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EXPRESSION NOTE

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Endogenous β-galactosidase activity in amphioxus: a useful histochemical marker for the digestive system

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Abstract Endogenous β-galactosidase activity has been shown in the digestive tract of amphioxus from the larval to the adult stage and it can be easily followed as a histochemical marker. Enzymatic activity first appeared in 30-h larvae, became evident in 36-h larvae and remained in adults. In situ detection of β-galactosidase activity was used to monitor morphological and functional differentiation of the digestive system and the posteriorization of the endodermal structures in retinoic-acid treated embryos. The endogenous β-galactosidase activity was distinguished from the bacterial *lacZ* reporter by incubation at low pH.

Keywords Amphioxus · β-Galactosidase activity · Endodermal marker

Introduction

β-Galactosidases (β-gal) are lysosomal enzymes that cleave non-reducing β-D-galactose residues in β-D-galactosides. They contribute to glycolipid metabolism and their deficiency is associated with G_{M1} gangliosidosis, an inherited metabolic disorder (Gossrau et al. 1991). Endogenous β-gal activity has been reported in *Drosophila* and mammals (Lodja 1970; Schnetzer and Tyler 1996). Although there is some intra-specific variation, tissues that are rich in β-gal include intestine, kidney and epididymis (Conchie et al. 1958; Pearson et al. 1963). Furthermore, β-gal activity at pH 6.0 has been reported as a marker for cell senescence (Dimri et al. 1995).

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Results and discussion

In the research reported here, endogenous β-gal activity has been detected in the chordate amphioxus, the closest living relative to vertebrates. This enzymatic activity appeared after staining incubation for over 48 h, at the ventral posterior endoderm of *Branchiostoma floridae* early larvae (30 h; Fig. 1A,B).

This was before larval feeding since the mouth had not yet opened. At 36 h, all the larvae showed β-gal activity in the midgut (Fig. 1C), and a strong signal was observed from the midgut to the anus at 50 h, whereas the pharyngeal portion remained unstained (Fig. 1D). In addition, a conspicuous signal was detected at the mid-hindgut junction, where the iliocolon ring develops. Although the digestive tract morphologically appeared as an uncompartmentalized tube the uneven staining pattern of 6-day and 15-day larvae revealed functional differentiation (Fig. 1E–H). The high β-gal activity at the mid-hindgut junction was located in two circular segments boundering the iliocolon ring (Fig. 1H). An intensely stained region was observed in the esophagus-midgut junction, from which the hepatic diverticulum will differentiate after metamorphosis. Another intensely stained small group of cells in the posterior left wall of the midgut could correspond to a new landmark of asymmetric amphioxus development (Fig. 1G,H), which could be used to draw new body part homologies between amphioxus and vertebrates (Holland and Holland 1999). The appearance of the 11th gill slit marks the beginning of amphioxus metamorphosis. Observations from this time onwards showed that the β-gal pattern remained in the gut and expanded into the developing hepatic diverticulum (Fig. 1F).

The involvement of retinoic acid (RA) in chordate anterior-posterior axis formation is well established. Exogenous RA affects endodermal development in ascidians

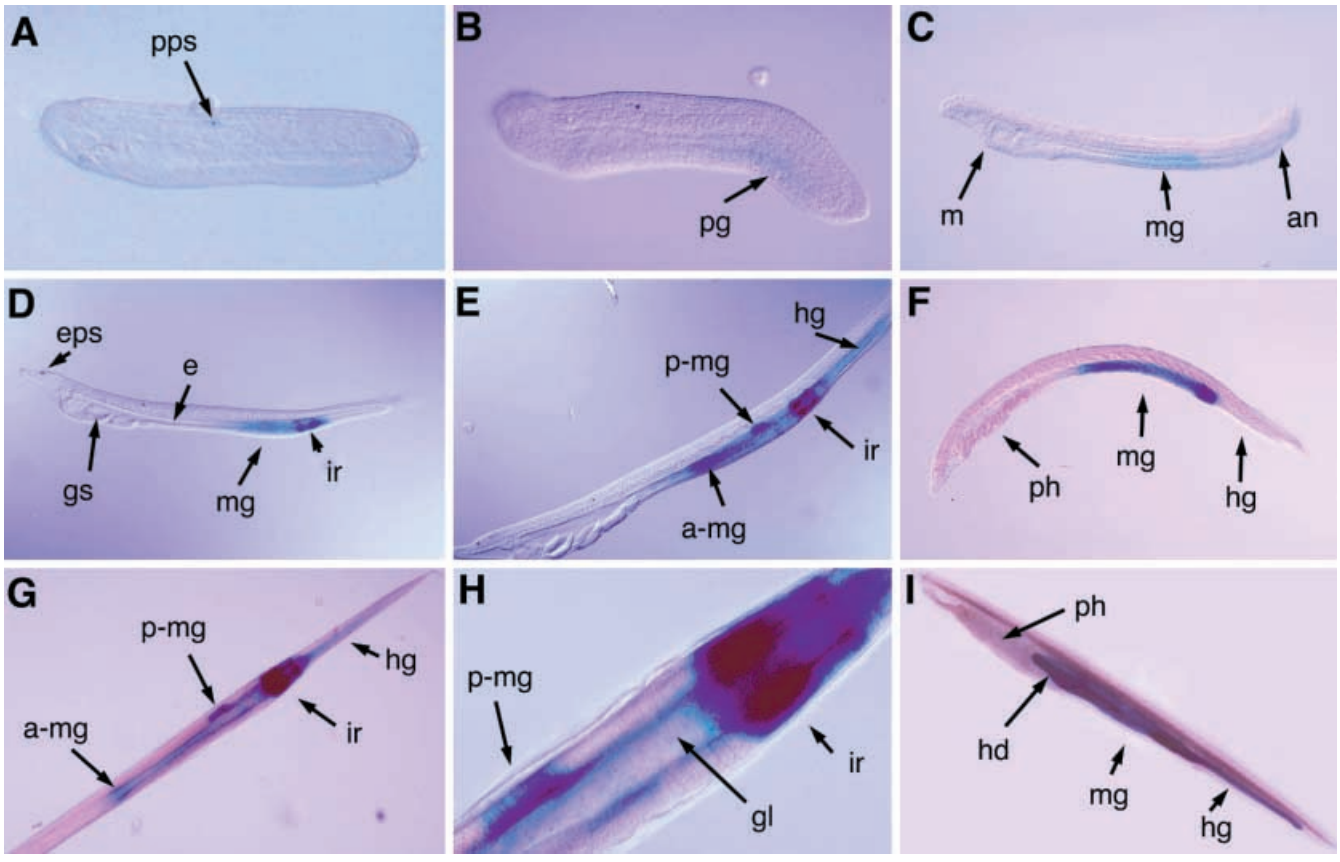

Fig. 1A–I β-Galactosidase (β-gal) activity detection during *Branchiostoma floridae* development. Animals were fixed in 0.2% glutaraldehyde in calcium/magnesium-free diluted artificial sea water (308 mM NaCl, 7.3 mM KCl, 1.4 mM $NaHCO_3$ and 17 mM Na_2SO_4) for 30 min at room temperature. After two washes in PBT (phosphate-buffered saline pH 7.2, Tween-20 0.1%) for 10 min each and once in staining buffer [1 mM $MgCl_26H_2O$, 3 mM $K_4Fe(CN)_63H_2O$ and 3 mM $K_3Fe(CN)_6$ in PBT] the samples were stained in fresh staining buffer with 0.4 mg/ml X-gal at 37°C. At alkaline pHs, 100 mM Tris-HCl buffer for the staining solution, instead of PBT, was used. The samples were fixed in 4% paraformaldehyde in PBT overnight at 4°C, washed three times in PBT and mounted in 80% glycerol in PBT. All specimens were oriented with the anterior end of the animal toward the left; in lateral views dorsal is to the *top*. **A** At the 24-h larval stage, β-gal activity was not detected. **B** β-Gal activity was weakly detected in the presumptive gut of 30-h larvae. **C** β-Gal stained the midgut of 36-h larvae uniformly. **D–F** Uneven staining showed gut compartmentalization in 4-day (**D**), 6-day (**E**) and 15-day (**F**) larvae. **G–H** Ventral view of 8-gill-slit larvae. Asymmetrical differentiation was detected on the left posterior midgut. **I** Strong β-gal activity was detected in the hepatic diverticulum of adult animals. *a-mg* Anterior midgut, *an* anus, *gs* gill slits, *gl* gut light, *hd* hepatic diverticulum, *hg* hindgut, *e* esophagus, *eps* eye pigment spot, *ir* iliocolon ring, *mg* mid-gut, *m* mouth, *ph* pharynx, *p-mg* posterior midgut, *pps* primary pigment spot

(urochordates; Hinman and Degnan 1998) and induces the loss of pharyngeal arches in vertebrates, due to *Hox* code alterations in neural crest migrating cells (Kraft et al. 1994; Lee et al. 1995). In amphioxus, RA treatment affects the pharyngeal development not by changing the *Hox* code but by inducing the overexpression of *Pax-1* and possibly other genes (Holland and Holland 1996). This posteriorization effect could be followed by the β-gal pattern of treated animals (Fig. 2A,B): their digestive tract resembled a blue tube that extended along the anterior-posterior axis (Fig. 2C,D). β-gal activity was detected in the digestive tract but not in the pharynx of wild-type embryos. Detection of β-gal activity in the presumptive pharyngeal region of treated animals indicated that RA not only prevented the formation of gill slit and mouth but probably induced an alteration of gene expression in the anterior endoderm and hence functional posteriorization.

The β-gal of amphioxus and mammals have acidic pH optima. They both behave poorly at weakly alkaline conditions, which are very favorable to the prokaryotic enzymes. On the other hand, an extensive antibiotic treatment (0.5 µg/ml penicillin, 0.5 µg/ml gentamycin and 1 µg/ml streptomycin) did not alter β-gal staining. Besides, β-gal activity detection preceded the mouth opening. Taken together, these findings indicated that β-gal activity is endogenous in amphioxus.

The *Lac Z* gene has been extensively used as a marker in gene transfer assays. In this case, histochemical discrimination of the prokaryotic activity from endogenous β-gal activity would be attainable at pH >8.5 under the conditions already described for mammals (Weiss et al. 1999). At these pHs the amphioxus β-gal activity was always negligible whereas *Lac-Z* expression of transformed *Escherichia coli DH5α* was clearly identified (data not shown). The discrimination between these two activities, β-gal endogenous and bacterial *Lac-Z*, is of relevance before using this prokaryotic reporter in gene transfer assays.

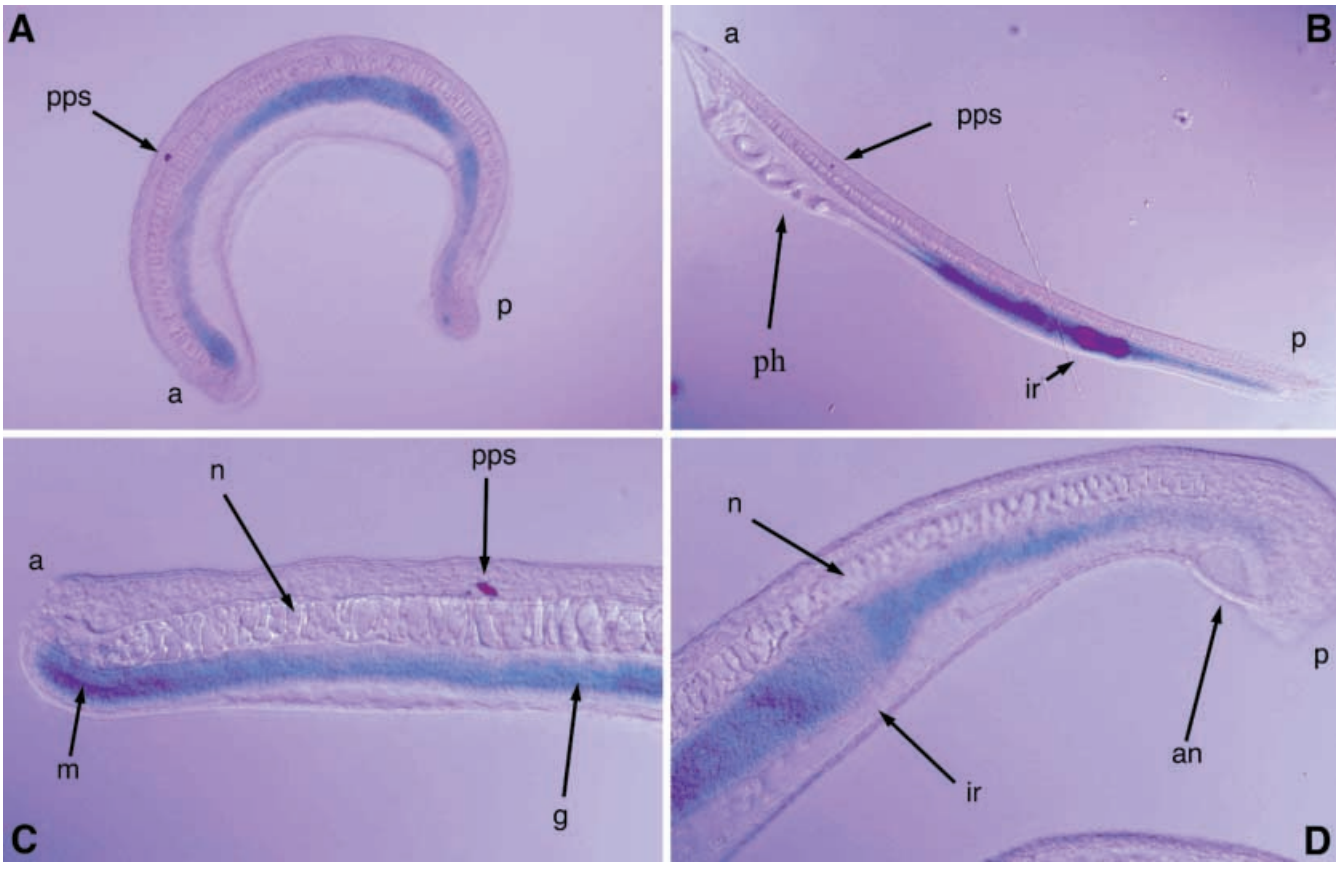

Fig. 2A–D β-Gal activity in retinoic acid (RA)-treated 6-day larvae: 2.5-h embryos were continuously treated in starvation with 10^{-6} M RA (1:1,000 dilution of 10^{-3} M RA in 100% DMSO) until processed, as described in Holland and Holland (1996). **A,B** RA-treated and untreated 6-day larvae, respectively. **C,D** Magnified view of the anterior and posterior ends of an RA-treated animal, respectively. *a* Anterior end, *an* anus, *g* gut, *ir* iliocolon ring, *n* notochord, *ph* pharynx, *p* posterior end, *pps* primary pigment spot

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