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ABSTRACT

Several homeobox-containing genes related to Distal-less (Dlx/Dll) have been isolated from a wide variety of organisms and have been shown to function as developmental regulators. In Drosophila only one Distal-less gene has been identified so far, and in vertebrates many components of the Distal-less family have been characterized. This suggests that, during the evolution of the Chordate phylum, the Dlx genes arose from an ancestral Distal-less gene via gene duplication. Three Dll homeoboxes have been isolated from the protochordate *Ciona intestinalis* and their clustered arrangement has been described. Since Ciona is regarded as one of the most primitive extant chordates, the present analysis gives us an insight into how these fundamental biological genes are evolved or are conserved during chordate evolution. The goal of this project was to clone coding sequences from the DllA/DllB cluster of *Ciona intestinalis* in order to observe the gene expression using in situ hybridization.

During my study of DllA and DllB genes in Ciona, I used various techniques including PCR, restriction digest, sticky-end ligation, and transformation, to obtain cDNA from each gene. These cDNAs, DNA copies of the mRNA sequences, were used to create digoxigenin-labeled antisense RNA probes by in vitro transcription with T7 RNA polymerase. The in-situ hybridization documented gene expression in the epithelial cells and neural tissue, which is consistent with the expression patterns found in other species. The DllA probe was expressed in the anterior epithelial tissue in mid-tail stage embryos, on the adhesive papillae of late-tail stage embryos, and in the bilateral primordia of the atrial siphon of larvae. DllB probe in blastula through early tail stages showed expression patterns in epidermal and neuroectodermal cells.

INTRODUCTION

The model that is being used to study gene expression is Ciona intestinalis, one of the most primitive of chordates. In their juvenile stage they retain all of the basic chordate characteristics, and subsequently lose their notochord once they reach their adult stage. They serve as a fitting model for this study because their genome has already been sequenced, they have a relatively small genome, a fast embryonic development, and most importantly, their embryos are easily used for in situ hybridizations (Corbo et. al. 2001).

Developmental genes, such as the Distal-less genes, are being studied because of their repercussions on human health. Disruption of cis-regulatory DNA have been shown to cause birth defects, abnormalities, and could be the cause of some types of cancer. The Dlx genes have been shown to be the cause of Tricho-dento-osseous syndrome, which causes abnormalities in the hair, teeth, and cranial bones of humans (Hart et. al. 1998).

The Dlx genes are homeobox genes that play a role in skeletal formation and in the development of the central nervous

system. Dlx genes are expressed during all stages of craniofacial, axial, and appendicular skeletal formation. In mouse embryos the Dlx gene is expressed in multiple tissues including placenta, brachial arches, developing hair follicle, differentiating odontoblasts, and keratinocytes.

MATERIALS AND METHODS

Construction of the probes

Obtaining the cDNA involved cloning two pieces of the Distalless homeobox cluster. In order to perform this task, primers were constructed from the known sequence of the Ciona Distalless-B/A gene cluster. Through PCR (polymerase chain reaction) of the primers, amplified fragments corresponding to the DllB (.3Kb) and DllA (1.3Kb) coding regions were made and named MLC1 and MLC2 respectively. These fragments were then purified of extra PCR product and cut with restriction enzymes. MLC1 was cut with HindIII and BamHI in order to correspond to the PstBlue vector that it is eventually ligated into. MLC2 was cut with HindIII and SalI to correspond to the pBKS vector. Both of these vectors contain the LacZ gene, which is used for staining in the Ciona embryos. Once cut with enzymes the fragments were run on an agarose gel by electrophoresis, and the DNA bands that resulted were cut out of the gel and purified. The fragments were then ligated into their respective vector. After, they were transformed into E. Coli, plated on an agar plates, and allowed to grow overnight. Once the ligation and transformation process was completed, the E. Coli were lysed and the transformed DNA was purified of any impurities that remain. The DNA was then checked for the MLC1 and MLC2 inserts by another restriction digest and electrophoresis to ensure that the transformation worked correctly. These cDNA pieces were used to create digoxigenin-labeled antisense RNA probes by in vitro transcription with T7 RNA polymerase.

In situ Hybridization

Embryos were cultured at 18°C and chemically dechorionated at spawning. Embryos were fixed in 4% paraformaldehyde in .5M NaCl and .1M MOPS for thrity minutes. They were then transferred to 100% ethanol and stored at 4°C. Once the in situ began the embryos were rehydrated with one wash in 75%ethanol, 50% ethanol, and 30% ethanol and then three times with PTw. The blastula to early tail embryos used for the DllB probes were treated with 2ug/ml final concentration of Proteinase K at 37°C for 4 minutes. The early tail to late tail embryos for DllA were treated at the same concentration for eight minutes. Half the larvae used for DllA probes were treated at the 2ug/ml concentration for 12 minutes at 37°C while the other half were treated with 4ug/ml concentration for 15 minutes. They were all post fixed in 4% paraformaldehyde for a half an hour at room temperature. The hybe temperature was 60°C using a modified Corbo hybe solution. After the pre-hybe period of two hours, 300ng of probe was placed in hybe solution and heated at 80°-90°C for eight minutes before being added to the embryos. The embryos were washed using the Martindale washing regime. This consists of washings at hybe temperature for twenty minutes of 75%hybe/25% 2x SSC/0.1% Tw, 50%hybe/50% 2x SSC/0.1% Tw, 25%hybe/75% 2x SSC/0.1% Tw, 100% 2x

SSC/0.1% Tw, and three in 0.1x SSC/0.1% Tw. The next washes were at room temperature for ten minutes of 75% 0.1x SSC/0.1% Tw/ 25% Ptw, 50% 0.1x SSC/0.1% Tw/ 50% Ptw, 25% 0.1x SSC/0.1% Tw/ 75% Ptw, and two in 100% Ptw. They were then washed twice in B-M Block in PTw and 1ml of 2X anti-Dig antibody was added to incubate overnight without rocking at 12°C. They were washed twice for five minutes each with AP Staining Buffer (100mM NaCl, 50mM MgCl, 100mM Tris pH9.5, 0.5% Tween 20). They were developed with 2X AP Substrate Solution (6.6ul/ml of NBT 100mg/ml in DMF, and 6.6ul/ml of BCIP 50mg/ml in DMF). The staining reaction took anywhere from 3-96 hours. Specimens were moun ted in 50% glycerol/Ptw and photographed.

RESULTS

The whole mount in situ hybridizations of DllA showed there was no signal observed before the mid-tail stage embryos which is in agreement with present data (Caracciolo et. al. 2000). The mid tail stage embryos have definite staining in the focal anterior epidermal cells (Fig.1q). The late tail have staining in the anterior epidermis (Fig.1s) where the adhesive papillae would form. The adhesive papillae have developmental similarities to the branchial arches of amphibians and other vertebrates (Nishida, 1987). The larvae have staining in the ectodermal tissue in two places posterior to the eye spot (Fig.1 r,t), which based on current literature is the bilateral primordia of the atrial siphon (Caracciolo et. al. 2000).

In DllB the staining was seen as early as the 64 cell blastula stage embryos. Staining occurred in all of the animal cells (Fig.1 a,e) of the embryo and in two cells on the vegetal side (Fig.1 b,f), which are part of the B lineage of cells. The two cells on the vegetal pole are specifically the B7.6 cells and curiously the only staining that is not ectoderm. These cells give rise to muscle/endoderm (Nicol et. al. 1988).

In gastrula and early neurula stage embryos (Fig.1 c,d,g-j,m,n) there was some general epidermis and ectodermal staining, which is consistent with the cell lineage patterns seen in the literature (Nishida, 1987). The staining follows the neural plate development. During gastrulation, the group of animal cells most anteriorly located comes up dorsally and overlays the anterior vegetal group of cells that is in the presumptive notochordal region and responsible for neural inducing activity (Takahashi et. al. 1998). This movement of cells is seen in the staining patterns of the late gastrula embryos which shows dark staining on the anterior vegetal pole (Fig.1 c,g) and lighter staining on the anterior animal pole (Fig.1 d,h).

The late neurula (Fig.1 l,p) developed a linear smear over the remnants of row VI of the neural plate. They are specifically cells a10.72 and a10.80 (Nicol et. al. 1988). The progeny of these cells will appear in epidermis and palps. The linear smear localizes into two spots in early tail embryos (Fig.1o) which are progeny of the a line and will form the anterior palps (Nicol et. al. 1988).
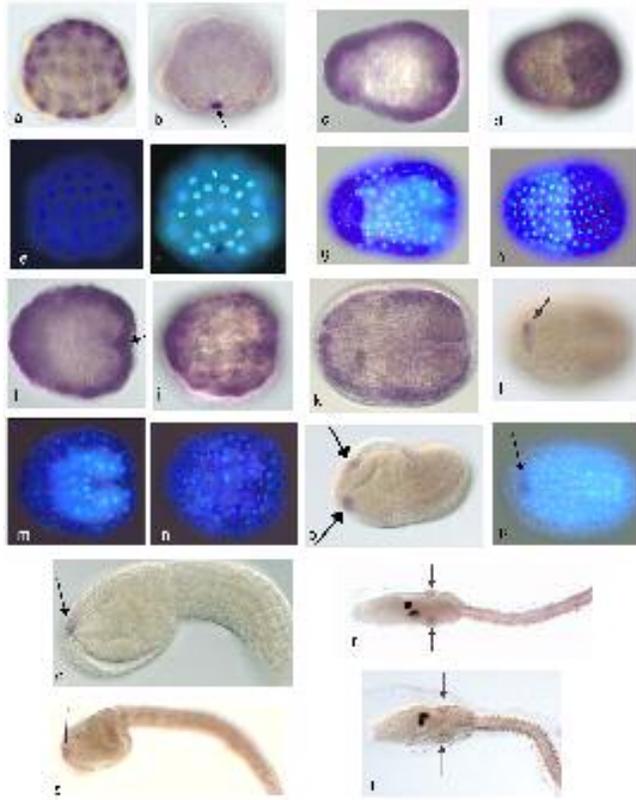
Figure 1. In situ photographs of DllA and DllB. All picture have anterior facing left except the 64-cell embryos in which anterior is up. (a-p) DllB staining. (q-t) DllA staining. (a-b) 64-cell viewed from the dorsal and ventral poles respectively. (e-f) DAPI staining of those embryos. (c-d) late gastrula ventral and dorsal sides (g-h) DAPI staining of those embryos. (i-j) early gastrula ventral and dorsal sides. (m-n) DAPI staining of those embryos. (k) early neurula (l+p) late neurula (o) early tail. (q) mid tail (s) late tail (r+t) larvae.

DISCUSSION

The study of Distal-less genes in ascidians could have implications on understanding how the gene evolved. The roles of the Dlx genes in vertebrate development have primarily been ascertained through the analysis of loss-of-function mutations in zebra fish and mice. (Panganiban et. al. 2002) In studies of both these animals Dlx1 and Dlx2 were shown to have effects in the branchial arches. The in situ of DllA showed staining in the anterior region where the adhesive papillae form (Fig.1q,s). The adhesive papillae are similar to the branchial arches in higher organisms. Therefore the Dlx1 and Dlx2 genes in higher organisms could have evolved from the DllA gene. The primitive ectoderm gives rise to a neural plate encircled by surface ectoderm/epidermis. At the border of the neural plate and epidermis lie cells that give rise to neural crest and the dorsal midline of the neural tube. Several of the Dlx genes are required for development of these tissues. The Dlx3 and Dlx5 were seen in gastrulation in the neural plate and surface ectoderm in zebrafish (Panganiban et. al. 2002). The in situ of DllB (Fig.1 c,d,g-j) showed staining in the same areas in the same stage. This shows a strong correlation between DllB and Dlx3 and Dlx5. Those genes most likely evolved from the DllB gene.

LITERATURE CITED

Caracciolo A, Di Gregorio A, Aniello F, Di Lauro R, Branno M. Identification and developmental expression of three *Distal-less* homeobox containing genes in the ascidian *Ciona intestinalis*. *Mechanisms of Development.* 2000;99:173-176.

Corbo JC, Di Gregorio A, Levine M. The Ascidian as a Model Organism in Developmental and Evolutionary Biology. *Cell.* 2001;106:5:535-538.

Hart TC, Bowden DW, Price JA, Kula K, Hall K, Wright JT. A common Dlx3 gene mutation is responsible for tricho-dento-osseous syndrome in Virginia and North Carolina. *Journal of Medical Genetics.* 1998;35:825-828.

Nicol D, Meinertzhagen IA. Lineages of Ascidian Neural Plate. *Developmental Biology.* 1988;130:2:721-736.

Nishida H. Cell lineage analysis in ascidian embryos by intracellular injection of a tracer enzyme. *Developmental Biology.* 1987;121:2:526-541.

Panganiban G, Rubenstein JLR. Developmental functions of the *Distal-less*/Dlx homeobox genes. *Development*. 2002;129:4371-4386.

Takahashi K, Okamura Y. Ion Channels and Early Development of Neural Cells. Physiological Reviews. 1998;78:2:307-337.