Belg. J. Zool., 131 (Supplement 1): 63-64 April 2001

SHORT NOTES

Analysis of alkaline phosphatase expression during embryogenesis of *Pseudostylochus intermedius* (Platyhelminthes Polycladida)

Keisuke Shojima[1, 2], **Kuniko Yukita**[1] **and Sachiko Ishida**[1]

[1] **Department of Biofunctional Science, Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan**
[2] **Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan**

The Platyhelminthes have traditionally been considered to be extant representatives of the ancestral triploblastic animals and thus are an important group in studies of phylogeny and germ layer differentiation during development. Both direct and indirect polyclad developments characterize different species of polyclad turbellarian platyhelminths. The cell lineage of the indirect-developing polyclad *Hoploplana inquilina* has been traced using fluorescent markers by BOYER et al. (1, 2). However, direct-developing polyclad embryos have been examined only by observation of living material (3, 4).

The presence of alkaline phosphatase (ALP) as an endoderm-specific marker has been observed during embryogenesis of a number of invertebrates, including ascidians, sea urchins, and starfish (5, 6, 7). The purpose of this study was to characterize ALP expression as a marker of endodermal differentiation in the direct-developing polyclad *Pseudostylochus intermedius* Kato, 1939.

Adult worms were collected in the Natsudomari Peninsula, Aomori Prefecture. Fertilized eggs were obtained by poking the receptaculum seminis of starved mature worms with a needle. The eggs measured approximately 150 μm and developed to juveniles in about three weeks at room temperature. ALP expression was detected using the method of WITTAKER & MEEDEL (8) with some modifications.

ALP expression was observed in the periphery of the nuclei from the fertilized egg to the 4-cell stage. From the 8-cell stage, when the spiral cleavage pattern is first clearly detectable, to the formation of the mesentoblast, ALP is ubiquitously expressed in the micromere. This suggests that maternal alkaline phosphatase may be active in the micromeres at this time. During gastrulation ALP expression in the micromeres disappears but is detected strongly in two groups of cells bilaterally situated between the ectoderm and the yolk mass. This expression may be the initiation of zygotic ALP expression. After gastrulation, only the bilateral, strong expression can be seen in the embryo (Fig. 1A). In the small yolk mass stage, the bilateral staining has extended toward the animal pole and expression also appears in the dividing endodermal cells in the centre of the embryo (Fig.1 B). At the two eye-spot stage, ALP expression is seen in the developing intestines and in the pharynx, as well as in the bilateral regions first observed during gastrulation (Fig. 1C). In the juvenile, expression is obviously observed in the intestine, pharynx and protonephridia (Fig. 1D). Thus, polyclad embryos also exhibit endoderm-specific ALP.

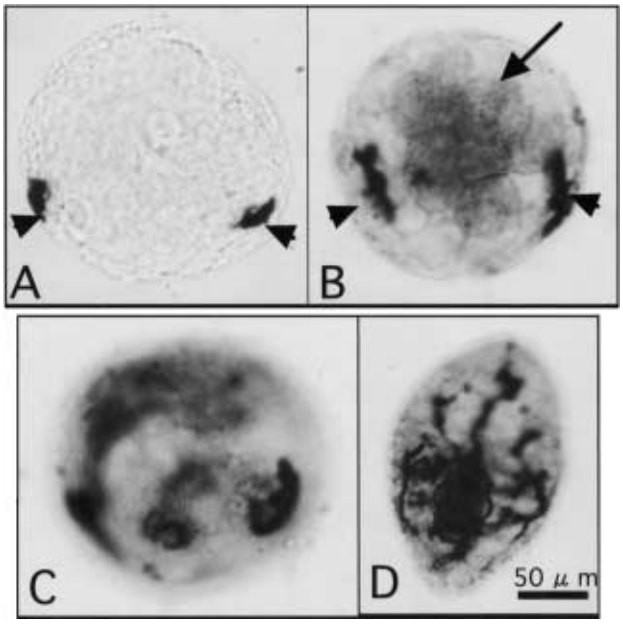

Fig. 1. – ALP expression during embryogenesis of *P. intermedius*.
(A) Bilateral, strong expression after gastrulation. (B) Small yolk mass stage. Expression also appears in the dividing endodermal cells in the centre of the embryo. (C) Two eye-spot stage. Expression is also observed in the developing intestines and in the pharynx. (D) Juvenile. Expression is seen in the intestine, pharynx and protonephridia. Arrowheads in A and B are expression in two groups of cells. Arrow in B indicates the dividing endodermal cell mass. A and B, dorsal view. C and D, ventral view.

Corresponding author: S. Ishida, e-mail: sachikoi@cc.hirosaki-u.ac.jp

ALP expression in *P. intermedius* is interesting in that it is not confined to the endodermal tissue. The Gomori-Clark staining method indicates that the adults have flame cells that correspond in position to the bilateral regions of ALP expression in the embryo. Furthermore, the typical flame cell was observed at these regions with a transmission electron microscope (Fig. 2). The expression of ALP in nephridia has also been reported in annelids (9, 10). Thus we suggest that the bilaterally-stained regions, which first appear during gastrulation, are the primordia of protonephridia.

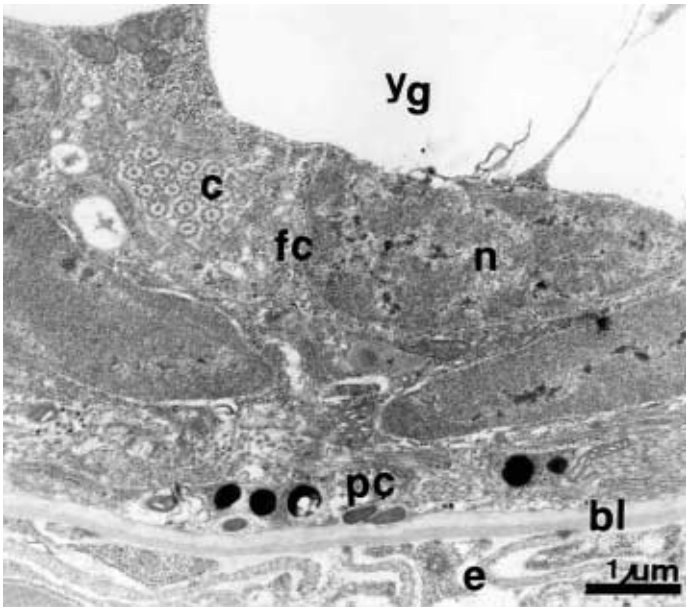

Fig. 2. – Electron micrograph of one side of bilateral ALP expressive region in juvenile (cross section).
A typical flame cell (fc) is situated near yolk granules (yg) in lateral sub-epidermal region. bl: basal layer, c: cilia, e: epidermis, n: nucleus, pc: pigmented cell.

This study provides evidence of ALP expression in both endodermal and mesodermal (protonephridial) tissues, suggesting that it can be used as tissue-specific marker for specific derivatives of both germ layers.

REFERENCES

1. Boyer, B.C., J.Q. Henry & M.Q. Martindale (1996). Dual origins of mesoderm in a basal spiralian: Cell lineage analyses in the polyclad turbellarian *Hoploplana inquilina*. *Dev. Biol.,* 179: 329-338.
2. Boyer, B.C., J.J. Henry & M.Q. Martindale (1998). The cell lineage of a polyclad turbellarian embryo reveals close similarity to coelomate spiralians. *Dev. Biol.,* 204: 111-123.
3. K. Kato (1940). On the development of some Japanese polyclads. *Jpn. J. Zool.,* 8: 537-573.
4. Teshirogi, W., S. Ishida & K. Jatani (1981). On the early development of some Japanese polyclads. *Rep. Fukaura Mar. Biol. Lab., Hirosaki Univ.*, 9: 2-31. (*in Japanese*).
5. Nishida, H. & G. Kumano (1997). Analysis of the temporal expression of endoderm-specific alkaline phosphatase during development of the ascidian *Halocynthia roretzi. Dev. Growth Differ.*, 39: 199-205.
6. Khaner, O. & F. Wilt (1990). The influence of cell interaction and tissue mass on differentiation of sea urchin mesomeres. *Development*, 109: 625-634.
7. Kuraishi, R. & K. Osanai (1994). Contribution of maternal factors and cellular interaction to determination of archenteron in the starfish embryo. *Development*, 120: 2619-2628.
8. Whittaker, J.R. & T.H. Meedel (1989). Two histospecific enzyme expressions in the same cleavage-arrested one-celled ascidian embryos. *J. Exp. Zool.*,250: 168-175.
9. Myohara, M., C. Yoshida-Noro, F. Kobari & S. Tochinai (1999). Fragmenting oligochaete *Encytraceus japonensis*: A new material for regeneration study. *Dev. Growth Differ.*, 41: 549-555.
10. Kitamura, K. & T. Shimizu (2000). Analyses of segment-specific of alkaline phosphatase activity in the mesoderm of the oligochaete annelid *Tubefex*; Implication for specification of segmental identity. *Dev. Biol.*, 219: 214-223.