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Characterization and expression of a sea star *otx* ortholog (*Protxβ1/2*) in the larva of *Patiriella regularis*

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ABSTRACT

A transcript of *otx* from the sea star *Patiriella regularis* (*Protxβ1/2*) was characterized and its expression in early bipinnaria larvae was documented by whole mount in situ hybridization (WMISH). The nucleotide sequence exhibited 94% identity with *Amotxβ1/2* from the closely related species *Patiria miniata*. *Protxβ1/2* was expressed strongly in the developing archenteron in the future fore and mid-gut regions. This was followed by expression of *Protxβ1/2* in the developing enterocoels, mesodermal derivatives. This suggests a role for *Protx* in endomesoderm development. In coelom development, *Protxβ1/2* was first expressed in the left coelom. Subsequently expression was evident in the right coelom, but localization was never as strong as in the left coelom. This asymmetry in *Protxβ1/2* expression in the coeloms was evident up to the stage when they started to extend posteriorly. These data indicate that *Protxβ1/2* may have a role in coelom development, particularly in the left coelom, a definitive adult structure.

1. Results and discussion

Expression of the homeobox orthodenticle-related gene, *otx* in archenteron development has been reported in asteroid, holothuroid and hemichordate embryos (Harada et al., 2000; Shoguchi et al., 2000; Hinman et al., 2003a). This gene appears to play a key role in the gene regulatory network for endomesoderm specification (Hinman et al., 2003a,b, 2007). In later development, *otx* is expressed in the ciliary band ectoderm of holothuroid and asteroid larvae (Shoguchi et al., 2000; Lowe et al., 2002; Hinman et al., 2003a) and in the developing nervous system of juvenile echinoids (Nielsen et al., 2003; Morris et al., 2004; Morris and Byrne 2005). Here we characterized an *otx* transcript from the sea star *Patiriella regularis* and documented its expression in development in the early bipinnaria larva to the formation of the coeloms, mesodermal derivatives.

In the Ambulacraria (Echinodermata + Hemichordata) *otx* orthologs have been characterized for asteroids, echinoids and hemichordates (Li et al., 1997; Kiyama et al., 1998; Hinman et al., 2003a,b; Lowe et al., 2003). Echinoderms have a single copy of *otx* (Li et al., 1997; Kiyama et al., 1998; Hinman et al., 2003a). Two Otx proteins are generated from multiple transcripts of this gene in echinoids (Li et al., 1997; Mitsunaga-Nakatsubo et al., 1998) and three proteins are generated in asteroids (Hinman et al., 2003a). Here we characterized an *otx* transcript from the sea star *Patiriella regularis* and documented its expression in larval development.

1.1. Characterization of the Protx ortholog

The transcript identified, 1113 nucleotides long encoded 276 amino acid residues in the open reading frame (ORF) region (Fig. 1A). It had 285 nucleotides in the 5′ untranslated region (5′ UTR), upstream of the 60 amino acid residues in the homeodomain and 492 nucleotides in the C-terminal region (Fig. 1A). Sequence characteristic of *otx* (Germot et al., 2001) were present (Fig. 1A). These included the highly conserved motif, DPPRK (contains a splice site), the homeodomain and two motifs (S/A)(I/L)WSPA and (D/E)CL(D/E)YK(D/E)(Q/P) located 3′ of the homeodomain.

Comparison of the *Protx* sequence with the analysis of echinoderm *otx* in Hinman et al. (2003a) revealed that the N-terminal of *Protx* contained two of the three 5′ UTR predicted exons definitive for asteroid *Amotxβ*-a-1 sequence (Fig. 1B). The *Protx* sequence begins at nucleotide 79 of the *Amotxβ*-a-1 sequence and showed 94% identity to the *Amotxβ*-a-1 transcript (Hinman et al., 2003a). This *Patiria miniata* sequence is entered as *Amotxβ-a* in GenBank (Accession No.: AY263967.1). Naming of this sequence was subsumed into *Amotxβ1/2* (in combination with *Amotxβ*-a-2 and

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(A)

ACTTTAGTTGATCACTTTATACCTGGAACATACTACACAGAGAGCTTATAGCACGCCCCT

CAGGCTAGTGTAAAACCCCTGTATGTCATGAACTGGGCTTGAGGCAAATCTCATCATTAA

CTCTGCACAGAACAGTTTTTGAAGTGAATCACCAAAAAAGACAAGGAGACAGCGAGTATA

CGCGCATCCAAAGTCACCATCTCTGTCTTGTTTTGATCATCCCTTTGCAGTGCCCTAACG

GCAGTGGAGCGACACGGTATCGACACGGCCTTCCCAATCCGCAGC

1 ATGAGTGGTATGGCGTACACTATACCCCCAGTGCACGGGCCACCGAACATGAAGGGGCCC

M S G M A Y T I P P V H G P P N M K G P

61 CCTTTCTCGGTCAACGGGATCAGTCTAGCGGCCCACAACGTCGAGTCCCTCCACCCAGCA

P F S V N G I S L A A H N V E S L H P A

121 ATGAACTACCACAGTTGTTTTACAGACCCGCCGAGGAAA**ACCAGACGGGAGAGAACCACG**

M N Y H S C F T D P P R K T R R E R T T

181 **TTCACCCGGGCGCAGCTGGACGTCTTGGAGACGCTGTTCAGCAAGACGCGNTACCCGGAC**

F T R A Q L D V L E T L F S K T R Y P D

241 **ATCTTTATGAGGGAAGAGGTCGCCTTGAAGATCAACTTACCGGAGTCAAGAGTACAGGTT**

I F M R E E V A L K I N L P E S R V Q V

301 **TGGTTTAAGAACAGAAGGGCAAAGTGTCGCCAGCAG**GCACAGCAACAACAGAACGGTACG

W F K N R R A K C R Q Q A Q Q Q Q N G T

361 CCCTCCAAGCCACGCCCAGTCAAGAAGAAGTCCCCCTCCCGGGACACACCCCCGAGCAGT

P S K P R P V K K K S P S R D T P P S S

421 GACTCCTCCCCCTCCTACAAGTCGCTGCCCATCACCTCTATGCCCAACAACAACAGCATC

D S S P S Y K S L P I T S M P N N N S I

481 TGGAGCCCCGCCTCCATCCCGCCCCCGCCGCCAATGGGTGACTCCATCTCGTCCAATAAC

W S P A S I P P P P P M G D S I S S N N

541 AGCTGTATGCAGCACCAGTACTCCATGGCCAACGCCCACCAGGGGGCCTACGCCCAGGGC

S C M Q H Q Y S M A N A H Q G A Y A Q G

601 TACCAGTCCGCCCCTTACTTTGGCAACCCCATGGACTACCTCTCGGGGGTGCCGCAGTTC

Y Q S A P Y F G N P M D Y L S G V P Q F

661 CCTGGAGGGGGCAGCCTGAGTCAGATGACGTCGTCCATGAATAGCCCGCACATGAGCACA

P G G G S L S Q M T S S M N S P H M S T

721 GCGCCCTCACAGCTCACACCCACACACCCGAGTATGGGCGGGTCCATGCCCAGCGCCAGT

A P S Q L T P T H P S M G G S M P S A S

781 GCAGACTGTTTAGAAAGCAAGGACCCATCGTGGAAGTTCCAAGTCCTG

A D C L E S K D P S W K F Q V L

(B)

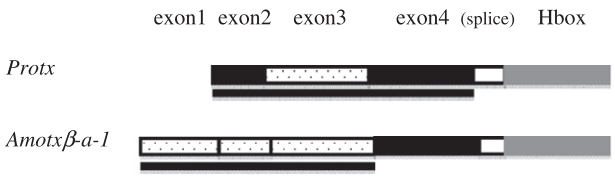

Fig. 1. (A) Partial nucleotide and deduced amino acid sequences of *Protxβ1/2* showing the main features of the transcript. The area corresponding to the homeodomain is shown in bold. The amino acid residues with double underline indicate the highly conserved motif, DPPRK (also contains a splice site) of *Amotx1/2*, upstream of the homeodomain. Diagnostic motifs (SIWSPA and DCLESKDPSWKFQVL) located 3′ of the homeodomain are underlined. The dotted and solid underlines, and shaded sequences correspond to the predicted exons (second third and fourth exons, respectively) in *Amotxβ-a-1* (Hinman et al., 2003a). (B) Top, the location of the in situ hybridization probe used in this study (underline) spans the 5′ UTR and the first 144 nucleotides in the coding region, illustrated with respect to predicted exons of *Amotxβ-a-1* sequence. Bottom, the location of the in situ probe used by Hinman et al. (2003a,b) for comparison (underline).

Amotxβ-a-3) to follow the naming of *otx* transcripts in echinoids (Hinman et al., 2007). We thus named the *Patiriella regularis* transcript *Protxβ1/2* (Accession No.: **GU064913.1**).

The probe used for whole mount in situ hybridization (WMISH) and RT-PCR of *Protxβ1/2* included the start of the 5′ UTR and the first 144 nucleotides of the coding region (Fig. 1B, see below). In