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The ligand-gated chloride channel gene family of *Drosophila melanogaster*

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ABSTRACT

The antagonism of cellular excitability in insects is mediated by a family of ionotropic receptors, the ligand-gated chloride channels. In insects these inhibitory receptors include synaptic GABA and histamine receptors, functionally analogous to vertebrate GABA and glycine receptors, and glutamate receptors that appear to be unique to invertebrates. The ligand-gated chloride channel family in insects includes two well-validated targets for insect control agents: GABA receptors, which are the targets for a structural variety of small-molecule insecticides (polychlorocycloalkanes such as dieldrin and phenylpyrazoles such as fipronil) that have been used widely in agriculture; and glutamate receptors, which are the targets of macrocyclic lactone natural products (the avermectins) that have yielded not only commercial insecticides but also anthelminthic agents (i.e., ivermectin) employed in animal and human health. The availability of the complete genome sequence of the insect model system *Drosophila melanogaster* has permitted the identification of all of the genes encoding proteins with structural similarity to known ligand-gated chloride channel subunits (ligand-gated chloride channel homologs or LCCHs). Here, we review the present status of knowledge of the structure and function of the proteins encoded by this compact gene family. The 12 LCCH genes of *D. melanogaster* exhibit a surprising degree of structural diversity, which is further enhanced for some subunits by a variety of post-transcriptional and post-translational modifications. Although the structures of the gene products encoded by this small gene family are now well characterized, surprisingly little is known of the biological functions of the majority of them and the structures of most native receptors remain unknown.

1. Introduction

Ligand-gated chloride channels gated by GABA, glutamate and histamine mediate synaptic inhibition and regulate cellular excitability in insects and other invertebrates [1–3]. These receptors are part of a larger superfamily of ligand-gated ion channels that includes excitatory receptors gated by acetylcholine and are similar in structure, function and pharmacology to the inhibitory GABA and glycine receptors in vertebrates [4–6].

Insect GABA- and glutamate-gated chloride channels are important targets for the action of insecticides [7]. Polychlorocycloalkane insecticides (e.g., dieldrin) and phenylpyrazole-type compounds (e.g., fipronil) block GABA- and glutamate-gated chloride channels [8,9], producing indirect excitation of the nervous system by the removal of inhibitory input. The macrocyclic lactone insecticides (e.g., avermectins and related compounds) are allosteric activators of glutamate-gated chloride channels, causing flaccid paralysis of insects by hyperpolarizing nerve and muscle cells.

Vertebrate GABA and glycine receptors are heteropentamers, with each receptor comprised of subunits from two or three different structural subgroups [5,6]. Each subunit contains a large, extracellular domain and four hydrophobic transmembrane helices. Assembly of the extracellular domains forms binding sites for activating ligands, whereas assembly of the transmembrane domains forms a chloride-selective ion pore that crosses the cell membrane. During the 1990s, four genes (*Rdl*, *Grd*, *Lcch3* and *GluCl*) that code for subunits of ligand-gated chloride channels were identified in *Drosophila melanogaster* [10–14]. Whereas the predicted products of these genes exhibited structural homology to vertebrate ligand-gated chloride channel subunits, each appeared to be a component of a distinct receptor, and the native subunit compositions of the receptors containing these subunits is unknown.

The publication of the complete genomic sequence of *D. melanogaster* [15] and the subsequent expansion of genomic tools for insect neurobiology have provided new opportunities to characterize all the ligand-gated chloride channel homologs (LCCHs) encoded in the *D. melanogaster* genome. Here, we focus on available information on the LCCH gene family of *D. melanogaster*. We identify the members of this gene family and discuss the predicted structures and properties of the putative LCCH subunits that they encode, and we assess progress that has been made toward defining the native molecular structure and functional properties of individual LCCHs in *D. melanogaster*.

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2. LCCH subunits identified by conventional molecular and genetic techniques

2.1. Rdl

A *D. melanogaster* strain having resistance to dieldrin [16] and reduced neuronal sensitivity to dieldrin and picrotoxinin [17], both of which are known to block GABA receptors, provided a point of entry to identify putative GABA receptor subunit genes in this species. The resistance gene, called *Rdl* (resistance to dieldrin), was mapped to cytogenetic region 66F [18]. A chromosomal walk encompassing ~200 kb from this region yielded a predicted cDNA transcript encoding a peptide with a high degree of amino acid sequence similarity to vertebrate GABA receptor β subunits [10]. The restoration of insecticide susceptibility by transformation of dieldrin-resistant flies with the wildtype *Rdl* gene provided genetic proof that resistance was conferred by a mutation in this putative GABA receptor subunit [10]. Subsequent studies identified a single point mutation, A302S in the second hydrophobic transmembrane helix, that was correlated with resistance [19].

2.2. Lcch3

A second strategy for the isolation of LCCH sequences from *D. melanogaster* employed PCR-based homology probing for a conserved amino acid sequence "signature motif" found in transmembrane domain M2 of mammalian GABA and glycine receptor subunits. A single-site PCR screening strategy [20] employing pools of degenerate primers capable of coding for this heptapeptide motif (–TTVLTMT–) yielded three genomic DNA fragments (designated *Lcch1*, *Lcch2* and *Lcch3*) with sequence homology to mammalian GABA and glycine receptor sequences [21]. *Lcch1* was identical to *Rdl* whereas *Lcch2* and *Lcch3* represented previously-uncharacterized genes. The full-length cDNA for *Lcch3* encodes a polypeptide with a high degree of sequence similarity to vertebrate GABA receptor β subunits [11]. The *Lcch3* gene was localized to cytogenetic region 13F on the X chromosome by *in situ* hybridization of oligonucleotide probes to polytene chromosome preparations [21].

2.3. Grd/Lcch2

The independent identification of a GABA receptor-like sequence element from *D. melanogaster* led to the isolation of a full-length cDNA for *Grd* (GABA and glycine receptor-like subunit of *Drosophila*), a third putative insect GABA receptor subunit [12]. The *Grd* sequence, which was derived from the same locus as the *Lcch2* fragment, includes a 73-amino acid insertion in the extracellular domain that is not present in any other member of the sequence family. The Lcch2 protein was less similar to various vertebrate GABA and glycine receptor subunits than either Rdl or Lcch3. The *Grd* gene was localized to cytogenetic region 75A on chromosome 3L by *in situ* hybridization [12,21].

2.4. GluCl

Evidence for the action of avermectins on invertebrate glutamate-gated chloride channels [7] stimulated the search for glutamate receptor subunit genes in *D. melanogaster*. PCR screening with degenerate primer pools targeting conserved sequences in transmembrane domains M1 and M3 of the *GluCl-α* gene of *Caenorhabditis elegans* yielded a single novel *D. melanogaster* sequence, designated *GluCl* [14]. The inferred sequence of the *D. melanogaster* GluCl protein was most similar to vertebrate glycine receptor α subunits and more similar to the GluCl-α and GluCl-β subunits of *C. elegans* than to Rdl. Like vertebrate glycine receptor subunits and the *C. elegans* GluCl-α and GluCl-β subunits, the *D. melanogaster* GluCl sequence contains a second pair of cysteine residues in the extracellular domain that are absent in vertebrate GABA receptor subunits and the three putative *D. melanogaster* GABA receptor subunits. The *GluCl-α* gene was localized to cytogenetic region 92A-B by *in situ* hybridization [22].

3. Genome-wide screens for LCCH subunit homologs

3.1. Identification of additional putative LCCH subunit genes

The publication of the complete *D. melanogaster* genome [15] and the availability of associated bioinformatic tools provided the opportunity to identify all members of the LCCH gene family of *D. melanogaster*. An initial survey of ion channel families [23] suggested the existence of 10 distinct LCCH sequences and related family of 10 sequences homologous to nicotinic acetylcholine receptor (nAChR) subunits. Subsequent rigorous homology searches using the BLAST algorithm [24] with various combinations of known vertebrate and invertebrate ligand-gated chloride channel subunits as query sequences consistently returned a set of 12 genes comprising the *D. melanogaster* LCCH gene family (Table 1) [25–27]. Expansion of this group further by relaxation of the criteria for similarity returned members of the nAChR subunit family (e.g., nAcRβ-21C) [25], indicating that the LCCH gene family is limited to 12 members. The LCCH family includes the four genes identified previously (*Rdl*, *Lcch3*, *Grd* and *GluCl*), two sequences subsequently identified as subunits of histamine-gated chloride channels (*HisCl1* and *HisCl2*), one sequence subsequently identified as encoding a protein that forms pH-sensitive chloride channels (*pHCl*), and five sequences of unknown function. For the purposes of this review, the latter five sequences are identified by their unique cytogenetic locations (*Lcch-4D*, *Lcch-14A*, *Lcch-47C*, *Lcch-74C* and *Lcch-100C*).

Comparison of cytogenetic locations suggests that the *Lcch3* and *Lcch-14A* genes occur as a tandem duplication separated by less than 500 bp of DNA in cytogenetic region 14A1 on the X chromosome. Two other pairs of genes of this gene family have relatively close genetic linkage: *HisCl2* and *GluCl* map to cytogenetic regions 92A13 and 92B1-2 on chromosome 3R, respectively, and are separated by about 67 kb of DNA; and, *Lcch-74C* and *Grd* map to cytogenetic regions 74C3 and 75A4 on chromosome 3L, respectively, and are separated by about 354 kb of DNA. The others members of the gene family occur singly at dispersed locations throughout the genome (Table 1).

Analysis of the gene sequences identified by BLAST identified open reading frames of postulated transcripts and their deduced amino acid sequences, thereby providing the technical basis for isolating the corresponding cDNAs of the previously uncharacterized members of this gene family [26–29] (D.C. Knipple and D.M. Soderlund, unpublished results). Homology relationships based on deduced full-length amino acid sequences of the *D. melanogaster* LCCH family are illustrated in a sequence tree (Fig. 1), consisting of two major branches supporting multiple subunits (one containing GluCl, HisCl1, HisCl2, Rdl, Grd, Lcch3, Lcch-14A and Lcch-47C; and, one containing Lcch-4D, Lcch-74C and Lcch-100C) and a branch containing only pHCl. This gene family shows extensive phylogenetic conservation, as exemplified by the identification of 10 of 12 orthologous genes in the *Apis mellifera* genome, with only one homolog of the Lcch-4D/Lcch-74C/Lcch-100C subfamily being present [30].

3.2. Structural features of LCCH gene products

An alignment of the amino acid sequences of the *D. melanogaster* LCCH subunits (Fig. 2) illustrates conserved structural features of