ELSEVIER

Mechanisms of Development 115 (2002) 177–179

www.elsevier.com/locate/modo

Gene expression pattern

The *Abdominal-B*-like gene expression during larval development of *Nereis virens* (polychaeta)

Milana A. Kulakova, Roman P. Kostyuchenko, Tatiana F. Andreeva, Archil K. Dondua*

Department of Embryology, State University of St. Petersburg, Universitetskaya nab. 7/9, 199034 St. Petersburg, Russia

Received 12 February 2002; received in revised form 4 April 2002; accepted 4 April 2002

Abstract

We have studied the posterior *Hox* gene *Nvi-Post1* expression in the early development of the polychaete *Nereis virens*. This is the first evidence of the posterior group *Hox* genes expression during the larval development of a Lophotrochozoan. The expression begins in the trochophore hyposphere at the prospective sites of larval parapodia. As the larva develops the expression weakens and finally becomes undetectable in the nectochaete stage and juvenile worm. The *Nvi-Post1* expression appears to be important for larval, but not postlarval development.

Keywords: Posterior *Hox* genes; Expression pattern; *Nvi-Post1*; Larval segments; *Nereis virens*; Polychaeta; Lophotrochozoa

1. Results and discussion

Hox genes have been found in all the three clades of bilaterian animals – Lophotrochozoa (Kourakis et al., 1997; De Rosa et al., 1999; Irvine and Martindale, 2000; Andreeva et al., 2001; Nogi and Watanabe, 2001), Ecdysozoa (Averof and Patel, 1997; Averof and Akam, 1995) and Deuterostomia (Krumlauf, 1994; Burke et al., 1995). These clustered genes are well-known to play an important morphogenetic role in specifying anterior-posterior (A-P) axis in Ecdysozoa and Chordata (Akam, 1995). The function of *Hox* genes remains poorly understood in Lophotrochozoa. Irvine and Martindale (2000) have studied expression patterns for five anterior *Hox* genes in larvae of Chaetopterus, a polychaete annelid with a tagmatized body plan. They showed two important features: (1) the colinearity of expression; and (2) the permanent expression of all genes they studied in the growth zone of larvae.

In our work we focused on the not tagmatized, homonomously segmented White Sea polychaete *Nereis virens*. With the use of whole-mount in situ hybridization we analyzed the expression of one of the two posterior group *Hox* genes of *N. virens*, *Nvi-Post1* (De Rosa et al., 1999; Andreeva et al., 2001). The expression of this gene was studied during the cleavage period, in unsegmented spherical trochophore larva, segmenting metatrochophore, nectochaete with three larval segments, and in young eight to 12 segmented worms.

Nvi-Post1 expression is first detected at the trochophore stage (64 h after fertilization) as two diffuse bilaterally symmetric spots in the hyposphere of the larva (Fig. 1). *Nvi-Post1* expression in the episphere was not found. At the next stage (72 h) when the trochophore slightly elongates in the A-P direction, the domains of *Nvi-Post1* expression become more distinct (Fig. 2). At the stage of early metatrochophore (86 h) each domain of expression divides into three isolated subdomains along the A-P axis. Each of the subdomains contains clear ventral and dorsal parts (Figs. 3 and 4). Thus at this stage when the larva still does not have morphological segmentation *Nvi-Post1* is expressed in the hyposphere, forming a pattern of six coupled rounded zones. On semi-thin sections we showed that the cells expressing *Nvi-Post1* (Figs. 5 and 6) are localized under the ectoderm and form spherical conglomerates probably corresponding to primordia of the larval parapodiae. At the metatrochophore stage, when the body of the larva is visibly subdivided into three larval segments and three pairs of schizocoelic coeloms form, *Nvi-Post1* is expressed at the bases of developing parapodiae (Figs. 7 and 8). The intensity of expression becomes weaker in the two anterior segments. Strong signal remains only in the posterior segment. Later at the nectochaete stage (Fig. 9) and in juvenile worms (Fig. 10) *Nvi-Post1* expression is not detectable. We also tested expression of *Nvi-Post1* in the posterior part of older worms by

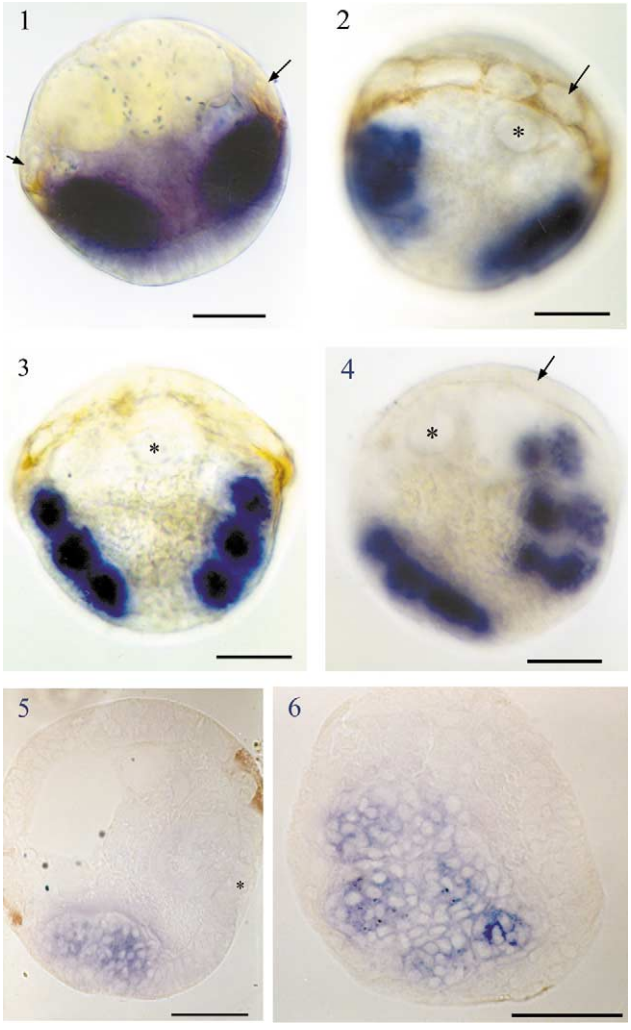
* Corresponding author. Tel.: +7-812-328-9453; fax: +7-812-328-9530.
E-mail address: akdondua@yahoo.co.uk (A.K. Dondua).

0925-4773/02/$ - see front matter
PII: S0925-4773(02)00113-2

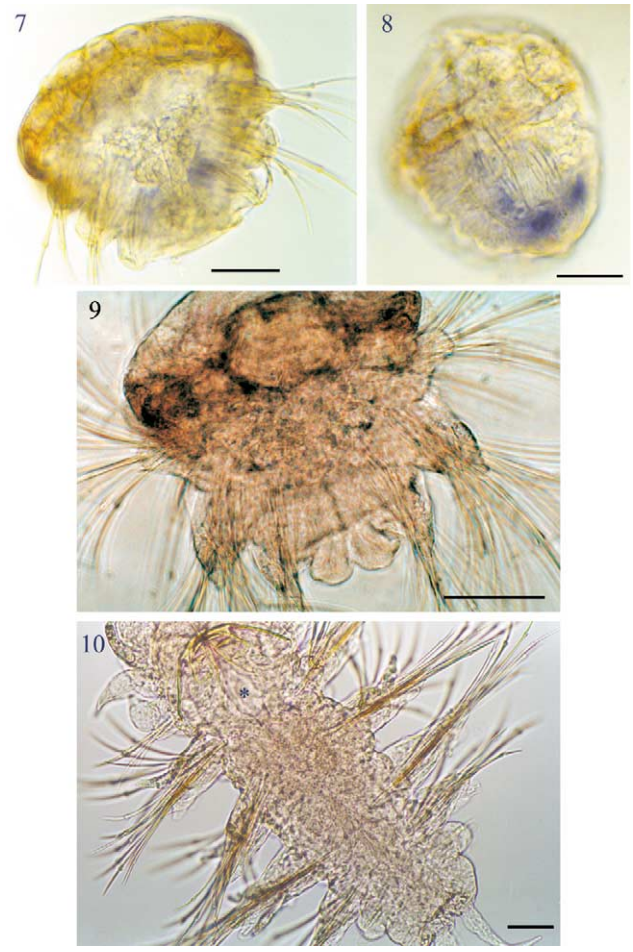
reverse transcription-polymerase chain reaction (PCR), but could detect no expression at late stages (data not shown).

Posterior group *Hox* genes are employed in a variety of morphogenetic processes in the Ecdysozoans and Vertebrates. They specify rear parts of the body (Peterson et al., 1994) and the proximal-distal axis in vertebrate limb (Zakany and Duboule, 1999). However, the expression of *Nvi-Post1* in specialized cells of anterior segments of *Nereis* bears no obvious relation to posterior *Hox* expression in other groups.

Our data provide the only evidence for expression of a *Post1* class *Hox* gene in a Lophotrochozoan to date. Our finding that *Nvi-Post1* expression is connected with formation of larval but not postlarval segments gives evidence in favour of an old concept, the primary heteronomicity of segments in polychaetes (Iwanoff, 1928).



Figs. 1–6. In situ hybridization to *Nereis virens* posterior Hox gene *Nvi-Post1* at the different stages. Scale bar 50 μm. (1) Trochophore 64 h after fertilization. Domains of expression are detected in hyposphere only. Prototroch is visible (arrow). (2) Stage 72 h after fertilization. Mouth (asterisk) and prototroch (arrow) can be seen. Figs. 3–4 (total) and 5–6 (semi-thin sections). Trochophore 84 h of development. On the semi-thin sections the strong signal is seen in cytoplasma. Asterisk and arrow denote mouth and prototroch, respectively.



Figs. 7–10. (7,8) Metatrochophore stage. Strong signal is present only in the posterior larval segment. (9) Nectochaete stage. No signal is detected. (10) Juvenile worm. No domains of expression are detected. Asterisk denotes the pharynx.

2. Materials and methods

2.1. Animals

Adult *N. virens* were collected at the Marine Biological Station of the St. Petersburg State University, at the White Sea. Males and females were kept separately. Artificial fertilization and cultivation of the embryos were carried out at 11°C (Dondua, 1975). The material was fixed with 4% PFA (paraformaldehyde) in 2 × phosphate-buffered saline.

2.2. Cloning

A 376 bp *Nvi-Post1* fragment was amplified by PCR. The primers were constructed based on the sequence of iPCR-fragments of this gene. The cloned fragment was used for preparation of Dig-RNA probes.

2.3. Whole-mount in situ hybridization

Digoxigenin-labeled RNA probes were prepared according to the manufacturer's protocol (Boehringer Mannheim GmbH, Mannheim, Germany), with the *Nvi-Post1* cloned DNA as template. Hybridization was carried out at 65°C for 24 h. BM-purple (Roche/BMB) was used as a chromogenic substrate to localize the hybridized probe.

Acknowledgements

We are grateful to Dr Charles E. Cook for his help in analyzing *Nvi-Post1* iPCR clones and Dr Michael Akam for the opportunity to perform iPCR and clone the products in his laboratory at the University Museum of Zoology, Cambridge, UK. This work was supported by the Russian Foundation for Basic Research (00-04-49404 and 01-04-63077).

References

Akam, M., 1995. *Hox* genes and the evolution of diverse body plans. Philos. Trans. R. Soc. Lond. 349, 313–319.

Andreeva, T.F., Cook, Ch., Korchagina, N.M., Akam, M., Dondua, A.K., 2001. Cloning and analysis of structural organization of *Hox* genes in the polychaete *Nereis virens*. Rus. J. Dev. Biol. (Ontogenez) 32, 225–233.

Averof, M., Akam, M., 1995. *Hox* genes and the diversification of insect and crustacean body plans. Nature 376, 420–423.

Averof, M., Patel, N.H., 1997. Crustacean appendage evolution associated with changes in *Hox* gene expression. Nature 388, 682–686.

Burke, A.C., Nelson, C.E., Morgan, B.A., Tabin, C., 1995. *Hox* genes and the evolution of vertebrate axial morphology. Development 121, 333–346.

De Rosa, R., Grenier, J.K., Andreeva, T.F., Cook, C.E., Adoutte, A., Akam, M., Carroll, S.B., Balavoine, G., 1999. *Hox* genes in brachiopods, priapulids and protostome evolution. Nature 399, 772–776.

Dondua, A.K., 1975. Effect of actinomycin D and sibiromycin on the embryonic and larval development of *Nereis virens* (Sars.). Ontogenez 6, 475–484.

Irvine, S.Q., Martindale, M.Q., 2000. Expression patterns of anterior Hox genes in the polychaete Chaetopterus: correlation with morphological boundaries. Dev. Biol. 217, 333–351.

Iwanoff, P.P., 1928. Die Entwicklung der Larvalsegmente bei den Annelides. Z. Morph. Ökol. Tiere. 10, 62–161.

Kourakis, M.J., Master, V.A., Lokhorst, D.K., Nardelli-Haefliger, D., Wedeen, C.J., Martindale, M.Q., Shankland, M., 1997. Conserved anterior boundaries of Hox gene expression in the central nervous system of the leech Helobdella. Dev. Biol. 190, 284–300.

Krumlauf, R., 1994. *Hox* genes in vertebrate development. Cell 7, 191–201.

Nogi, T., Watanabe, K., 2001. Position-specific and non-colinear expression of the planarian posterior (Abdominal-B-like) gene. Dev. Growth Differ. 43, 177–184.

Peterson, R.L., Papenbrock, T., Davda, M.M., Awgulewitsch, A., 1994. The murine Hoxc cluster contains five neighboring AbdB related genes that show unique spatially co-ordinated expression in posterior embryonic subregions. Mech. Dev. 47, 253–260.

Zakany, J., Duboule, D., 1999. *Hox* genes in digit development and evolution. Cell Tissue Res. 296, 19–25.