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Embryonic expression of engrailed in sea urchins

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Abstract

Neural patterning genes that are expressed along the anterior–posterior axis of deuterostomes are expressed late in larval development in echinoderms and are thought to function in establishing the highly-derived, adult body plan. We have used genomic resources to clone an engrailed gene (SpEn) from *Strongylocentrotus purpuratus*, and with this we have developed an antibody specific for SpEn. SpEn is expressed late in embryogenesis in the developing larval nervous system. At the prism stage, a small number of neuroblasts in the oral ectoderm on the edge of the larval mouth begin expressing SpEn. The cells are in bilaterally symmetric positions. The expression of SpEn precedes the expression of the neural markers, synaptotagmin and serotonin in the SpEn immunoreactive cells. The SpEn cells are located on the margin of the domain of cells expressing SpNK2.1, but they do not have nuclear SpNK2.1. Expression of engrailed in a pair of bilateral neural structures in early development appears to be a shared feature of bilaterians.

Keywords: Deuterostome; Embryonic development; Evolution; Echinoderm; Gene expression; Engrailed; Neural development; Larval nervous system; Sea urchin; Echinoid

Shared deuterostome characters include similar patterns of expression of several neural specification genes (Holland, 2002; Lowe et al., 2003). However, in Echinoderms expression of several of these genes is reported to be predominantly in late larval stages and thought to be co-opted to function in formation of the radially symmetric adult form (Lowe and Wray, 1997; Arenas-Mena et al., 1998, 2000). As a consequence, it has been proposed that these genes do not reveal underlying homologies and reinforce the idea that echinoderms are highly derived deuterostomes.

The larval nervous system of echinoids is comprised of neurons that are principally associated with the ciliary bands that surround the oral field and the ciliary band that forms the rim of the larval mouth (Burke, 1978, 1983; Bisgrove and Burke, 1986; 1987; Nakajima, 1986; Beer et al., 2001). The neurons arise in the ectoderm at the end of gastrulation and differentiate into several types of neurons in prism stage embryos (Nakajima et al., 2004). During neurogenesis, neurons interconnect and project neurites to form a basiepithelial nerve net underlying the aboral epidermis.

Access to genomic data has allowed development of reagents that are specific for echinoid gene products (http://www.hgsc.bcm.tmc.edu). BLAST results of the 17 Aug 2005 assembly of the sea urchin genome have identified sequences that appear to encode a *Strongylocentrotus purpuratus* orthologue of engrailed (Fig. 1). SpEn is present as a single copy gene comprised of two exons on NW_813110.1. The first exon comprises nucleotides 1–356, and the second exon contains nucleotides 357–750 and spans the homeodomain. Alignments and phylogenetic trees indicate that the putative protein is similar to engrailed proteins of other echinoids and deuterostomes (Fig. 1) (Dolecki and Humphreys, 1988). The gene predicts a cDNA containing an open reading frame of 750 nucleotides encoding a protein of 249 amino acids with a predicted molecular weight of 28,250 Da. PCR primers allowed us to amplify and sequence a 365 bp product that spans the intron–exon boundaries and indicates that the predicted gene is expressed. A 372 bp portion of the second exon was amplified, cloned, sequenced and subcloned to permit bacterial expression of the protein (Fig. 1). Serum from a rat immunized with the protein recognizes a 30 kD protein in immunoblots of neural tissues from adult *S. purpuratus* and has permitted immunolocalization of SpEn in embryos. Pre-absorption of the antibody with the expressed protein abolishes immunoreactivity in immunofluorescence preparations.

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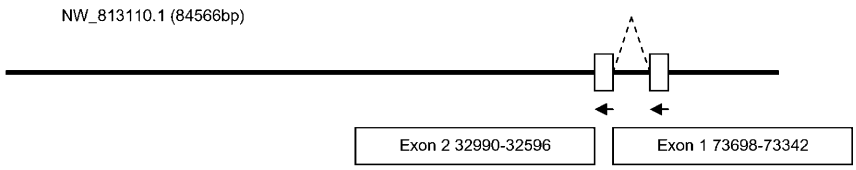
A



B

MSALSLGGRE PRSPTPHHHP LHVPRSHSDN DLAVKTRFTD FFIETILGPE

FGGSRKPSKA HHRKEDEHEV ERQDRTNVAK RCDEARPVGA GEKASTKSSS

SQSWPAWVYC TRYSDRPSS**G PRTRKVKRRE KKADEKRPRT AFSASQLQRL**

KQEFQQSNYL TEQRRRALAK ELTLSESQIK IWFQNKRAKI KKATGLKNGL

ARQLMAQGLY NHSTVPLDGD DMDTKLMNAS GDCSRSDYTS DSDGDSLTH

C

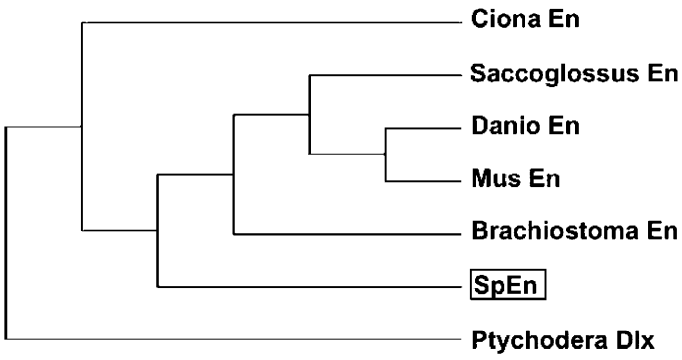


Fig. 1. (A) Diagram of the *Strongylocentrotus purpuratus* engrailed orthologue (SpEn) gene organization; (B) conceptual translation of the SpEN protein. The homeodomain is underlined and the region of SpEn that was expressed to produce anti-serum is in bold type; (C) phylogenetic tree computed using neighbor-joining (Neighbor) from an alignment of the conserved homeodomain sequences of engrailed from representative deuterostomes. Accession numbers: *Saccoglossus kowalevskii* gi|32307803; *Ciona intestinalis*, gi|26017194; *Brachiostoma floridae* gi|1772909; Danio rerio gi|62517.Mouse gi|462292.*Strongylocentrotus purpuratus* (SpEn) gi|72021185|ref|XP_794753.1|. The Distaless gene from *Ptychodera flava* has been included as an outgroup gi|6683068|dbj|BAA89014.1|.

The first cells that are immunoreactive with anti-SpEn appear between 60 and 72 h (Fig. 2). SpEn is detected in 1 or 2 cells, on either side of the larval mouth (Fig. 2C). The cells are in the thickened epithelium lateral to the larval mouth, where the adoral ciliary band will form. The SpEn expressing cells are round, lack processes or extensions, and at this stage, are not immunoreactive with the neural marker anti-synaptotagmin B (1E11) or anti-serotonin. In 96 h embryos, the number of SpEn immunoreactive cells ranges from 1 to 5 with the median number of three cells (mean = 3.3, $N = 44$) (Fig. 2D). In 5 day plutei, the range of SpEn immunoreactive cells is from 2 to 5 and the median number is three (mean = 3.95, $N = 20$) (Fig. 2E). The cells align with the lateral edges of the larval mouth and are positioned symmetrically, although there is not always the same number of cells on either side of the mouth. The SpEn immunoreactive cells frequently have a clear region in the cytoplasm and colocalization with nuclear markers (Sytox or DAPI) indicates that there are high levels of the SpEn protein in the cytoplasm (Fig. 2F).

In 96 h plutei, cells with neurites expressing synaptotagmin B (1E11) have differentiated in the adoral ciliary band, which forms the rims of the larval mouth (Fig. 3A–C). The cell bodies of these neurons form the oral ganglia, and the neurites extend into a plexus underlying the circumesophageal muscles. In co-localization experiments, it is clear that some of these neurons are the cells expressing SpEn. The SpEn immunoreactive neurons are invariably aligned in a row midway between the upper and lower lips on either side of the larval mouth. The SpEn immunoreactive neurons contribute neurites to the neuropil underlying the oral ganglion. Some of the cells of the oral ganglion contain serotonin (Fig. 3D–F). These serotonin containing neurons differentiate later than the serotonergic neurons of the apical organ and appear not to express tryptophan hydroxylase (Yaguchi and Katow, 2003).