

Gene expression pattern

Identification and developmental expression of *Ci-msxb*: a novel homologue of *Drosophila msh* gene in *Ciona intestinalis*

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Abstract

We report the cloning and expression pattern of *Ci-msxb* the second *Ciona intestinalis* homeobox gene homologue to the *Drosophila* muscle segment homeobox (*msh*) gene. Northern blot analysis showed that transcripts appeared at gastrula stage, peaked in the early tailbud and decreased during the tailed stages. Whole mount in situ hybridization showed that the *Ci-msxb* expression first is detected at 110 cell-stage in the blastomeres that are precursors of different tissue (muscle, spinal cord, endodermal strand, brain, mesenchyme, pigmented cells and primordial pharynx). Transcript level declined in mesoderm cells after the completion of gastrulation, but mRNAs were still present in the folding neural plate during neurulation and in the pigmented cells. Later, at larval stage, transcripts were present around the otolith and ocellus, in a restricted part of the nervous system and in the primordial pharynx; the gene expression was conserved after metamorphosis in the juvenile. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Urochordata; *Ciona intestinalis*; Homeobox-containing gene; Msx; Development; Nervous system; Pharynx; Otolith; Ocellus; Neural gland

1. Results

Members of the *Msx/msh* gene family, that exhibit an extraordinary degree of sequence conservation, have been isolated from a variety of organisms from basal metazoans to human (Ivens et al., 1990; Seimya et al., 1994). Primitive organisms appeared to have only one *msh* gene, whereas in mammals there are at least three family members. The duplications of the *msh/Msx* family members appeared to have occurred sometimes around the origins of the vertebrate lineage (Davidson, 1995). In ascidian the characterization of a closely related *msh* gene has been already reported in literature. Particularly, Ma et al. (1996) reported the isolation and the expression pattern of a *Msx-a* in the ascidians *Molgula oculata* and *Molgula citrina*, while Holland (1991) reported only the isolation of a *msh-like* gene homeobox in *Ciona intestinalis*, using polymerase chain reaction on genomic DNA.

We identified an additional gene belonging to the *msx* gene family by screening a *C. intestinalis* larvae cDNA library. The nucleotide sequence of *Ci-msxb* cDNA,

completed by 5'RACE up to the transcription start site, encodes a protein of 327 amino acid residues. Alignment of the predicted *Ci-msxb* homodomain sequence with the corresponding region of various *Msx/msh* homodomains confirmed its identification as an ascidian *msx* protein. This alignment was used for phylogenetic analysis using the program from the PHYLIP 3.52 computer package (Felsenstein, 1989). The unrooted phylogenetic tree obtained (Fig. 1) shows that *Ci-msxb* falls within a proto-stome/invertebrate cluster, distinct from the deuterostome/vertebrate cluster. It should be noted that *Ci-msxb* is more similar to the *Mocu msxa* (Ma et al., 1996) than to the *Ci-msh* isolated by Holland (1991), even if they can be considered to be members of the *msh-like* family, in accordance with the classification made by Bell et al. (1993).

Northern blot hybridized with the *Ci-msxb* probe showed a single 1.4 kb RNA band (Fig. 2). The expression is strictly zygotic, in fact the band first appeared at gastrula stage, peaked in the early tailbud and decreased during the later stages, indicating that it may play its major role during embryogenesis in post-gastrulation stages.

The spatial localization of *Ci-msxb* transcripts in embryos at different developmental stages was performed by in situ hybridization of whole-mount specimens.

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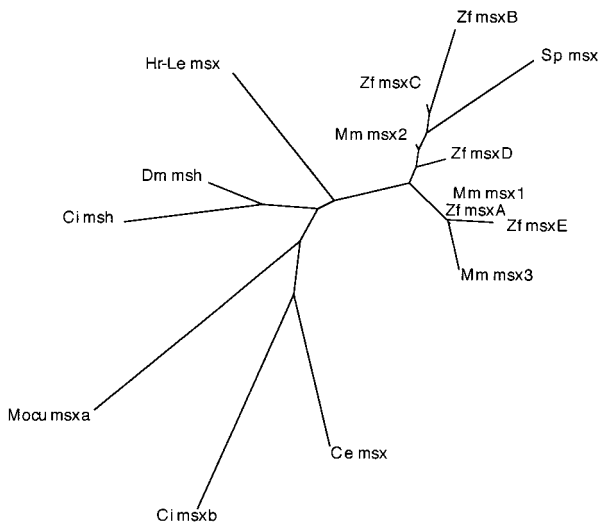


Fig. 1. Relationship of *msxB* gene family members. Only the homeodomain was used to constructing the phylogenetic tree, because the 8 amino acids at 5' and the 13 amino acids at 3' of the homeodomain were not always available. Sources for the sequences are: *Ci msxB*: ascidian *Ciona intestinalis*, EMBL: AJ132758; *Ce msxB*: nematode *Caenorhabditis elegans*, Swissprot: Q09604; *Zf msxB* A–D: zebrafish *Brachydanio rerio*, Swissprot: Q03357, Q03356, Q01703, Q01704; *Zf msxB* E: zebrafish *Danio rerio*, EMBL: U50563; *Dm msxB*: fruit fly *Drosophila melanogaster*, Swissprot: Q03372; *Mm msxB* 1–3: mouse *Mus musculus*, Swissprot: P13297, Q03358; EMBL: M38577; *Mocu msxA*: ascidian *Molgula oculata*, Ma et al., 1996; *Sp msxB*: sea urchin *Strongylocentrotus purpuratus*, Dobias et al., 1997; *Ci msxB*: ascidian *Ciona intestinalis*, EMBL: M38581; *Hr-Le msxB*: leech *Helobdella robusta*, EMBL: U61846.

At 110 cell-stage transcripts were detected in the B7.7 and B8.5 blastomeres, that are precursors of mesenchymal

cells, as well as in the b8.17 and b8.19 blastomeres that will give rise to the muscle, spinal cord and endodermal strand (Fig. 3A,B). The signal was present in the a8.25 blastomere that will give rise to the otolith and ocellus and in the a8.17 and a8.19 blastomeres that are the precursors of brain and primordial pharynx (Fig. 3C,D).

At neurula stage three different sites of expression of *Ci-msxB* can be detected (Fig. 3E): one signal along the neural tube, in the dorsal region of the embryo; the second one in two cells of the embryo, that will give rise to the otolith and ocellus; the third signal was detected in the posterior-ventral epidermis.

The same pattern of expression is conserved at the early tailbud stage (Fig. 3F,G).

At larva stage (Fig. 3H) the expression of *Ci-msxB* is clearly evident around the ocellus, in a restricted part of the nervous system, specifically in a thin neck connecting the sensory vesicle and the visceral ganglion and in primordial pharynx.

In situ hybridization experiments on sectioned young adult were carried out in order to identify adult tissues expressing *Ci-msxB*. Signals were evident in the branchial sac or pharynx (Satoh, 1994), in particular in the internal longitudinal bars, present on the inner wall of the organism (Fig. 4A) and in the neural gland that lies between the two siphons associated to the cerebral ganglion (Fig. 4C). Hybridization of the corresponding sections with the sense probe were reported as control and no signals are detected (Fig. 4B,D).

This paper reports on the presence of the second *msxB* gene in *C. intestinalis* and the presence of two genes in *Ciona intestinalis*, if confirmed in other urochordates, would

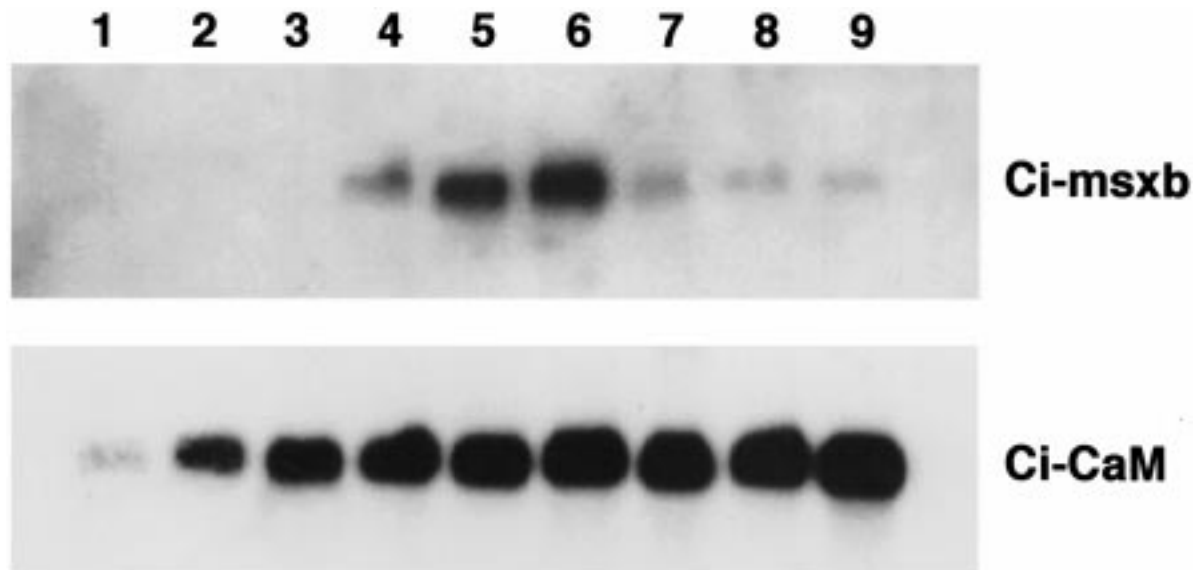


Fig. 2. Expression of *Ci-msxB* during *Ciona* embryonic development. Northern blot of RNA poly (A)⁺ (10 µg for lane) prepared from several embryological stages. Upper panel: hybridization with ³²P-labelled *Ci-msxB* cDNA probe. Lower panel: the same blot was further hybridized with *Ci-CaM* cDNA probe (Di Gregorio et al., 1998). Lane 1: RNA from unfertilized eggs; lanes 2-9: RNA from 16 blastomeres, blastula, gastrula, neurula, early tailbud, middle tailbud, late tailbud, larva stage embryos, respectively.

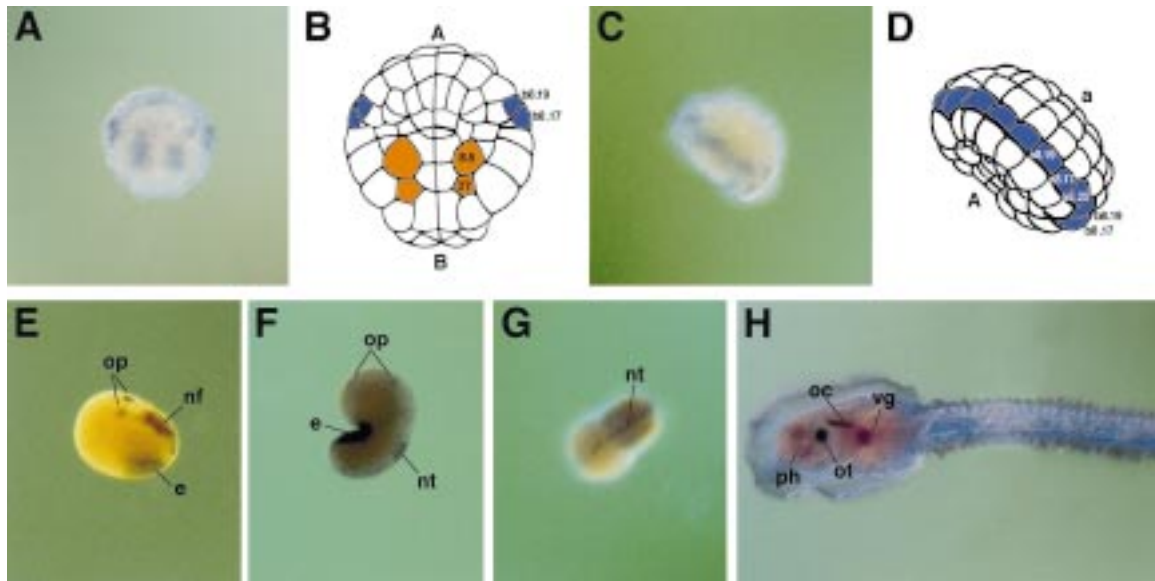


Fig. 3. Spatial expression of *Ci-msxb* transcript as revealed by whole-mount in situ hybridization. (A) The 110 cell-stage embryo viewed from the vegetal pole and (B) diagrammatic drawing of A. (C) The 110 cell-stage embryo viewed from the lateral pole and (D) diagrammatic drawing of C. (E) An embryo at neurula stage from lateral view. (F-G) An early taubud embryo viewed from the lateral and dorsal side respectively. (H) A larva stage from dorsal view. Control embryos hybridized with sense probe did not show signals (data not shown). e, epidermis; nf, neural folds; nt, notochord; oc, ocellus; op, otolith-ocellus precursors; ot, otolith; ph, primordial pharynx; vg, visceral ganglion.

suggest that the gene duplication event occurred before urochordates diverged unless the phenomenon was due to convergent evolution.

Moreover the restricted area of gene expression suggests a major role of the *Ci-msxb* in the development of structures derived mainly from the ectodermal layer.

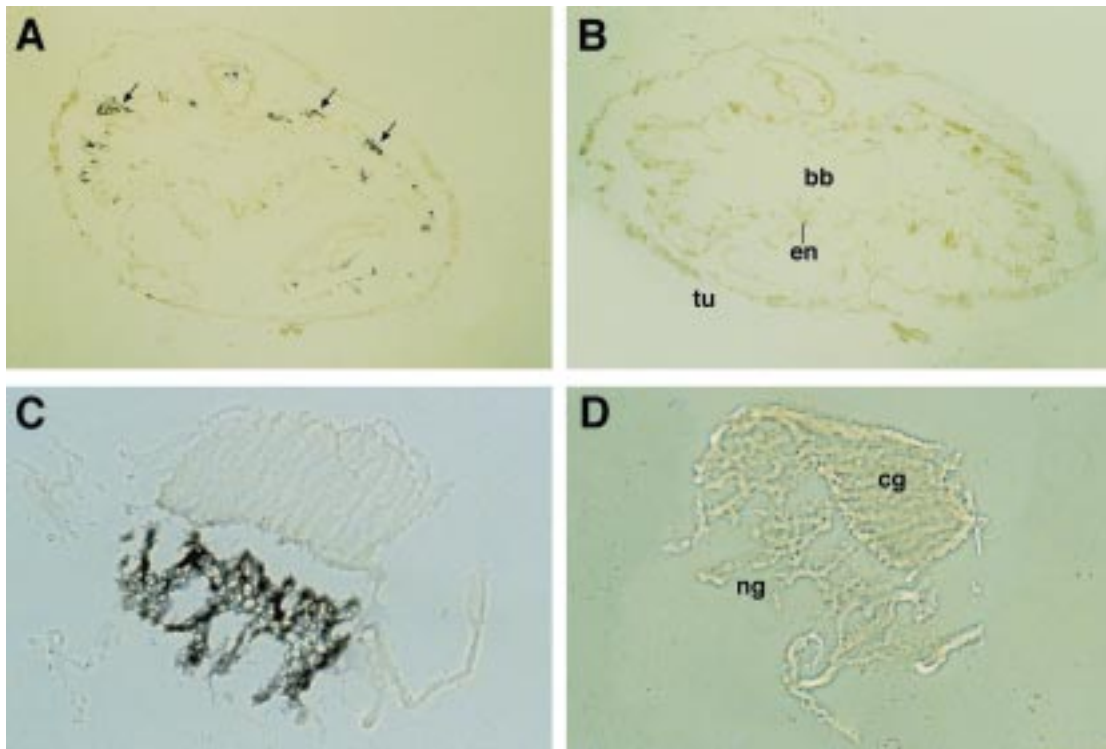


Fig. 4. Spatial expression of *Ci-msxb* in juvenile. Semithin transversal section of the pharynx hybridized with antisense (A) and sense (B) *Ci-msxb* probe. Semithin transversal section of the nervous system hybridized with antisense (C) and sense (D) *Ci-msxb* probe. The arrows indicate the internal longitudinal bars. bb, branchial sac; cg, cerebral ganglion; en, endostyle; ng, neural gland; tu, tunic.

2. Materials and methods

2.1. Cloning of the *C. intestinalis* *msx* cDNA

cDNA library from *C. intestinalis* larvae was prepared as already reported in Di Gregorio et al. (1995), screening and sequencing were performed as in Di Gregorio et al. (1998).

2.2. Whole mount *in situ* hybridization

The *Ci-msxb* cDNA insert in pBluescript SK(–) was used as a template for the synthesis of DIG-11-UTP labelled RNA sense or antisense riboprobes as described by manufacturers in the Digoxigenin RNA labelling kit (Boehringer Mannheim). Whole mount *in situ* hybridization on *Ciona* embryos and larvae were carried out as described in Caracciolo et al. (1997).

2.3. Expression of *Ci-mshb* in the adult

Young adult and dissected neural complex were fixed in 4% paraformaldehyde in 75% sea water at 4°C. Samples were dehydrated, embedded in paraffin, sectioned at 7.5 µm and processed for *in situ* hybridization according to Simeone et al. (1995).

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