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Authors: Harada, Yoshito, Okai, Noko, Taguchi, Shunsuke, Shoguchi, Eiichi, Tagawa, Kunifumi, et al.

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[SHORT COMMUNICATION]

Embryonic Expression of a Hemichordate *distal-less* Gene

Yoshito Harada¹, Noko Okai², Shunsuke Taguchi³, Eiichi Shoguchi,
Kunifumi Tagawa, Tom Humphreys and Nori Satoh*

Department of Zoology, Graduate School of Science, Kyoto University,
Sakyo-ku, Kyoto 606-8502, Japan
Kewalo Marine Laboratory, Pacific Biomedical Research Center,
University of Hawaii, Honolulu, HI 96813, USA

ABSTRACT—Hemichordates occupy a critical phylogenetic position among deuterostomes because they exhibit echinoderm-like larval morphology and chordate-like adult morphology. Analyses of the expression and function of hemichordate developmental genes will therefore provide insight into the evolution of deuterostome body plans. The *distal-less/dlx* gene encodes a homeodomain transcription factor and plays roles in the development of appendages and the brain in a variety of animals. Here we have characterized a *distal-less* gene (*Pf-dlx*) of the hemichordate *Ptychodera flava*. During embryogenesis, *Pf-dlx* is expressed in the whole aboral ectoderm of the blastula and gastrula. Later, its expression appears in several cells in the boundary region between the oral and aboral ectoderm. The tornaria larvae express *Pf-dlx* in some specific cells of the ciliary band. The results are discussed in terms of an ancestral function of the *distal-less/dlx* gene in the formation of the nervous system.

INTRODUCTION

Our present knowledge of the molecular mechanisms of evolutionary developmental processes has mainly been obtained from investigations using model animals such as flies (*Drosophila melanogaster*), nematodes (*Caenorhabditis elegans*), fish (*Danio rerio*), frogs (*Xenopus laevis*) and mice (*Mus musculus*). From comparative studies of these animals, a number of conserved regulatory systems have been found to play roles in putatively comparable developmental processes among such model organisms. For example, the HOM-C/Hox complex has been shown to play a central role in the antero-posterior axis formation in a variety of animals (Miller and Miles, 1993).

Interestingly, such correspondences are seen even in the formation of structures which are not necessarily thought to involve plegiomorphy. For example, the formation of the *Drosophila* 'wing' and the chick 'wing' utilizes some shared developmental regulatory systems (Laufer *et al.*, 1997), although these structures are thought of as a good instance of evolutionary convergence (see Hall, 1998). Such similarity of gene expression pattern between vertebrates and insects appears to be surprising for several reasons. One reason is that these two phyla are phylogenetically distant from each other. Another reason is that modes of their development may not have persisted well after evolving from their ancestral condition, but rather they have been altered, since most invertebrate taxa display different modes of development than vertebrate taxa, particularly concerning the initial process of cell specification (reviewed by Davidson, 1991). Therefore, in order to understand the evolution of animal body plans, we need to accumulate much more basic molecular information on the development of animals, including small phyla of marine invertebrates, because they display a major part of the biodiversity of metazoa.

Hemichordates, acorn worms, occupy a unique phylogenetic location because they exhibit echinoderm-like larval morphology as well as chordate-like adult morphology (e.g.,

* Corresponding author: Tel. +81-75-753-4081;

FAX. +81-75-705-1113.

E-mail: satoh@ascidian.zool.kyoto-u.ac.jp

¹ Present address: Kuroda Chiromorphology Project ERATO, Japan Science and Technology Corporation, Park Bldg., 4-7-6 Komaba, Meguro-ku, Tokyo 153-0041, Japan

² Present address: Undergraduate School of Medicine, Hiroshima University, Higashi Hiroshima 739-8526, Japan

³ Present address: Glaxo Wellcome Ltd. Tsukuba Research Lab., Tsukuba 305-8500, Japan

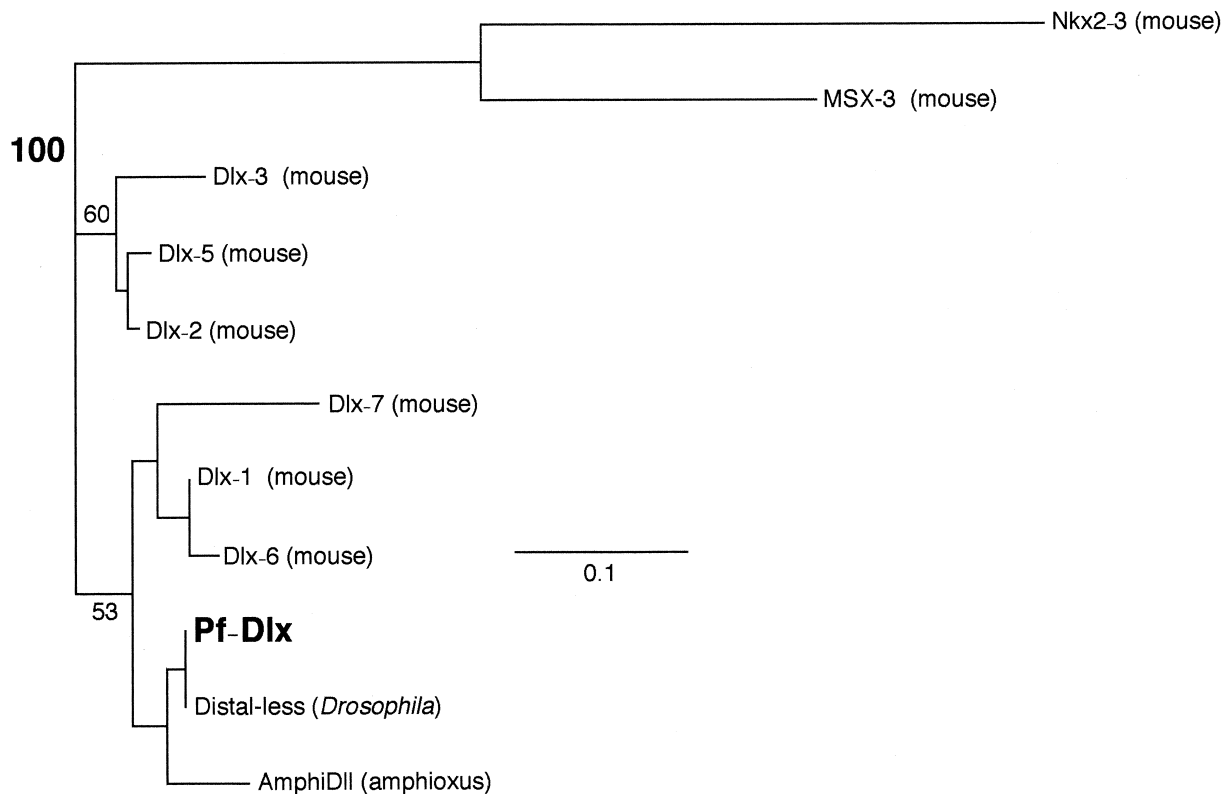


Fig. 2. Molecular phylogenetic analysis of relationships of *distal-less/dlx* subclass and related-class homeobox gene products using the homeodomain amino acid sequences. Branch lengths are proportional to evolutionary distance corrected for multiple substitutions with the scale denoting 0.1 amino acid substitutions per site. The numbers indicate the relative robustness of each node as assessed by bootstrap analysis (100 replications).

of the related homeobox gene products by using mouse *Nkx2-3* and *Msx-3* gene products as outgroups. As shown in Fig. 2, the clade of Dlx proteins, including Pf-Dlx, was supported with a 100% bootstrap value. Thus, we concluded that *Pf-dlx* is a hemichordate ortholog of the *distal-less/dlx* gene. The vertebrate *dlx* family is divided into two major subgroups as a consequence of tandem gene duplication, and each subgroup contains several subclass members (Fig. 2; Stock *et al.*, 1996). However, *Pf-dlx* does not show any affinity to a specific subclass, in contrast to amphioxus *AmphiDII* and *distal-less* of *Drosophila*.

***Pf-dlx* is expressed in the aboral ectoderm of the blastula and gastrula and in cells of the oral/aboral boundary of the tornaria larva**

The expression of *Pf-dlx* was detected as early as the blastula stage. It was expressed in half of the ectodermal region of the blastula and early gastrula (Fig. 3A, B). The *Pf-dlx*-positive half corresponds to what will become the aboral side, and *in situ* hybridization signal was stronger in the aboral side than in the oral/aboral boundary side. This expression pattern suggests that the ectoderm of the acorn worm blastula can be subdivided into oral and aboral halves, as in the case of the ectoderm of the sea urchin blastula (reviewed by Davidson *et al.*, 1998). Since the tornaria larva of acorn worms and the larva of echinoderms show remarkable morphological simi-

larity to each other, it will be interesting to examine whether the ectoderm of acorn worm embryos is specified by mechanisms similar to those for the ectoderm of echinoderm embryos (discussed in Davidson *et al.*, 1998).

The expression of *Pf-dlx* in the aboral ectoderm decreased during gastrulation, except in cells on the edge of the expression domain, or else expression appeared in this region *de novo*. At the late gastrula stage, cells with strong *Pf-dlx* expression were observed in the oral/aboral boundary (Fig. 3C). Figure 3D-G shows *Pf-dlx* expression in the tornaria larva. Judging from specimens viewed from the animal pole, the aboral ectodermal expression continued at this stage (Fig. 3E), although it became weaker. *Pf-dlx* signals were evident in cells located discontinuously in the line of the oral/aboral boundary (Fig. 3D, F). This boundary may correspond to the aboral part of the post-oral ciliary band, which contains cells of the nervous system, by analogy to the sea urchin embryo (Nakajima, 1986; Cameron *et al.*, 1993). Figure 3D shows an aboral view of the early tornaria; *Pf-dlx*-positive cells are buried in the epidermis, and distribution of cells with *Pf-dlx* expression in the larva is not left-and-right symmetrical (Fig. 3D). This pattern of *Pf-dlx* expression persisted in the 6-day-old larva, the last stage we observed.

The *distal-less/dlx* gene is expressed in the appendages of animals in many phyla (e.g., Panganiban *et al.*, 1997). However, it is thought that the *distal-less/dlx* gene is older than the

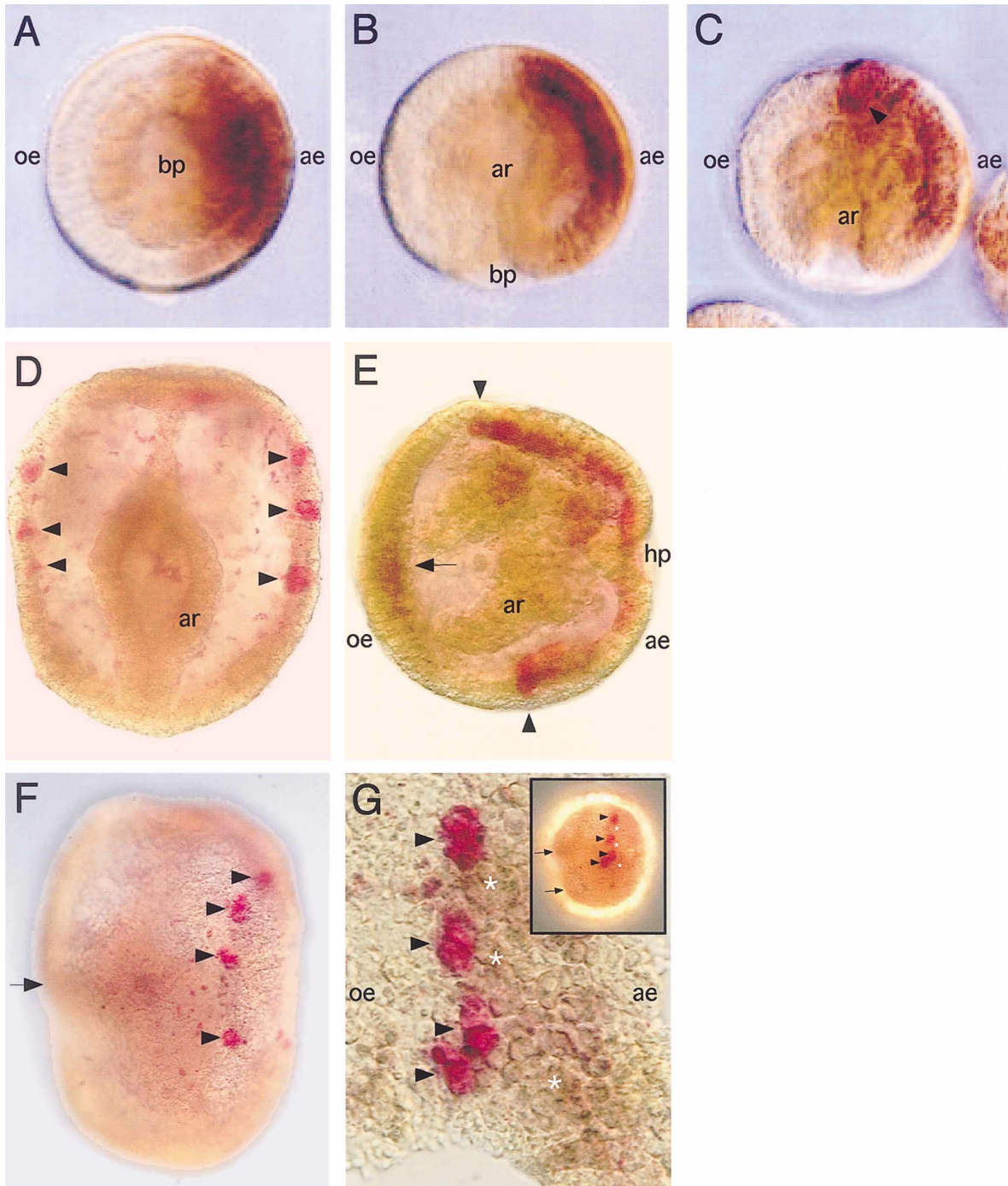


Fig. 3. Spatial expression of *Pf-dlx*. (A, B) An early gastrula (18 hr of development), vegetal pole view (A) and lateral view (B). ae, aboral ectoderm; ar, archenteron; bp, blastopore; oe, oral ectoderm. (C) A late gastrula (24 hr of development). The intensity of the signal in the aboral ectoderm decreases, while a distinct signal is detected in the oral/aboral boundary (arrowhead). (D–F) Simultaneous detection of *Pf-dlx* (red signals, arrowheads) and *Pf-otx* expression (brown signals, arrows) in early tornaria larvae (3 days of development), viewed from the aboral side (D), animal pole (E) and lateral side (F), respectively. D and E show the same larva. hp, hydropore. (G) An enlargement of the left lateral ectoderm region of a tornaria larva shown in the insert. Red signals of *Pf-dlx* mRNA are detected in several cells at the oral/aboral boundary, which partially overlap the region with brown signals of *Pf-otx* expression (white asterisks).

putative outgrowth-bearing ancestor. In addition, the genes are expressed in the CNS and peripheral nervous system, including parts of the brain involved in optic function (Price *et al.*, 1991; Kaphingst and Kunes, 1994). The *distal-less/dlx* gene is also expressed in the CNS of nematode embryos (Pan-

ganiban *et al.*, 1997). Therefore, it has been suggested that the ancestral function of the gene is associated with the CNS formation. Although the function of *Pf-dlx* in the ciliary band remains to be elucidated, the pattern of its expression suggests its role in the nervous system formation. We recently

found that the hemichordate *otx* gene (*Pf-otx*) is expressed along the ciliary band (Harada *et al.*, 2000). To determine whether the expression domains of *Pf-dlx* and *Pf-otx* are overlapping, larvae were double-hybridized with two probes, one for *Pf-dlx* and the other for *Pf-otx*, and the relative locations of the regions showing expression of these genes were monitored simultaneously. As shown in Fig. 3G, cells with *Pf-dlx* expression were located within the region of cells with *Pf-otx* expression. However, it was not determined whether individual cells express both *Pf-dlx* and *Pf-otx* simultaneously. These molecular probes can be used as tools for examining further a putative relationship between the ciliary bands of the tornaria larva and the CNS of chordates, an issue which has long been debated by numerous researchers (Garstang, 1928; Crowther and Whittaker, 1992; Nielsen, 1999).

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REFERENCES

- Brusca RC, Brusca GJ (1990) "Invertebrates." Sinauer Associates, Inc., Sunderland, MA, USA
- Cameron RA, Britten RJ, Davidson EH (1993) The embryonic ciliated band of the sea urchin, *Strongylocentrotus purpuratus*, derives from both oral and aboral ectoderm territories. *Dev Biol* 160: 369–376
- Cohen SM (1990) Specification of limb development in the *Drosophila* embryo by positional cues from segmentation genes. *Nature* 343: 173–177
- Crowther RJ, Whittaker JR (1992) Structure of the caudal neural tube in an ascidian larva: vestiges of its possible evolutionary origin from a ciliated band. *J Neurobiol* 23: 280–292
- Davidson EH (1991) Spatial mechanisms of gene regulation in metazoan embryos. *Development* 113: 1–26
- Davidson EH, Cameron RA, Ransick A (1998) Specification of cell fate in the sea urchin embryo: summary and some proposed mechanisms. *Development* 125: 3269–3290
- Dollé P, Price M, Duboule D (1992) Expression of the murine *Dlx-1* homeobox gene during facial, ocular and limb development. *Differentiation* 49: 93–99
- Duboule D (1994) "Guidebook to the Homeobox Genes." Oxford University Press, New York
- Felsenstein J (1993) PHYLIP ver. 3.5. University of Washington, Seattle
- Garstang W (1928) The morphology of the tunicata, and its bearings on the phylogeny of the Chordata. *Q J Microsc Sci* 72: 51–187
- Hall BK (1998) "Evolutionary Developmental Biology, Second ed." Chapman & Hall, London
- Harada Y, Okai N, Taguchi S, Tagawa K, Humphreys T, Satoh N (2000) Developmental expression of the hemichordate *otx* ortholog. *Mech Dev* 91: 337–339
- Holland ND, Panganiban G, Henyey EL, Holland LZ (1996) Sequence and developmental expression of *AmphiDlx*, an amphioxus *Distal-less* gene transcribed in the ectoderm, epidermis and nervous system: insights into evolution of craniate forebrain and neural crest. *Development* 122: 2911–2920
- Kaphingst K, Kunes S (1994) Pattern formation in the visual centers of the *Drosophila* brain: *wingless* acts via *decapentaplegic* to specify the dorsoventral axis. *Cell* 78: 437–448
- Laufer E, Dahn R, Orozco OE, Yeo CY, Pisenti J, Henrique D, Abbott UK, Fallon JF, Tabin C (1997) Expression of *Radical fringe* in limb-bud ectoderm regulates apical ectodermal ridge formation. *Nature* 386: 366–373
- Lowe CJ, Wray GA (1997) Radical alterations in the roles of homeobox genes during echinoderm evolution. *Nature* 389: 718–721
- Miller DJ, Miles A (1993) Homeobox genes and the zootype. *Nature* 365: 215–216
- Nakajima Y (1986) Development of the nervous system of sea urchin embryos: formation of ciliary bands and the appearance of two types of ectoneural cells in the pluteus. *Dev Growth Differ* 28: 531–542
- Nielsen C (1999) Origin of the chordate central nervous system - and the origin of chordates. *Dev Genes Evol* 209: 198–205
- Panganiban G, Irvine SM *et al.* (1997) The origin and evolution of animal appendages. *Proc Natl Acad Sci USA* 94: 5162–5166
- Peterson KJ, Cameron RA, Tagawa K, Satoh N, Davidson EH (1999) A comparative molecular approach to mesodermal patterning in basal deuterostomes: the expression pattern of *Brachyury* in the enteropneust hemichordate *Ptychodera flava*. *Development* 126: 85–95
- Price M, Lemaistre M, Pischetola M, Di Lauro R, Duboule D (1991) A mouse gene related to *Distal-less* shows a restricted expression in the developing forebrain. *Nature* 351: 748–751
- Shoguchi E, Satoh N, Maruyama YK (2000) A starfish homolog of mouse *T-brain-1* is expressed in the archenteron of *Asterina pectinifera* embryos: Possible involvement of two T-box genes in starfish gastrulation. *Dev Growth Differ* 42: 61–68
- Stock DW, Ellies DL, Zhao Z, Ekker M, Ruddle FH, Weiss KM (1996) The evolution of the vertebrate *Dlx* gene family. *Proc Natl Acad Sci USA* 93: 10858–10863
- Tagawa K, Nishino A, Humphreys T, Satoh N (1998a) The spawning and early development of the Hawaiian acorn worm (hemichordate), *Ptychodera flava*. *Zool Sci* 15: 85–91
- Tagawa K, Humphreys T, Satoh N (1998b) Novel pattern of *Brachyury* gene expression in hemichordate embryos. *Mech Dev* 75: 139–143
- Tagawa K, Humphreys T, Satoh N (2000) *T-Brain* expression in the apical organ of hemichordate tornaria larvae suggests its evolutionary link to the vertebrate forebrain. *J Exp Zool* 288: 23–31
- Taguchi S, Tagawa K, Humphreys T, Nishino A, Satoh N, Harada Y (2000) Characterization of a hemichordate *fork head/HNF-3* gene expression. *Dev Genes Evol* 210: 11–17

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