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Review

Recent findings in evolution and function of insect innexins

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ABSTRACT

The past decade has seen significant advances in the field of *innexin* biology, particularly in the model invertebrate organisms, the nematode *Caenorhabditis elegans* and the fly *Drosophila melanogaster*. However, advances in genomics and functional techniques during this same period are ushering in a period of comparative *innexin* biology. Insects are the most diverse metazoan taxa in terms of species number, as well as in developmental, physiological, and morphological processes. Combined with genomics data, the study of *innexins* should rapidly advance. In this review, we consider the current state of knowledge regarding *innexins* in insects, focusing on *innexin* diversity, both evolutionary and functional. We also consider an unusual set of *innexins*, known as *vinnexins*, that have been isolated from mutualistic viruses of some parasitoid wasps. We conclude with a call to study insect *innexins* from a broader, evolutionary perspective. Knowledge derived from such comparative studies will offer significant insight into developmental and evolutionary physiology, as well as specific functional processes in a taxon that has huge biomedical and ecological impact on humans.

1. Introduction

Gap junctions and their structural genes have been identified in nearly all metazoan taxa. In the 1980s, connexin genes were identified as the molecular basis for gap junctions in rats and other mammals [1,2], although numerous studies failed to identify connexin genes in invertebrates such as the nematode *Caenorhabditis elegans* and the fly *Drosophila melanogaster*. Genetic screens and heterologous functional studies with these two model invertebrates identified the *innexin* genes and confirmed that they form gap junctions [3,4]. Sequence analyses later identified genes that were similar to *innexins* in chordate genomes, termed pannexins [5]; subsequently, phylogenetic analyses have supported that *innexins* and pannexins are evolutionarily homologous, supporting their evolutionarily common origin, while connexins are unrelated [6–8].

Insects (and the remainder of the phylum Arthropoda) demonstrate incredibly diverse structure and function. Insects initially appear in the fossil record approximately 400 million years ago [9], and arthropods more than 525 million years ago [10]. Arthropods account for almost 85% of animal species described [11], and exhibit a wide range of morphologies, physiologies, and niche inhabitance. Insects alone account for more than 75% of known metazoan species [12], and exhibit tremendous morphological diversity, ranging from major variations on the insect body plan, to incomplete and complete metamorphosis (that is, minor to major morphology differences through ontogeny), to subtler changes including polyphenisms [13,14]. Insects colonize, inhabit, and alter essentially all niches on Earth, with the exception of the deep ocean, and as such demonstrate a wide range of physiological adaptations. They (particularly flies, or the Diptera) also exhibit faster genomic divergence rates than mammals and other vertebrates [16]. Innexins and gap junctions have been hypothesized to play major roles in contributing to the morphological and physiological variation [15], although to date little systematic analysis examining this relationship has been performed in this major taxon. Rather, the overwhelming majority of work in insects on *innexins* and gap junctions has been performed in *D. melanogaster*, due to the genetic, genomic, and molecular tool chest available for this model organism. Given the long evolutionary history, the breadth of morphological and physiological diversity, and the rate of genome evolution, our current understanding of the diversity of insect *innexins* is likely a very limited representation of the diversity that is present. Recent work in numerous non-model insects supports this observation [17–22].

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Beyond simple diversity, there are other reasons to take a comparative approach to studying insect *innexins*. As stated, insect species, morphological, and even genomic diversity outstrips that of other phylogenetic lineages. The i5K initiative, which proposes to leverage community resources to sequence and annotate the genome of 5000 insect species representing the breadth of insect biodiversity [23], will generate tremendous amounts of genomic data. These data will facilitate a range of comparative genomics projects, and eventually comparative functional genomics. Relevant to *innexins*, this project initially will facilitate identification of the genomic complement of insect *innexins*; that is, the increasing availability of genomic sequences will permit the identification of the core *innexin* genes of insects. In parallel, genome sequences will permit identification of genomic novelty in the *innexin* gene family – sequences that exist in certain lineages and not in others. As the complement of *innexins* is determined for insect lineages, characterization studies will permit inferences to be drawn regarding the role of Innexins in functional diversification.

Currently, the insect *innexin* field is dominated by knowledge and studies in *D. melanogaster*. Eight *Drosophila innexin* loci have been isolated and transcript patterns analyzed through development [24], with reverse-genetic approaches allowing targeted analysis of Innexin function. A subsequent section of this review briefly will consider knowledge regarding function of *innexin* orthologues, recognizing that although the majority of these data stem from *Drosophila*, there is still much to learn from this model insect. But, the i5K project will greatly expand the possibilities for functional genomics, beyond *Drosophila*, in addition to evolutionary genomics. There is tremendous heterogeneity across insect taxa in regards to manipulability for functional studies, including logistics (e.g., rearing), tool development, and biological susceptibility to manipulation. For example, RNAi functions well, if in varied fashion, in Diptera (flies), Coleoptera (beetles), and Isoptera (termites) [25,26], but generally very poorly in Lepidoptera (moths, butterflies) [27]. As genomic resources relative to *innexins* increase, organisms that are more amenable to (or interesting for) functional studies will be identified. This will permit comparative functional studies, allowing the development of insight into conservation and divergence of *innexin* orthologues, their interactions with cellular partners, and so forth.

In this review, we will discuss the current state of understanding regarding the phylogenetic pattern of insect *innexins* relative to other *innexin* lineages. From this, we infer what appears to be the basal complement of *innexin* genes in insects. From there, we identify what appear to be unique clades of insect *innexins*. In considering these *innexin* clades, we briefly review the roles associated with Innexins, particularly in considering the potential for conservation and divergence within insect evolutionary lineages. Finally, we discuss *innexin* homologues within the Polydnaviridae, a family of mutualistic insect viruses. Together, these data point to a rich future for *innexin* work, promising many exciting insights into both gap junction roles and the pathways underlying many physiological processes in insects.

2. Phylogeny of insect innexins

Gap junction genes have now been identified in the genome of all Eumetazoa that have been examined, with the exception of echinoderms, and at least one *innexin* is encoded by the genome of the parasitic dicyemid mesozoans, *Dicyema japonicum* and *Dicyema koshidai* [28]. However, in line with previous reports, a BLAST search of the Placazoa and the Parazoa (Porifera) revealed no *innexin* homologues (BLAST search, December, 2013), as is expected given the absence of intercellular junctions in the Porifera. The pattern of *innexin* genes reflects deep evolutionary relationships within metazoans, implying that the pattern may be useful in phylogenetic studies and that *innexins* possibly play a role(s) in major evolutionary advances [28]. As previously reported [6,7,28], and demonstrated in Fig. 1, *innexins* exhibit phylum-specific diversification. It appears that *innexins* originated early in metazoan evolution, predating the divergence of the Lophotrochozoa (including the phyla Annelida and Mollusca) and Ecdysozoa (the molting phyla, including the phyla Nematoda and Arthropoda), which would account for the occurrence of pannexins in Deuterostomia (including mammals and other vertebrates). Following this initial genesis, *innexins* have undergone diversification within the phyla, including Arthropoda (including insects), Nematoda, Mollusca, and others represented in Fig. 1. The basis of this diversity is unclear. Based on conservation of only a single site across ecdysozoan lineages, *innexin* diversification was proposed to be the result of genetic drift [6]. However, the results of systematic selection analyses have not been reported, thus selection for functional variation cannot be ruled out. Indeed, alignments of insect *innexins* demonstrates multiple conserved sites, suggesting selection may vary at different phylogenetic levels.

The majority of physiological evidence of the role of Innexins comes from *D. melanogaster*, a member of the order Diptera (flies) (see below). Genomic analysis identified eight members of the *innexin* gene family in *D. melanogaster* [29]. Mutants have been identified for many members of the gene family, associating function with specific *innexin* lesions or alterations. Sequences of insect genomes are now permitting the identification of many more *innexin* loci, allowing for the development of a more robust insect *innexin* gene tree. Upon examination of the arthropod-specific clades of the tree (Fig. 1, "Insect" branches), several patterns emerge. Chiefly, the evolutionary pattern within orthologues (e.g., Inx2) largely is congruent with organismal patterns (Fig. 2). The Inx2 proteins form distinct organismal order-level clades, including the holometabolous Hymenoptera (bees, wasps, and

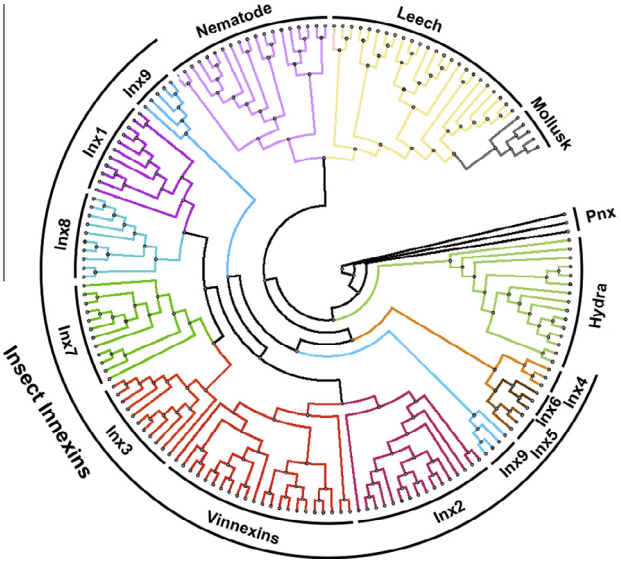

Fig. 1. Phylogenetic tree of conceptual Innexin and Pannexin translation products demonstrates phylum-specific diversification of the gene family. *Innexin* and pannexin sequences were downloaded from Genbank. Innexins were analyzed from phyla included Arthropoda (insects), Annelida (leech), Nematoda, Mollusca, and Cnidaria (Hydra). Pannexins representing mouse, rat, and human were included. Conceptual translated products were aligned using MUSCLE and a Neighbor-Joining tree created and visualized in Unipro UGENE. Innexins form phylum-specific clades, supporting gene diversification following, rather than preceding, diversification of phyla.