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Genomics, cys-loop ligand-gated ion channels and new targets for the control of insect pests and vectors

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Cys-loop ligand-gated ion channels (cysLGICs) play roles in the nervous system. They consist of five subunits arranged around a central ion channel with each subunit being encoded for by a separate gene. In insects, the cysLGIC superfamily commonly consists of 21–25 genes giving rise to several receptor classes such as nicotinic acetylcholine receptors and GABA receptors. Insect cysLGICs are of interest as they are the target of insecticides. Analyses of genome sequences have identified cysLGIC gene superfamilies from different species including crop pests, disease vectors and beneficial insects. This review explores recent studies that have pushed forward our knowledge about this superfamily and considers the potential of developing improved strategies to control insect pests whilst sparing non-target organisms.

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Introduction

Cys-loop ligand-gated ion channels (cysLGICs) make up a superfamily of receptors, for which the best-known role is to mediate the actions of neurotransmitters (sometimes referred to as agonists) in sending signals throughout the nervous system. CysLGICs act as molecular switches, which change conformation upon binding to an agonist to allow a net influx of ions into the cell [1]. They consist of five subunits arranged around a central ion channel (Figure 1). Each subunit has four transmembrane domains (TM1–4) and possesses an N-terminal extracellular domain containing the characteristic Cys-loop motif consisting of two disulphide bond-forming cysteines separated by 13 amino acid residues. In insects, neurotransmitters known to act on cysLGICs in this way include acetylcholine (ACh), γ-aminobutyric acid (GABA), glutamate and histamine. The neurotransmitter binding site is located at the interface of two adjacent subunits and is formed by six distinct regions (loops A–F [2]) in the N-terminal extracellular domain with loops A, B and C being contributed by one subunit and loops D, E and F by another. CysLGICs can exist either as homomers, where all five subunits are the same, or as heteromers consisting of at least two different subunits. The subunit composition determines the functional and pharmacological properties of the cysLGIC, thus receptor diversity is generated by multiple subunit-encoding genes in a given organism. As will become apparent throughout this review, insect cysLGICs are of interest as they are involved in various aspects of nervous system function as well as being the molecular targets of highly effective insecticides.

Sequencing of insect genomes have allowed for detailed comparisons of cysLGIC gene families from diverse species (Table 1). From Table 1, it can be seen that the number of cysLGIC genes are similar from one species to another, ranging from 21 to 26 genes. Many subunits are highly conserved in different species, which is highlighted by recently characterised cysLGIC superfamilies from the tiger mosquito, *Aedes aegypti* (BJ Matthews *et al.*, unpublished), the bumble bee *Bombus terrestris* [3] and the pea aphid *Acyrthosiphon pisum* (del Villar and Jones, unpublished) [4], which are shown in a phylogenetic tree in Figure 2. The cysLGICs have been grouped according to the neurotransmitter they respond to. These groups are briefly considered in turn below with specific focus on recent developments.

Nicotinic acetylcholine receptors

Nicotinic acetylcholine receptors (nAChRs) mediate the fast actions of acetylcholine (ACh). Subunits are denoted as α subunits due to the presence of two adjacent cysteine residues in loop C, which are important for ACh binding [5]. nAChR subunits lacking these two cysteines are referred to as β. To be functional, nAChRs require at least two of the subunits to be α. As shown in Figure 2, *A. pisum*, *Ae. aegypti* and *B. terrestris* possess 11, 15 and 11 nAChR subunit genes, respectively. Insects have core groups of subunits (α1–8, β1) that are highly conserved between species and therefore, presumably, play important roles [6]. Interestingly, *A. pisum* lacks an α5 subunit (Figure 2). The aphid cysLGIC gene superfamily is the most primitive one characterised to date, thus it has been speculated that the α5 subunit is the newest member of the insect core group of subunits appearing in more highly evolved species [4]. nAChRs have been long known to play important roles in the insect nervous system. For

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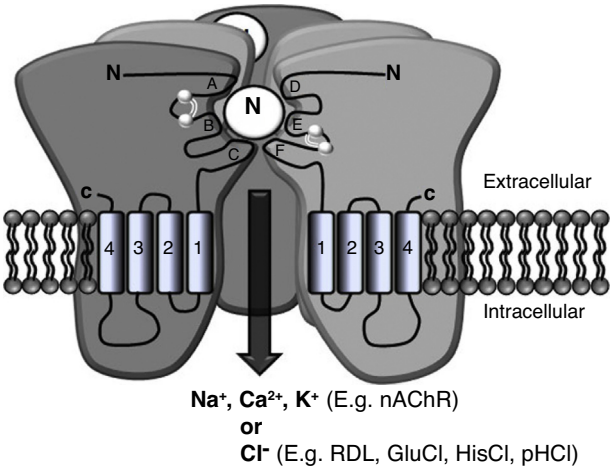
Figure 1



Structure of a cys-loop ligand-gated ion channel (cysLGIC). Schematic representation of a heteromeric receptor consisting of two different subunits (dark and light grey). The peptide layout of two subunits are shown highlighting the Cys-loop (two white circles connected by a white double line) and four transmembrane domains. The six binding loops (A–F) that contribute to ligand binding are shown and two neurotransmitter (N) molecules are bound. The five subunits that make up the receptor are arranged around a central ion-permeable channel. The ions passing through the channel depend on the type of cysLGIC.

example, a recent study showed, using RNA interference, that α1, α4, α5, and α6 (but not α3) nAChR subunits are involved in olfactory memory processing in *Drosophila* [7].

nAChRs are the molecular targets of neonicotinoid insecticides [8,9], three of which (imidacloprid, clothianidin and thiamethoxam) will be banned, most likely by the end of 2018, from outdoor use by the European Union amidst fears that they are contributing to the alarming decline of bees and other non-target organisms (BBC Science and Environment News; URL: https://www.bbc.co.uk/news/science-environment-43910536). Several other classes of insecticides, such as spinosyns, sulfoxamines and butenolides, act on nAChRs [9]. Also, natural peptides found in venom, such as that of the funnel-web spider, can act on insect nAChRs, with a higher selectivity than on vertebrate nAChRs [10].

Recent studies have highlighted particular nAChR subunits as being the targets of certain insecticides. For instance, the neonicotinoids nitenpyram and imidacloprid, stimulated dopamine release in nerve cords of *Drosophila melanogaster* larvae by acting as agonists on nAChRs [11]. This dopamine release was significantly lower in *Drosophila* with mutations in the α1 and β2 nAChRs subunits, indicating that these two subunits are important for the actions of both neonicotinoids. The β1 subunit has also been implicated as an important neonicotinoid target since the R81T mutation is associated with neonicotinoid resistance in aphids [12] although the mutation can affect neonicotinoids differentially depending on whether the insecticide has a cyano or nitro chemical group [13]. The use of RNAi and patch-clamp electrophysiology on cockroach (*Periplaneta americana*) dorsal unpaired median neurons indicated that the α3 and α8 subunits may also form part of nAChRs targeted by imidacloprid [14].

Another class of insectcides, spinosyns, acts on a different nAChR subunit, α6, as indicated by recent findings that there were significantly more truncated forms of the α6 subunit in the flower thrips, *Frankliniella occidentalis*, which were resistant to spinosad [15]. Also, altered α6 mRNA, which lacked exon 3, was found at higher levels in the tomato leaf miner, *Tuta absoluta*, that were resistant to spinosad [16]. The CRISPR/Cas9 system capable of making desired changes in genome sequences [17] was used to show that the G275E point mutation in α6 of *Drosophila* flies confers decreased sensitivity to spinosad [18••], demonstrating this subunit as being important for spinosyn action.

It remains to be seen which nAChR subunits are targeted by other insecticide classes. It will be of interest to see whether different insecticide classes act on the same handful of subunits or whether the whole nAChR gene family can effectively be targeted. Genome editing with CRISPR/

Table 1

Insect species that have their complete cysLGIC superfamily described

Species	Order	Significance	cysLGIC subunit gene number	Reference/s
Aedes aegypti	Diptera	Disease vector	25	(Matthews BJ *et al.*, unpublished)
Apis mellifera	Hymenoptera	Pollination, honey production	21	[49,50]
Acyrthosiphon pisum	Homoptera	Crop pest	22	[4], (del Villar and Jones, unpublished)
Bombus impatiens	Hymenoptera	Pollination	21	[3]
Bombus terrestris	Hymenoptera	Pollination	21	[3]
Drosophila melanogaster	Diptera	Genetic model organism	23	[50,51]
Musca domestica	Diptera	Disease vector	23	[52]
Nasonia vitripennis	Hymenoptera	Biological control of insect pests	26	[53]
Tribolium castaneum	Coleoptera	Pest of stored food	24	[39]

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