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Pr-lynx1, a modulator of nicotinic acetylcholine receptors in the insect

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Insect nicotinic acetylcholine receptors (nAChRs) are targets for insecticides. Despite the importance of the nAChR as a major target for insecticide action, modulators of nAChRs in insects remain unidentified. Here we describe the cloning and identification of a nAChR modulator gene in an insect. This gene was isolated by searching the firefly *Pyrocoelia rufa* cDNA library, and the gene itself encodes a protein 120 amino acids in length, named *Pr-lynx1*. *Pr-lynx1* shares all the features, including a cysteine-rich consensus motif and common gene structure, of the *Ly-6/neurotoxin* superfamily. The recombinant Pr-lynx1, which is expressed as a 12-kDa polypeptide in baculovirus-infected insect Sf9 cells, is normally present at the cell surface as a GPI-anchored protein. Northern and Western blot analyses revealed that Pr-lynx1 is expressed in various tissues, such as the ganglion, brain, mandibular muscle, proventriculus, leg muscle, and epidermis. This expression pattern is similar to the distribution of nAChRs as assayed by α3 nAChR immunoreactivity. Co-expression of Pr-lynx1 in *Xenopus* oocytes expressing α3β4 nAChRs results in an increase in acetylcholine-evoked macroscopic currents, indicating a functional role of Pr-lynx1 as a protein modulator for nAChRs. This study on *Pr-lynx1* is the first report of a modulator of nAChRs in an insect species.

Keywords: Ly-6/neurotoxin superfamily; Nicotinic acetylcholine receptors (nAChRs); nAChR modulator; insect

Introduction

The nicotinic acetylcholine receptor (nAChR), an agonist-gated ion channel complex for rapid excitatory neurotransmission, plays an important role in the mediation of synaptic signals in the nervous systems of animals. In mammals and other vertebrates, the nAChR functions in the peripheral as well as the central nervous system (CNS) (McGehee and Role, 1995; Lindstrom, 1996). Vertebrate neuronal nAChRs are assembled from α2–α10 and β2–β4 subunits, of which the α subunits are largely responsible for the binding of acetylcholine (ACh) (Lindstrom et al., 1995; Lukas et al., 1999; Alexander et al., 2001).

ACh is an excitatory neurotransmitter that binds to nAChRs and is released from axonal terminals distributed throughout the brain. nACHRs can also be activated by the drug nicotine. Activation of postsynaptic nAChRs induces depolarizing inward currents, whereas activation of nAChRs located on presynaptic terminals contributes to Ca^{2+} flux into the terminal, thereby augmenting neurotransmitter release (Dani, 2001). Thus, activation of nAChRs can shift the balance of competing inhibitory and excitatory inputs towards excitation, producing synaptic changes (Mansvelder and McGehee, 2000; Mann and Greenfield, 2003). The significance of maintaining the proper balance of cholinergic signaling has been previously reported in association with reductions in nAChR levels or activity (Lindstrom, 1997; Picciotto and Zoli, 2002) and with detrimental effects in response to overactivation of nAChRs (Damaj et al., 1999; Abrous et al., 2002; Broide et al., 2002; Fonck et al., 2003). Additionally, it has been shown that even subtle alterations in nAChR activity can result in important consequences for cholinergic function (Dani et al., 2000; Wooltorton et al., 2003).

A previous study reported the presence of a cholinergic modulator, lynx1 (Miwa et al., 1999), which can form stable associations with nAChRs and alter their function *in vitro* and *in vivo* (Ibanez-Tallon et al., 2002; Miwa et al., 1999, 2006). Lynx1, an evolutionary precursor to snake venom toxins, shares structural characteristics with toxins such as α- and κ-bungarotoxins, which bind tightly to nAChRs and inhibit their activation. Unlike α-bungarotoxin, lynx1 is not an inhibitor of nAChR function, but rather increases current amplitudes in response to ACh (Ibanez-Tallon et al., 2002). Thus, lynx1, which is a novel member of the Ly-6/neurotoxin superfamily, is likely to have a significant role in modulating cholinergic signaling by acting as an endogenous protoxin to modulate nAChRs (Miwa et al., 1999;

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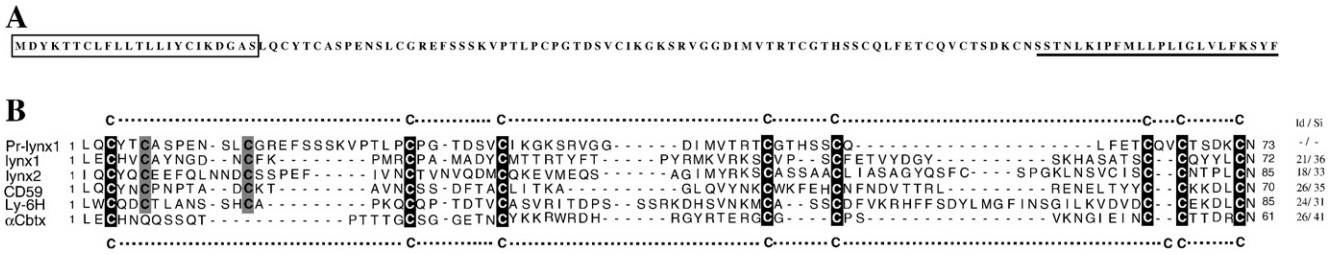

Fig. 1. Comparison of the deduced amino acid sequence of *Pr-lynx1* with the *Ly-6/neurotoxin* gene superfamily. (A) Deduced amino acid sequence of the *Pr-lynx1* ORF. The boxed N-terminus represents the putative signal sequence. The hydrophobic GPI consensus sequence at the C-terminus is underlined. GenBank accession number is EU022311 (*Pr-lynx1* cDNA). (B) Amino acid sequence homology between Pr-lynx1 and members of the *Ly-6/neurotoxin* gene superfamily. Family members comprise the lymphocyte cell surface antigens (Ly-6 and CD59), the snake venom toxin peptide α-cobratoxin (αCbtx), and the neuronal cell surface molecules lynx1 and lynx2. The conserved cysteines are shaded in black when conserved in all members of the superfamily, or in gray when they are absent in the neurotoxin. The Pr-lynx1 sequence was used as a reference for the identity/similarity (Id/Si) values.

Ibanez-Tallon et al., 2002, 2004). In addition, a previous study has demonstrated that lynx1 functions as an allosteric modulator of nAChR function *in vivo*, balancing neuronal activity and survival in the CNS (Miwa et al., 2006). Recently, lynx2, a novel member of the Ly-6/neurotoxin superfamily, has also been identified (Dessaud et al., 2006).

In insects, the nAChR is widely distributed in the CNS and constitutes a major target for insecticide action (Tomizawa and Casida, 2003). Ten nAChR subunit genes have been identified from the complete genome sequences of the fruit fly, *Drosophila melanogaster*, which has seven α (Dα1–7) and three β (Dβ1–3) subunit genes (Littleton and Ganetzky, 2000), and the malaria mosquito, *Anopheles gambiae*, which has nine α (Agamα1–9) and one β (Agamβ1) subunit gene(s) (Jones et al., 2005). Additionally, insect nAChRs are a subject of particular interest because they are the primary target of neonicotinoid insecticides (Matsuda et al., 2001). In light of this, the molecular characterization of insect nAChR subunit genes has been reported for various insects, such as the peach potato aphid (*Myzus persicae*), the locust (*Locusta migratoria* and *Schistocerca gregaria*), the tobacco hornworm (*Manduca sexta*), the honey bee (*Apis mellifera*), the brown planthopper (*Nilaparvata lugens*), the cat flea (*Ctenocephalides felis*), and the house fly (*Musca*

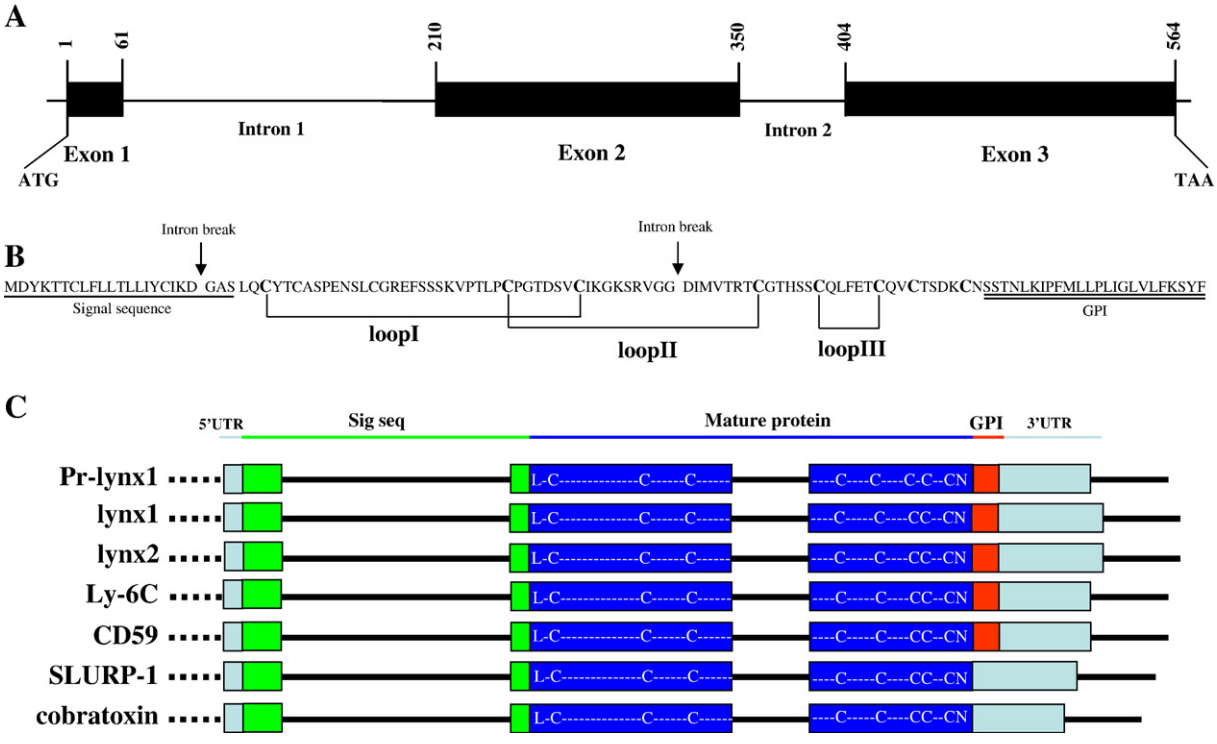

Fig. 2. Comparison of the gene organization of *Pr-lynx1* with the *Ly-6/neurotoxin* gene superfamily. (A) Gene organization of *Pr-lynx1*. Numbers indicate the position in the genomic sequences. GenBank accession number is EU022310 (*Pr-lynx1* genomic DNA). (B) Intron breaks and loops in *Pr-lynx1*. The arrow indicates the position of the intron break in the genomic sequences. The putative signal sequence is underlined and the GPI sequence is double-underlined. (C) Representation of the coding exons of the *Pr-lynx1* gene as compared to members of the *Ly-6/neurotoxin* gene superfamily: *lynx1* (Miwa et al., 1999), *lynx2* (Dessaud et al., 2006), *Ly-6C* (Fleming et al., 1993), *CD59* (Fletcher et al., 1994), *SLURP-1* (Chimienti et al., 2003), and *α-cobratoxin* (Chang et al., 1997). The exons are represented as boxes and are not to scale. 5′-UTR sequence, signal sequence (Sig seq), mature protein, hydrophobic GPI consensus sequence (GPI), and 3′-UTR sequence are shaded with different contrasts. Intron breaks are conserved within the superfamily, and exons 2 and 3 encode the mature protein.