducing appropriate gaps and percent identity is determined over the length of the aligned sequences (i.e., a gapped alignment). To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Research 25(17):3389-3402. In another embodiment, the alignment is optimized by introducing appropriate gaps and percent identity is determined over the entire length of the sequences aligned (i.e., a global alignment). A preferred, non-limiting example of a mathematical algorithm utilized for the global comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The invention also provides IDE and IRAP chimeric or fusion proteins. As used herein, an IDE or IRAP "chimeric protein" or "fusion protein" comprises an IDE or IRAP polypeptide operatively linked to a non-IDE polypeptide or non-IRAP polypeptide, respectively. A "IDE polypeptide" or "IRAP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to the IDE or IRAP protein, respectively, whereas a "non-IDE polypeptide" or "non-IRAP polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially identical to the IDE protein or IRAP protein. Within a fusion protein the IDE or IRAP polypeptide can correspond to all or a portion of an IDE or IRAP protein. In a preferred embodiment, an IDE or IRAP fusion protein comprises at least one biologically active portion of an IDE or IRAP protein, respectively. In another preferred embodiment, an IDE or IRAP fusion protein comprises at least two biologically active portions of an IDE or IRAP protein, respectively. In yet another preferred embodiment, a fusion protein can comprise IDE, or a bioactive portion thereof, operatively linked to IRAP, or a bioactive portion thereof, such that IDE and IRAP, or their respective bioactive portions are brought into close proximity. Within the fusion protein, the term "operatively linked" is intended to indicate that the IDE or IRAP polypeptide and the non-IDE polypeptide or non-IRAP polypeptide are fused in-frame to each other. The non-IDE polypeptide or non-IRAP polypeptide can be fused to the N-terminus or C-terminus of the IDE polypeptide or IRAP polypeptide, respectively.

For example, in one embodiment, the fusion protein is a GST-fusion protein in which the IDE or IRAP sequences are fused to the C-terminus of the GST sequences. In another embodiment, the fusion protein is a chitin binding domain (CBD) fusion protein in which the IDE or IRAP sequences are fused to the N-terminus of chitin binding
domain (CBD) sequences. Such fusion proteins can facilitate the purification of recombinant IDE or IRAP.

Preferably, a chimeric or fusion protein of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety. A IDE- or IRAP-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the IDE or IRAP polypeptide.

A IDE polypeptide or IRAP polypeptide, or a portion or fragment of IDE or IRAP, can also be used as an immunogen to generate antibodies that bind IDE or IRAP or that block IDE/IRAP binding using standard techniques for polyclonal and monoclonal antibody preparation. A full-length polypeptide can be used or, alternatively, the invention provides antigenic peptide fragments for use as immunogens. Preferably, an antigenic fragment comprises at least 8 amino acid residues of the amino acid sequence of IDE (as set forth in GenBank Accession no. NP_004960) or IRAP (as set forth in GenBank Accession no. NP_005566.1) and encompasses an epitope of IDE or IRAP such that an antibody raised against the peptide forms a specific immune complex with IDE or IRAP, respectively. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of IDE or IRAP that are located on the surface of the protein, e.g., hydrophilic regions. Antigenic determinants at the termini of IDE are preferred for the development of antibodies that do not interfere with the IDE:IRAP interaction. Alternatively, interfering antibodies can be generated towards antigenic determinants located within the IRAP interacting domain of IDE. The latter are preferred for therapeutic purposes.
A IDE or IRAP immunogen typically is used to prepare antibodies by immunizing a suitable subject, \( e.g., \) rabbit, goat, mouse or other mammal\) with the immunogen. An appropriate immunogenic preparation can contain, for example, recombinantly expressed IDE or IRAP polypeptide or a chemically synthesized IDE or IRAP polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent. Immunization of a suitable subject with an immunogenic IDE or IRAP preparation induces a polyclonal anti-IDE or anti-IRAP antibody response, respectively.

Accordingly, another aspect of the invention pertains to anti-IDE or anti-IRAP antibodies. The term "antibody\) as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, \( i.e., \) molecules that contain an antigen binding site which specifically binds (immunoreacts with) an antigen, such as IDE or IRAP. Examples of immunologically active portions of immunoglobulin molecules include \( F(ab) \) and \( F(ab')_2 \) fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies that bind IDE. The term "monoclonal antibody\) or "monoclonal antibody composition\), as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of IDE or IRAP. A monoclonal antibody composition thus typically displays a single binding affinity for a particular IDE or IRAP polypeptide with which it immunoreacts.

Polyclonal anti-IDE or anti-IRAP antibodies can be prepared as described above by immunizing a suitable subject with an IDE or IRAP immunogen, respectively. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized IDE or IRAP. If desired, the antibody molecules can be isolated from the mammal \( e.g., \) from the blood\) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction. At an appropriate time after immunization, \( e.g., \) when the anti-IDE or anti-IRAP antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) *Nature* 256:495-497\) \( \)see also, Brown *et al.\) (1981) *J. Immunol.* 127:539-46; Brown *et al.\) (1980) *J. Biol. Chem.* .255:4980-83; Yeh *et al.\) (1976) *PNAS* 76:2927-31; and Yeh *et al.\) (1982) *Int. J. Cancer* 29:269-75\)\), the more recent human B cell hybridoma technique (Kozbor *et al.\) (1983) *Immunol Today* 4:72\)\), the EBV-hybridoma technique (Cole *et al.\) (1985), *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96\) or trioma techniques.
The technology for producing monoclonal antibody hybridomas is well known (see generally R. H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); E. A. Lerner (1981) *Yale J. Biol. Med.*, 54:387-402; M. L. Gefter et al. (1977) *Somatic Cell Genet.* 3:231-36). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an IDE or IRAP immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds IDE or IRAP, respectively.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating an anti-IDE monoclonal antibody (see, e.g., G. Galfre et al. (1977) *Nature* 266:55052; Gefter et al. *Somatic Cell Genet.*, cited supra; Lerner, *Yale J. Biol. Med.*, cited supra; Kenneth, *Monoclonal Antibodies*, cited supra). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods which also would be useful. Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. For example, murine hybridomas can be made by fusing lymphocytes from a mouse immunized with an immunogenic preparation of the present invention with an immortalized mouse cell line. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). Any of a number of myeloma cell lines can be used as a fusion partner according to standard techniques, e.g., the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from ATCC. Typically, HAT-sensitive mouse myeloma cells are fused to mouse splenocytes using polyethylene glycol ("PEG"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind IDE or IRAP, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal anti-IDE or anti-IRAP antibody can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with IDE or IRAP to thereby isolate immunoglobulin library members that bind IDE or IRAP, respectively. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™ Phage Display Kit*, Catalog No.

An anti-IDE or anti-IRAP antibody (e.g., monoclonal antibody) can be used to isolate IDE or IRAP, bioactive portions thereof, or fusion proteins by standard techniques, such as affinity chromatography or immunoprecipitation. Anti-IRAP antibodies (or antibodies made according to any of the above-described techniques to any other GLUT4 vesicle component, e.g., GLUT4, or any other preferred donor fraction or acceptor fraction component, can be used to detect protein levels in donor or acceptor fractions as part of certain assay methodologies described herein. Detection can be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin or avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include $^{125}$I, $^{131}$I, $^{35}$S or $^{3}$H.
IIC. Recombinant Expression Vectors and Assay Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, for producing the fusion proteins reagents of the instant invention. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. A preferred vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector.

The recombinant expression vectors of the invention comprise a nucleic acid that encodes, for example IDE or IRAP or a bioactive fragment or IDE or IRAP, in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). The expression vectors can be introduced into host cells to thereby produce proteins, including fusion proteins or peptides. Alternatively, retroviral expression vectors and/or adenoviral expression vectors can be utilized to express the proteins of the present invention.

The recombinant expression vectors of the invention can be designed for expression of IDE or IRAP polypeptides in prokaryotic or eukaryotic cells. For example, IDE or IRAP polypeptides can be expressed in bacterial cells such as E. coli, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990).

Expression of proteins in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion
moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Purified fusion proteins are particularly useful in the cell-free assay methodologies of the present invention.

In yet another embodiment, an IDE or IRAP-encoding nucleic acid is expressed in mammalian cells, for example, for use in the cell-based assays described herein. When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid).

Another aspect of the invention pertains to assay cells into which a recombinant expression vector has been introduced. An assay cell can be prokaryotic or eukaryotic, but preferably is eukaryotic. A preferred assay cell is an adipocyte, for example, a human adipocyte. Adipocytes can be derived from human adipose tissue as undifferentiated cells and expanded ex vivo prior to differentiation for use in the assays of the present invention. Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), and other laboratory manuals.

An assay cell of the invention, can be contacted with a test compound and assayed for any IDE and/or IRAP biological activity in order to identify the compound as an insulin responsive modulator. IDE biological activities which can be assayed as part of the methodologies of the present invention include, but are not limited to, (1) modulation of cellular protein degradation (e.g., insulin degradation); (2) modulation of fat metabolism; (3) modulation of insulin clearance; (4) modulation of proteosome activation; (4) modulation of ubiquitination; and (5) peroxisome targeting activity. IRAP biological activities which can be assayed as part of the methodologies of the present invention include, but are not limited to, (1) interaction between IDE or a bioactive fragment thereof with IRAP or a bioactive fragment thereof; (2) modulation of GLUT4 translocation (e.g., exocytosis); (3) modulation of IRAP translocation (e.g., exocytosis); (4) modulation of translocation of another GLUT4 vesicle component; (5) modulation of sorting or retention of IRAP and/or GLUT4; (6) modulation of sorting or retention of another GLUT4 vesicle component; (7) modulation of the entry of IRAP and/or GLUT4 into recycling vesicles; (8) modulation of entry of another GLUT4
vesicle component into recycling vesicles; (9) regulation of intracellular trafficking; and (10) regulation of glucose uptake.

**IID. Translocation Assays**

The methods of the present invention feature identifying compounds having potential insulin response-modulatory activity (e.g., identifying lead compounds for drug discovery) based on the ability of the compound to modulate IRAP:IDE interactions or an activity associated with such interactions. In assaying for IDE activity and/or IRAP activities, it may be desirable in certain assay formats to determine the ability of a test compound to modulate translocation, for example IRAP and/or GLUT4 translocation. Translocation assays can be performed according to art-recognized cell-based techniques, including, detecting the presence or absence of vesicle components at the cell surface. Alternatively, translocation can be assayes utilizing various membrane and/or vesicle preparations. In one embodiment, the assay can comprise contacting a donor vesicle fraction comprising GLUT4 vesicles with a test compound and determining the ability of the test compound to modulate GLUT4 vesicle translocation, such that an insulin response modulator is identified. Determining the ability of the test compound to modulate GLUT4 vesicle translocation can include detecting translocation of a GLUT4 vesicle component to an acceptor vesicle fraction (e.g., detecting changes in GLUT4 levels or IRAP, for example, as compared to an appropriate control). Exemplary donor fractions include GLUT4 vesicle preparations and/or low density microsomal fraction. Exemplary acceptor fractions include plasma membrane fractions. In each of the embodiments described herein, IDE and/or IRAP can be used as full-length proteins. Alternatively, bioactive fragments of IDE and/or IRAP can be used.

**III. Additional IDE Activities**

In addition to or alternative to the IDE activities described above, IDE may further play a role in a "tethering" mechanism which is responsible for retaining GLUT4/IRAP-containing vesicles intracellularly, in the absence of insulin. IDE may itself function as a tethering protein or may exhibit an enzymatic activity on an IDE-tethering protein, thus altering the activity of the tethering protein. Accordingly, the invention also features assays for identifying insulin response modulators comprising contacting IDE or an IDE-responsive tether protein immobilized on a suitable assay membrane or in a suitable assay vessel with a GLUT4/IRAP vesicle preparation and a test compound and determining the ability of the test compound to modulate interaction of IDE or the IDE-responsive tether protein with IRAP in the vesicles, a compound that modulates such an interaction being identified as an insulin response modulator.
III. Pharmaceutical Compositions

This invention further pertains to insulin response modulators identified by the above-described screening assays. Insulin response modulators identified by the above-described screening assays can be tested in an appropriate animal model. For example, an insulin response modulator identified as described herein can be used in an animal model to determine the efficacy, toxicity, or side effects of treatment with such a modulator. Alternatively, a modulator identified as described herein can be used in an animal model to determine the mechanism of action of such an agent. Furthermore, this invention pertains to uses of insulin response modulators identified by the above-described screening assays for therapeutic treatments as described infra.

Accordingly, the insulin response modulators of the present invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, antibody, or modulatory compound and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.
Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should 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 bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmacologically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the
following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or 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 subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are
dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

IV. Methods of Treatment

The present invention also features methods of treatment or therapeutic methods.

In one embodiment, the invention features a method of treating a subject (e.g., a human subject in need thereof) with a modulatory compound identified according to the present invention, such that a desired therapeutic effect is achieved. In another embodiment, the method involves administering to an isolated tissue or cell line from the subject a modulatory compound identified according to the methodology described herein, such that a desired therapeutic effect is achieved. In a preferred embodiment, the invention features a method of treating a subject having an insulin response disorder, for example, reduced insulin sensitivity or insulin resistance or diabetes (e.g., Type II diabetes). The
present invention also provides for therapeutic methods of treating a subject having pre-diabetes or symptoms thereof, hyperglycemia and/or Type I diabetes. Desired therapeutic effects include a modulation of any IDE-, IRAP- or IDE/IRAP-associated activity, as described herein. A preferred therapeutic effect is modulation of glucose uptake and/or transport. Desired therapeutic effects also include, but are not limited to curing or healing the subject, alleviating, relieving, altering or ameliorating a disease or disorder in the subject or at least one symptom of said disease or disorder in the subject, or otherwise improving or affecting the health of the subject. A preferred aspect of the invention pertains to methods of modulating IDE/IRAP interactions for therapeutic purposes.

The effectiveness of treatment of a subject with an insulin response modulator can be accomplished by (i) detecting the level of insulin responsiveness or, alternatively, glucose tolerance in the subject prior to treating with an appropriate modulator; (ii) detecting the level of insulin responsiveness or, alternatively, glucose tolerance in the subject prior post treatment with the modulator; (iii) comparing the levels pre-administration and post administration; and (iv) altering the administration of the modulator to the subject accordingly. For example, increased administration of the modulator may be desirable if the subject continues to demonstrate insensitive insulin responsiveness.

Alternatively, the effectiveness of treatment of a subject with an insulin response modulator can be accomplished by (i) detecting the blood glucose or glucose tolerance in the subject prior to treating with an appropriate modulator; (ii) detecting the blood glucose level or, alternatively, glucose tolerance in the subject prior post treatment with the modulator; (iii) comparing the levels pre-administration and post administration; and (iv) altering the administration of the modulator to the subject accordingly. For example, increased or sustained administration of the modulator may be desirable if the subject fails to adequately clear blood glucose.

V. **Diagnostic Assays**

The present invention is based at least in part on the discovery that IDE and IRAP are binding partners and a role for this interaction in regulating normal insulin responsiveness in a subject is described. Based on the proposed role for IDE:IRAP in normal insulin responsiveness, aberrant IDE:IRAP interaction, expression and/or activity may be associated with abnormal insulin responsiveness. Accordingly, the present invention also features diagnostic assays, for determining aberrant IDE:IRAP interaction, expression or activity, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a
disease or disorder (e.g., abnormal insulin responsiveness), or is at risk of developing such a disorder. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing such a disorder (e.g., a disorder associated with aberrant IDE expression or activity). Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disease or disorder. A preferred agent for detecting an IDE or IRAP protein is an antibody capable of binding to IDE or IRAP, respectively, preferably an antibody with a detectable label. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. The invention also encompasses kits for the detection of aberrant IDE:IRAP interaction, expression or activity in a biological sample. For example, the kit can comprise a labeled compound or agent capable of detecting IDE and/or IRAP in a biological sample; means for determining the amount of IDE and/or IRAP in the sample; and/or means for comparing the amount of IDE in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

**Exemplification**

The present invention relates to the fields of diabetes and insulin resistance. Agents that would act as insulin-mimetics will permit restoration of insulin sensitivity and result in lower blood glucose levels. Insulin resistance results from the inability of normally insulin-responsive tissues to respond to the hormone. In normally functioning muscle and fat cells, insulin binds to its receptor on the surface of the cell and initiates a series of intracellular events including the transport of glucose into the cell. This glucose transport is the key event that regulates the level of glucose in the blood and maintains normoglycemia. The inability to take up glucose into these cells is a condition called insulin resistance and in often found in the diabetic or pre-diabetic state. The activity and regulation of the molecule that transports glucose into the cell has been widely studied in the hopes that understanding of its function may lead to the ability to alter that function and restore responsiveness to insulin. The insulin-responsive glucose transporter, GLUT4, is found in intracellular vesicles that are located in an insulin-sensitive intracellular compartment. In the absence of insulin, these GLUT4-containing vesicles (G4Vs) are retained in the cytosol of the cell. Upon insulin binding to its
receptor at the surface of the cell the G4Vs move from this compartment to the cell surface where GLUT4 is then at the cell surface and can transport glucose into the cell. The trafficking and regulation of GLUT4, as well as other proteins that are associated with G4Vs are beginning to be understood. One protein that has been shown to be present in G4Vs is the insulin-responsive aminopeptidase (IRAP). In addition to being co-localized with GLUT4, IRAP translocates to the plasma membrane in response to insulin like GLUT4 does. IRAP is a transmembrane protein with a large extracellular aminopeptidase domain and a smaller (109 amino acids) amino terminal domain that is intracellular. It has been hypothesized that G4Vs are retained intracellularly via a “tethering” mechanism. Insulin-stimulated events result in the release of this tether and subsequent movement of G4Vs to the cell surface. Moreover, IRAP has been implicated in this tethering of mechanism; the microinjection of the cytoplasmic domain of IRAP induces the translocation of G4Vs in the absence of insulin. To identify candidate proteins involved in this tethering of mechanism, a biochemical screen was set up to identify proteins from insulin responsive cells that interact with IRAP.

Example 1: Identification And Characterization of IDE as an IRAP binding protein

An IRAP fusion protein was generated, expressed in and purified from E. coli and used as an affinity reagent to bind proteins that interact with IRAP. The cDNA coding for the cytoplasmic domain of IRAP, corresponding to amino acids 1-109, was subcloned into the pTYB4 vector from the IMPACT T7 System (New England Biolabs). This system permits folding of the amino terminus of IRAP in a manner similar to the native conformation and is therefore accessible to potential binding proteins in the cytosol.

A single colony of E. coli strain ER2566 containing pIRAP-CBD or the empty pTYB4 vector coding for only the intein and CDB sequences was inoculated into LB/ampicillin and grown overnight at 37°C. Overnight cultures were used to inoculate (1:50) a fresh LB/amp culture which was grown at 37°C until OD600 was 0.5 – 0.6. IPTG (1 mM) was added to induce fusion protein expression. Induction of expression was overnight at room temperature. Following induction, cells were collected by centrifugation, resuspended in Buffer 2 [PBS, pH 7.0; 1 mM EDTA; 2 nM AEBSF; 0.1% Triton X-100; 1 M NaCl] and lysed by sonication. Lysates were centrifuged at 4°C 12,000 x g for 30 min. The supernatant was applied to a chitin bead column that was equilibrated in Buffer 2. The pellet was resuspended in Buffer 2 and sonication and centrifugation were repeated. The resulting supernatant was loaded onto the column and the column was washed with >15 column volumes Buffer 2 to reduce nonspecific
binding of E. coli proteins. Following washing at high salt concentration, the buffer was changed to Buffer 1 [PBS, pH 7.0; 1 mM EDTA; 2 nM AEBSF]. The beads containing IRAP-CBD or CBD alone were removed from the columns and stored at 4°C as a 25% slurry in PBS/0.02% sodium azide.

For cleavage of the N-terminus of IRAP from the intein-CBD portion of the fusion protein to yield a soluble peptide, the column was quickly flushed with 3 volumes Buffer 1 containing 50 mM DTT, added fresh. The flow was then stopped and the column was incubated for two days at 4°C to induce cleavage. Three column volumes of Buffer 1 were added to the beads and 1 ml fractions were collected and concentrated using a Microcon-3 device (Amicon). Protein concentration was determined by absorbance at 280 nm.

Cytosol was prepared from fully differentiated 3T3-L1 adipocytes. Cells were placed on ice, washed twice with cold PBS and once with cold HES [250 mM sucrose; 20 mM HEPES, pH 7.4; 5 mM EDTA; 10 μg/ml aprotinin; 1 μg/ml leupeptin, 200 μM AEBSF]. HES (1 ml) was added to each plate and cells were scraped, pooled and lysed by Potter-Elvehjem homogenization. The lysate was centrifuged at 16,000 xg at 4°C and the fat cake was removed. The supernatant was centrifuged at 220,000 xg for 60 min at 4°C to yield a pellet of internal membranes and the cytosol supernatant. Protein concentration was determined by Bradford assay (BioRad). CBD and IRAP-CBD beads (400 μl each) were added to PolyPrep columns (BioRad) and equilibrated with HES.

3T3-L1 cytosol was precleared by application to the CBD column, the flow-through was applied to the IRAP-CBD column and the column was washed with greater than ten volumes of HES. Proteins were eluted with three column volumes of HES containing 5 μM NT-IRAP peptide. The eluted material was concentrated using a Microcon-3 device (Amicon), solubilized in Laemml sample buffer, separated by 12% SDS-PAGE and visualized with Bio-Safe Coomassie Blue-G250 (BioRad). Samples of CBD and IRAP-CBD beads and IRAP-NT peptide also were subjected to SDS-PAGE.

The protein gel was washed in HPLC-grade water, stained with Bio-Safe Coomassie Blue-G250 (BioRad) and destained in HPLC-grade water. Protein bands in the 120 kDa region of the gel (and control regions of the gel containing no bands) were excised with a new razor blade and transferred to a 1.5 ml microfuge tube that was rinsed in HPLC-grade water. Gel slices were washed twice with 50% HPLC-grade acetonitrile/HPLC-grade water, the supernatant was removed and the tubes were stored at -80°C. Analysis was performed at the Harvard Microchemistry Facility according to protocols developed there. Tryptic digestion, HPLC and mass spectrometry. Peptide peaks were analyzed by comparison with the database and a protein was identified in the
material of approximately 120 kDa that bound to IRAP 1-109 previously identified in
the art as insulin degrading enzyme (IDE).

IDE is a metalloprotease that has been shown to degrade a number of
biologically important peptides, including insulin and the amyloid-beta protein
implicated in Alzheimer's disease (reviewed in Duckworth et al., 1998; Bennett et al.,
2000; Chesneau et al., 2000). IDE has been localized to endosomes and peroxisomes in
various cell types, however the peroxisomal location is not required for the insulin
degrading capacity of the enzyme (Chesneau et al., 1997; reviewed in Duckworth et al.,
1998). In addition, genetic analysis of the GK rat has identified the IDE gene as a
possible diabetes susceptibility locus (Fakhrai-Rad et al., 2000).

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than
routine experimentation, many equivalents to the specific embodiments of the invention
described herein. Such equivalents are intended to be encompassed by the following
claims.
What is claimed:

1. A method for identifying an insulin response modulator, comprising contacting a composition comprising insulin-responsive aminopeptidase (IRAP) or a bioactive fragment thereof and insulin degrading enzyme (IDE) or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate binding of IRAP or the IRAP bioactive fragment to IDE or the IDE bioactive fragment, such that an insulin response modulator is identified.

2. A method for identifying an insulin response modulator, comprising contacting a composition comprising insulin-responsive aminopeptidase (IRAP) or a bioactive fragment thereof and insulin degrading enzyme (IDE) or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate an activity of IRAP or the IRAP bioactive fragment, such that an insulin response modulator is identified.

3. A method for identifying an insulin response modulator, comprising contacting a composition comprising insulin-responsive aminopeptidase (IRAP) or a bioactive fragment thereof and insulin degrading enzyme (IDE) or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate an activity of IDE or the IDE bioactive fragment, such that an insulin response modulator is identified.

4. A method for identifying an insulin response modulator, comprising contacting a composition comprising insulin-responsive aminopeptidase- (IRAP)-containing vesicles and insulin degrading enzyme (IDE) or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate binding of IDE or a bioactive fragment thereof to the IRAP-containing vesicles, such that an insulin response modulator is identified.

5. A method for identifying an insulin response modulator, comprising contacting a composition comprising insulin-responsive aminopeptidase- (IRAP)-containing vesicles and insulin degrading enzyme (IDE) or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate an activity of IRAP, such that an insulin response modulator is identified.
6. A method for identifying an insulin response modulator, comprising contacting a composition comprising insulin-responsive aminopeptidase- (IRAP)-containing vesicles and insulin degrading enzyme (IDE) or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate an activity of IDE or the IDE bioactive fragment, such that an insulin response modulator is identified.

7. The method of any one of claims 1-3, wherein the IRAP bioactive fragment comprises at least 20 contiguous amino acids of amino acids 1-109 IRAP.

8. The method of claim 7, wherein the IRAP bioactive fragment comprises at least amino acids 55-82 of human IRAP.

9. The method of claim 7, wherein the IRAP bioactive fragment comprises at least amino acids 1-109 of human IRAP.

10. The method of any one of claims 1-7, wherein at least one of IRAP, the IRAP bioactive fragment, IDE or the IDE bioactive fragment is detectably labeled.

11. The method of any one of claims 1-7, wherein at least one of IRAP, the IRAP bioactive fragment, IDE or the IDE bioactive fragment is radioactively labeled.

12. The method of any one of claims 1-7, wherein at least one of IRAP, the IRAP bioactive fragment, IDE or the IDE bioactive fragment is fluorescently labeled.

13. The method of any one of claims 4-6, wherein the IRAP-containing vesicles are detectably labeled.

14. The method of any one of claims 4-6, wherein the IRAP-containing vesicles are radioactively labeled.

15. The method of any one of claims 4-6, wherein the IRAP-containing vesicles are fluorescently labeled.

16. The method of claim 1 or 4, wherein binding is compared to an appropriate control.
17. The method of any one of claims 2-3 or 5-6, wherein activity is compared to an appropriate control.

18. The method of any one of claims 1-7, wherein at least one of IRAP, the IRAP bioactive fragment, IDE or the IDE bioactive fragment is immobilized.

19. The method of any one of claims 4-5, wherein the IRAP-containing vesicles are immobilized.

20. The method of claim 2 or 5, wherein the IRAP activity is selected from the group consisting of IRAP translocation, extracellular aminopeptidase activity, regulation of intracellular GLUT4 trafficking and regulation of intracellular retention of GLUT4.

21. The method of claim 3 or 6, wherein the IDE activity is insulin degradation.

22. A method for identifying an insulin response modulator, comprising contacting a cell that expresses IDE or a bioactive fragment thereof and IRAP or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate binding of IDE or the IDE bioactive fragment to IRAP or the IRAP bioactive fragment, such that an insulin response modulator is identified.

23. A method for identifying an insulin response modulator, comprising contacting a cell that expresses IDE or a bioactive fragment and IRAP or a bioactive fragment thereof with a test compound and determining the ability of the test compound to modulate an IDE or IRAP activity, such that an insulin response modulator is identified.

24. The method of claim 23, wherein the IRAP activity is selected from the group consisting of IRAP translocation, extracellular aminopeptidase activity, regulation of intracellular GLUT4 trafficking and regulation of intracellular retention of GLUT4.

25. The method of claim 23, wherein the IDE activity is selected from the group consisting of modulation of cellular protein degradation, modulation of fat metabolism, modulation of insulin clearance, modulation of proteosome activation, modulation of ubiquitination and peroxisome targeting activity.
26. The method of claim 22 or 23, wherein said cell overexpresses IDE or the bioactive fragment thereof.

27. The method of claim 22 or 23, wherein said cell overexpresses IRAP or the bioactive fragment thereof.

28. The method of claim 22 or 23, wherein said cell overexpresses IDE or the IDE bioactive fragment and IRAP or the IRAP bioactive fragment.

29. The method of any one of the preceding claims, wherein the modulator identified is a positive modulator.

30. The method of any one of the preceding claims, wherein the modulator identified is a negative modulator.

31. A modulator identified by any one of the preceding claims.

32. A method for identifying an IRAP:IDE modulator, comprising contacting a composition comprising IRAP or bioactive fragment thereof and IDE or bioactive fragment thereof with a test compound and determining the ability of the test compound to enhance binding of the IRAP or bioactive fragment thereof to the IDE or bioactive fragment thereof, such that the modulator is identified.

33. A method for identifying an IRAP:IDE modulator, comprising contacting a composition comprising IRAP or bioactive fragment thereof and IDE or bioactive fragment thereof with a test compound and determining the ability of the test compound to inhibit binding of the IRAP or bioactive fragment thereof to the IDE or bioactive fragment thereof, such that the modulator is identified.

34. A method of modulating insulin responsiveness in a subject comprising administering to said subject an insulin response modulator identified according to the methods of any one of claims 1-30 or 32-33, such that insulin responsiveness is modulated.
35. A method of regulating blood glucose levels in a subject comprising administering to said subject an insulin response modulator identified according to the methods of any one of claims 1-30 or 32-33, such that insulin responsiveness is modulated.

36. An antibody that specifically binds to an IRAP-interacting domain of IDE, said antibody being capable of interfering with the IRAP:IDE interaction.

37. A pharmaceutical composition comprising the antibody of claim 36.

38. A pharmaceutical composition comprising the modulator of claim 31.

39. A pharmaceutical composition comprising an IRAP-interacting domain of IDE, said IRAP-interacting domain being capable of interfering with the IRAP:IDE interaction.
FIG 1B

Rat
KLFDGMASNGTQSLPTDVMTTVFKVGETKGLFLFSMYSSMGEAEKDKLIEALASS

Human
KLFDGMASNGTQSLPTDVMTTVFKVGETKGLFLFSMYSSMGEAEKDKLIEALASS

****,****************;***;***; **;*;*;*;*;*;*;*;*;*;

Rat
ADAHKLYNLKSSLEDIDDIERTQKLSLILRIRTGURQFPGHLLAWDFVKENWNLKLVHKFHLGS

Human
EDVRKLYNLKSSLEDIDDIERTQKLSLILRIRTGURQFPGHLLAWDFVKENWNLKLVFKFHLGS

*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;*;

Rat
YTIQSTVAGSTHLFSTKHLSSVQEFQFENQSBATLQYRCVQAFAEVETNIQWMAARNLKT

Human
YTIQSTVAGSTHLFSTKHLSSVQAEFFQFENQSBATFRLRCVQEAESVQNIQWMEKLNS

****,****************;***;***;*;*;*;*;*;*;*;**;*;

Rat
LTLNL

Human
LTWL

** **
FIG 2

mryrlawllh palpstfrev lgarlppper lcgfgkktys kmnnpaikri gnhitksped
kreyrglela ngikvliasd pttddksaal dvhigalsdp pniaglshfc ehmlflgtkk
ypkeneyesqf lsehagsna ftsgebynyy fdsvshehleq aldrfaqffl cplfdesckd
renavdseh eknvmndawr lfglekatgn pkhpfskfgt gnykyletrp nqegidvrqe
llkfhahys amlmacvlig resldltnl vvlkrlsen knvplpfepe hpfqeehlkq
lykivpiqdi xnlvtfpip dlgkyyksnp ghylhgh egpgsllsel sksgwntiv
ggkkgargsf mffiinvldlt eegllhvedi ilhmfgiyqk lraeggpswwr fgeckdlnav
afrrkeckp ipqysjqiag lhypleevel taeylleefr pdliemvlck lpwernrvai
vsksfekgtd rteewygtqy ksaeipdevi kkwqndlnq kflplktknef iptnfelpl
ekatpypal ikdvmeklw fkgddkkkpm kaclnfesf ssfaydpqihc nmaylyeell
kslnisayya aelagslydi qnitygmyls vkgynulkpi llkkiieka mfeidekrfe
iikeaymrsl nffraeqphq hammyvlrlm tewatkdele kealddvtp rrkafiqpl
srlhieallh gniktaaalg imqmdneli ehahtkpllt sqlyvryrevo ldpdrzgyvyq
qrrnvnmmc iyeyygotmqr stsenmnfeel fcqiisepcf ntlrtkeqgil yrifsgprra
ngigslrlfii qskipphyle srvaeaffitm eksiedmtee afghkhiqala irrldkpkkl
saecakywe eissqynfdr dnteyvlkt ltkediikfy kemladvapr rkxsvshvla
rmdscpvg sqpcqndlnl sqpalpgpe vqnmtefkr glplfplvkg hinmaakl