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Chemical transmission in the sea anemone *Nematostella vectensis*: A genomic perspective

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ABSTRACT

The sequencing of the starlet sea anemone (*Nematostella vectensis*) genome provides opportunities to investigate the function and evolution of genes associated with chemical neurotransmission and hormonal signaling. This is of particular interest because sea anemones are anthozoans, the phylogenetically basal cnidarians least changed from the common ancestors of cnidarians and bilaterian animals, and because cnidarians are considered the most basal metazoans possessing a nervous system. This analysis of the genome has yielded 20 orthologues of enzymes and nicotinic receptors associated with cholinergic function, an even larger number of genes encoding enzymes, receptors and transporters for glutamatergic (28) and GABAergic (34) transmission, and two orthologues of purinergic receptors. Numerous genes encoding enzymes (14), receptors (60) and transporters (5) for aminergic transmission were identified, along with four adenosine-like receptors and one nitric oxide synthase. Diverse neuropeptide and hormone families are also represented, mostly with genes encoding prepropeptides and receptors related to varying closeness to RFamide (17) and tachykinin (14), but also galanin (8), gonadotropin-releasing hormones and vasopressin/oxytocin (5), melanocortins (11), insulin-like peptides (5), glycoprotein hormones (7), and uniquely cnidarian peptide families (44). Surprisingly, no muscarinic acetylcholine receptors were identified and a large number of melatonin-related, but not serotonin, orthologues were found. Phylogenetic tree construction and inspection of multiple sequence alignments reveal how evolutionarily and functionally distant chemical transmitter-related proteins are from those of higher metazoans.

1. Introduction

Cnidarians are regarded as the most basal metazoans possessing nervous systems. Consequently, they have attracted considerable interest among neurobiologists as the evolutionary implications of studying their nervous systems became increasingly apparent. Cnidarian neurons are largely organized into planar nerve nets of varying density that are distributed in the ectoderm and, especially within the anthozoan class, in the endoderm (Pantin, 1952; Batham, 1965). As the neuronal processes cross over each other in the nerve net, they form more or less specialized synaptic junctions in which synaptic vesicles were identified (Anderson and Grünert, 1988; Westfall and Grimmelikhuijzen, 1993; Westfall et al., 1995). A large body of electrophysiological investigations showed that cnidarian neurons fundamentally work like neurons of physiologically more complex animals and that mechanisms of chemical neurotransmission in cnidarians are similar to those of higher invertebrates and of vertebrates (Anderson and Spencer, 1989; Mackie, 1990).

Although many forms of neuronal activity were documented in cnidarian nervous systems, the lack of strong evidence for classical transmitters (Martin and Spencer, 1983) coupled with evidence of widespread use of epithelial conduction and of electrical synapses between neurons in hydrozoan experimental models (Mackie, 2004), led to skepticism about the nature and extent of transmitter use in these animals. However, the last 25 years have witnessed the emergence of a large body of biochemical, immunohistochemical, molecular and physiological investigations demonstrating the presence in neurons and the biological activity of neuropeptides, biogenic amines and fast-acting small transmitters such as glutamic acid and GABA (Grimmelikhuijzen et al., 1996, 2002; Kass-Simon and Pierobon, 2007 for reviews). Yet, while the evidence for the role of both neuropeptides and classical transmitters in effector activities of all cnidarian classes is persuasive, the body of existing data is too incomplete and fragmented among several species to gain a satisfactory picture of the set of transmitter systems available to cnidarians.

The recent sequencing of the genome of the starlet sea anemone *Nematostella vectensis* (Putnam et al., 2007) provides a unique opportunity to explore the full repertoire of putative gene products known to be

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involved in transmitter biosynthesis, transport and receptors, all within a single cnidarian species. Recently, the morphological organization of the nervous system of this anemone was described (Marlow et al., 2009). The starlet sea anemone is a representative of the Anthozoa, the most basal cnidarian class which includes also corals and sea pens. Representatives of this class are considered to be closer to the ancestor of bilaterian animals than are other cnidarians (Bridge et al., 1992, 1995). Therefore, any analysis of protein families from the genome of *N. vectensis* is likely to provide insights on the evolution of protein structure and function in the context of the eumetazoan ancestry of cnidarians and of their predating the emergence of bilaterian animals. In addition, it is the intention of this genomic approach to provide a resource for investigators to explore new avenues and hypotheses in their efforts to understand various aspects of transmitter function in cnidarians.

Transmitter-associated proteins belong to different classes of proteins (enzymes, transporters, receptors), all of which contributing to transmitter function while potentially exhibiting distinctive features that reflect their evolutionary history. The following analysis of the repertoire of putative transmitter-related genes in the genome of *N. vectensis* aims at assessing for the first time the range, functional capability and evolutionary implications of transmitter systems in a cnidarian. For the purpose of this analysis, the word «transmitter» is used in a broad sense to include all substances that may be released from neurons and that act as *bona fide* neuroactive substances (triggering a post-synaptic response), modulators (modulating a pre- or post-synaptic event) or hormones (triggering a response significantly away from the release site).

2. Methods

Protein sequences were searched from the US Department of Energy Joint Genome Institute website for *N. vectensis* (http://genome.jgi-psf.org/Nemve1/Nemve1.home.html). Searches were conducted using annotation keywords or search engines such as KOG and BLAST available on the site. PHI-BLAST was also used to improve hit returns on queries of neuropeptide precursor proteins, using PHI patterns for the various neuropeptide families. All hits with an *e*-value below e^{-10} were selected. The selected sequences were downloaded through the MEGA v.4 software (Tamura et al., 2007) and directed to the RPS-BLAST alignment tool for inspection. Sequences of interest that were deemed too short or that included incomplete expected conserved domains were discarded. Duplicates and splice variants were identified by manually inspecting all aligned sequences and were removed from the pool of analysed sequences.

For phylogenetic analyses each protein transcript of interest was aligned with BLAST against the entire nr database. Sequences among the first 100 hits were selected and used to construct phylogenetic trees. The hits and related *N. vectensis* sequences were next aligned using ClustalW with MegAlign (Lasergene, DNASTAR) and trees were constructed with the MEGA implementation of distance neighbor-joining with complete deletion of gaps. Manual deletions were also performed to emphasize conserved domains. For sequences of membrane proteins, the vast majority of which are G protein-coupled receptors (GPCR), the N- and C-tails were removed. Consensus trees were obtained by bootstrapping the data (1000 replicates). To further validate some of the phylogenies, maximum likelihood analyses were conducted with PUZZLE (Strimmer and von Haeseler, 1996).

In addition, sequences were selected from the constructed trees of each protein class to create alignments designed to assess the extent to which the sea anemone proteins retained aa residues important for functional features of the corresponding protein class. For this purpose multiple alignments of proteins and their putative sea anemone orthologues were generated with MegAlign using the ClustalW algorithm and were displayed with the GeneDoc editor program (version 2.7; http://www.psc.edu/biomed/genedoc). The Statistics report function of GeneDoc was also used to evaluate percentages of residue similarity and identity in pairwise alignments. For this purpose the N- and C-tails of all membrane protein sequences were removed. SignalP was used to detect signal peptides of neuropeptide precursor proteins and NeuroPred for prediction of cleavage sites.

3. Results and discussion

3.1. An overview of the repertoire

Nearly 280 *N. vectensis* sequences of appropriate length and/or including integral functional domains were retained for analysis. Receptors represent nearly 70% of these sequences. The remaining sequences are distributed among biosynthetic or inactivating enzymes, cell membrane or vesicular membrane transporters and neuropeptide precursors. Although every effort was made to cull from the genome all transcripts relevant to transmitters, it is likely that some were missed due to oversight or to their belonging to hitherto unknown transmitter categories.

For convenience candidate genes are classified in three categories: small transmitters acting through both ionotropic and metabotropic receptors such as acetylcholine, amino acid and purinergic transmitters (Table 1), aminergic and other small transmitters such as adenosine and nitric oxide (Table 2) and neuropeptides/hormones

Table 1
Genes of *N. vectensis* predicted to code for proteins associated with acetylcholine, amino acids and ATP.

Transmitter type	Protein ID number	EST ID number
Acetylcholine		
Choline acetyltransferase	Nv_416, Nv_95805, Nv_203043	Nv_163430
Acetylcholinesterase	Nv_31599, Nv_87444, Nv_119959, Nv_209664 Nv_211382	Nv_160761, Nv_171912 Nv_239659, Nv_244109
Nicotinic receptors	Nv_40919, Nv_85091, Nv_91696, Nv_91941 Nv_110265, Nv_198343, Nv_198927, v_199721 Nv_200917, Nv_205808, Nv_205855, Nv_214990	Nv_240779, Nv_247410
Amino acids		
Glutamate		
AMPA receptors	Nv_13877, Nv_24412, Nv_50912, Nv_104623 Nv_117160, Nv_132356	
Kainate receptors	Nv_141731	Nv_239847
NMDA receptors	Nv_11315, Nv_31895, Nv_51517, Nv_211456	Nv_171792
Metabotropic receptors	Nv_31331, Nv_40374, Nv_105783, Nv_197524 Nv_198894, Nv_201378, Nv_218792, Nv_229374	Nv_241281, Nv_244506
Glutamate transporters	Nv_110362, Nv_210965, Nv_230013	Nv_173595
Vesicular transporters	Nv_10128, Nv_11440, Nv_81701, Nv_123866 Nv_138860, Nv_231086	
GABA		
Glutamate decarboxylase	Nv_60452, Nv_60834, Nv_70014	
GABAa receptors	Nv_40863, Nv_60804, Nv_93322, Nv_103931 Nv_111552, Nv_114921, Nv_201724, Nv_204447 Nv_211643, Nv_215047, Nv_230093	
Glycine receptor	Nv_22284	
GABAb receptors	Nv_86565, Nv_87697, Nv_206093, Nv_223171	Nv_158857, Nv_176489 Nv_239821, Nv_243252 Nv_244104
GABA transporters	Nv_60521, Nv_79255, Nv_79785, Nv_81637 Nv_109800, Nv_228010	Nv_161287, Nv_187148 Nv_236066, Nv_247614
Vesicular transporters	Nv_1064, Nv_22306, Nv_33294, Nv_60758 Nv_96724, Nv_142801, Nv_206710, Nv_214632 Nv_222356	Nv_241391, Nv_241392 Nv_247861
ATP		
Purinergic receptors (P2X)	Nv_102596, Nv_104653	