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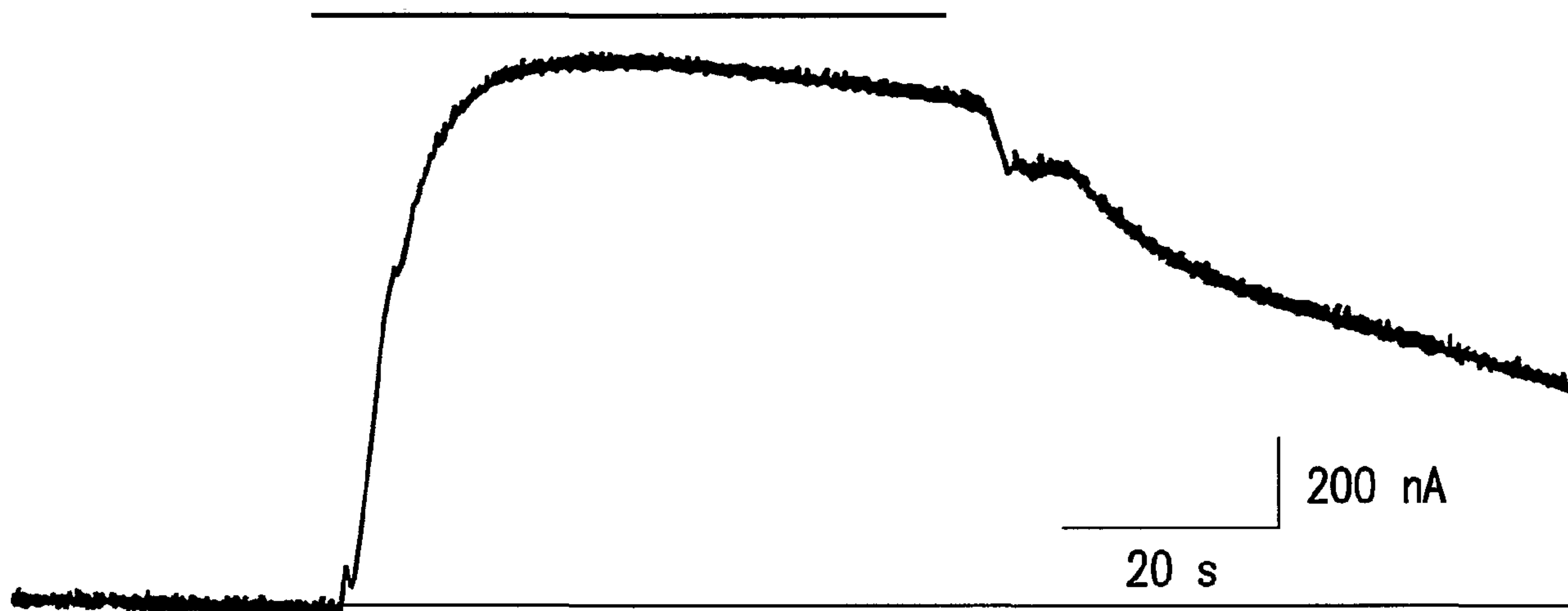
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MELANOGASTER

(54) Title: DNA MOLECULES ENCODING A GLUTAMATE-GATED CHLORIDE CHANNEL IN DROSOPHILA
MELANOGASTER

AC05 is expressed in Oocytes and
activated by Histamine

10 μ M Histamine



(57) Abrégé/Abstract:

The present invention relates in part to isolated nucleic acid molecules (polynucleotides) which encode *Drosophila melanogaster* ligand-gated ion channel proteins. The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding *Drosophila* ligand-gated ion channel proteins, substantially purified forms of associated *Drosophila* ligand-gated ion channel proteins and recombinant membrane fractions comprising these proteins, associated mutant proteins, and methods associated with identifying compounds which modulate associated *Drosophila melanogaster* ligand-gated ion channel proteins, which will be useful as insecticides.

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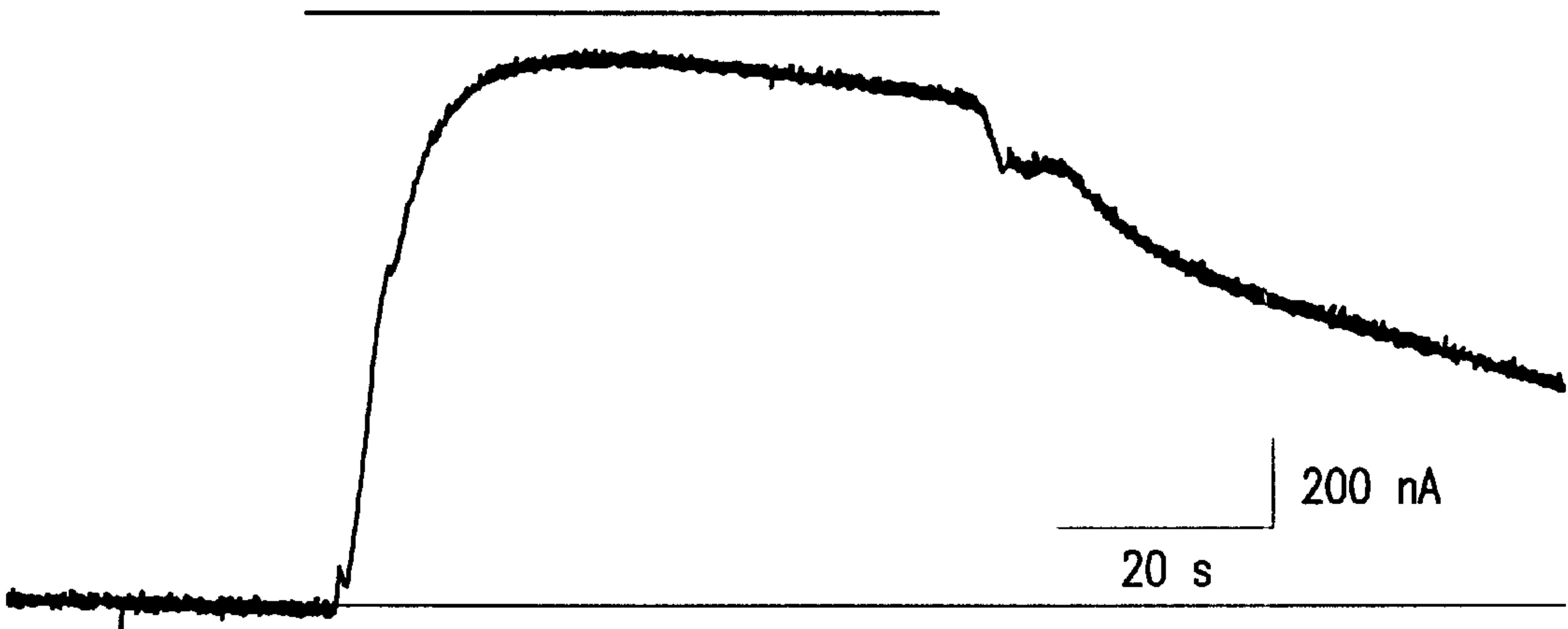
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ning of each regular issue of the PCT Gazette.*

(54) Title: DNA MOLECULES ENCODING A GLUTAMATE-GATED CHLORIDE CHANNEL IN DROSOPHILA
MELANOGASTER

AC05 is expressed in Oocytes and
activated by Histamine

10 μ M Histamine



(57) Abstract: The present invention relates in part to isolated nucleic acid molecules (polynucleotides) which encode *Drosophila melanogaster* ligand-gated ion channel proteins. The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding *Drosophila* ligand-gated ion channel proteins, substantially purified forms of associated *Drosophila* ligand-gated ion channel proteins and recombinant membrane fractions comprising these proteins, associated mutant proteins, and methods associated with identifying compounds which modulate associated *Drosophila melanogaster* ligand-gated ion channel proteins, which will be useful as insecticides.

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5 TITLE OF THE INVENTION

DNA MOLECULES ENCODING A GLUTAMATE-GATED CHLORIDE
CHANNEL IN DROSOPHILA MELANOGASTER

10 CROSS-REFERENCE TO RELATED APPLICATIONS

Not Applicable

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

15 Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

20

FIELD OF THE INVENTION

The present invention relates in part to isolated nucleic acid molecules (polynucleotides) which encode *Drosophila melanogaster* ligand-gated ion channels.

25 The present invention also relates to recombinant vectors and recombinant hosts which contain a DNA fragment encoding *Drosophila* ligand-gated ion channels, substantially purified forms of associated *Drosophila* ligand-gated ion channels and recombinant membrane fractions comprising these proteins, associated mutant proteins, and methods associated with identifying compounds which modulate
30 associated *Drosophila melanogaster* ligand-gated ion channels, which will be useful as insecticides.

BACKGROUND OF THE INVENTION

Glutamate-gated chloride channels, or H-receptors, have been identified in arthropod nerve and muscle (Lingle et al, 1981, *Brain Res.* 212: 481-488; Horseman
5 et al., 1988, *Neurosci. Lett.* 85: 65-70; Wafford and Sattelle, 1989, *J. Exp. Bio.* 144: 449-462; Lea and Usherwood, 1973, *Comp. Gen. Pharmacol.* 4: 333-350; and Cull-Candy, 1976, *J. Physiol.* 255: 449-464).

Invertebrate glutamate-gated chloride channels are important targets for the widely used avermectin class of anthelmintic and insecticidal compounds. The
10 avermectins are a family of macrocyclic lactones originally isolated from the actinomycete *Streptomyces avermitilis*. The semisynthetic avermectin derivative, ivermectin (22,23-dihydro-avermectin B_{1a}), is used throughout the world to treat parasitic helminths and insect pests of man and animals. The avermectins remain the most potent broad spectrum endectocides exhibiting low toxicity to the host. After
15 many years of use in the field, there remains little resistance to avermectin in the insect population. The combination of good therapeutic index and low resistance strongly suggests that the glutamate-gated chloride (GluCl) channels remain good targets for insecticide development.

Glutamate-gated chloride channels have been cloned from the soil nematode
20 *Caenorhabditis elegans* (Cully et al., 1994, *Nature* 371: 707-711; see also U.S. Patent No. 5,527,703 and Arena et al., 1992, *Molecular Brain Research.* 15: 339-348) and *Ctenocephalides felis* (flea; see WO 99/07828).

In addition, a gene encoding a glutamate-gated chloride channel from *Drosophila melanogaster* was previously identified (Cully et al., 1996, *J. Biol. Chem.*
25 271: 20187-20191; see also U.S. Patent No. 5,693,492).

O'Tousa et al. (1989, *J. Neurogenetics* 6: 41-52) map photoreceptor mutations to the ChIII 92B region of the *Drosophila* genome.

Stuart, 1999, *Neuron* 22:431-433 reviews the art which suggests that histamine is an invertebrate neurotransmitter.

30 Despite the identification of the aforementioned cDNA clones encoding GluCls, including a previous identification of a *Drosophila* GluCl gene (see U.S. Patent No. 5,693,492), it would be advantageous to identify additional genes which encode invertebrate ligand-gated ion channels, including but not limited to additional GluCls or other ligand-gated channels, such as a ligand-gated ion channel (LGIC)

which is activated by histamine, which may provide additional targets for effective insecticides, in turn allowing for improved screening to identify novel LGIC modulators that may have insecticidal, mitacidal and/or nematocidal activity for animal health or crop protection. The present invention addresses and meets these
5 needs by disclosing novel genes which express a *Drosophila melanogaster* ligand-gated ion channel, wherein expression of the respective *Drosophila* gene in *Xenopus* oocytes or other appropriate host cell results in an active LGIC. Heterologous expression of a respective LGIC(s) of the present invention will allow the pharmacological analysis of compounds active against parasitic invertebrate species
10 relevant to animal and human health. Such species include worms, fleas, tick, and lice. Heterologous cell lines expressing an active LGIC can be used to establish functional or binding assays to identify novel LGIC modulators that may be useful in control of the aforementioned species groups.

15 SUMMARY OF THE INVENTION

The present invention relates to an isolated or purified nucleic acid molecule (polynucleotide) which encodes a novel *Drosophila melanogaster* invertebrate ligand-gated ion channel (LGIC) protein which comprises at least a portion of a LGIC receptor. The DNA molecules disclosed herein may be transfected into a host cell of
20 choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional single, homomultimer or heteromultimer LGIC channel. The cDNA clones described herein express a functional single channel protein, both of which are activated by histamine. Therefore, these DmLGIC channels form receptors which provide for additional screening targets to identify modulators of these
25 channels, modulators which may act as effective insecticidal, mitacidal and/or nematocidal treatment for use in animal and human health and/or crop protection.

The present invention further relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel *Drosophila melanogaster* LGIC protein, this DNA molecule comprising the nucleotide sequence
30 disclosed herein as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5 and SEQ ID NO:6.

The present invention also relates to biologically active fragments or mutants of SEQ ID NOs:1, 3, 5 and 6 which encode mRNA expressing a novel *Drosophila melanogaster* invertebrate LGIC protein. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least

substantially mimics the pharmacological properties of the respective *Drosophila* LGIC protein, including but not limited to the *Drosophila* LGIC proteins as set forth in SEQ ID NO:2, SEQ ID NO:4, and SEQ ID NO:7. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions,
5 amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a functional *Drosophila* LGIC in a eukaryotic cell, such as *Xenopus* oocytes, so as to be useful for screening for agonists and/or antagonists of *Drosophila* LGIC activity.

A preferred aspect of this portion of the present invention is disclosed in
10 Figure 1 (SEQ ID NO:1; designated AC05-10), Figure 3 (SEQ ID NO:3; designated AC05-11), Figure 5 (SEQ ID NO:5; designated AC15-4) and Figure 6 (SEQ ID NO:6) encoding a novel *Drosophila melanogaster* LGIC protein.

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary
15 DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

The present invention also relates to recombinant vectors and recombinant
20 host cells, both prokaryotic and eukaryotic, which contain the nucleic acid molecules disclosed throughout this specification.

The present invention also relates to a substantially purified form of a *Drosophila* LGIC protein, which comprises the amino acid sequence disclosed in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4) and Figure 7 (SEQ ID NO:7).

25 A preferred aspect of this portion of the present invention is a *Drosophila* LGIC protein which consists of the amino acid sequence disclosed in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4) and Figure 7 (SEQ ID NO:7).

Another preferred aspect of the present invention relates to a substantially purified, fully processed (including proteolytic processing, glycosylation and/or
30 phosphorylation), mature LGIC protein obtained from a recombinant host cell containing a DNA expression vector comprising nucleotide sequence as set forth in SEQ ID NOs: 1, 3, 5 and/or 6 which express the respective DmLGIC protein. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line, or *Xenopus* oocytes, as noted above.

Another preferred aspect of the present invention relates to a substantially purified membrane preparation, partially purified membrane preparation, or cell lysate which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete
5 open reading frame as set forth in SEQ ID NOs: 1, 3, 5 and/or 6, resulting in a functional form of the respective DmLGIC. The subcellular membrane fractions and/or membrane-containing cell lysates from the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) contain the functional and processed proteins encoded by the nucleic acids of the
10 present invention. This recombinant-based membrane preparation may comprise a *Drosophila* LGIC and is essentially free from contaminating proteins, including but not limited to other *Drosophila* source proteins or host proteins from a recombinant cell which expresses the AC05-10 (SEQ ID NO:2), AC05-11 (SEQ ID NO:4) and/or AC15-4/AC15-25 (SEQ ID NO:7) LGIC protein. Therefore, a preferred aspect of the
15 invention is a membrane preparation which contains a *Drosophila* LGIC comprising the functional form of the full length LGIC proteins as disclosed in Figure 2 (SEQ ID NO:2, AC05-10), Figure 4 (SEQ ID NO:4, AC05-11), and Figure 7 (SEQ ID NO:7, AC15-4/AC14-25). These subcellular membrane fractions will comprise either wild type and/or mutant variations which are biologically functional forms of the
20 *Drosophila* LGIC (including but not limited to functional channels generated by a single polypeptide, or any homomultimer or heteromultimer channel combinations thereof) at levels substantially above endogenous levels. Any such channel will be useful in various assays described throughout this specification to select for modulators of the respective LGIC channel. A preferred eukaryotic host cell of
25 choice to express the LGICs of the present invention is a mammalian cell line, or *Xenopus* oocytes.

The present invention also relates to biologically active fragments and/or mutants of an *Drosophila* LGIC protein, comprising the amino acid sequence as set forth in SEQ ID NOs: 2, 4 and/or 7, including but not necessarily limited to amino
30 acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators, including but not limited to agonists and/or antagonists for *Drosophila* ligand-gated ion channel pharmacology.

A preferred aspect of the present invention is disclosed in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4) and Figure 7 (SEQ ID NO:7), respective amino acid sequences which compose the *Drosophila* LGIC proteins of the present invention. Characterization of one or more of these channel proteins allows for screening to
5 identify novel LGIC modulators that may have insecticidal, mitacidal and/or nematocidal activity for animal health or crop protection. As noted above, heterologous expression of functional single channel, homomultimer and/or heteromultimer combination of *Drosophila melanogaster* LGICs disclosed herein is contemplated at levels substantially above endogenous levels and will allow for the
10 pharmacological analysis of compounds active against parasitic invertebrate species relevant to animal and human health. Such species include worms, fleas, tick, and lice. Heterologous cell lines expressing a functional DmLGIC channel (e.g., functional forms of SEQ ID NOs: 2, 4 and/or 7), can be used to establish functional or binding assays to identify novel LGIC modulators that may be useful in control of the
15 aforementioned species groups.

The present invention also relates to polyclonal and monoclonal antibodies raised against forms of DmLGIC, or a biologically active fragment thereof.

The present invention also relates to DmLGIC fusion constructs, including but not limited to fusion constructs which express a portion of the DmLGIC linked to
20 various markers, including but in no way limited to GFP (Green fluorescent protein), the MYC epitope, GST, and Fc. Any such fusion constructs may be expressed in the cell line of interest and used to screen for modulators of one or more of the DmLGIC proteins disclosed herein.

The present invention relates to methods of expressing *Drosophila* LGIC
25 proteins and biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of LGIC activity.

It is an object of the present invention to provide an isolated nucleic acid
30 molecule (e.g., SEQ ID NOs:1, 3, 5 and 6) which encodes a novel form of *Drosophila* LGIC, or fragments, mutants or derivatives DmLGIC, as set forth in SEQ ID NOs: 2, 4, and 7, respectively. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express

a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for selective modulators for invertebrate ligand-gated ion channel pharmacology.

5 It is a further object of the present invention to provide the *Drosophila* LGIC proteins or protein fragments encoded by the nucleic acid molecules referred to in the preceding paragraph.

It is a further object of the present invention to provide recombinant vectors and recombinant host cells which comprise a nucleic acid sequence encoding *Drosophila* LGIC proteins or a biological equivalent thereof.

10 It is an object of the present invention to provide a substantially purified form of *Drosophila* LGIC proteins, as set forth in SEQ ID NOs: 2, 4, and 7.

Is another object of the present invention to provide a substantially purified recombinant form of a *Drosophila* LGIC protein which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which
15 comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NOs: 1, 3, 5 and 6, resulting in a functional, processed form of the respective DmLGIC. It is especially preferred is that the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line.

It is an object of the present invention to provide for biologically active
20 fragments and/or mutants of *Drosophila* LGIC proteins, such as set forth in SEQ ID NOs: 2, 4, and 7, including but not necessarily limited to amino acid substitutions, deletions, additions, amino terminal truncations and carboxy-terminal truncations such that these mutations provide for proteins or protein fragments of diagnostic, therapeutic and/or prophylactic use.

25 It is further an object of the present invention to provide for substantially purified subcellular membrane preparations, partially purified subcellular membrane preparations, or crude lysates from recombinant cells which comprise pharmacologically active *Drosophila* LGICs, especially subcellular fractions obtained from a host cell transfected or transformed with a DNA vector comprising a
30 nucleotide sequence which encodes a protein which comprises the amino acid as set forth in Figure 2 (SEQ ID NO:2), Figure 4 (SEQ ID NO:4) and/or Figure 7 (SEQ ID NO:7).

It is another object of the present invention to provide a substantially purified membrane preparation, partially purified subcellular membrane preparations, and/or

crude lysates obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NOs: 1, 3, 5 and/or 6, resulting in a functional, processed form of the respective DmLGIC. It is especially preferred that the recombinant host cell be a
 5 eukaryotic host cell, such as a mammalian cell line, or *Xenopus* oocytes.

It is also an object of the present invention to use *Drosophila* LGIC proteins or membrane preparations containing *Drosophila* LGIC proteins or a biological equivalent to screen for modulator, preferably selective modulators, of *Drosophila* LGIC activity. Any such protein or membrane associated protein may be useful in screening and selecting these
 10 modulators active against parasitic invertebrate species relevant to animal and human health. Such species include worms, fleas, tick, and lice. These membrane preparations may be generated from heterologous cell lines expressing these LGICs and may constitute full length protein, biologically active fragments of the full length protein or may rely on fusion proteins expressed from various fusion constructs which may be constructed with materials available in
 15 the art.

There is also provided herein a method of identifying a compound that modulates histamine-gated channel protein activity, which comprises:

- (a) injecting into a host cell solution a population of nucleic acid molecules, at least a portion of which encodes a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein selected from the group consisting of SEQ ID NO:2, SEQ
 20 ID NO:4 and SEQ ID NO:7, such that expression of said portion of nucleic acid molecules results in an active histamine-gated channel;
- (b) adding a test compound into said solution; and
- (c) measuring host cell membrane current at a holding potential more positive
 25 than the reversal potential for chloride.

The nucleic acid molecules are preferably selected from the group consisting of complementary DNA, poly A+ messenger RNA and complementary RNA.

As used herein, "substantially free from other nucleic acids" means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic
 30 acids. As used interchangeably with the terms "substantially free from other nucleic acids" or "substantially purified" or "isolated nucleic acid" or "purified nucleic acid" also refer to aDNA molecules which comprises a coding region for a *Drosophila* LGIC protein that has

been purified away from other cellular components. Thus, a *Drosophila* LGIC DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-*Drosophila* LGIC nucleic acids. Whether a given *Drosophila* LGIC DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as , *e.g.*, agarose gel electrophoresis combined with appropriate staining methods, *e.g.*, ethidium bromide staining, or by sequencing.

As used herein, “substantially free from other proteins” or “substantially purified” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus *Drosophila* LGIC protein preparation that is substantially free from other proteins will contain, as a percent of

its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-*Drosophila* LGIC proteins. Whether a given *Drosophila* LGIC protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, *e.g.*, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, *e.g.*, silver staining or immunoblotting. As used interchangeably with the terms "substantially free from other proteins" or "substantially purified", the terms "isolated *Drosophila* LGIC protein" or "purified *Drosophila* LGIC protein" also refer to *Drosophila* LGIC protein that has been isolated from a natural source. Use of the term "isolated" or "purified" indicates that *Drosophila* LGIC protein has been removed from its normal cellular environment. Thus, an isolated *Drosophila* LGIC protein may be in a cell-free solution or placed in a different cellular environment from that in which it occurs naturally. The term isolated does not imply that an isolated *Drosophila* LGIC protein is the only protein present, but instead means that an isolated *Drosophila* LGIC protein is substantially free of other proteins and non-amino acid material (*e.g.*, nucleic acids, lipids, carbohydrates) naturally associated with the *Drosophila* LGIC protein *in vivo*. Thus, a *Drosophila* LGIC protein that is recombinantly expressed in a prokaryotic or eukaryotic cell and substantially purified from this host cell which does not naturally (*i.e.*, without intervention) express this LGIC protein is of course "isolated *Drosophila* LGIC protein" under any circumstances referred to herein. As noted above, a *Drosophila* LGIC protein preparation that is an isolated or purified *Drosophila* LGIC protein will be substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-*Drosophila* LGIC proteins.

As used interchangeably herein, "functional equivalent" or "biologically active equivalent" means a protein which does not have exactly the same amino acid sequence as naturally occurring *Drosophila* LGIC, due to alternative splicing, deletions, mutations, substitutions, or additions, but retains substantially the same biological activity as *Drosophila* LGIC. Such functional equivalents will have significant amino acid sequence identity with naturally occurring *Drosophila* LGIC and genes and cDNA encoding such functional equivalents can be detected by reduced stringency hybridization with a DNA sequence encoding naturally occurring

Drosophila LGIC. For example, a naturally occurring *Drosophila* LGIC disclosed herein comprises the amino acid sequence shown as SEQ ID NO:2 and is encoded by SEQ ID NO:1. A nucleic acid encoding a functional equivalent has at least about 50% identity at the nucleotide level to SEQ ID NO:1.

5 As used herein, "a conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g.,
10 arginine for lysine; glutamic acid for aspartic acid).

As used herein, "LGIC" refers to a --ligand-gated ion channel --

As used herein, "DmLGIC" refers to a -- *Drosophila melanogaster* ligand-gated ion channel --.

As used herein, "GluCl" refers to -- L-glutamate gated chloride channel --.

15 As used herein, "DmGluCl" refers to -- *Drosophila melanogaster* L-glutamate gated chloride channel --.

As used herein, the term "mammalian" will refer to any mammal, including a human being.

20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequence which of the *Drosophila* LGIC clone, AC05-10, as set forth in SEQ ID NO:1.

Figure 2 shows the amino acid sequence of the *Drosophila* LGIC AC05-10 protein, as set forth in SEQ ID NO:2.

25 Figure 3 shows the nucleotide sequence which of the *Drosophila* LGIC clone, AC05-11, as set forth in SEQ ID NO:3.

Figure 4 shows the amino acid sequence of the *Drosophila* LGIC AC05-11 protein, as set forth in SEQ ID NO:4.

30 Figure 5 shows the nucleotide sequence which of the *Drosophila* LGIC clone, AC15-4, as set forth in SEQ ID NO:5.

Figure 6 shows the nucleotide sequence which of the *Drosophila* LGIC clone, AC15-25, as set forth in SEQ ID NO:6.

Figure 7 shows the amino acid sequence of the *Drosophila* LGIC AC15-4/AC15-25 protein, as set forth in SEQ ID NO:7.

Figure 8 shows the activation of a recombinant DmLGIC (AC05-10) by histamine in transfected *Xenopus* oocytes.

DETAILED DESCRIPTION OF THE INVENTION

5 The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes a *Drosophila melanogaster* invertebrate LGIC protein, which are phylogenetically related to known DmGluCl proteins but which show alternative pharmacology, and hence, represent novel insecticide targets. The isolated or purified nucleic acid molecules of the present invention are substantially
10 free from other nucleic acids. For most cloning purposes, DNA is a preferred nucleic acid. As noted above, the DNA molecules disclosed herein it may be transfected into a host cell of choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional single, homomultimer or heteromultimer LGIC channel. The cDNA clones described herein express a functional single
15 channel protein, both of which are activated by histamine. Therefore, these DmLGIC channels form receptors which provide for additional screening targets to identify modulators of these channels, modulators which may act as effective insecticidal, mitacidal and/or nematocidal treatment for use in animal and human health and/or crop protection. The DNA molecules disclosed herein are transfected into a host cell
20 of choice wherein the recombinant host cell provides a source for substantial levels of an expressed functional single, homomultimer or heteromultimer LGIC channel.

 The present invention relates to an isolated nucleic acid molecule (polynucleotide) which encodes mRNA which expresses a novel *Drosophila melanogaster* invertebrate LGIC protein, this DNA molecule comprising the
25 nucleotide sequence disclosed herein as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, and SEQ ID NO:6. The isolation and characterization of the DmLGIC nucleic acid molecules of the present invention were identified as described in detail in Example Section 1.

 Invertebrate glutamate-gated chloride channels (GluCls) are related to the
30 glycine- and GABA- gated chloride channels and are distinct from the excitatory glutamate receptors (e.g. NMDA or AMPA receptors). The first two members of the GluCl family were identified in the nematode *C. elegans*, following a functional screen for the receptor of the anthelmintic drug ivermectin. Several additional GluCls have now been cloned in other invertebrate species. However, there is no evidence

yet for GluCl counterparts in vertebrates; because of this, GluCls and any related ligand-gated channels are potentially excellent targets for anthelmintics, insecticides, acaricides, etc. Specific GluCl modulators, such as nodulisporic acid and its derivatives, indeed have an ideal safety profile because they lack mechanism-based toxicity in vertebrates. The present invention relates in part to four novel *Drosophila* LGIC clones, AC05-10, AC05-11, AC15-4 and AC15-25, which show homology to the earlier identified DmGluCl α and to *C. felis* CfGluCl DNA.

The present invention relates to the isolated or purified DNA molecule described in Figure 1 (AC05-10) and set forth as SEQ ID NO:1, which encodes the *Drosophila* LGIC protein described in Figure 2 and set forth as SEQ ID NO:2, the nucleotide sequence of AC05-10 is as follows:

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CAATCGTCGC GATAACTCTG CCGTTTCTTT ATTGGTTTTT GCTGCGCGAC GAGTAAAATA
TAATTCCTCG CTTACTAATC CTCCGAGCAA GTTCATTCTC AAGCGCACCC AGAGATGAGC
TACTTTGGGA ATTGACATGG ACTGCGGAGC AATGAGTGCC AGAGGAACAA TATCAAAGCC
15 GAAGGTAGTG TGTTCATAT AT GCCAAAGCCCA ACTAGCAAAT TGGTAGAATT CAGGTGCCTT
ATTGCGTTGG CAATATATTT GCACGCGCTG GAGCAATCGA TCCAGCACTG CCATTGTGTT
CATGGTTACA GAAATAACAC GGAGAGCGCC GAGCTGGTCT CCCACTACGA GTCGAGTCTT
TCGCTCCCGG ACATTTTGCC CATTCCTCA AAGACGTACG ACAAGAACCG GGCTCCCAAG
CTCCTCGGCC AGCCACAGT AGTCTACTTC CATGTCACGG TCCTCTCCCT GGACTCCATT
20 AACGAGGAGT CTATGACCTA TGTGACGGAC ATCTTCCTTG CACAAAGCTG GCGTGATCCT
CGCCTGCGGT TGCCTGAGAA CATGAGTGAG CAGTATCGCA TATTGGATGT CGACTGGTTG
CACAGCATTT GGCGGCCCGA TTGCTTCTTT AAGAACGCCA AAAAGGTCAC CTTCCATGAG
ATGAGCATTC CCAATCACTA TCTCTGGCTG TACCACGACA AAACGCTGCT CTATATGTCC
AAACTCACGT TGGTCCTGTC GTGCGCCATG AAGTTTGAGT CCTATCCGCA TGACACGCAA
25 ATCTGCTCCA TGATGATCGA GAGTTTATCC CATACGGTGG AAGATTTGGT TTTCATTTGG
AACATGACCG ACCCACTTGT GGTAAACACG GAGATTGAGT TGCCGCAGCT AGACATATCA
AATAACTACA CAACCGACTG TACTATAGAG TACTCAACAG GTAACTTCAC CTGCCTGGCC
ATTGTGTTCA ACCTGCGCCG ACGCCTGGGT TACCATTTGT TCCACACCTA CATCCCCTCG
GCTCTGATTG TGGTCATGTC TTGGATATCG TTTTGGATAA AACCAGAAGC GATACCCGCC
30 CGTGTAACTC TGGGAGTGAC CTCACTGCTA ACCCTGGCCA CCCAGAATAC CCAGTCGCAA
CAATCGCTGC CGCCGGTTTC GTATGTCAAG GCTATAGACG TCTGGATGTC GTCCTGTTTCG
GTGTTTGTAT TCCTTTCTCT GATGGAATTT GCAGTGGTCA ACAATTTTAT GGGACCGGTG
GCCACAAAGG CAATGAAGGG GTATTCGGAC GAGAACATCA GTGATCTGGA CGACCTAAAG
TCTGCACTAC AGCATCATCG GGAATCGATT ATTGAGCCCC AGTACGACAC TTTCTGCCAT

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GGCCATGCCA CAGCCATTTA TATAGACAAA TTCTCGCGCT TTTTCTTCCC GTTTTCGTTC
 TTTATACTCA ATATTGTCTA TTGGACAACG TTCCTATTGAT GGATGGAAAA GTTTCTCCGA
 AGGAATAGAG CGTAAACA (SEQ ID NO:1).

The present invention also relates to the isolated or purified DNA molecule
 5 described in Figure 3 (AC05-11) and set forth as SEQ ID NO:3, which encodes the
 Drosophila LGIC protein described in Figure 4 and set forth as SEQ ID NO:4, the
 nucleotide sequence AC05-11 as follows:

CAATCGTCGC GATAACTCTG CCGTTTCTTT ATTGGTTTTT GCTGCGCGAC GAGTAAAATA
 TAATTCCTCG CTTACTAATC CTCCGAGCAA GTTCATTCTC AAGCGCACCC AGAGATGAGC
 10 TACTTTGGGA ATTGACATGG ACTGCGGAGC AATGAGTGCC AGAGGAACAA TATCAAAGCC
 GAAGGTAGTG TGTTCATAAT GCAAAGCCCA ACTAGCAAAT TGGTAGAATT CAGGTGCCTT
 ATTGCGTTGG CAATATATTT GCACGCGCTG GAGCAATCGA TCCAGCACTG CCATTGTGTT
 CATGGTTACA GAAATAACAC GGAGAGCGCC GAGCTGGTCT CCCACTACGA GTCGAGTCTT
 TCGCTCCCGG ACATTTTGCC CATTCCCTCA AAGACGTACG ACAAGAACCG GGCTCCCAAG
 15 CTCCTCGGCC AGCCACAGT AGTCTACTTC CATGTCACGG TCCTCTCCCT GGACTCCATT
 AACGAGGAGT CTATGACCTA TGTGACGGAC ATCTTCCTTG CACAAAGCTG GCGTGATCCT
 CGCCTGCGGT TGCCTGAGAA CATGAGTGAG CAGTATCGCA TATTGGATGT CGACTGGTTG
 CACAGCATTT GGCGGCCCGA TTGCTTCTTT AAGAACGCCA AAAAGGTCAC CTTCCATGAG
 ATGAGCATTC CCAATCACTA TCTCTGGCTG TACCACGACA AAACGCTGCT CTATATGTCC
 20 AAACTCACGT TGGTCCTGTC GTGCGCCATG AAGTTTGAGT CCTATCCGCA TGACACGCAA
 ATCTGCTCCA TGATGATCGA GAGTTTATCC CATA CGGTGG AAGATTTGGT TTTCATTTGG
 AACATGACCG ACCCACTTGT GGTAAACACG GAGATTGAGT TGCCGCAGCT AGACATATCA
 AATAACTACA CAACCGACTG TACTATAGAG TACTCAACAG GTAAC TTCAC CTGCCTGGCC
 ATTGTGTTCA ACCTGCGCCG ACGCCTGGGT TACCATTTGT TCCACACCTA CATCCCCTCG
 25 GCTCTGATTG TGGTCATGTC TTGGATATCG TTTTGGATAA AACCAGAAGC GATACCCGCC
 CGTGTAAC TC TCGGAGTGAC CTCACTGCTA ACCCTGGCCA CCCAGAATAC CCAGTCGCAA
 CAATCGCTGC CGCCGGTTTC GTATGTCAAG GCTATAGACG TCTGGATGTC GTCCTGTTTCG
 GTGTTTGTAT TCCTTTCTCT GATGGAATTT GCAGTGGTCA ACAATTTTAT GGGACCGGTG
 GCCACAAAGG CAATGAAGGG GTATTCGGAC GAGAACATCA GTGATCTGGA CGACCTAAAG
 30 CATCATCGGG AATCGATTAT TGAGCCCCAG TACGACACTT TCTGCCATGG CCATGCCACA
 GCCATTTATA TAGACAAATT CTCGCGCTTT TTCTTCCCGT TTTCGTTCTT TATACTCAAT
 ATTGTCTATT GGACAACGTT CCTATTGATGG ATGGAAAAGT TTCTCCGAAG GAATAGAGCG
 TAAACA (SEQ ID NO:3).

The present invention also relates to the isolated or purified DNA molecule described in Figure 5 (AC15-4) and set forth as SEQ ID NO:5, which encodes the Drosophila LGIC protein described in Figure 7 and set forth as SEQ ID NO:7, the nucleotide sequence AC15-4 as follows:

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5  AACTGCCAAG ACGTTTAGAA CGGAAAAACT GAATTTTCAA AAATATTTTCG AGTAAACTGT
   TAAATGCTGA AGTGTTCTGA AATATTCCTT AAAACATAGA AACCTTCTTT GACATCTTTA
   TAAAGCAATA AAATTCATTC GGGAAGTTTA TGAATAGTGG TGTTATTAAT CATGCCATTT
   GTGGCGTCAA GCTGATGGTT ATGTAATCTC TGTGAAGATT CTAGAAATCC AACAGAAATA
   TATTGCTTCG AAAACCAAGC AAAGATTACT TGACTGGAGA GGAAAGCTAT TTCGAATTCA
10 TCTAAAAACT GTAAAGAGTT CACATTAAAA TGGTGTTCCA AATAATAATC CTGGTGATCT
   GCACCATCTG CATGAAGCAC TACGCCAAAG GGGAGTTTCA ACAAAGTCTG GCCATAACCG
   ACATCCTGCC CGAGGACATC AAGCGTTACG ACAAGATGAG ACCGCCGAAG AAAGAGGGTC
   AGCCGACGAT AGTCTACTTC CATGTGACTG TGATGGGTCT GGACTCCATT GATGAGAACT
   CGATGACTTA TGTGGCGGAT GTGTTCTTTG CACAGACGTG GAAGGATCAT CGCCTGCGAT
15 TGCCGGAGAA TATGACACAG GAATACCGCC TGCTCGAGGT GGACTGGCTA AAAAATATGT
   GGCGCCCGGA TTCGTTTTTC AAAAACGCCA AATCGGTGAC CTTTCAGACC ATGACAATAC
   CCAATCACTA TATGTGGCTG TACAAGGATA AGACCATTCT CTATATGGTC AAGCTAACAC
   TGAAGCTGTC CTGCATCATG AATTTGCGCA TTTATCCTCA TGACACACAG GAGTGCAAGC
   TGCAAATGGA AAGCCTGTCC CACACCACGG ATGACTTGAT ATTCCAGTGG GATCCAACAA
20 CGCCCCTTGT GGTGATGAA AACATCGAAC TGCCGCAGGT GGCCCTCATC CGGAATGAAA
   CGGCGGACTG CACCCAGGTT TATTCCACTG GCAACTTCAC ATGCCTGGAG GTGGTGTTCA
   CCCTTAAGCG TCGTTTGGTT TACTACGTTT TCAACACCTA CATTCCCACC TGCATGATAG
   TGATCATGTC ATGGGTATCC TTCTGGATCA AACCGGAGGC GGCACCAGCC CGTGTGACTC
   TGGGTGTCAC CTCCTTGCTA ACGCTTTCCA CGCAACACGC CAAATCGCAG TCGTCTTTGC
25 CACCTGTTTC CTATCTCAAG GCAGTGGACG CCTTTATGTC CGTTTGCACG GTGTTTCGTGT
   TTATGGCCCT CATGGAGTAT TGTCTAATAA ACATCGTCCT GAGCGACACG CCCATTCCCA
   AGCCGATGGC TTATCCACCC AAACCTGTGG CGGGCGATGG GCCCAAGAAA GAGGGCGAGG
   GTGCTCCTCC TGGGGGCAGC AACTCGACGG CCAGCAAGCA ACAAGCCACC ATGTTGCCAC
   TGGCCGATGA AAAGATCGAG AAAATTGAGA AGATCTTTGA CGAGATGACC AAGAATAGAA
30 GGATTGTAAC CACCACACGC CGCGTGGTGC GTCCACCATT GGACGCCGAT GGTCCGTGGA
   TTCCGCGACA GGAGTCGCGG ATAATACTGA CCCC GACTAT CGCTCCGCCG CCACCGCCCC
   CTCAGCCAGC GGCACCGGAA CCGGA ACTAC CCAAGCCGAA ACTCACACCC GCCCAGGAGC
   GGCTCAAGCG GGCTATATAT ATAGATCGGT CCTCGCGCGT CCTTTTCCCC GCCCTCTTCG
   CCAGTCTGAA TGGCATCTAC TGGTGTGTGT TCTACGAGTA TCTATAAGGA CTACGACGAC

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TGTGCCCTGT AAATACTTTC GCTAGCTCTC TGGCACTCCA TCCGAGTGTT AAACGTTGAT
 TGTTCGCATA TATCGAAACG TGTATCGCAA ATTTAATCTT AAGCTTTCAC GCACAAGCTT
 TAAGTCAATG AATTTTAAAC ATAGATTATT GTTAAACCAG AAGGAAGGAA TAATGGTACA
 GATGGAGATC TGATTACAGG ATAAATTACA AATTATCAAT TCAATTCCTA AAATGCTTAA
 5 AGTTAATCAA GTGACGTAGT AGCTGATGTA GCC (SEQ ID NO:5) .

The present invention also relates to the isolated or purified DNA molecule
 described in Figure 6 (AC15-25), as set forth as SEQ ID NO:6, which also encodes
 the Drosophila LGIC protein described in Figure 7 and set forth as SEQ ID NO:7, the
 nucleotide sequence AC15-25 as follows:

10 CGAGTAAACT GTTAAATGCT GAAGTGTCTT GAAATATTCC TTAAAACATA GAAACCTTCT
 TTGACATCTT TATAAAGCAA TAAAATTCAT TCGGGAAGTT TATGAATAGT GGTGTTATTA
 ATCATGCCAT TTGTGGCGTC AAGCTGATGG TTATGTAATC TCTGTGAAGA TTCTAGAAAT
 CCAACAGAAA TATATTGCTT CGAAAACCAA GCAAAGATTA CTTGACTGGA GAGGAAAGCT
 ATTTTCGAATT CATCTAAAAA CTGTAGCTCA CATTAAATG GTGTTCCAAA TAATAATCCT
 15 GGTGATCTGC ACCATCTGCA TGAAGCACTA CGCCAAAGGG GAGTTTCAAC AAAGTCTGGC
 CATAACCGAC ATCCTGCCCCG AGGACATCAA GCGTTACGAC AAGATGAGAC CGCCGAAGAA
 AGAGGGTCAG CCGACGATAG TCTACTTCCA TGTGACTGTG ATGGGTCTGG ACTCCATTGA
 TGAGAACTCG ATGACTTATG TGGCGGATGT GTTCTTTGCA CAGACGTGGA AGGATCATCG
 CCTGCGATTG CCGGAGAATA TGACACAGGA ATACCGCCTG CTCGAGGTGG ACTGGCTAAA
 20 AAATATGTGG CGGCCGGATT CGTTTTTCAA AAACGCCAAA TCGGTGACCT TTCAGACCAT
 GACAATACCC AATCACTATA TGTGGCTGTA CAAGGATAAG ACCATTCTGT ACATGGTCAA
 ACTAACACTG AAGCTGTCCT GCATCATGAA CTTGCGCCATT TATCCTCATG ATACACAGGA
 GTGCAAGCTG CAAATGGAAA GCCTGTCCCA CACCACGGAT GACTTGATAT TTCAGTGGGA
 TCCAACGACG CCCCTTGTGG TTGATGAAAA CATCGAGCTG CCGCAGGTGG CCCTCATCCG
 25 AAATGAAACG GCGGACTGTA CCCAAGTTTA TTCCACTGGC AACTTCACAT GCCTGGAGGT
 GGTGTTCAAC CTTAAGCGTC GTTTGGTTTA CTACGTTTTT AACACCTACA TTCCACCTG
 CATGATAGTG ATCATGTCAT GGGTATCCTT CTGGATCAAA CCGGAGGCGG CACCAGCCCCG
 TGTGACTCTG GGTGTCACCT CCTTGCTAAC GCTTTCCACG CAACACGCCA AATCGCAGTC
 GTCTTTGCCA CCTGTTTCCT ATCTCAAGGC AGTGGACGCC TTTATGTCCG TTTGCACGGT
 30 GTTCGTGTTT ATGGCCCTCA TGGAGTATTG TCTAATAAAC ATCGTCCTGA GCGACACGCC
 CATTCCCAAG CCGATGGCCT ATCCACCCAA ACCTGTGGCG GGAGATGGGC CCAAGAAAGA
 GGGCGAGGGT GCTCCTCCTG GGGGCAGCAA CTCGACGGCC AGCAAGCAAC AAGCCACCAT
 GTTGCCACTG GCCGATGAAA AGATCGAGAA AATTGAGAAG ATCTTTGACG AGATGACCAA
 GAATAGAAGG ATTGTAACCA CCACACGCCG CGTGGTGCGT CCGCCATTGG ACGCCGATGG

TCCGTGGATT CCGCGACAGG AGTCGCGGAT AATACTGACC CCGACTATCG CTCCGCCGCC
 ACCGCCCCCT CAGCCAGCGG CACCGGAACC GGAAGTGGCC AAGCCGAAAC TCACACCCGC
 CCAGGAGCGG CTCAAGCGGG CTATATATAT AGATCGGTCC TCGCGCGTCC TTTTCCCCGC
 CCTCTTCGCC AGTCTGAATG GCATCTACTG GTGTGTGTTC TACGAGTATC **TATAAGGACT**
 5 ACGACGACTG TGCCCTGTAA ATACTTTCGC TAGCTCTCTG GCACTCCATC CGAGTGTAA
 ACGTTGATTG TTCGCATATA TCGAAACGTG TATCGCAAAT TTAATCTTAA GCTTTCACGC
 ACAAGCTTTA AGTCAATGAA TTTTAAACAT AGATTATTGT TAAACCAGAA GGAAGGAATA
 ATGGTACAGA TGGAGATCTG ATTACAGGAT AAATTACAAA TTATCAATTC AATTCCTAAA
 ATGCTTAAAG TTAATCAAGT GACGTAGTAG CTGATGTAGC CTAAGTGAAT TGTA (SEQ ID
 10 NO:6) .

The above-exemplified isolated DNA molecules, shown in Figure 1, 3 and 5, respectively, comprise the following characteristics:

AC05-10 (SEQ ID NO:1):

1518 nuc. :initiating Met (nuc. 199-201) and "TGA" term. codon (nuc.1477-1479),
 15 the open reading frame resuting in an expressed protein of 426 amino acids, as set forth in SEQ ID NO:2.

AC05-11 (SEQ ID NO:3):

1506 nuc.:initiating Met (nuc. 199-201) and "TGA" term. codon (nuc. 1465-1467),
 the open reading frame resuting in an expressed protein of 422 amino acids, as set
 20 forth in SEQ ID NO:4.

AC15-4 (SEQ ID NO:5):

2133 nuc. :initiating Met (nuc. 330-332) and "TAA" term. codon (nuc. 1785-1787),
 the open reading frame resuting in an expressed protein of 485 amino acids, as set
 forth in SEQ ID NO:7.

25 AC15-25 (SEQ ID NO:6):

2034 nuc. :initiating Met (nuc. 278-280) and "TAA" term. codon (nuc. 1733-1735),
 the open reading frame resulting in an expressed protein of 485 amino acids, as set
 forth in SEQ ID NO:7.

The Ac5-10 and Ac5-11 open reading frames are identical, save for a 12
 30 nucleotide insertion within Ac5-10 which encodes a 4 amino acid insertion within the
 M3-M4 intracellular loop in Ac05-10 (a Ser-Ala-Leu-Gln insertion from amino acid
 residue 375 through amino acid residue 378, as set forth in SEQ ID NO:2).
 Therefore, the Ac5-10 protein is 426 amino acids in length while the Ac5-11 protein

is 422 amino acids in length. Expression of Ac5-10 in *Xenopus* results in a functional ion channel that responds to the addition of histamine.

Two clones of Ac15 are disclosed. Ac15-4 (2073 bp) and Ac15-25 (2034 bp) predict the same protein sequence but differ in 16 silent nucleotide changes within the 1455 nucleotide open reading frame. The expressed protein from Ac15-4/Ac15-25 is 485 amino acids in length.

The present invention also relates to biologically active fragments or mutants of SEQ ID NOs: 1, 3, 5 and 6 which encode mRNA expressing DmLGIC. Any such biologically active fragment and/or mutant will encode either a protein or protein fragment which at least substantially mimics the wild type protein, including but not limited to the wild type forms as set forth in SEQ ID NOs: 2, 4, and/or 7. Any such polynucleotide includes but is not necessarily limited to nucleotide substitutions, deletions, additions, amino-terminal truncations and carboxy-terminal truncations such that these mutations encode mRNA which express a protein or protein fragment of diagnostic, therapeutic or prophylactic use and would be useful for screening for agonists and/or antagonists for DmLGIC function.

A preferred aspect of this portion of the present invention is disclosed in Figures 1, 3, 5 and 6, which describes the four novel DNA molecules which encode three forms of DmLGIC proteins.

The isolated nucleic acid molecules of the present invention may include a deoxyribonucleic acid molecule (DNA), such as genomic DNA and complementary DNA (cDNA), which may be single (coding or noncoding strand) or double stranded, as well as synthetic DNA, such as a synthesized, single stranded polynucleotide. The isolated nucleic acid molecule of the present invention may also include a ribonucleic acid molecule (RNA).

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the construction of synthetic DNA that encodes the DmLGIC protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ ID NOs: 1, 3, 5 and 6 but still encodes the same DmLGIC protein as SEQ ID NOs: 1, 3, 5 and 6. Such synthetic DNAs are intended to be within the scope of the present invention. If it is desired to express such synthetic DNAs in a particular host cell or organism, the codon usage of such synthetic DNAs can be adjusted to reflect the codon usage of that particular host, thus

leading to higher levels of expression of the DmLGIC protein in the host. In other words, this redundancy in the various codons which code for specific amino acids is within the scope of the present invention. Therefore, this invention is also directed to those DNA sequences which encode RNA comprising alternative
 5 codons which code for the eventual translation of the identical amino acid, as shown below:

- A=Ala=Alanine: codons GCA, GCC, GCG, GCU
- C=Cys=Cysteine: codons UGC, UGU
- D=Asp=Aspartic acid: codons GAC, GAU
- 10 E=Glu=Glutamic acid: codons GAA, GAG
- F=Phe=Phenylalanine: codons UUC, UUU
- G=Gly=Glycine: codons GGA, GGC, GGG, GGU
- H=His =Histidine: codons CAC, CAU
- I=Ile =Isoleucine: codons AUA, AUC, AUU
- 15 K=Lys=Lysine: codons AAA, AAG
- L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
- M=Met=Methionine: codon AUG
- N=Asp=Asparagine: codons AAC, AAU
- P=Pro=Proline: codons CCA, CCC, CCG, CCU
- 20 Q=Gln=Glutamine: codons CAA, CAG
- R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU
- S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU
- T=Thr=Threonine: codons ACA, ACC, ACG, ACU
- V=Val=Valine: codons GUA, GUC, GUG, GUU
- 25 W=Trp=Tryptophan: codon UGG
- Y=Tyr=Tyrosine: codons UAC, UAU

Therefore, the present invention discloses codon redundancy which may result in differing DNA molecules expressing an identical protein. For purposes of this specification, a sequence bearing one or more replaced codons will be defined as a
 30 degenerate variation. Another source of sequence variation may occur through RNA editing, as discussed *infra*. Such RNA editing may result in another form of codon redundancy, wherein a change in the open reading frame does not result in an altered amino acid residue in the expressed protein. For whatever biological reason, a example can be found within the present disclosure. The cDNA clones

Ac15-4 and Ac15-25 encode a 485 amino acid protein as set forth in SEQ ID NO: 7, but 16 silent nucleotide changes occur when comparing the Ac15-25 open reading frame sequence to the Ac15-4 open reading frame sequence. Also included within the scope of this invention are mutations either in the DNA
5 sequence or the translated protein which do not substantially alter the ultimate physical properties of the expressed protein. For example, substitution of valine for leucine, arginine for lysine, or asparagine for glutamine may not cause a change in functionality of the polypeptide.

It is known that DNA sequences coding for a peptide may be altered so as
10 to code for a peptide having properties that are different than those of the naturally occurring peptide. Methods of altering the DNA sequences include but are not limited to site directed mutagenesis. Examples of altered properties include but are not limited to changes in the affinity of an enzyme for a substrate or a receptor for a ligand.

15 The present invention also relates to recombinant vectors and recombinant hosts, both prokaryotic and eukaryotic, which contain the substantially purified nucleic acid molecules disclosed throughout this specification. The nucleic acid molecules of the present invention encoding a DmLGIC protein, in whole or in part, can be linked with other DNA molecules, i.e, DNA molecules to which the DmLGIC
20 coding sequence are not naturally linked, to form "recombinant DNA molecules" which encode a respective DmLGIC protein. The novel DNA sequences of the present invention can be inserted into vectors which comprise nucleic acids encoding DmLGIC or a functional equivalent. These vectors may be comprised of DNA or RNA; for most cloning purposes DNA vectors are preferred. Typical vectors include
25 plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA that can encode a DmLGIC protein. It is well within the purview of the skilled artisan to determine an appropriate vector for a particular gene transfer or other use.

Included in the present invention are DNA sequences that hybridize to SEQ ID
30 NOs:1, 3, 5 and 6 under moderate to highly stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows: Prehybridization of filters containing DNA is carried out for 2 hours to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in

prehybridization mixture containing 100 $\mu\text{g/ml}$ denatured salmon sperm DNA and 5×10^6 cpm of ^{32}P -labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography. Other procedures using conditions of high stringency would include either a hybridization step carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 65°C for 30 to 60 minutes. Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, e.g., Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. See, e.g.,: (Computational Molecular Biology, Lesk, A. M., ed. Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exists a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo and Lipton, 1988, *SIAM J Applied Math* 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo and Lipton, 1988, *SIAM J Applied Math* 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, et al, 1984, *Nucleic Acids Research* 12(1):387), BLASTN, and FASTA (Altschul, et al., 1990, *J Mol. Biol.* 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO:1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations or alternative nucleotides per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO:1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations or alternative nucleotide substitutions of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. One source of such a "mutation" or change which results in a less than 100% identity may occur through RNA editing. The process of RNA editing results in modification of an mRNA molecule such that use of that modified mRNA as a template to generate a cloned cDNA may result in one or more nucleotide changes, which may or may not result in a codon change. This RNA editing is known to be catalyzed by an RNA editase. Such an RNA editase is RNA adenosine deaminase, which converts an adenosine residue to an inosine residue, which tends to mimic a cytosine residue. To this end, conversion of an mRNA residue from A to I will result in A to G transitions in the coding and noncoding regions of a cloned cDNA (e.g., see Hanrahan et al, 1999, *Annals New York Acad. Sci.* 868:51-66; for a review see Bass, 1997, *TIBS* 22: 157-162). Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% identity to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO:2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the

reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence of anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. Again, as noted above, RNA editing may result in a codon change which will result in an expressed protein which differs in "identity" from other proteins expressed from "non-RNA edited" transcripts, which correspond directly to the open reading frame of the genomic sequence.

The present invention also relates to a substantially purified form of a respective DmLGIC protein, which comprises the amino acid sequence disclosed in Figure 2, Figure 4 and Figure 6, and as set forth in SEQ ID NOs :2, 4 and 6, respectively. The disclosed DmLGIC proteins contain an open reading frame of 426 (SEQ ID NO: 2), 422 amino acids (SEQ ID NO: 4) and 485 (SEQ ID NO:6) amino acids in length, as shown in Figures 2, 4 and 6, and as follows:

15 MQSPTSKLVE FRCLIALAIY LHALEQSIQH CHCVHGYRNN TESAELVSHY
 ESSLSLPDIL PIPSKTYDKN RAPKLLGQPT VVYFHVTVLS LDSINEESMT
 YVTDIFLAQS WRDPRLRLPE NMSEQYRILD VDWLHSIWRP DCFFKNAKKV
 TFHEMSIPNH YLWLYHDKTL LYMSKLTLVL SCAMKFESYP HDTQICSMMI
 ELSHTVEDL VFIWNMTDPL VVNTIEIQLPQ LDISNNYTTD CTIEYSTGNF
 20 TCLAIVFNLR RRLGYHLFHT YIPSALIVVM SWISFWIKPE AIPARVTLGV
 TSLTTLATQN TQSQQSLPPV SYVKAIDVWM SSCSVFVFLS LMEFAVVNNF
 MGPVATKAMK GYSDENISDL DDLKSALQHH RESIIEPQYD TFCHGHATAI
 YIDKFSRFFF PFSFFILNIV YWTTFL* (SEQ ID NO:2);

25 MQSPTSKLVE FRCLIALAIY LHALEQSIQH CHCVHGYRNN TESAELVSHY
 ESSLSLPDIL PIPSKTYDKN RAPKLLGQPT VVYFHVTVLS LDSINEESMT
 YVTDIFLAQS WRDPRLRLPE NMSEQYRILD VDWLHSIWRP DCFFKNAKKV
 TFHEMSIPNH YLWLYHDKTL LYMSKLTLVL SCAMKFESYP HDTQICSMMI
 ELSHTVEDL VFIWNMTDPL VVNTIEIQLPQ LDISNNYTTD CTIEYSTGNF
 30 TCLAIVFNLR RRLGYHLFHT YIPSALIVVM SWISFWIKPE AIPARVTLGV
 TSLTTLATQN TQSQQSLPPV SYVKAIDVWM SSCSVFVFLS LMEFAVVNNF
 MGPVATKAMK GYSDENISDL DDLKHHRESI IEPQYDTFCH GHATAIYIDK
 FSRFFFPFSF FILNIVYWTT FL* (SEQ ID NO:4); and,

MVFQIIILVI CTICMKHYAK GEFQQSLAIT DILPEDIKRY DKMRPPKKEG
 OPTIVYFHVT VMGLDSIDEN SMTYVADVFF AQTWKDHRLR LPENMTQEYR
 LLEVDWLKNM WRPDSFFKNA KSVTFQTMTI PNHYMWLYKD KTILYMKLT
 LKLSCIMNFA IYPHDTQECK LQMESLSHTT DDLIFQWDPT TPLVVDENIE
 5 LPQVALIRNE TADCTQVYST GNFTCLEVVF TLKRRLVYYV FNTYIPTCMI
 VIMSWVSFWI KPEAAPARVT LGVTSLLTLS TQHAKSQSSL PPVSYLKAVD
 AFMSVCTVFV FMALMEYCLI NIVLSDTPIP KPMAYPPKPV AGDGPKKEGE
 GAPPGGSNST ASKQQATMLP LADEKIEKIE KIFDEMTKNR RIVTTTRRVV
 RPPLDADGPW IPRQESRIIL TPTIAPPPPP PQPAAPEPEL PKPKLTPAQE
 10 RLKRAIYIDR SSRVLFPALE ASLNGIYWCV FYEYL* (SEQ ID NO:7) .

The present invention also relates to biologically active fragments and/or
 mutants of the DmLGIC protein comprising the amino acid sequence as set forth in
 SEQ ID NOs:2, 4, and 7, including but not necessarily limited to amino acid
 substitutions, deletions, additions, amino terminal truncations and carboxy-terminal
 15 truncations such that these mutations provide for proteins or protein fragments of
 diagnostic, therapeutic or prophylactic use and would be useful for screening for
 agonists and/or antagonists of DmLGIC function.

Another preferred aspect of the present invention relates to a substantially
 purified, fully processed LGIC protein obtained from a recombinant host cell
 20 containing a DNA expression vector comprises a nucleotide sequence as set forth in
 SEQ ID NOs: 1, 3, 5, and/or 6 and expresses the respective DmLGIC precursor
 protein. It is especially preferred that the recombinant host cell be a eukaryotic host
 cell, such as a mammalian cell line, or *Xenopus* oocytes, as noted above.

As with many proteins, it is possible to modify many of the amino acids of
 25 DmLGIC protein and still retain substantially the same biological activity as the wild
 type protein. Thus this invention includes modified DmLGIC polypeptides which
 have amino acid deletions, additions, or substitutions but that still retain substantially
 the same biological activity as a respective, corresponding DmLGIC. It is generally
 accepted that single amino acid substitutions do not usually alter the biological
 30 activity of a protein (see, e.g., *Molecular Biology of the Gene*, Watson *et al.*, 1987,
 Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and
 Cunningham & Wells, 1989, *Science* 244:1081-1085). Accordingly, the present
 invention includes polypeptides where one amino acid substitution has been made in
 SEQ ID NO:2, 4, and/or 7 wherein the polypeptides still retain substantially the same

biological activity as a corresponding DmLGIC protein. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ ID NO:2, 4, or 7 wherein the polypeptides still retain substantially the same biological activity as a corresponding DmLGIC protein. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions.

One skilled in the art would also recognize that polypeptides that are functional equivalents of DmLGIC and have changes from the DmLGIC amino acid sequence that are small deletions or insertions of amino acids could also be produced by following the same guidelines, (i.e, minimizing the differences in amino acid sequence between DmLGIC and related proteins. Small deletions or insertions are generally in the range of about 1 to 5 amino acids. The effect of such small deletions or insertions on the biological activity of the modified DmLGIC polypeptide can easily be assayed by producing the polypeptide synthetically or by making the required changes in DNA encoding DmLGIC and then expressing the DNA recombinantly and assaying the protein produced by such recombinant expression.

The present invention also includes truncated forms of DmLGIC which contain the region comprising the active site of the enzyme. Such truncated proteins are useful in various assays described herein, for crystallization studies, and for structure-activity-relationship studies.

The present invention also relates to membrane-containing crude lysates or substantially purified subcellular membrane fractions from the recombinant host cells (both prokaryotic and eukaryotic as well as both stably and transiently transformed cells) which contain the nucleic acid molecules of the present invention. These recombinant host cells express DmLGIC or a functional equivalent, which becomes post translationally associated with the cell membrane in a biologically active fashion. These subcellular membrane fractions will comprise either wild-type or mutant forms of DmLGIC at levels substantially above endogenous levels and hence will be useful in assays to select modulators of DmLGIC proteins or channels. In other words, a specific use for such subcellular membranes involves expression of DmLGIC within the recombinant cell followed by isolation and substantial purification of the membranes away from other cellular components and subsequent use in assays to select for modulators, such as agonist or antagonists of the protein or biologically active channel comprising one or more of the proteins disclosed herein. Alternatively,

the lysed cells, containing the membranes, may be used directly in assays to select for modulators of the recombinantly expressed protein(s) disclosed herein. Therefore, another preferred aspect of the present invention relates to a substantially purified membrane preparation or lysed recombinant cell components which include
5 membranes, which has been obtained from a recombinant host cell transformed or transfected with a DNA expression vector which comprises and appropriately expresses a complete open reading frame as set forth in SEQ ID NOs: 1, 3, 5, and/or 6, resulting in a functional, processed form of the respective single, homomultimer or heteromultimer DmLGIC receptor. It is especially preferred is that
10 the recombinant host cell be a eukaryotic host cell, such as a mammalian cell line, or *Xenopus* oocytes, as noted above.

To this end, a preferred aspect of the present invention is a functional DmLGIC channel receptor, comprised of either a single channel protein or a channel comprising multiple subunits, referred to herein as a homomultimer channel or a
15 heteromultimer channel. Therefore, a single channel may be comprised of a protein as disclosed in SEQ ID NOs: 2, 4 or 7, or a biologically active equivalent thereof (i.e., a altered channel protein which still functions in a similar fashion to that of a wild-type channel receptor). A homomultimer channel receptor complex will comprise more than one polypeptide selected from the disclosed group of SEQ ID NOs: 2, 4
20 and 7, as well as biologically active equivalents. A heteromultimer channel receptor complex will comprise multiple subunits wherein at least 2 of the 3 proteins disclosed herein contribute to channel formation, or where at least one of the proteins associates with additional proteins or channel components to provide for an active channel receptor complex. Therefore, the present invention additionally relates to
25 substantially purified channels as described herein, as well as substantially purified membrane preparations, partially purified membrane preparations, or cell lysates which contain the functional single, homomultimer or heteromultimer channels described herein.

The present invention also relates to isolated nucleic acid molecules which are
30 fusion constructions expressing fusion proteins useful in assays to identify compounds which modulate wild-type DmLGIC activity, as well as generating antibodies against DmLGIC. One aspect of this portion of the invention includes, but is not limited to, glutathione S-transferase (GST)-DmLGIC fusion constructs. Recombinant GST-DmLGIC fusion proteins may be expressed in various expression systems, including

Spodoptera frugiperda (Sf21) insect cells (Invitrogen) using a baculovirus expression vector (pAcG2T, Pharmingen). Another aspect involves DmLGIC fusion constructs linked to various markers, including but not limited to GFP (Green fluorescent protein), the MYC epitope, and GST. Again, any such fusion constructs may be
5 expressed in the cell line of interest and used to screen for modulators of one or more of the DmLGIC proteins disclosed herein.

A preferred aspect for screening for modulators of DmLGIC activity is an expression system for the electrophysiological-based assays for measuring ligand-gated ion channel activity comprising injecting the DNA molecules of the present
10 invention into *Xenopus laevis* oocytes. The general use of *Xenopus* oocytes in the study of ion channel activity is known in the art (Dascal, 1987, *Crit. Rev. Biochem.* 22: 317-317; Lester, 1988, *Science* 241: 1057-1063; see also *Methods of Enzymology*, Vol. 207, 1992, Ch. 14-25, Rudy and Iverson, ed., Academic Press, Inc., New York). An improved method exists for measuring channel activity and modulation by
15 agonists and/or antagonists which is several-fold more sensitive than previous techniques. The *Xenopus* oocytes are injected with nucleic acid material, including but not limited to DNA, mRNA or cRNA which encode a gated-channel, wherein channel activity may be measured as well as response of the channel to various modulators. Ion channel activity is measured by utilizing a holding potential more
20 positive than the reversal potential for chloride (i.e., greater than -30 mV), preferably about 0 mV. This alteration in assay measurement conditions has resulting in a 10-fold increase in sensitivity of the assay to modulation by ivermectin phosphate. Therefore, this improved assay allows screening and selecting for compounds which modulate LGIC activity at levels which were previously thought to be undetectable.

25 Any of a variety of procedures may be used to clone DmLGIC. These methods include, but are not limited to, (1) a RACE PCR cloning technique (Frohman, et al., 1988, *Proc. Natl. Acad. Sci. USA* 85: 8998-9002). 5' and/or 3' RACE may be performed to generate a full-length cDNA sequence. This strategy involves using gene-specific oligonucleotide primers for PCR amplification of
30 DmLGIC cDNA. These gene-specific primers are designed through identification of an expressed sequence tag (EST) nucleotide sequence which has been identified by searching any number of publicly available nucleic acid and protein databases; (2) direct functional expression of the DmLGIC cDNA following the construction of a DmLGIC-containing cDNA library in an appropriate expression vector system;

(3) screening a DmLGIC-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labeled degenerate oligonucleotide probe designed from the amino acid sequence of the DmLGIC protein; (4) screening a DmLGIC-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding the DmLGIC protein. This partial cDNA is obtained by the specific PCR amplification of DmLGIC DNA fragments through the design of degenerate oligonucleotide primers from the amino acid sequence known for other kinases which are related to the DmLGIC protein; (5) screening a DmLGIC-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA or oligonucleotide with homology to a mammalian DmLGIC protein. This strategy may also involve using gene-specific oligonucleotide primers for PCR amplification of DmLGIC cDNA identified as an EST as described above; or (6) designing 5' and 3' gene specific oligonucleotides using SEQ ID NO: 1, 3, and 5 as a template so that either the full-length cDNA may be generated by known RACE techniques, or a portion of the coding region may be generated by these same known RACE techniques to generate and isolate a portion of the coding region to use as a probe to screen one of numerous types of cDNA and/or genomic libraries in order to isolate a full-length version of the nucleotide sequence encoding DmLGIC. Alternatively, the DmLGIC cDNA may be cloned as described in Example Section 1. Briefly, partial sequences potentially encoding two novel ligand gated ion channel genes, AC05 and AC15, were identified in the *Drosophila* genome sequencing project using the Extended Smith Waterman algorithm. The query sequence was the *C. elegans* glutamate gated ion channel *avr-15a* peptide sequence (accession number-AJ000538), and the DNA database searched was publicly available *Drosophila* high throughput genomic sequences. The search was performed on a Compugen Biocel XLP hardware search engine (Petach Tikva, Israel). Both sequences entered into the database contained predicted introns. Primers specific to either Ac05 or Ac15 were designed based on the database sequences. With these primer combinations, RT-PCR on whole fly total RNA followed by TA cloning was performed for both genes. Fragments of approximately 500bp in length for both Ac05 and Ac15 were isolated and verified by DNA sequencing. PolyA⁺ RNA was purified from whole body Oregon R *Drosophila* and used to generate the double-stranded cDNA. 5' and 3' RACE fragments were obtained for both genes by 1st round PCR and nested PCR. The resulting fragment sizes were ~1.3 kb for Ac05 and ~1.8 kb for Ac15 in 3'-

RACE. In 5'- RACE Ac05 and Ac15 both have fragment sizes of ~1kb. The PCR products were cloned into a pCR2.1-TOPO vector. Miniprep DNA samples were screened by restriction digestion to separate spliced from unspliced clones. Using the sequences obtained from the 5' and 3' RACE products, PCR primers for both genes were designed to generate full-length clones. cDNA clones Ac05-10 and Ac05-11 were generated using primers Ac05 F1 and R1 for 1st round PCR and primers Ac05 F1 and R2 for 2nd round PCR. cDNA clones Ac15-4 and Ac15-25 were generated using primers Ac15 F1 and R1 for 1st round PCR.. The PCR products were cloned into pCR2.1-TOPO vector. Two clones of Ac05 were identified: Ac05-10 (1518 bp) and Ac05-11 (1506 bp). The clones are identical but for a 4 amino acid insertion within the M3-M4 intracellular loop in Ac05-10 (a Ser-Ala-Leu-Gln insertion from amino acid residue 375 through amino acid residue 378, as set forth in SEQ ID NO:2). Two clones of Ac15 were identified: Ac15-4 (2073 bp) and Ac15-25 (2034 bp), which predict the same protein sequence but differ in 16 nucleotides within 1455 nucleotide open reading frame.

It is readily apparent to those skilled in the art that other types of libraries, as well as libraries constructed from other cell types-or species types, may be useful for isolating a DmLGIC-encoding DNA or a DmLGIC homologue. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have DmLGIC activity. The selection of cells or cell lines for use in preparing a cDNA library to isolate a cDNA encoding DmLGIC may be done by first measuring cell-associated DmLGIC activity using any known assay available for such a purpose.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. Complementary DNA libraries may also be obtained from numerous commercial sources, including but not limited to Clontech Laboratories, Inc. and Stratagene.

It is also readily apparent to those skilled in the art that DNA encoding DmLGIC may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techniques can be found in

Sambrook, et al., *supra*. One may prepare genomic libraries, especially in P1 artificial chromosome vectors, from which genomic clones containing the DmLGIC can be isolated, using probes based upon the DmLGIC nucleotide sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou et al., 1994, 5 *Nature Genet.* 6:84-89).

In order to clone a DmLGIC gene by one of the preferred methods, the amino acid sequence or DNA sequence of a DmLGIC or a homologous protein may be necessary. To accomplish this, a respective DmLGIC protein may be purified and the partial amino acid sequence determined by automated sequenators. It is not necessary 10 to determine the entire amino acid sequence, but the linear sequence of two regions of 6 to 8 amino acids can be determined for the PCR amplification of a partial DmLGIC DNA fragment. Once suitable amino acid sequences have been identified, the DNA sequences capable of encoding them are synthesized. Because the genetic code is degenerate, more than one codon may be used to encode a particular amino acid, and 15 therefore, the amino acid sequence can be encoded by any of a set of similar DNA oligonucleotides. Only one member of the set will be identical to the DmLGIC sequence but others in the set will be capable of hybridizing to DmLGIC DNA even in the presence of DNA oligonucleotides with mismatches. The mismatched DNA oligonucleotides may still sufficiently hybridize to the DmLGIC DNA to permit 20 identification and isolation of DmLGIC encoding DNA. Alternatively, the nucleotide sequence of a region of an expressed sequence may be identified by searching one or more available genomic databases. Gene-specific primers may be used to perform PCR amplification of a cDNA of interest from either a cDNA library or a population of cDNAs. As noted above, the appropriate nucleotide sequence for use in a PCR- 25 based method may be obtained from SEQ ID NO: 1, 3, 5 or 6 either for the purpose of isolating overlapping 5' and 3' RACE products for generation of a full-length sequence coding for DmLGIC, or to isolate a portion of the nucleotide sequence coding for DmLGIC for use as a probe to screen one or more cDNA- or genomic-based libraries to isolate a full-length sequence encoding DmLGIC or DmLGIC-like 30 proteins.

This invention also includes vectors containing a DmLGIC gene, host cells containing the vectors, and methods of making substantially pure DmLGIC protein comprising the steps of introducing the DmLGIC gene into a host cell, and cultivating the host cell under appropriate conditions such that DmLGIC is produced. The

DmLGIC so produced may be harvested from the host cells in conventional ways. Therefore, the present invention also relates to methods of expressing the DmLGIC protein and biological equivalents disclosed herein, assays employing these gene products, recombinant host cells which comprise DNA constructs which express these proteins, and compounds identified through these assays which act as agonists or antagonists of DmLGIC activity.

The cloned DmLGIC cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector (such as pcDNA3.neo, pcDNA3.1, pCR2.1, pCR2.1-TOPO, pBlueBacHis2 or pLITMUS28, as well as other examples, listed *infra*) containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant DmLGIC. Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned DNA and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic DNA in a variety of hosts such as bacteria, blue green algae, plant cells, insect cells and animal cells. Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast or bacteria-animal cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. To determine the DmLGIC cDNA sequence(s) that yields optimal levels of DmLGIC, cDNA molecules including but not limited to the following can be constructed: a cDNA fragment containing the full-length open reading frame for DmLGIC as well as various constructs containing portions of the cDNA encoding only specific domains of the protein or rearranged domains of the protein. All constructs can be designed to contain none, all or portions of the 5' and/or 3' untranslated region of a DmLGIC cDNA. The expression levels and activity of DmLGIC can be determined following the introduction, both singly and in combination, of these constructs into appropriate host cells. Following determination of the DmLGIC cDNA cassette yielding optimal expression in transient assays, this DmLGIC cDNA construct is transferred to a variety of expression vectors (including recombinant viruses), including but not

limited to those for mammalian cells, plant cells, insect cells, oocytes, bacteria, and yeast cells. Techniques for such manipulations can be found described in Sambrook, et al., *supra*, are well known and available to the artisan of ordinary skill in the art. Therefore, another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding the DmLGIC. An expression vector containing DNA encoding a DmLGIC-like protein may be used for expression of DmLGIC in a recombinant host cell. Such recombinant host cells can be cultured under suitable conditions to produce DmLGIC or a biologically equivalent form. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses. Commercially available mammalian expression vectors which may be suitable for recombinant DmLGIC expression, include but are not limited to, pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pCI-neo (Promega), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Biolabs), pcDNAI, pcDNAIamp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lZD35 (ATCC 37565). Also, a variety of bacterial expression vectors may be used to express recombinant DmLGIC in bacterial cells. Commercially available bacterial expression vectors which may be suitable for recombinant DmLGIC expression include, but are not limited to pCR2.1 (Invitrogen), pET11a (Novagen), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia). In addition, a variety of fungal cell expression vectors may be used to express recombinant DmLGIC in fungal cells. Commercially available fungal cell expression vectors which may be suitable for recombinant DmLGIC expression include but are not limited to pYES2 (Invitrogen) and *Pichia* expression vector (Invitrogen). Also, a variety of insect cell expression vectors may be used to express recombinant protein in insect cells. Commercially available insect cell expression vectors which may be suitable for recombinant expression of DmLGIC include but are not limited to pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (PharMingen).

Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey and rodent origin;

and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. For instance, one insect expression system utilizes *Spodoptera frugiperda* (Sf21) insect cells (Invitrogen) in tandem with a baculovirus expression vector (pAcG2T, Pharmingen). Also, mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), Saos-2 (ATCC HTB-85), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26), MRC-5 (ATCC CCL 171) and CPAE (ATCC CCL 209).

The specificity of binding of compounds showing affinity for DmLGIC is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to DmLGIC or that inhibit the binding of a known, radiolabeled ligand of DmLGIC to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for DmLGIC. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of DmLGIC and may be peptides, proteins, or non-proteinaceous organic molecules.

Accordingly, the present invention is directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a DmLGIC protein as well as compounds which effect the function of the DmLGIC protein. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of a DmLGIC channel. For example, Cascieri et al. (1992, *Molec. Pharmacol.* 41:1096-1099) describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled agonist of the neurokinin receptors either in the presence or the absence of the

substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor. Where binding of the substance such as an agonist or antagonist to DmLGIC is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

As noted above in regard to the use of *Xenopus* oocytes to express a DmLGIC gene of interest, the present invention is directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a DmLGIC protein. Compounds which modulate these activities may be DNA, RNA, peptides, proteins, or non-proteinaceous organic molecules. Compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding DmLGIC, or the function of the DmLGIC-based channels. Compounds that modulate the expression of DNA or RNA encoding DmLGIC or the biological function (i.e., channel activation by histamine or other ligands and/or compounds which activate the wild type channel) thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function (i.e., effect of channel activity) of a test sample with the levels of expression or function in a standard sample. Kits containing DmLGIC, antibodies to DmLGIC, or modified DmLGIC may be prepared by known methods for such uses.

To this end, the present invention relates in part to methods of identifying a substance which modulates DmLGIC receptor activity, which involves:

- (a) combining a test substance in the presence and absence of a DmLGIC receptor protein wherein said DmLGIC receptor protein comprises the amino acid sequence as set forth in SEQ ID NO: 4, and/or 7; and,
- (b) measuring and comparing the effect of the test substance in the presence and absence of the DmLGIC receptor protein.

In addition, several specific embodiments are disclosed herein to show the diverse type of screening or selection assay which the skilled artisan may utilize in tandem with an expression vector directing the expression of the DmLGIC receptor protein. Methods for identifying agonists and antagonists of other receptors are well

known in the art and can be adapted to identify agonists and antagonists of DmLGIC. Therefore, these embodiments are presented as examples and not as limitations. To this end, the present invention includes assays by which DmLGIC modulators (such as agonists and antagonists) may be identified. Accordingly, the present invention
5 includes a method for determining whether a substance is a potential agonist or antagonist of DmLGIC that comprises:

- (a) . transfecting or transforming cells with an expression vector that directs expression of DmLGIC in the cells, resulting in test cells;
 - (b) allowing the test cells to grow for a time sufficient to allow DmLGIC
10 to be expressed and for a functional channel to be generated;
 - (c) exposing the cells to a labeled ligand of DmLGIC in the presence and in the absence of the substance;
 - (d) measuring the binding of the labeled ligand to the DmLGIC channel;
- where if the amount of binding of the labeled ligand is less in the presence of the
15 substance than in the absence of the substance, then the substance is a potential agonist or antagonist of DmLGIC.

The conditions under which step (c) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used
20 buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C. The test cells may be harvested and resuspended in the presence of the substance and the labeled ligand. In a modification of the above-described method, step (c) is modified in that the cells are not harvested and resuspended but rather the radioactively labeled known agonist and the substance are contacted with the cells
25 while the cells are attached to a substratum, e.g., tissue culture plates.

The present invention also includes a method for determining whether a substance is capable of binding to DmLGIC, i.e., whether the substance is a potential agonist or an antagonist of DmLGIC channel activation, where the method comprises:

- (a) transfecting or transforming cells with an expression vector that directs
30 the expression of DmLGIC in the cells, resulting in test cells;
- (b) exposing the test cells to the substance;
- (c) measuring the amount of binding of the substance to DmLGIC;

(d) comparing the amount of binding of the substance to DmLGIC in the test cells with the amount of binding of the substance to control cells that have not been transfected with DmLGIC;

wherein if the amount of binding of the substance is greater in the test cells as compared to the control cells, the substance is capable of binding to DmLGIC.

Determining whether the substance is actually an agonist or antagonist can then be accomplished by the use of functional assays such as, e.g., the assay involving the use of promiscuous G-proteins described below.

The conditions under which step (b) of the method is practiced are conditions that are typically used in the art for the study of protein-ligand interactions: e.g., physiological pH; salt conditions such as those represented by such commonly used buffers as PBS or in tissue culture media; a temperature of about 4°C to about 55°C. The test cells are harvested and resuspended in the presence of the substance.

The above described assays may be functional assays, where electrophysiological assays (e.g., see Example 2) may be carried out in transfected mammalian cell lines as well as *Xenopus* oocytes to measure the various effects test compounds may have on the ability of a known ligand (such as histamine or glutamate) to activate the channel, or for a test compound to modulate activity in and of itself (similar to the effect of ivermectin on known GluCl channels). Therefore, the skilled artisan will be comfortable adapting the cDNA clones of the present invention to known methodology to both initially and secondary screens to select for compounds that bind and/or activate the functional DmLGIC channels of the present invention.

The DNA molecules, RNA molecules, recombinant protein and antibodies of the present invention may be used to screen and measure levels of DmLGIC. The recombinant proteins, DNA molecules, RNA molecules and antibodies lend themselves to the formulation of kits suitable for the detection and typing of DmLGIC. Such a kit would comprise a compartmentalized carrier suitable to hold in close confinement at least one container. The carrier would further comprise reagents such as recombinant DmLGIC or anti-DmLGIC antibodies suitable for detecting DmLGIC. The carrier may also contain a means for detection such as labeled antigen or enzyme substrates or the like.

The assays described above can be carried out with cells that have been transiently or stably transfected with DmLGIC. The expression vector may be

introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, protoplast fusion, and electroporation. Transfection is meant to include any method known in the art for introducing DmLGIC into the test cells. For example, transfection includes calcium phosphate or calcium chloride mediated transfection, lipofection, infection with a retroviral construct containing DmLGIC, and electroporation. The expression vector-containing cells are individually analyzed to determine whether they produce DmLGIC protein. Identification of DmLGIC expressing cells may be done by several means, including but not limited to immunological reactivity with anti-DmLGIC antibodies, labeled ligand binding, and/or the presence of host cell-associated DmLGIC activity.

The specificity of binding of compounds showing affinity for DmLGIC is shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to DmLGIC or that inhibit the binding of a known, radiolabeled ligand of DmLGIC to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for DmLGIC. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method are likely to be agonists or antagonists of DmLGIC and may be peptides, proteins, or non-proteinaceous organic molecules.

Accordingly, the present invention is directed to methods for screening for compounds which modulate the expression of DNA or RNA encoding a DmLGIC protein as well as compounds which effect the function of the DmLGIC protein. Methods for identifying agonists and antagonists of other receptors are well known in the art and can be adapted to identify agonists and antagonists of DmLGIC. For example, Cascieri et al. (1992, *Molec. Pharmacol.* 41:1096-1099) describe a method for identifying substances that inhibit agonist binding to rat neurokinin receptors and thus are potential agonists or antagonists of neurokinin receptors. The method involves transfecting COS cells with expression vectors containing rat neurokinin receptors, allowing the transfected cells to grow for a time sufficient to allow the neurokinin receptors to be expressed, harvesting the transfected cells and resuspending the cells in assay buffer containing a known radioactively labeled

agonist of the neurokinin receptors either in the presence or the absence of the substance, and then measuring the binding of the radioactively labeled known agonist of the neurokinin receptor to the neurokinin receptor. If the amount of binding of the known agonist is less in the presence of the substance than in the absence of the substance, then the substance is a potential agonist or antagonist of the neurokinin receptor. Where binding of the substance such as an agonist or antagonist to is measured, such binding can be measured by employing a labeled substance or agonist. The substance or agonist can be labeled in any convenient manner known to the art, e.g., radioactively, fluorescently, enzymatically.

Therefore, the specificity of binding of compounds having affinity for DmLGIC shown by measuring the affinity of the compounds for recombinant cells expressing the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that bind to DmLGIC or that inhibit the binding of a known, radiolabeled ligand of DmLGIC (such as glutamate, ivermectin or nodulasporic acid) to these cells, or membranes prepared from these cells, provides an effective method for the rapid selection of compounds with high affinity for DmLGIC. Such ligands need not necessarily be radiolabeled but can also be nonisotopic compounds that can be used to displace bound radiolabeled compounds or that can be used as activators in functional assays. Compounds identified by the above method again are likely to be agonists or antagonists of DmLGIC and may be peptides, proteins, or non-proteinaceous organic molecules. As noted elsewhere in this specification, compounds may modulate by increasing or attenuating the expression of DNA or RNA encoding DmLGIC, or by acting as an agonist or antagonist of the DmLGIC receptor protein. Again, these compounds that modulate the expression of DNA or RNA encoding DmLGIC or the biological function thereof may be detected by a variety of assays. The assay may be a simple "yes/no" assay to determine whether there is a change in expression or function. The assay may be made quantitative by comparing the expression or function of a test sample with the levels of expression or function in a standard sample.

Expression of DmLGIC DNA may also be performed using *in vitro* produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being

preferred.

Following expression of DmLGIC in a host cell, DmLGIC protein may be recovered to provide DmLGIC protein in active form. Several DmLGIC protein purification procedures are available and suitable for use. Recombinant DmLGIC protein may be purified from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography and hydrophobic interaction chromatography. In addition, recombinant DmLGIC protein can be separated from other cellular proteins by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for full-length DmLGIC protein, or polypeptide fragments of DmLGIC protein.

Polyclonal or monoclonal antibodies may be raised against DmLGIC or a synthetic peptide (usually from about 9 to about 25 amino acids in length) from a portion of DmLGIC as disclosed in SEQ ID NOs:2, 4, and/or 7. Monospecific antibodies to DmLGIC are purified from mammalian antisera containing antibodies reactive against DmLGIC or are prepared as monoclonal antibodies reactive with DmLGIC using the technique of Kohler and Milstein (1975, *Nature* 256: 495-497). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for DmLGIC. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with DmLGIC, as described above. Human DmLGIC-specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with an appropriate concentration of DmLGIC protein or a synthetic peptide generated from a portion of DmLGIC with or without an immune adjuvant.

Preimmune serum is collected prior to the first immunization. Each animal receives between about 0.1 mg and about 1000 mg of DmLGIC protein associated with an acceptable immune adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete, alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of DmLGIC protein or peptide fragment thereof in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the

initial immunization. Those animals receiving booster injections are generally given an equal amount of DmLGIC in Freund's incomplete adjuvant by the same route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a
5 single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20°C.

Monoclonal antibodies (mAb) reactive with DmLGIC are prepared by immunizing inbred mice, preferably Balb/c, with DmLGIC protein. The mice are immunized by the IP or SC route with about 1 mg to about 100 mg, preferably about
10 10 mg, of DmLGIC protein in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 1 to about 100 mg of DmLGIC in a buffer solution such as phosphate
15 buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable
20 hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody producing cells and myeloma cells are fused in polyethylene glycol, about 1000 mol. wt., at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin
25 supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid phase immunoradioassay (SPIRA) using DmLGIC as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype
30 of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, 1973, Soft Agar Techniques, in *Tissue Culture Methods and Applications*, Kruse and Paterson, Eds., Academic Press.

Monoclonal antibodies are produced *in vivo* by injection of pristine primed Balb/c mice, approximately 0.5 ml per mouse, with about 2×10^6 to about 6×10^6

hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art.

5 *In vitro* production of anti-DmLGIC mAb is carried out by growing the hybridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, but are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of DmLGIC in body fluids or tissue and cell extracts.

It is readily apparent to those skilled in the art that the above described methods for producing monospecific antibodies may be utilized to produce antibodies specific for DmLGIC peptide fragments, or a respective full-length DmLGIC.

15 DmLGIC antibody affinity columns are made, for example, by adding the antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M
20 ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in phosphate buffered saline (pH 7.3) and the cell culture supernatants or cell extracts containing full-length DmLGIC or DmLGIC protein fragments are slowly passed through the column. The column is then washed
25 with phosphate buffered saline until the optical density (A_{280}) falls to background, then the protein is eluted with 0.23 M glycine-HCl (pH 2.6). The purified DmLGIC protein is then dialyzed against phosphate buffered saline.

The present invention also relates to a non-human transgenic animal which is useful for studying the ability of a variety of compounds to act as modulators of
30 DmLGIC, or any alternative functional DmLGIC *in vivo* by providing cells for culture, *in vitro*. In reference to the transgenic animals of this invention, reference is made to transgenes and genes. As used herein, a transgene is a genetic construct including a gene. The transgene is integrated into one or more chromosomes in the cells in an animal by methods known in the art. Once integrated, the transgene is

carried in at least one place in the chromosomes of a transgenic animal. Of course, a gene is a nucleotide sequence that encodes a protein, such as one or a combination of the cDNA clones described herein. The gene and/or transgene may also include genetic regulatory elements and/or structural elements known in the art. A type of target cell for transgene introduction is the embryonic stem cell (ES). ES cells can be obtained from pre-implantation embryos cultured *in vitro* and fused with embryos (Evans et al., 1981, *Nature* 292:154-156; Bradley et al., 1984, *Nature* 309:255-258; Gossler et al., 1986, *Proc. Natl. Acad. Sci. USA* 83:9065-9069; and Robertson et al., 1986 *Nature* 322:445-448). Transgenes can be efficiently introduced into the ES cells by a variety of standard techniques such as DNA transfection, microinjection, or by retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal (Jaenisch, 1988, *Science* 240: 1468-1474). It will also be within the purview of the skilled artisan to produce transgenic or knock-out invertebrate animals (e.g., *C. elegans*) which express the DmLGIC transgene in a wild type *C. elegans* LGIC background as well in *C. elegans* mutants knocked out for one or both of the *C. elegans* LGIC subunits.

Pharmaceutically useful compositions comprising modulators of DmLGIC may be formulated according to known methods such as by the admixture of a pharmaceutically acceptable carrier. Examples of such carriers and methods of formulation may be found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the protein, DNA, RNA, modified DmLGIC, or either DmLGIC agonists or antagonists including tyrosine kinase activators or inhibitors.

Therapeutic or diagnostic compositions of the invention are administered to an individual in amounts sufficient to treat or diagnose disorders. The effective amount may vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration.

The pharmaceutical compositions may be provided to the individual by a variety of routes such as subcutaneous, topical, oral and intramuscular.

The term "chemical derivative" describes a molecule that contains additional chemical moieties which are not normally a part of the base molecule. Such moieties

may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Examples of such moieties are described in a variety of texts, such as Remington's Pharmaceutical Sciences.

5 Compounds identified according to the methods disclosed herein may be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents may be desirable.

 The present invention also has the objective of providing suitable topical, oral, systemic and parenteral pharmaceutical formulations for use in the novel methods of
10 treatment of the present invention. The compositions containing compounds identified according to this invention as the active ingredient can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compounds can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations),
15 pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection. Likewise, they may also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

20 Advantageously, compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily. Furthermore, compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to
25 those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen.

 For combination treatment with more than one active agent, where the active agents are in separate dosage formulations, the active agents can be administered
30 concurrently, or they each can be administered at separately staggered times.

 The dosage regimen utilizing the compounds of the present invention is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal, hepatic and cardiovascular function of the

patient; and the particular compound thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

The following examples are provided to illustrate the present invention without, however, limiting the same hereto.

10

EXAMPLE 1

Isolation and Characterization of DNA Molecules Encoding DmLGIC

The molecular procedures were performed following standard procedures well known in the art available in references such as Ausubel et. al. (1992, Short protocols in molecular biology. F.M. Ausubel et al., -2nd. ed. (John Wiley & Sons)) and Sambrook et al.(1989, Molecular cloning. A laboratory manual. J. Sambrook, E.F. Fritsch, and T. Maniatis -2nd ed. (Cold Spring Harbor Laboratory Press)).

Ac05 and Ac15 Database Search - Partial sequences potentially encoding two novel ligand gated ion channel genes, AC05 and AC15, were identified in the *Drosophila* genome sequencing project using the Extended Smith Waterman algorithm. The query sequence was the *C. elegans* glutamate gated ion channel *avr-15a* peptide sequence (accession number-AJ000538), and the DNA database searched was publicly available *Drosophila* high throughput genomic sequences. The search was performed on a Compugen Biocel XLP hardware search engine (Petach Tikva, Israel).

Both sequences entered into the database contained predicted introns. Primers specific to either 05 or 15 were designed based on the database sequences and synthesized. They are as follows::

1. ac05F1: 5'- CTT GCA CAA AGC TGG CGT G -3' [SEQ ID NO:8],
(ac007805)
2. ac05F2: 5'- GTG AGC AGT ATC GCA TAT TG -3' [SEQ ID NO:9],
(ac007805;
3. ac05R1: 5'- GTA GTT ATT TGA TAT GTC TAG C -3' [SEQ ID NO:10],
(ac007805);

30

4. ac05R2: 5'- ACC TGT TGA GTA CTC TAT AG -3' [SEQ ID NO:11],
(ac007805);
5. ac15F1 5'- TTT GCA CAG ACG TGG AAG G - 3' [SEQ ID NO:12],
(ac007815);
- 5 6. ac15F2 5'- ACA GGA ATA CCG CCT GCT C -3' [SEQ ID NO:13],
(ac007815); and,
7. ac15R1 5'- TTC ATT TCG GAT GAG GGC CAC -3' [SEQ ID NO:14]
(ac007815);

With these primer combinations, RT-PCR on whole fly total RNA followed by TA
10 cloning was performed for both genes. Fragment of approximately 500bp for both
Ac05 and Ac15 were isolated and verified by sequencing.

5' and 3' RACE for Ac05 and Ac15 - The Marathon[®] cDNA Amplification Kit
from Clontech (Palo Alto, CA) was used as the primary tool for both 5'- and 3'-
RACE reactions. PolyA⁺ RNA was purified from whole body Oregon R *Drosophila*
15 by Oligotex[®] mRNA Midi Kit (Qiagen, Santa Clarita, CA) and used to generate the
double-stranded cDNA following the manufacturer's protocol. The following primers
were used for RACE reactions:

3'-RACE forward primers:

Ac05:

- 20 1. Ac05GSPF1 - 5'-CAT CTT CCT TGC ACA AAG CTG GCG TG-3' [SEQ ID
NO:15], (ac007805);
2. Ac05NGSPF2 - 5'- CAT GAG TGA GCA GTA TCG CAT ATT G -3' [SEQ ID
NO:16], (ac007805);

Ac15:

- 25 1. Ac15GSPF1 - 5'-TGT GTT CTT TGC ACA GAC GTG GAA GG -3' [SEQ ID
NO:17] (ac007815).
2. Ac15NGSPF2 5'- TAT GAC ACA GGA ATA CCG CCT GCT C -3' [SEQ ID
NO:18] (ac007815).

5'-RACE reverse primers:

30 Ac05:

1. Ac05GSPR1: 5'- GTC TAG CTG CGG CAA CTC AAT CTC CGT G -3' [SEQ ID
NO:19], (ac007805);
2. Ac05NGSPR2: 5'- CTC GAT CAT CAT GGA GCA GAT TTG CGT G -3' [SEQ
ID NO:20], (ac007805).

Ac15:

1. Ac15GSPR1: 5'- CGC CGT TTC ATT TCG GAT GAG GGC CAC-3' [SEQ ID NO:21], (ac007815 84958 bp - 84984 bp);
2. Ac15NGSPR2: 5'- CAG GCT TTC CAT TTG CAG CTT GCA CTC C-3' [SEQ ID NO:22], (ac007815). This primer spans a splice junction, and the existence of this continuous sequence was available only from the sequence data of the Ac15 fragment described above.

5' and 3' RACE fragments were obtained for both genes by 1st round PCR and nested PCR based on the protocol of the Marathon[®] Kit, with a modification of the 5'RACE PCR cycle: 1 cycle of 1 minute at 94°C; 5 cycles of 1 minute at 94°C and 4 minutes at 72°C; and 25 cycles of 1 minute at 94°C, 1 minutes at 68°C, and 3 minute at 72°C. The resulting fragment sizes were ~1.3 kb for Ac05 and ~1.8 kb for Ac15 in 3'- RACE. In 5'- RACE Ac05 and Ac15 both have fragment sizes of ~1kb. The PCR products were cloned into pCR2.1-TOPO vector using the TOPO[®] TA Cloning Kit (Invitrogen, Carlsbad, CA). Miniprep DNA samples were screened by restriction digestion to separate spliced from unspliced clones. For the 3' ends, 6 and 8 samples of Ac05 and Ac15, respectively, were sequenced. For the 5' ends, 5 and 8 samples of Ac05 and Ac15 were sequenced.

Generation of Full-Length Clones - Using the sequences obtained from the 5' and 3' RACE products, PCR primers for both genes were designed to generate full-length clones. Forward primers and reverse primers for both Ac05 and Ac15 were designed as follows::

Ac05FullF1: 5'-CAA TCG TCG CGA TAA CTC TGC CG-3' [SEQ ID NO:23];
 Ac05FullR1: 5'-CCT TTA TTT ATA CAC TAC ATG GTA ATC-3' [SEQ ID NO:24]; Ac05FullR2: 5'-TGT TTA CGC TCT ATT CCT TCG GAG-3' [SEQ ID NO:25]; Ac15FullF1: 5'-AAC TGC CAA GAC GTT TAG AAC GG-3' [SEQ ID NO:26]; Ac15FullF2: 5'-CGA GTA AAC TGT TAA ATG CTG AAG TG-3' [SEQ ID NO:27]; Ac15FullR1: 5'-TAC AAT TCA CTT AGG CTA CAT CAG C-3' [SEQ ID NO:28]; and, Ac15FullR2: 5'-GGC TAC ATC AGC TAC TAC GTC AC-3' [SEQ ID NO:29].

The Advantage[®] 2 PCR Kit (Clontech, Palo Alto, CA) was used for 1st and 2nd round PCR. cDNA clones Ac05-10 and Ac05-11 were generated using primers Ac05 F1 and R1 for 1st round PCR and primers Ac05 F1 and R2 for 2nd round PCR. cDNA clones Ac15-4 and Ac15-25 were generated using primers Ac15 F1 and R1 for

1st round PCR. 1st round PCR conditions were as follows: 1 cycle of 2 min at 94° C; 5 cycles of 30sec at 94° C, and 2min 30sec at 72° C; 25 cycles at 30sec at 94° C, 1min at 68° C, and 1min 30sec at 72° C; and 1 cycle at 5min at 72° C. For 2nd round PCR, primers Ac15 F1 and R2 were used for clone Ac15-4, and primers Ac15 F2 and R1 were used for clone Ac15-25. 2µl of the first PCR product were used as template in a total reaction volume of 50 µl. 2nd round PCR conditions were as follows: 1 cycle of 2 min at 94° C; 5 cycles of 30sec at 94° C, 1min at 68° C, and 1min 30sec at 72° C; 5 cycles of 30sec at 94° C, 1min at 65° C, and 1min 30sec at 72° C; 20 cycles of 30sec at 94° C, 1min at 60° C, and 1min 30sec at 72° C; and 1 cycle of 5min at 72° C. One major band of ~1.5kb was isolated for Ac05 and ~2kb for Ac15. The PCR products were cloned into pCR2.1-TOPO vector using the TOPO[®] TA Cloning Kit (Invitrogene, Carlsbad, CA).

Two clones of Ac05 were identified: Ac05-10 (1518 bp) and Ac05-11 (1506 bp). The clones are identical but for a 4 amino acid insertion within the M3-M4 intracellular loop in Ac05-10. Two clones of Ac15 have been identified: Ac15-4 (2073 bp) and Ac15-25 (2034 bp), which predict the same protein sequence but differ in 16 nucleotides.

Synthesis of in vitro transcribed capped RNA - A PCR strategy was used to add both the T7 promoter upstream of the initiating methionine (ATG), and a polyA⁺ tail following the stop codon (TGA and TAA for Ac05 and Ac15) of the open reading frame (ORF) of clones Ac05-10, Ac05-11 and Ac15-4, Ac15-25. The primers employed are:

Ac05:

Ac05T7: 5'-TAA TAC GAC TCA CTA TAG GGA GGG TGT TCA TAA TGC AAA GCC -3' [SEQ ID NO:30]; and, Ac05dT, 5'-TTT TTT TTT TTT TTT TTT TTC ATA GGA ACG TTG TCC AAT AGA C-3' [SEQ ID NO:31].

Ac15:

AC15T7: 5'-TAA TAC GAC TCA CTA TAG GGA GGC ACA TTA AAA TGG TGT TC-3' [SEQ ID NO:32]; and, AC15dT: 5'-TTT TTT TTT TTT TTT TTT TTC CTT ATA GAT ACT CGT AGA AC-3' [SEQ ID NO:33].

Amplified ORFs which contained both the T7 promoter and polyA⁺ tail were purified using the Qiaquick PCR Purification Kit (Qiagen, Germany), and used directly as templates in the *in vitro* transcription reaction (mMessage mMachine[™], Ambion, Austin, TX) following the manufacturer's protocol. After removal of the DNA

template, the RNA was extracted with phenol/CHCl₃, precipitated with LiCl, and resuspended in nuclease-free water at a storage concentration of 0.5 µg/µl.

EXAMPLE 2

5 Functional expression of DmLGICs clones in *Xenopus* oocytes

Full length cDNA clones corresponding to the selected RT-PCR sequences were used as template for synthesis of *in vitro* transcribed RNA (Ambion Inc.). The capped cRNA transcripts are synthesized using appropriate oligonucleotide primers and the mMESSAGE mMACHINE *in vitro* RNA transcription kit from Ambion.

10 *Xenopus laevis* oocytes were prepared and injected using standard methods as described (Arena et al., 1991, *Mol. Pharmacol.* 40: 368-374; Arena et al, 1992, *Mol. Brain Res.* 15: 339-348). Adult female *Xenopus laevis* were anesthetized with 0.17% tricaine methanesulfonate and the ovaries were surgically removed and placed in a dish consisting of (mM): NaCl 82.5, KCl 2, MgCl₂ 1, CaCl₂ 1.8, HEPES 5, adjusted to pH 7.5 with NaOH (OR-2). Ovarian lobes were broken open, rinsed several times, and gently shaken in OR-2 containing 0.2% collagenase (Sigma, Type 1A) for 2-5 hours. When approximately 50% of the follicular layers were removed, Stage V and VI oocytes were selected and placed in media consisting of (mM): NaCl 86, KCl 2, MgCl₂ 1, CaCl₂ 1.8, HEPES 5, Na pyruvate 2.5, theophylline 0.5, gentamicin 0.1

15 adjusted to pH 7.5 with NaOH (ND-96) for 24-48 hours before injection. For most experiments, oocytes were injected with 10 ng of cRNA in 50 nl of RNase free water. Control oocytes were injected with 50 nl of water. Oocytes were incubated for 1-5 days in ND-96 supplemented with 50 mg/ml gentamycin, 2.5 mM Na pyruvate and 0.5 mM theophylline before recording. Incubations and collagenase digestion were

25 carried out at 18° C.

Voltage-clamp studies were conducted with the two microelectrode voltage clamp technique using a Dagan CA1 amplifier (Dagan Instruments, Minneapolis, MN). The current passing microelectrodes were filled with 0.7 M KCl plus 1.7 M K₃-citrate and the voltage recording microelectrodes were filled with 1.0 M KCl. The

30 extracellular solution for most experiments was saline consisting of (mM): NaCl 96, BaCl₂ 3.5, MgCl₂ 0.5, CaCl₂ 0.1, HEPES 5, adjusted to pH 7.5 with NaOH. The extracellular chloride concentration was reduced in some experiments by equimolar replacement of NaCl with the sodium salt of the indicated anion. Experiments were conducted at 21-24° C. Data were acquired using the program Pulse and most

analysis was performed with the companion program Pulsefit (Instrutech Instruments, Great Neck, NY) or with Igor Pro (Wavemetrics, Lake Oswego, OR). Data were filtered (f_c , -3db) at 1 kHz, unless otherwise indicated. Figure 8 shows the results of the experiment in which the clone DmLGIC AC05 clone was expressed in a *Xenopus* oocyte. The measurement was made as described in this Example with the two microelectrode voltage clamp technique and the membrane potential was held at 0 mV. The bar at top shows the duration of application of histamine. This indicates that expression of this protein reconstitutes a functional ion channel that responds to the addition of histamine.

Expression of the AC15 clone in a *Xenopus* oocyte also forms a functional single channel protein which, as with AC05, responds to the addition of histamine.

EXAMPLE 3

Functional expression of DmLGICs clones in Mammalian Cells

A DmLGIC may be subcloned into a mammalian expression vector and used to transfect the mammalian cell line of choice. Stable cell clones are selected by growth in the presence of G418. Single G418 resistant clones are isolated and tested to confirm the presence of an intact DmLGIC gene. Clones containing the DmLGICs are then analyzed for expression using immunological techniques, such as immunoprecipitation, Western blot, and immunofluorescence using antibodies specific to the DmLGIC proteins. Antibody is obtained from rabbits inoculated with peptides that are synthesized from the amino acid sequence predicted from the DmLGIC sequences. Expression is also analyzed using patch clamp electrophysiological techniques and an anion flux assay.

Cells that are expressing DmLGIC stably or transiently, are used to test for expression of active channel proteins. These cells are used to identify and examine other compounds for their ability to modulate, inhibit or activate the respective channel.

Cassettes containing the DmLGIC cDNA in the positive orientation with respect to the promoter are ligated into appropriate restriction sites 3' of the promoter and identified by restriction site mapping and/or sequencing. These cDNA expression vectors may be introduced into fibroblastic host cells, for example, COS-7 (ATCC# CRL1651), and CV-1 tat [Sackevitz et al., 1987, *Science* 238: 1575], 293, L (ATCC# CRL6362) by standard methods including but not

limited to electroporation, or chemical procedures (cationic liposomes, DEAE dextran, calcium phosphate). Transfected cells and cell culture supernatants can be harvested and analyzed for DmLGIC expression as described herein.

5 All of the vectors used for mammalian transient expression can be used to establish stable cell lines expressing DmLGIC. Unaltered DmLGIC cDNA constructs cloned into expression vectors are expected to program host cells to make DmLGIC protein. In addition, DmLGIC is expressed extracellularly as a secreted protein by ligating DmLGIC cDNA constructs to DNA encoding the signal sequence of a secreted protein. The transfection host cells include, but are
10 not limited to, CV-1-P [Sackevitz et al., 1987, *Science* 238: 1575], tk-L [Wigler, et al., 1977, *Cell* 11: 223], NS/0, and dHFr- CHO [Kaufman and Sharp, 1982, *J. Mol. Biol.* 159: 601].

Co-transfection of any vector containing a DmLGIC cDNA with a drug selection plasmid including, but not limited to G418, aminoglycoside
15 phosphotransferase; hygromycin, hygromycin-B phosphotransferase; APRT, xanthine-guanine phosphoribosyl-transferase, will allow for the selection of stably transfected clones. Levels of DmLGIC are quantitated by the assays described herein. DmLGIC cDNA constructs may also be ligated into vectors containing amplifiable drug-resistance markers for the production of mammalian cell clones
20 synthesizing the highest possible levels of DmLGIC. Following introduction of these constructs into cells, clones containing the plasmid are selected with the appropriate agent, and isolation of an over-expressing clone with a high copy number of plasmids is accomplished by selection with increasing doses of the agent. The expression of recombinant DmLGIC is achieved by transfection of
25 full-length DmLGIC cDNA into a mammalian host cell.

EXAMPLE 4

Cloning of DmLGIC cDNA into a Baculovirus Expression Vector for Expression in Insect Cells

30 Baculovirus vectors, which are derived from the genome of the AcNPV virus, are designed to provide high level expression of cDNA in the Sf9 line of insect cells (ATCC CRL# 1711). A recombinant baculovirus expressing DmLGIC cDNA is produced by the following standard methods (In Vitrogen Maxbac Manual): the DmLGIC cDNA constructs are ligated into the polyhedrin

gene in a variety of baculovirus transfer vectors, including the pAC360 and the BlueBac vector (InVitrogen). Recombinant baculoviruses are generated by homologous recombination following co-transfection of the baculovirus transfer vector and linearized AcNPV genomic DNA [Kitts, 1990, *Nuc. Acid. Res.* 18: 5667] into Sf9-cells. Recombinant pAC360 viruses are identified by the absence of inclusion bodies in infected cells and recombinant pBlueBac viruses are identified on the basis of b-galactosidase expression (Summers, M. D. and Smith, G. E., Texas Agriculture Exp. Station Bulletin No. 1555). Following plaque purification, DmLGIC expression is measured by the assays described herein.

10 The cDNA encoding the entire open reading frame for DmLGIC is inserted into the BamHI site of pBlueBacII. Constructs in the positive orientation are identified by sequence analysis and used to transfect Sf9 cells in the presence of linear AcNPV mild type DNA.

Authentic, active DmLGIC is found in the cytoplasm of infected cells.
15 Active DmLGIC is extracted from infected cells by hypotonic or detergent lysis.

EXAMPLE 5

Cloning of DmLGIC cDNA into a yeast expression vector

Recombinant DmLGIC is produced in the yeast *S. cerevisiae* following the insertion of the optimal DmLGIC cDNA cistron into expression vectors designed to direct the intracellular or extracellular expression of heterologous proteins. In the case of intracellular expression, vectors such as EmBLyex4 or the like are ligated to the DmLGIC cistron [Rinas, et al., 1990, *Biotechnology* 8: 543-545; Horowitz B. et al., 1989, *J. Biol. Chem.* 265: 4189-4192]. For extracellular
25 expression, the DmLGIC cistron is ligated into yeast expression vectors which fuse a secretion signal (a yeast or mammalian peptide) to the NH₂ terminus of the DmLGIC protein [Jacobson, 1989, *Gene* 85: 511-516; Riett and Bellon, 1989, *Biochem.* 28: 2941-2949].

These vectors include, but are not limited to pAVE1-6, which fuses the human serum albumin signal to the expressed cDNA [Steep, 1990, *Biotechnology* 8: 42-46], and the vector pL8PL which fuses the human lysozyme signal to the expressed cDNA [Yamamoto, *Biochem.* 28: 2728-2732)]. In addition, DmLGIC is expressed in yeast as a fusion protein conjugated to ubiquitin utilizing the vector pVEP [Ecker, 1989, *J. Biol. Chem.* 264: 7715-7719,

Sabin, 1989 *Biotechnology* 7: 705-709, McDonnell, 1989, *Mol. Cell Biol.* 9: 5517-5523 (1989)]. The levels of expressed DmLGIC are determined by the assays described herein.

EXAMPLE 6

5 Purification of Recombinant DmLGIC

Recombinantly produced DmLGIC may be purified by antibody affinity chromatography. DmLGIC antibody affinity columns are made by adding the anti-DmLGIC antibodies to Affigel-10 (Biorad), a gel support which is pre-activated with N-hydroxysuccinimide esters such that the antibodies form
10 covalent linkages with the agarose gel bead support. The antibodies are then coupled to the gel via amide bonds with the spacer arm. The remaining activated esters are then quenched with 1M ethanolamine HCl (pH 8). The column is washed with water followed by 0.23 M glycine HCl (pH 2.6) to remove any non-conjugated antibody or extraneous protein. The column is then equilibrated in
15 phosphate buffered saline (pH 7.3) together with appropriate membrane solubilizing agents such as detergents and the cell culture supernatants or cell extracts containing solubilized DmLGIC are slowly passed through the column. The column is then washed with phosphate- buffered saline together with detergents until the optical density (A280) falls to background, then the protein is
20 eluted with 0.23 M glycine-HCl (pH 2.6) together with detergents. The purified DmLGIC protein is then dialyzed against phosphate buffered saline.

EXAMPLE 7

Purification of Recombinant DmLGIC

25 According to the Drosophila genome sequencing project, Ac007815 [Ac15] and Ac007805 [Ac05] map to chromosome III; specifically Ac15 to ChIII 92B and Ac05 to ChIII 87B. DrosGluClalpha1 (glc-1), maps to ChIII 92B, as does Ac15. O'Tousa et al. (1989, *J. Neurogenetics* 6: 41-52) map photoreceptor mutations to the ChIII 92B region of the Drosophila genome. In addition, Stuart (1999, *Neuron* 22:431-433)
30 notes that histamine is a potential invertebrate retinal neurotransmitter. Therefore, the data generated in Example section 2 herein in combination with the chromosomal location of Ac15 suggests that the LGIC disclosed herein are at least partially effective as being responsive to histamine.

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CLAIMS:

1. A purified nucleic acid molecule encoding a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein, wherein said protein comprises an amino acid sequence as set forth in SEQ ID NO:2.
2. An expression vector for expressing a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 1.
3. A host cell which expresses a recombinant *Drosophila* Ligand-Gated Ion Channel (LGIC) protein wherein said host cell contains the expression vector of claim 2.
4. A process for expressing a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein in a recombinant host cell, comprising:
 - (a) transfecting the expression vector of claim 2 into a suitable host cell; and,
 - (b) culturing the host cells of step (a) under conditions which allow expression of said *Drosophila* (LGIC) protein from said expression vector.
5. A purified DNA molecule encoding a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein which comprises a nucleotide sequence as set forth in SEQ ID NO:1.
6. The DNA molecule of claim 5 containing from nucleotide 199 to nucleotide 1479 of SEQ ID NO:1.
7. A purified nucleic acid molecule encoding a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein, wherein said protein comprises an amino acid sequence as set forth in SEQ ID NO:4.
8. An expression vector for expressing a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein in a recombinant host cell wherein said expression vector comprises a DNA molecule of claim 7.

9. A host cell which expresses a recombinant *Drosophila* Ligand-Gated Ion Channel (LGIC) protein wherein said host cell contains the expression vector of claim 8.
10. A process for expressing a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein in a recombinant host cell, comprising:
 - (a) transfecting the expression vector of claim 8 into a suitable host cell; and,
 - (b) culturing the host cells of step (a) under conditions which allow expression of said *Drosophila* (LGIC) protein from said expression vector.
11. A purified DNA molecule encoding a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein which comprises a nucleotide sequence as set forth in SEQ ID NO:3.
12. The DNA molecule of claim 11 containing from nucleotide 199 to nucleotide 1467 of SEQ ID NO:3.
13. A *Drosophila* Ligand-Gated Ion Channel (LGIC) protein substantially free from other proteins which comprise an amino acid sequence as set forth in SEQ ID NO:2.
14. A *Drosophila* Ligand-Gated Ion Channel (LGIC) protein of claim 13 which is a product of a DNA expression vector contained within a recombinant host cell.
15. A substantially pure membrane preparation comprising the *Drosophila* Ligand-Gated Ion Channel (LGIC) protein purified from the recombinant host cell of claim 14.
16. A *Drosophila* Ligand-Gated Ion Channel (LGIC) protein substantially free from other proteins which comprise an amino acid sequence as set forth in SEQ ID NO:4.

17. A *Drosophila* Ligand-Gated Ion Channel (LGIC) protein of claim 16 which is a product of a DNA expression vector contained within a recombinant host cell.
18. A substantially pure membrane preparation comprising the *Drosophila* Ligand-Gated Ion Channel (LGIC) protein purified from the recombinant host cell of claim 17.
19. A *Drosophila* Ligand-Gated Ion Channel (LGIC) protein which consists of an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO:2 and SEQ ID NO:4.
20. A *Drosophila* heteromultimer Ligand-Gated Ion Channel (LGIC) substantially free from other proteins which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.
21. A *Drosophila* Ligand-Gated Ion Channel (LGIC) protein of claim 20 which is a product of a DNA expression vector contained within a recombinant host cell..
22. A substantially pure membrane preparation comprising the *Drosophila* Ligand-Gated Ion Channel (LGIC) protein purified from the recombinant host cell of claim 21.
23. A *Drosophila* heteromultimer Ligand-Gated Ion Channel (LGIC) receptor substantially free from other proteins which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.
24. A *Drosophila* Ligand-Gated Ion Channel (LGIC) protein of claim 23 which is a product of a DNA expression vector contained within a recombinant host cell.
25. A substantially pure membrane preparation comprising the *Drosophila* Ligand-Gated Ion Channel (LGIC) protein purified from the recombinant host cell of claim 24.

26. A method of identifying a modulator of a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein, comprising:

- (a) contacting a labelled ligand of a *Drosophila* LGIC protein selected from the group consisting of SEQ ID NO:2 and SEQ ID NO: 4 in the presence and in the absence of a test compound; and
- (b) measuring the binding of the labelled ligand to the LGIC protein wherein if the amount of binding of the labelled ligand is less in the presence of the test compound than in the absence of the test compound, the test compound is a potential modulator of the LGIC.

27. The method of claim 26 wherein the *Drosophila* Ligand-Gated Ion Channel (LGIC) protein of step (a) is a product of a DNA expression vector contained within a recombinant host cell.

28. A method of identifying a compound that modulates histamine-gated channel protein activity, which comprises:

- (a) injecting into a host cell solution a population of nucleic acid molecules, at least a portion of which encodes a *Drosophila* Ligand-Gated Ion Channel (LGIC) protein selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4, such that expression of said portion of nucleic acid molecules results in an active histamine-gated channel;
- (b) adding a test compound into said solution; and
- (c) measuring host cell membrane current at a holding potential more positive than the reversal potential for chloride.

29. The method of claim 28 wherein said nucleic acid molecules are selected from the group consisting of complementary DNA, poly A⁺ messenger RNA and complementary RNA.

1/8

1 CAATCGTCGC GATAACTCTG CCGTTTCTTT ATTGGTTTTT GCTGCGCGAC
51 GAGTAAAATA TAATTCCTCG CTTACTAATC CTCCGAGCAA GTTCATTCTC
101 AAGCGCACCC AGAGATGAGC TACTTTGGGA ATTGACATGG ACTGCGGAGC
151 AATGAGTGCC AGAGGAACAA TATCAAAGCC GAAGGTAGTG TGTTCATAAT
201 GCAAAGCCCA ACTAGCAAAT TGGTAGAATT CAGGTGCCTT ATTGCGTTGG
251 CAATATATTT GCACGCGCTG GAGCAATCGA TCCAGCACTG CCATTGTGTT
301 CATGGTTACA GAAATAACAC GGAGAGCGCC GAGCTGGTCT CCCACTACGA
351 GTCGAGTCTT TCGCTCCCGG ACATTTTGCC CATTCCCTCA AAGACGTACG
401 ACAAGAACCG GGCTCCCAAG CTCCTCGGCC AGCCACAGT AGTCTACTTC
451 CATGTCACGG TCCTCTCCCT GGACTCCATT AACGAGGAGT CTATGACCTA
501 TGTGACGGAC ATCTTCCTTG CACAAAGCTG GCGTGATCCT CGCCTGCGGT
551 TGCCTGAGAA CATGAGTGAG CAGTATCGCA TATTGGATGT CGACTGGTTG
601 CACAGCATTT GGCGGCCCGA TTGCTTCTTT AAGAACGCCA AAAAGGTCAC
651 CTTCCATGAG ATGAGCATTC CCAATCACTA TCTCTGGCTG TACCACGACA
701 AAACGCTGCT CTATATGTCC AAATCACGT TGGTCCTGTC GTGCGCCATG
751 AAGTTTGAGT CCTATCCGCA TGACACGCAA ATCTGCTCCA TGATGATCGA
801 GAGTTTATCC CACACGGTGG AAGATTTGGT TTTCATTTGG AACATGACCG
851 ACCCACTTGT GGTTAACACG GAGATTGAGT TGCCGCAGCT AGACATATCA
901 AATAACTACA CAACCGACTG TACTATAGAG TACTCAACAG GTAAC TTCAC
951 CTGCCTGGCC ATTGTGTTCA ACCTGCGCCG ACGCCTGGGT TACCATTTGT
1001 TCCACACCTA CATCCCCTCG GCTCTGATTG TGGTCATGTC TTGGATATCG
1051 TTTTGGATAA AACCAGAAGC GATACCCGCC CGTGTAATC TGGGAGTGAC
1101 CTCACTGCTA ACCCTGGCCA CCCAGAATAC CCAGTCGCAA CAATCGCTGC
1151 CGCCGGTTTC GTATGTCAAG GCTATAGACG TCTGGATGTC GTCCTGTTTCG
1201 GTGTTTGTAT TCCTTTCTCT GATGGAATTT GCAGTGGTCA ACAATTTTAT
1251 GGGACCGGTG GCCACAAAGG CAATGAAGGG GTATTCGGAC GAGAACATCA
1301 GTGATCTGGA CGACCTAAAG TCTGCACTAC AGCATCATCG GGAATCGATT
1351 ATTGAGCCCC AGTACGACAC TTTCTGCCAT GGCCATGCCA CAGCCATTTA
1401 TATAGACAAA TTCTCGCGCT TTTTCTTCCC GTTTTCGTTC TTTATACTCA
1451 ATATTGTCTA TTGGACAACG TTCCTATGAT GGATGGAAAA GTTTCTCCGA
1501 AGGAATAGAG CGTAAACA (SEQ ID NO:1).

FIG. 1

2/8

1 MQSPTSKLVE FRCLIALAIY LHALEQSIQH CHCVHGYRNN TESAELVSHY
51 ESSLSLPDIL PIPSKTYDKN RAPKLLGQPT VVYFHVTVLS LDSINEESMT
101 YVTDIFLAQS WRDPRLRLPE NMSEQYRILD VDWLHSIWRP DCFFKNAKKV
151 TFHEMSIPNH YLWLYHDKTL LYMSKLTLVL SCAMKFESYP HDTQICSMMI
201 ESLSHTVEDL VFIWNMTDPL VVNTEIELPQ LDISNNYTTD CTIEYSTGNF
251 TCLAIVFNLR RRLGYHLFHT YIPSALIVVM SWISFWIKPE AIPARVTLGV
301 TSLLTLATQN TQSQQSLPPV SYVKAIDVWM SSCSVFVFLS LMEFAVVNNF
351 MGPVATKAMK GYSDENISDL DDLKSALQHH RESIIEPQYD TFCHGHATAI
401 YIDKFSRFFF PFSFFILNIV YWTTFL* (SEQ ID NO:2).

FIG.2

3/8

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1   CAATCGTCGC GATAACTCTG CCGTTTCTTT ATTGGTTTTT GCTGCGCGAC
51  GAGTAAAATA TAATTCCTCG CTTACTAATC CTCCGAGCAA GTTCATTCTC
101 AAGCGCACCC AGAGATGAGC TACTTTGGGA ATTGACATGG ACTGCGGAGC
151 AATGAGTGCC AGAGGAACAA TATCAAAGCC GAAGGTAGTG TGTTCATAAT
201 GCAAAGCCCA ACTAGCAAAT TGGTAGAATT CAGGTGCCTT ATTGCGTTGG
251 CAATATATTT GCACGCGCTG GAGCAATCGA TCCAGCACTG CCATTGTGTT
301 CATGGTTACA GAAATAACAC GGAGAGCGCC GAGCTGGTCT CCCACTACGA
351 GTCGAGTCTT TCGCTCCCGG ACATTTTGCC CATTCCCTCA AAGACGTACG
401 ACAAGAACCG GGCTCCCAAG CTCCTCGGCC AGCCCACAGT AGTCTACTTC
451 CATGTCACGG TCCTCTCCCT GGACTCCATT AACGAGGAGT CTATGACCTA
501 TGTGACGGAC ATCTTCCTTG CACAAAGCTG GCGTGATCCT CGCCTGCGGT
551 TGCCTGAGAA CATGAGTGAG CAGTATCGCA TATTGGATGT CGACTGGTTG
601 CACAGCATTT GGCGGCCCGA TTGCTTCTTT AAGAACGCCA AAAAGGTCAC
651 CTTCCATGAG ATGAGCATTC CCAATCACTA TCTCTGGCTG TACCACGACA
701 AAACGCTGCT CTATATGTCC AACTCACGT TGGTCCTGTC GTGCGCCATG
751 AAGTTTGAGT CCTATCCGCA TGACACGCAA ATCTGCTCCA TGATGATCGA
801 GAGTTTATCC CACACGGTGG AAGATTTGGT TTTCATTTGG AACATGACCG
851 ACCCACTTGT GGTTAACACG GAGATTGAGT TGCCGCAGCT AGACATATCA
901 AATAACTACA CAACCGACTG TACTATAGAG TACTCAACAG GTAAETTCAC
951 CTGCCTGGCC ATTGTGTTCA ACCTGCGCCG ACGCCTGGGT TACCATTTGT
1001 TCCACACCTA CATCCCCTCG GCTCTGATTG TGGTCATGTC TTGGATATCG
1051 TTTTGGATAA AACCAGAAGC GATACCCGCC CGTGTAAGTC TGGGAGTGAC
1101 CTCACTGCTA ACCCTGGCCA CCCAGAATAC CCAGTCGCAA CAATCGCTGC
1151 CGCCGGTTTC GTATGTCAAG GCTATAGACG TCTGGATGTC GTCCTGTTTCG
1201 GTGTTTGTAT TCCTTTCTCT GATGGAATTT GCAGTGGTCA ACAATTTTAT
1251 GGGACCGGTG GCCACAAAGG CAATGAAGGG GTATTCGGAC GAGAACATCA
1301 GTGATCTGGA CGACCTAAAG CATCATCGGG AATCGATTAT TGAGCCCCAG
1351 TACGACACTT TCTGCCATGG CCATGCCACA GCCATTTATA TAGACAAATT
1401 CTCGCGCTTT TTCTTCCCGT TTTCGTTCTT TATACTCAAT ATTGTCTATT
1451 GGACAACGTT CCTATGATGG ATGGAAAAGT TTCTCCGAAG GAATAGAGCG
1501 TAAACA (SEQ ID NO:3).
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FIG.3

4/8

1 MQSPTSKLVE FRCLIALAIY LHALEQSIQH CHCVHGYRNN TESAELVSHY
51 ESSLSPDIL PIPSKTYDKN RAPKLLGQPT VVYFHVTVLS LDSINEESMT
101 YVTDIFLAQS WRDPRLRLPE NMSEQYRILD VDWLHSIWRP DCFFKNAKKV
151 TFHEMSIPNH YLWLYHDKTL LYMSKLTLLV SCAMKFESYP HDTQICSMMI
201 ESSLHTVEDL VFIWNMTDPL VVNTIEIQLPQ LDISNNYTTD CTIEYSTGNF
251 TCLAIIVNLR RRLGYHLFHT YIPSALIVVM SWISFWIKPE AIPARVTLGV
301 TSLTTLATQN TQSQQSLPPV SYVKAIDVWM SSCSVFVFLS LMEFAVVNNF
351 MGPVATKAMK GYSDENISDL DDLKHHRESI IEPQYDTFCH GHATAIYIDK
401 FSRFFFPFSF FILNIVYWTT FL* (SEQ ID NO:4).

FIG.4

5/8

AC15-4

AACTGCCAAG ACGTTTAGAA CGGAAAAACT GAATTTTCAA AAATATTTTCG AGTAAACTGT
TAAATGCTGA AGTGTTCTGA AATATTCCTT AAAACATAGA AACCTTCTTT GACATCTTTA
TAAAGCAATA AAATTCATTC GGGAAAGTTTA TGAATAGTGG TGTTATTAAT CATGCCATTT
GTGGCGTCAA GCTGATGGTT ATGTAATCTC TGTGAAGATT CTAGAAATCC AACAGAAATA
TATTGCTTCG AAAACCAAGC AAAGATTACT TGACTGGAGA GGAAAGCTAT TTCGAATTCA
TCTAAAAACT GTAAAGAGTT CACATTAAAA **TGGTGTTC**CA AATAATAATC CTGGTGATCT
GCACCATCTG CATGAAGCAC TACGCCAAAG GGGAGTTTCA ACAAAGTCTG GCCATAACCG
ACATCCTGCC CGAGGACATC AAGCGTTACG ACAAGATGAG ACCGCCGAAG AAAGAGGGTC
AGCCGACGAT AGTCTACTTC CATGTGACTG TGATGGGTCT GGACTCCATT GATGAGAACT
CGATGACTTA TGTGGCGGAT GTGTTCTTTG CACAGACGTG GAAGGATCAT CGCCTGCGAT
TGCCGGAGAA TATGACACAG GAATACCGCC TGCTCGAGGT GGACTGGCTA AAAAATATGT
GGCGCCCGGA TTCGTTTTTC AAAAACGCCA AATCGGTGAC CTTTCAGACC ATGACAATAC
CCAATCACTA TATGTGGCTG TACAAGGATA AGACCATTCT CTATATGGTC AAGCTAACAC
TGAAGCTGTC CTGCATCATG AATTTCGCCA TTTATCCTCA TGACACACAG GAGTGCAAGC
TGCAAATGGA AAGCCTGTCC CACACCACGG ATGACTTGAT ATTCCAGTGG GATCCAACAA
CGCCCCTTGT GGTTGATGAA AACATCGAAC TGCCGCAGGT GGCCCTCATC CGGAATGAAA
CGGCGGACTG CACCCAGGTT TATTCCACTG GCAACTTCAC ATGCCTGGAG GTGGTGTTCA
CCCTTAAGCG TCGTTTGGTT TACTACGTTT TCAACACCTA CATTCCCACC TGCATGATAG
TGATCATGTC ATGGGTATCC TTCTGGATCA AACC GGAGGC GGCACCAGCC CGTGTGACTC
TGGGTGTCAC CTCCTTGCTA ACGCTTTCCA CGCAACACGC CAAATCGCAG TCGTCTTTGC
CACCTGTTTC CTATCTCAAG GCAGTGGACG CCTTTATGTC CGTTTGCACG GTGTTCGTGT
TTATGGCCCT CATGGAGTAT TGTCTAATAA ACATCGTCCT GAGCGACACG CCCATTCCCA
AGCCGATGGC TTATCCACCC AAACCTGTGG CGGGCGATGG GCCCAAGAAA GAGGGCGAGG
GTGCTCCTCC TGGGGGCAGC AACTCGACGG CCAGCAAGCA ACAAGCCACC ATGTTGCCAC
TGGCCGATGA AAAGATCGAG AAAATTGAGA AGATCTTTGA CGAGATGACC AAGAATAGAA
GGATTGTAAC CACCACACGC CGCGTGGTGC GTCCACCATT GGACGCCGAT GGTCCGTGGA
TTCCGCGACA GGAGTCGCGG ATAATACTGA CCCC GACTAT CGCTCCGCCG CCACCGCCCC
CTCAGCCAGC GGCACCGGAA CCGGA ACTAC CCAAGCCGAA ACTCACACCC GCCCAGGAGC
GGCTCAAGCG GGCTATATAT ATAGATCGGT CCTCGCGCGT CCTTTTCCCC GCCCTCTTCG
CCAGTCTGAA TGGCATCTAC TGGTGTGTGT TCTACGAGTA TCTAT**A**AGGA CTACGACGAC
TGTGCCCTGT AAATACTTTC GCTAGCTCTC TGGCACTCCA TCCGAGTGTT AAACGTTGAT
TGTTTCGCATA TATCGAAACG TGTATCGCAA ATTTAATCTT AAGCTTTCAC GCACAAGCTT
TAAGTCAATG AATTTTAAAC ATAGATTATT GTTAAACCAG AAGGAAGGAA TAATGGTACA
GATGGAGATC TGATTACAGG ATAAATTACA AATTATCAAT TCAATTCCTA AAATGCTTAA
AGTTAATCAA GTGACGTAGT AGCTGATGTA GCC (SEQ ID NO:5).

FIG.5

6/8

AC15-25

CGAGTAAACT GTTAAATGCT GAAGTGTTCT GAAATATTCC TTAAAACATA GAAACCTTCT
 TTGACATCTT TATAAAGCAA TAAAATTCAT TCGGGAAGTT TATGAATAGT GGTGTTATTA
 ATCATGCCAT TTGTGGCGTC AAGCTGATGG TTATGTAATC TCTGTGAAGA TTCTAGAAAT
 CCAACAGAAA TATATTGCTT CGAAAACCAA GCAAAGATTA CTTGACTGGA GAGGAAAGCT
 ATTTCGAATT CATCTAAAAA CTGTAGCTCA CATTAAA**ATG** GTGTTCCAAA TAATAATCCT
 GGTGATCTGC ACCATCTGCA TGAAGCACTA CGCCAAAGGG GAGTTTCAAC AAAGTCTGGC
 CATAACCGAC ATCCTGCCCC AGGACATCAA GCGTTACGAC AAGATGAGAC CGCCGAAGAA
 AGAGGGTCAG CCGACGATAG TCTACTTCCA TGTGACTGTG ATGGGTCTGG ACTCCATTGA
 TGAGAACTCG ATGACTTATG TGGCGGATGT GTTCTTTGCA CAGACGTGGA AGGATCATCG
 CCTGCGATTG CCGGAGAATA TGACACAGGA ATACCGCCTG CTCGAGGTGG ACTGGCTAAA
 AAATATGTGG CGGCCGGATT CGTTTTTCAA AAACGCCAAA TCGGTGACCT TTCAGACCAT
 GACAATACCC AATCACTATA TGTGGCTGTA CAAGGATAAG ACCATTCTGT ACATGGTCAA
 ACTAACACTG AAGCTGTCCT GCATCATGAA CTTCGCCATT TATCCTCATG ATACACAGGA
 GTGCAAGCTG CAAATGGAAA GCCTGTCCCA CACCACGGAT GACTTGATAT TTCAGTGGGA
 TCCAACGACG CCCCTTGTGG TTGATGAAAA CATCGAGCTG CCGCAGGTGG CCCTCATCCG
 AAATGAAACG GCGGACTGTA CCAAGTTTA TTCCACTGGC AACTTCACAT GCCTGGAGGT
 GGTGTTCAAC CTTAAGCGTC GTTTGGTTTA CTACGTTTTT AACACCTACA TTCCCACCTG
 CATGATAGTG ATCATGTCAT GGGTATCCTT CTGGATCAAA CCGGAGGCGG CACCAGCCCG
 TGTGACTCTG GGTGTCACCT CTTGCTAAC GCTTTCCACG CAACACGCCA AATCGCAGTC
 GTCTTTGCCA CCTGTTTCCT ATCTCAAGGC AGTGGACGCC TTTATGTCCG TTTGCACGGT
 GTTCGTGTTT ATGGCCCTCA TGGAGTATTG TCTAATAAAC ATCGTCCTGA GCGACACGCC
 CATTCCCAAG CCGATGGCCT ATCCACCCAA ACCTGTGGCG GGAGATGGGC CCAAGAAAGA
 GGGCGAGGGT GCTCCTCCTG GGGGCAGCAA CTCGACGGCC AGCAAGCAAC AAGCCACCAT
 GTTGCCACTG GCCGATGAAA AGATCGAGAA AATTGAGAAG ATCTTTGACG AGATGACCAA
 GAATAGAAGG ATTGTAACCA CCACACGCCG CGTGGTGCGT CCGCCATTGG ACGCCGATGG
 TCCGTGGATT CCGCGACAGG AGTCGCGGAT AATACTGACC CCGACTATCG CTCCGCCGCC
 ACCGCCCCCT CAGCCAGCGG CACCGGAACC GGAAGTGGCC AAGCCGAAAC TCACACCCGC
 CCAGGAGCGG CTCAAGCGGG CTATATATAT AGATCGGTCC TCGCGCGTCC TTTTCCCCGC
 CCTCTTCGCC AGTCTGAATG GCATCTACTG GTGTGTGTTT TACGAGTATC **TATAAGG**ACT
 ACGACGACTG TGCCCTGTAA ATACTTTCGC TAGCTCTCTG GCACTCCATC CGAGTGTTAA
 ACGTTGATTG TTCGCATATA TCGAAACGTG TATCGCAAAT TTAATCTTAA GCTTTCACGC
 ACAAGCTTTA AGTCAATGAA TTTTAAACAT AGATTATTGT TAAACCAGAA GGAAGGAATA
 ATGGTACAGA TGGAGATCTG ATTACAGGAT AAATTACAAA TTATCAATTC AATTCCTAAA
 ATGCTTAAAG TTAATCAAGT GACGTAGTAG CTGATGTAGC CTAAGTGAAT TGTA (SEQ ID
 NO:6) .

FIG.6

7/8

MVFQIIILVI CTICMKHYAK GEFQQSLAIT DILPEDIKRY DKMRPPKKEG
QPTIVYFHVT VMGLDSIDEN SMTYVADVFF AQTWKDHRLR LPENMTQEYR
LLEVDWLKNM WRPDSFFKNA KSVTFQTMTI PNHYMWLYKD KTILYMKLT
LKLSCIMNFA IYPHDTQECK LQMESLSHTT DDLIFQWDPT TPLVVDENIE
LPQVALIRNE TADCTQVYST GNFTCLEVVF TLKRRLVYYV FNTYIPTCMI
VIMSWVSFWI KPEAAPARVT LGVTSLLTLS TQHAKSQSSL PPVSYLKAVD
AFMSVCTVFV FMALMEYCLI NIVLSDTPIP KPMAYPPKPV AGDGPKKEGE
GAPPGGSNST ASKQQATMLP LADEKIEKIE KIFDEMTKNR RIVTTTTRRVV
RPPLDADGPW IPRQESRIIL TPTIAPPPPP PQPAAPEPEL PKPKLTPAQE
RLKRAIYIDR SSRVLPALF ASLNGIYWCV FYEYL* (SEQ ID NO:7).

FIG.7

8/8

AC05 is expressed in Oocytes and
activated by Histamine

10 μ M Histamine

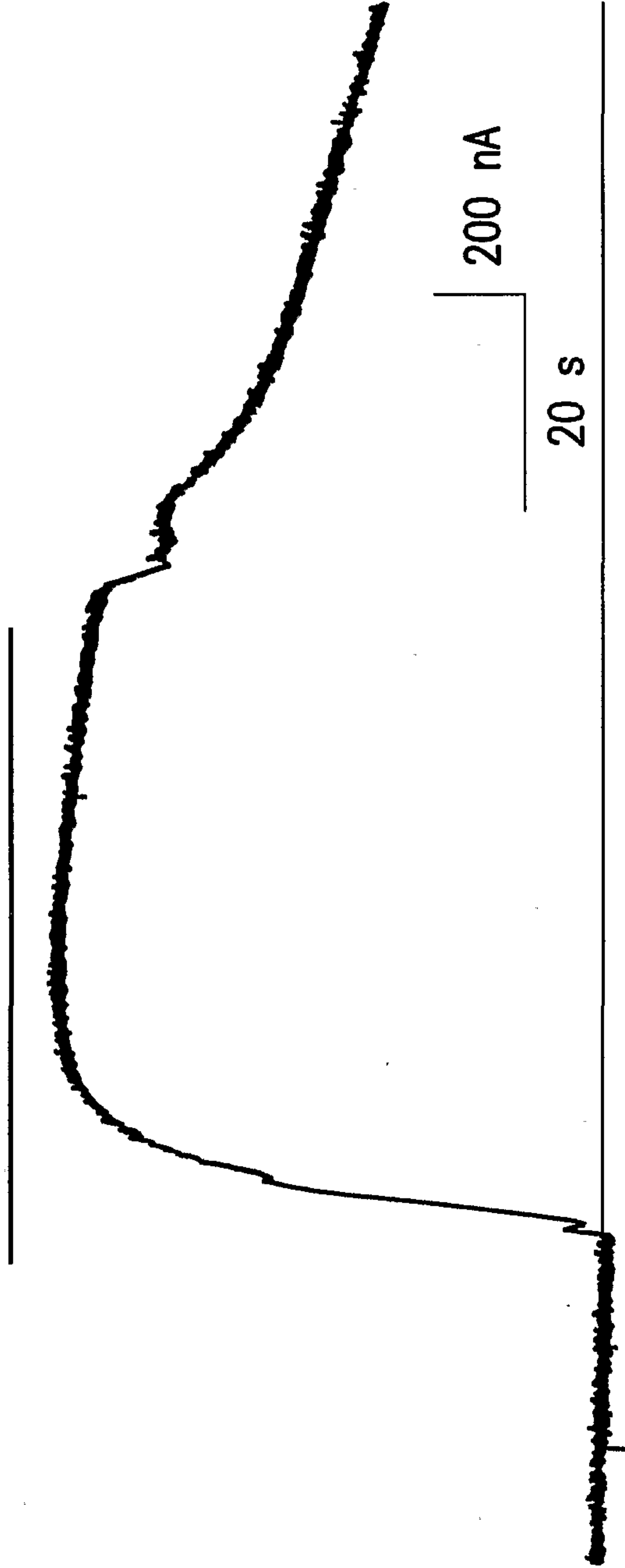


FIG.8

AC05 is expressed in Oocytes and
activated by Histamine

10 μ M Histamine

