Dev Genes Evol (2009) 219:523–530
DOI 10.1007/s00427-009-0308-6

SHORT COMMUNICATION

Involvement of Hox genes in shell morphogenesis in the encapsulated development of a top shell gastropod (*Gibbula varia* L.)

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Received: 1 September 2009 / Accepted: 13 November 2009 / Published online: 1 December 2009

Abstract Regulatory gene expression during the patterning of molluscan shells has only recently drawn the attention of scientists. We show that several Hox genes are expressed in association with the shell gland and the mantle in the marine vetigastropod *Gibbula varia* (L.). The expression of *Gva-Hox1*, *Gva-Post2*, and *Gva-Post1* is initially detected in the trochophore larval stage in the area of the shell field during formation of embryonic shell. Later, during development, these genes are expressed in the mantle demonstrating their continuous role in larval shell formation and differentiation of mantle edge that secretes the adult shell. *Gva-Hox4* is expressed only late during the development of the veliger-like larva and may also be involved in the adult shell morphogenesis. Additionally, this gene also seems to be associated with secretion of another extracellular structure, the operculum. Our data provide further support for association of Hox genes with shell formation which suggest that the molecular mechanisms underlying shell synthesis may consist of numerous conserved pattern-formation genes. In cephalopods, the only other molluscan class in which Hox gene expression has been studied, no involvement of Hox genes in shell formation has been reported. Thus, our results suggest that Hox genes are coopted to various functions in molluscs.

Communicated by D.A. Weisblat

Electronic supplementary material The online version of this article (doi:10.1007/s00427-009-0308-6) contains supplementary material, which is available to authorized users.

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Keywords Gastropod · Hox genes · Shell morphogenesis · Trochophore larva · Veliger larva

Introduction

A key feature of the conchiferan molluscs (i.e., Gastropoda, Bivalvia, Scaphopoda, Cephalopoda, and tryblidian Monoplacophora) is the possession of highly diverse calcareous shells (Salvini-Plawen and Steiner 1996) that offer an excellent fossil record, suggesting a molluscan origin in the late Precambrian before the "Cambrian explosion" (Runnegar 1996). The first episode in conchiferan shell ontogeny is an invagination of the dorsal ectoderm differentiating the so-called "shell gland" that later evaginates to form the shell field, which then forms the mantle in the trochophore larvae (Eyster 1986). The epithelial cells of shell field secrete a thin organic layer, termed periostracum that give rise to the embryonic shell (Kniprath 1981), or protoconch I (Jablonski and Lutz 1980). Depending on the type of larval development and the time of mineralization, protoconch II may be formed during the veliger stage. The adult shell or teleoconch is added after metamorphosis (Jablonski and Lutz 1980). The molecular mechanisms regulating the differentiation of these shell structures are only recently beginning to be elucidated (Jackson et al. 2007). Jackson et al. (2007) show that over 25% of the genes expressed in the mantle of the vetigastropod *Haliotis asinina* encode structural proteins contributing to shell formation and patterning. A majority of the shell secretome encodes novel proteins. Additionally, it has been shown that the homeobox gene *engrailed* is expressed in tissues involved in embryonic shell formation in molluscs (Moshel et al. 1998; Jacobs et al. 2000; Wanninger and Haszprunar 2001; Nederbragt et al. 2002; Baratte et al. 2007; Iijima et al. 2008). Jacobs et al. (2000) detected expression of

engrailed around the margin of the shell and along the hinge in the bivalve *Transennella tantilla* and in transverse stripes associated with each developing shell plate, as well as in girdle area surrounding the region of plate formation in the polyplacophoran *Lepidochitona caverna*. Therefore, the authors suggested *engrailed* is required for the shell formation in molluscs (i.e., the skeletogenesis). As cephalopods undergo a direct development without larval stages, investigation of *engrailed* in cephalopods by Baratte et al. (2007) provided new insights at the role of this gene in shell formation. In the cephalopod *Sepia officinalis*, *engrailed* is first located in the shell area (before invagination) and in the mantle but it is no longer detected in the shell sac when shell formation starts (Baratte et al. 2007). This is consistent with observations made in gastropods and a scaphopod (Moshel et al. 1998; Wanninger and Haszprunar 2001; Nederbragt et al. 2002; Iijima et al. 2008) providing additional evidence that *engrailed* in molluscs is probably not required for the shell formation (i.e., the skeletogenesis) but rather for delimiting the shell compartment boundary, as proposed originally by Nederbragt et al. (2002).

Hox genes are a clustered family of homeobox genes whose characteristic genomic organization seems to be linked to their ancestral function along the head-tail body axis of animals, the so-called "spatial colinearity" (Lewis 1978). The fact that Hox genes are found in animals with radically different body plans offers an opportunity to understand the nature of organism complexity and evolution. Hinman et al. (2003) demonstrated that of the five most anterior Hox genes they studied, two of them are involved in adult or larval shell formation in the abalone *H. asinina*. *Has-Hox2*, *-Hox3*, *-Hox4*, and *-Hox5* follow conserved pattern of Hox gene expression during neurogangliogenesis in the abalone (Giusti et al. 2000; Hinman et al. 2003). However, *Has–Hox1* has coopted to function in the larval shell gland in the trochophore and *Has-Hox1* and *Has-Hox4* take part in shell morphogenesis of the adult mollusc (Hinman et al. 2003).

Although there are a few publications on the homeobox sequences of Hox genes in seven of eight molluscan classes (e.g., Iijima et al. 2006), the only other molluscan species for which Hox gene expression data is available is the sepiolid squid: *Euprymna scolopes* (Lee et al. 2003). In *E. scolopes* embryos, six Hox genes are expressed in the central nervous system (CNS), consistent with the ancestral role for these genes in axial patterning of CNS (Lee et al. 2003). Apart from CNS, Hox genes are expressed in ectodermally derived structures of the brachial and buccal crowns, funnel tube, stellate ganglia, metabrachial vesicles, and the light organ of the squid indicating that Hox expression is developmentally correlated with the morphological novelties in the cephalopod (Lee et al. 2003). None of the eight Hox genes tested in the squid are reported to be involved in shell formation (Lee et al. 2003).

In this paper, we present the first report of posterior Hox genes (*Gva-Post2* and *Gva-Post1*) being involved in shell morphogenesis in the intracapsular larva of the vetigastropod *Gibbula varia*. We show that *Gva-Hox1*, *Gva-Post2*, and *Gva-Post1* are involved first in formation of embryonic shell and later in formation of larval and adult shells. Additionally, we demonstrate that *Gva-Hox4* is expressed in the cells secreting the extracellular material forming the operculum. In the post-torsional veliger-like larvae, *Gva-Hox4* is also expressed in the mantle. Our results provide further evidence that Hox genes are coopted to carry out novel functions in larval and adult morphological structures in gastropods as well as shedding more light on understanding gene regulatory networks involved in shell formation.

Materials and methods

Collection and fertilization of adults

The adults of *Gibbula varia* (L.) were collected in Crete, Greece and cultured in the lab in artificial sea water in 22°C (salinity 28°). Copulation was induced by lowering the salinity a few degrees by adding fresh water to the aquarium at 17°C (personal observation of Achim Meyer, The Johannes Gutenberg University of Mainz). Embryos and larvae develop inside the gelatinous egg masses.

Development

The snail embryos undergo indirect development inside the egg capsule (lecithotrophic), producing first a trochophore with a prototroch, shell field, and mantle (Fig. 1a), and a foot rudiment. Then it grows into a veliger-like larva comprising a velum, a foot rudiment, embryonic or first larval shell (protoconch), mantle cavity, and the mantle fold (Fig. 1b, note that veliger larva is defined as a free-swimming planktotrophic larva, so we use the term "veliger-like" larva for the lecithotrophic encapsulated larva of *G. varia* throughout this paper). During this stage, torsion takes place by rotating the visceropallium (visceral mass/mantle) by 180° relative to cephalopodium (head/foot) (Page 2006). In pre-torsional veliger-like larva, the visceropallium is dorsal and posterior to the foot rudiment (Fig. 1b). The future mouth (stomodeum) and the operculum rudiment become obvious for the first time at this stage (Fig. 1b). After the torsion, a post-torsional veliger-like larva is produced in which the mantle is in anterior position over the back of the head (Fig. 1c). The velum reduces in size and splits ventrally to form two bands of velar cells running dorso-ventrally and meeting around the dorsal region of the head.

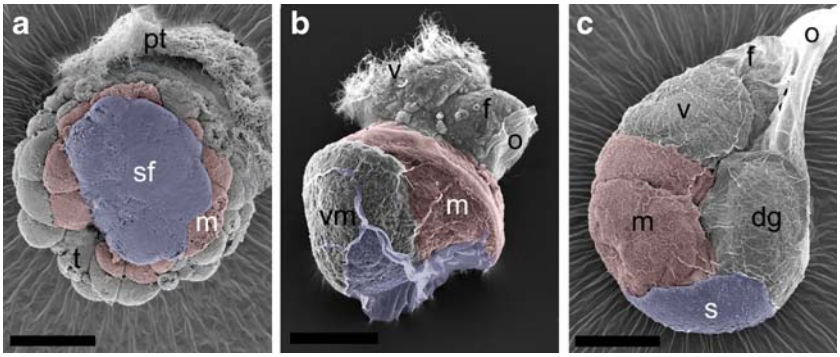

Fig. 1 Scanning electron microscopy of larval stages of *G. varia*. **a** Trochophore about 10 h after fertilization (*dorsal view*); **b** pre-torsional veliger-like larvae about 36 h after fertilization (*lateral view*); and **c** post-torsional veliger-like larvae about 60 h after fertilization (*lateral view*). The *red shade* marks the area of mantle and the *blue shade* is the shell field in **a**, periostracum in **b**, and larval shell in **c**. *dg* Digestive gland, *f* foot, *m* mantle, *o* operculum, *pt* prototroch, *s* shell, *sf* shell field, *t* telotroch (future anal region), *v* velum, *vm* visceral mass. *Scale bars* **a** 25 µm; **b** and **c** 50 µm

Cloning of Hox genes

RNA was extracted from blastula, gastrula, trochophore larva, and pre- and post-torsional veliger-like larvae using RNeasy Mini Kit (QIAGEN Vertriebs GmbH, Vienna, Austria). The cDNA was synthesized using SuperScript® III reverse transcriptase from each developmental stage (Invitrogen GmbH, Karlsruhe, Germany). The homeobox fragments of Hox genes were obtained by polymerase chain reaction using degenerate primers from cDNA of each stage (for sequences and references of degenerated primers used in this study, see supplementary material 1 Table S1 in electronic supplementary material). In total, 285 clones were sequenced and all 11 Hox genes and the three paraHox genes were recovered (Samadi and Steiner, unpublished data; see supplementary material 1 Table S2 in electronic supplementary material for number of clones sequenced for each primer pairs and number of unique fragments recovered). The homeobox fragments were used to design primers for rapid amplification of cDNA ends (primer sequences are available on request). The rapid amplification of cDNA ends (RACE) was performed with modifications according to Schramm et al. 2000 (see supplementary material 2 for further details on RACE protocol in electronic supplementary material). The RACE products were cloned by Topo-TA cloning kit (Invitrogen GmbH, Karlsruhe, Germany) and sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3130xl DNA analyser automated capillary sequencer.

Orthology assignment

Sequences obtained from RACE clones were matched against GenBank non-redundant protein databases using the BlastX algorithm. After automated aligning by Clustal X v.1.81 software, the homeobox were used as anchor and the flanking regions were realigned and checked manually. Assignment of gene fragments was made according to both the presence of diagnostic residues in the homeodomain and flanking regions and phylogenetic analyses. The species and the GenBank accession numbers of the sequences used in the phylogenetic analyses are listed in supplementary material 1 Table S3 in electronic supplementary material. Bayesian likelihood analyses were conducted using MrBayes version 3.1.1 (Huelsenbeck and Ronquist 2001) with five million generations.

Whole-mount in situ hybridization

To make a single fragment from 3′ and 5′ RACE polymerase chain reaction (PCR) products, gene-specific primers were designed for each Hox gene (one of primer pairs from 3′ and the other from 5′ RACE fragments) and PCR was carried out on *G. varia* mixed cDNA of larval stages. The sense and anti-sense probes were synthesized from these PCR products using the Maxiscript T7 and SP6 RNA polymerase kit (Ambion, Austin, USA) and the Dig RNA labelling kit (Roche Molecular Biochemicals, Vienna, Austria).

For whole-mount in situ hybridization (WMISH), several holes were made in the gelatinous masses of embryos and larvae using insect needles, then the masses were fixed for 4 h with MEMPFA-T (0.1 M MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO4, 4% paraformaldehyde, and 0.1% Tween-20) on a rotating wheel, washed four times, 15 min each, in MEM-T buffer (0.1 M MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO4, and 0.1% Tween-20) and dehydrated with 10-min washes with a graded methanol/MEM-T series (25/75, 50/50, 75/25, 100/0) followed by three times 15-min wash with 100% methanol and were stored at –20°C in methanol. Prior to

performing the WMISH, the embryos and larvae were pulled out of the egg masses by insect needles. The rest of WMISH method was performed according to Lespinet et al. 2002.

Results and discussion

Phylogenetic analysis

The initial orthology of the Hox fragments were designated by searching against GenBank non-redundant protein databases using the BlastX algorithm and the genes were named *Gva-Hox1*, *Gva-Hox4*, *Gva-Post1*, and *Gva-Post2* (accession numbers GU056183, GU056184, GU056185, and GU056186, respectively). The RACE PCR allowed the isolation of a cDNA of 1010 bp for *Gva-Hox1* that contained the complete open reading frame (ORF) of 316 amino acids (948 bp) with an inframe stop codon at the position of 978 bp (supplementary material 3, Fig. S1a in electronic supplementary material). The 3′ and 5′ RACE together yielded in a fragment of 472 bp that gave rise to a partial ORF of 157 amino acids containing the homeodomain of *Gva-Hox4* (supplementary material 3, Fig. S1b in electronic supplementary material). The full-length cDNA of 611 bp was obtained for *Gva-Post2,* containing a 148 bp 5′UTR and a 99 bp 3′UTR. The full-length cDNA contained the complete ORF of 115 amino acids (345 bp) with an inframe stop codon at the position of 494 bp (supplementary material 3, Fig. S1c in electronic supplementary material). For *Gva-Post1*, a full-length cDNA of 999 bp was recovered that contained a 144 bp 5′ UTR and a 78 bp 3′ UTR, with the complete ORF of 252 amino acids (756 bp) with an inframe stop codon at the position of 903 bp (supplementary material 3, Fig. S1d in electronic supplementary material). Results from phylogenetic analysis of Hox gene sequences are presented in Fig. 2. The dataset included 60 amino acids of homeodomain and the flanking regions. To check for the presence of diagnostic residues, the homeodomains and flanking peptides from orthologous genes of representative lophotrochozoans, ecdysozoans, and deuterostomes were aligned to the *Drosophila Antennapedia* homeodomain (see supplementary material 4, Fig. S2 in electronic supplementary material).

Hox gene expression

The expression patterns of *Gva-Hox1*, *Gva-Hox4*, *Gva-Post2*, and *Gva-Post1* were examined through the embryonic and larval developmental stages: blastula, gastrula, and the three larval stages. No expression of *Gva-Hox* transcripts was observed before the early trochophore stage (about 10 h after fertilization).

Expression of Gva-Hox1 In early trochophore (10 h after fertilization), *Gva-Hox1* is expressed in the expanding shell field (Fig. 3a). The transcripts are detected as a circle in the forming mantle edge (Fig. 3b) in the late trochophore larvae (18 h after fertilization). In pre-torsional veliger-like larvae, *Gva-Hox1* is expressed in the anterior margin of the mantle (Fig. 3c). This expression is consistent in the post-torsional veliger-like larvae with the difference that there is higher level of transcripts detected in this stage (Fig. 3d).

Expression of Gva-Post2 Gva-Post2 is expressed in a single layer of ectodermal cells around the evaginating shell field (in the cells forming the future mantle edge) in early trochophore (Fig. 3e). *Gva-Post2* expression overlaps with the expression of *Gva-Hox1* in the forming mantle in late trochophore stage (Fig. 3f). In both pre-torsional and post-torsional veliger-like larva, *Gva-Post2* expression is detected in the mantle fold (Fig. 3g,h).

Expression of Gva-Post1 In the early trochophore, *Gva-Post1* transcripts are first detected in the entire posttrochal dorsal ectoderm where the shell field and mantle will form (Fig. 3i). This expression overlaps with the in situ hybridization pattern of *Gva-Hox1* and *Gva-Post2* in evaginating shell field and the forming mantle. Later in trochophore, the expression in the center of shell field disappears and *Gva-Post1* is now detected in the ectodermal cells associated with the lateral edges of the expanding shell field and in the mantle (Fig. 2j). The expression pattern of *Gva-Post1* overlaps with that of *Gva-Hox1* and *Gva-Post2* in the forming mantle. In veliger-like larvae, *Gva-Post1* is expressed in the whole area of the mantle, including the outer and inner mantle folds, overlapping with expression domains of both *Gva-Hox1* and *Gva-Post2* (Fig. 3k,l) with the difference that *Gva-Hox1* is expressed only in the anterior margin of mantle and *Gva-Post2* only in the mantle fold but *Gva-Post1* expression covers the complete mantle area.

Expression of Gva-Hox4 Gva-Hox4 expression was first detected in the late trochophore stage. Expression of *Gva-Hox4* does not appear to be related to shell formation in the trochophore since the transcripts are detected in two ventral posttrochal ectodermal cells in left and right side of the foot rudiment where the future left and right larval kidneys are going to form (Fig. 3m). In veliger-like larvae, *Gva-Hox4* is expressed in the foot, immediately adjacent to the newly forming operculum, suggesting that it is necessary for controlling the construction of this extracellular structure (Fig. 3n). The expression of *Gva-Hox4* in operculum is retained with lower intensity in post-torsional veliger-like stage in which *Gva-Hox4* is additionally expressed in the mantle fold (Fig. 3o). The expression pattern of *Gva-Hox4* is similar to that of *Gva-Post2* in the mantle fold of

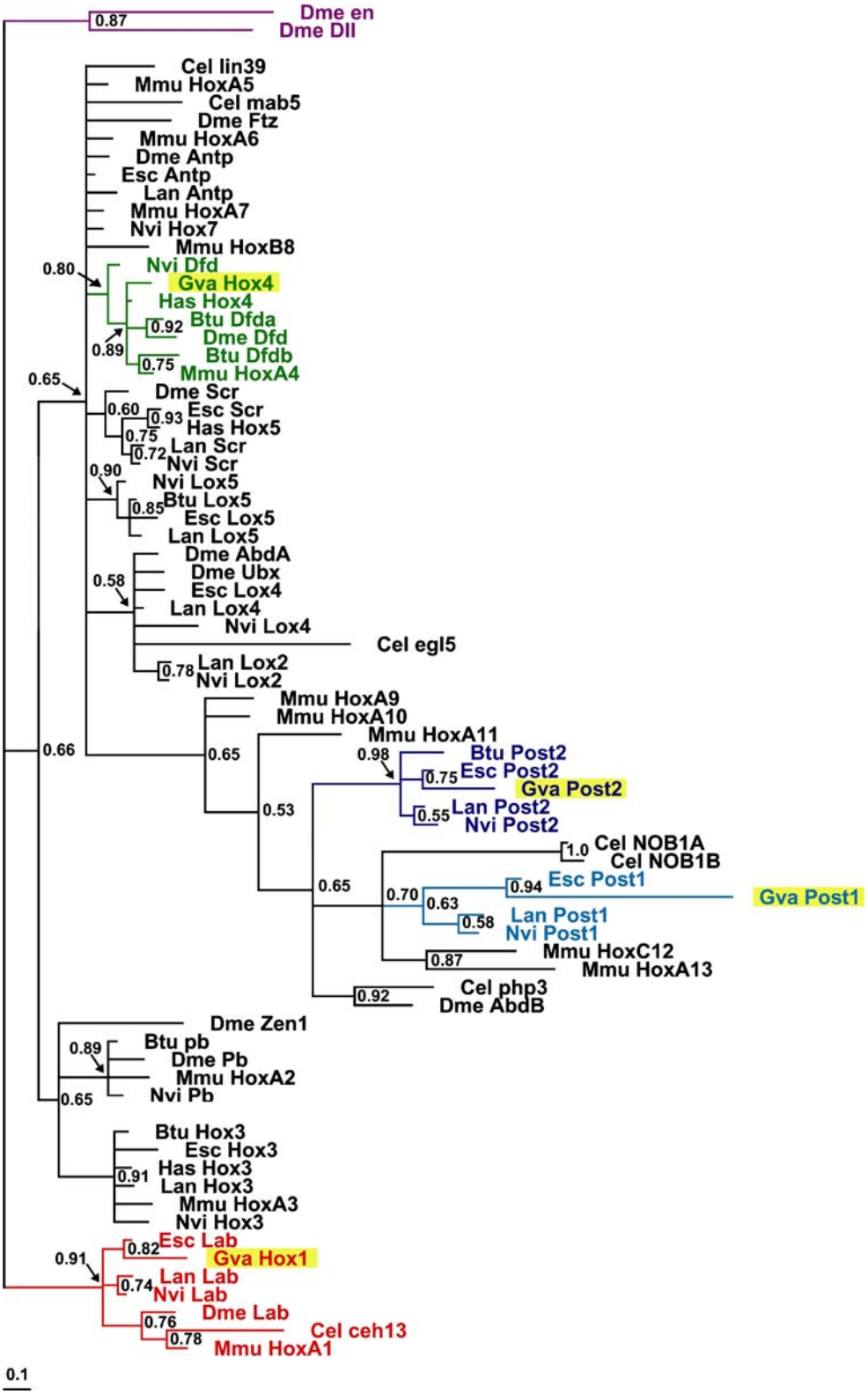

Fig. 2 Phylogenetic reconstruction of Hox gene relationships. Paralog group 1 is shown in *red*, paralog group 4 in *green*, and posterior genes in *blue* (*Post2* is shown with *dark blue* and *Post1* with *light blue*). *Engrailed* (*en*) and *distalless* (*Dll*) are used as outgroup (*purple*). *Yellow rectangles* highlight *G. varia* sequences. Tree is from Bayesian likelihood analysis using MrBayes: half compatibility consensus from 5,000,000 replicates, burn-in of 20,000 replicates. Support values of branches are posterior probability of Bayesian likelihood. *Bugula turrita* (*Btu*), *Caenorhabditis elegans* (*Cel*), *Drosophila melanogaster* (*Dme*), *Euprymna scolopes* (*Esc*), *Gibbula varia* (*Gva*), *Haliotis asinina* (*Has*), *Lingula anatine* (*Lan*), *Mus musculus* (*Mmu*), *Nereis virens* (Nvi)

post-torsional veliger-like larvae. Moreover, *Gva-Hox4* is expressed in the ectodermal cells giving rise to CNS (Fig. 3n,o arrow heads). The expression in the nervous system is initially detected in pleural ganglia in pre-torsional veliger-like larvae (Fig. 3n the arrow head). After torsion, *Gva-Hox4* is expressed in pleuropedal ganglia and to a lesser extent in esophageal ganglia (Fig. 3o arrow heads). This expression resembles that of *Has-Hox4* in the veliger nervous system of the abalone *H. asinina* (Hinman et al. 2003).

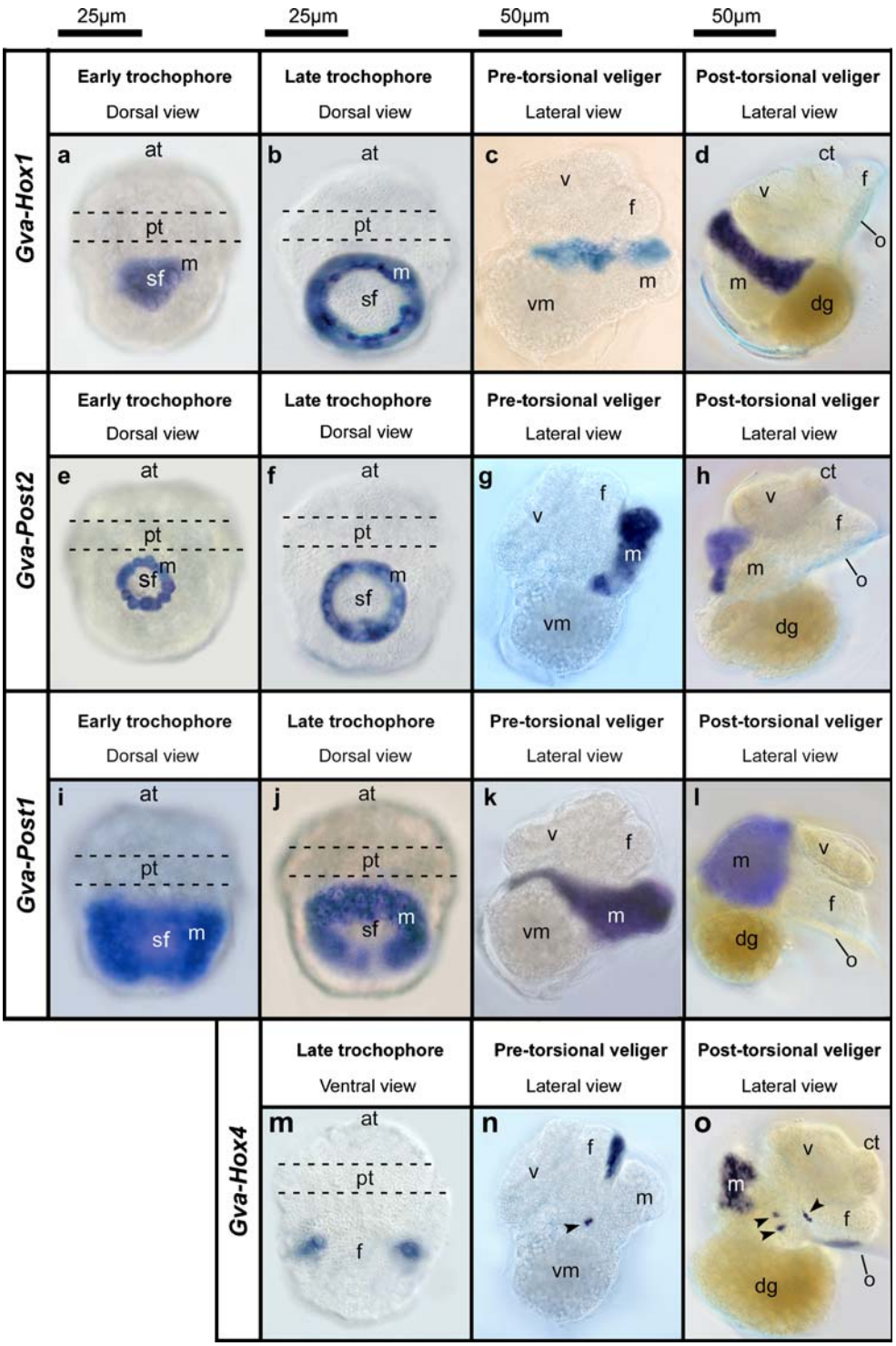

Fig. 3 Result of whole-mount in situ hybridization of Hox genes involved in shell formation in *G. varia*. **a-d** expression of *Gva-Hox1*: in the expanding shell field of early trochophore (10 h after fertilization) (**a**) in the mantle of late trochophore (18 h after fertilization) (**b**), in anterior margin of the mantle in pre-torsional veliger-like larva (**c**), and post-torsional veliger-like larva (**d**). **e-h** Expression of *Gva-Post2*: in inner margin of evaginating shell field in early trochophore (**e**), in the mantle in late trochophore (**f**) and in the mantle fold in pre-torsional (**g**), and post-torsional veliger-like larvae (**h**). **i-l** expression patterns of *Gva-Post1*: in the dorsal posttrochal area of the early trochophore (**i**), in the outer margin of the shell field and the mantle of late trochophore (**j**), and in the mantle of pre-torsional (**k**) and post-torsional veliger-like larvae (**l**). **m-o** Expression of *Gva-Hox4*: in two ectodermal cells at the sides of foot rudiment (**m**), in the cells adjacent to operculum in the foot of pre-torsional veliger-like larva (**n**), and in the mantle fold of post-torsional veliger-like larva (**o**). *Arrows* indicate the expression of *Gva-Hox4* in nervous system. *at* Apical tuft, *ct* cerebral tentacles, *dg* digestive gland, *f* foot, *m* mantle, *o* operculum, *pt* prototroch, *s* shell, *sf* shell field, *v* velum, *vm* visceral mass

Role of Hox genes in shell formation in gastropods

Our results confirm the involvement of *Hox1* and *Hox4* in shell formation in gastropods originally observed by Hinman et al. (2003) in the tropical abalone. In addition, we show for the first time that posterior Hox genes (*Post2* and *Post1*) are engaged in shell patterning of the larva and adult in a gastropod (Fig. 3). Expression of *Gva-Hox1*, *Gva-Post2*, and *Gva-Post1* in the shell field or in cells at the leading edge of the shell field (Fig. 3b,f,j) and later in the larval mantle (Fig. 3c,g,k) are consistent with the idea that these regulatory genes are playing a role in both protoconch (larval shell) and

teleoconch (adult shell) synthesis. In contrast, *Has-Hox4* and *Gva-Hox4* are not detected in the shell field or mantle in earlier larval stages (trochophore and pre-torsional veliger) but are expressed in the mantle of the post-torsional veliger larvae (Hinman et al. 2003, and Fig. 3o), after the embryonic shell has been formed. Therefore, *Hox4* seems to be engaged in late larval to adult shell formation. However, further analyses of *Gva-Hox* gene expression during postlarval development are necessary to address this assumption.

In the squid *Euprymna*, *Esc-lab* (*Hox1*) is expressed in the CNS, in the developing brachial crown and in the stellate ganglia (Lee et al. 2003). *Esc-Post2* expression is detected in the stellate ganglia and nerves, and *Esc-Post1* in the light organ (Lee et al. 2003). Hence, Hox genes, shown here to be involved in shell formation in gastropods, are implicated in formation of several diverse structures not related to shell structure in cephalopods. Our observations may serve as an example demonstrating the evolvability and plasticity of gene function in Mollusca.

Hox4 seems to control the genes that are involved in the secretion of the extracellular substances forming the operculum. In the abalone, two of the shell secretome genes: ubiquitin-like fold modifying proteins (*Has-ubfm*) and a gene encoding a protein similar to ferritin proteins (*Has-ferrt*) are expressed in the operculum in a pattern similar to *Hox4* (Jackson et al. 2007) suggesting possible connection between Hox genes and novel shell secretome genes in the snails.

Possible mechanisms involved in the shell formation in gastropods

Our data on association of Hox genes with shell formation raises the possibility that the molecular circuitry underlying shell synthesis may consist of numerous conserved pattern formation genes. As was mentioned in the introduction, the homeobox gene *engrailed* is expressed in the larval mantle margin of a range of molluscs suggesting that its signals may regulate larval shell compartmental boundary (Nederbragt et al. 2002). However, *engrailed* expression is not detected in the mantle of veliger larvae, showing that this gene is not involved in the adult shell program. Additionally, *Dpp-BMP2/4* genes, belonging to the transforming growth factor β family (TGF-β), are expressed in association with the shell field in gastropods (Nederbragt et al. 2002; Iijima et al. 2008). Specifically, in the limpet *Patella vulgata*, *dpp* is expressed in a circular band in the cells adjacent to and outside of the shell-forming cells that express *engrailed* (Nederbragt et al. 2002). In the snail *Lymnaea stagnalis*, by contrast, *dpp* is localized in the shell gland ectoderm with the preferential expression in the right-hand side, inside of the shell gland margin that expresses *engrailed* gene (Iijima et al. 2008). This observation suggests that *dpp* gene may not be directly involved in shell synthesis but rather it is associated with the signaling pathway of the chirality in the ectoderm of shell gland and possibly for the coiling direction of the adult shell, i.e., dextrality or sinistrality. Additionally, another member of TGF-β family *nodal* and one of the targets of Nodal signaling pathway (*Pitx*) have been shown to be involved in shell chirality of gastropods (Grande and Patel 2009). In the dextral species *Lottia gigantea*, both *nodal* and *Pitx* genes are expressed on the right side of the embryo near the shell field, whereas both genes are detected on the left side of shell field in the sinistral species *Biomphalaria glabrata* (Grande and Patel 2009). *Engrailed*, *dpp*, and *nodal* genes appear to be involved in delimitation of shell boundary or shell coiling process rather than direct involvement in shell secretion program. Therefore, Hox genes may be suggested as better candidates for regulating the process of shell formation.

Up to now, there is no information about the target genes that Hox genes may regulate during shell formation program. Several novel genes have been identified in the abalone *Haliotis* that have expression patterns similar to Hox genes during shell ontogenesis. The expression of *Has-ubfm*, *Has-ferrt*, *Has-tsfgr1* (encoding a protein with glycine, leucine, and tyrosine repeats), *Has-vm1* (encoding a protein with no significant similarity to proteins sequences within public databases), *Has-vm2* (encoding a protein with proline-rich repeats) and two maternal genes *Has-calmbp1* (encoding a protein with sequence similarity to proteins with Ca^{2+} binding sites) and *Has-cam1* (encoding a protein similar to calmodulin proteins) is restricted to ectodermal cells associated with the lateral edges of the expanding shell within trochophores (Jackson et al. 2007). Later in veliger larvae, the transcripts of several of these genes are detected consistently along the mantle edge or in inner and outer mantle folds (Jackson et al. 2007). These genes may be proposed as potential candidates for Hox gene targets. It is hope that gene function experiments will ultimately reveal the possible cascade of these conserved and novel genes involved in a complex gene network generating the larval and adult shells in snails.

Acknowledgments The authors are grateful to two anonymous reviewers for their constructive comments. We are thankful to Achim Meyer at the Johannes Gutenberg University of Mainz, Germany for his advice with collecting and rearing of animals and to Prof. Michael Akam and Dr. Joakim Eriksson for offering LS the opportunity of training in expression experiments at University Museum of Zoology, University of Cambridge, UK. LS was supported by an EC fellowship within the MOLMORPH network under the 6th Framework Programme "Marie Curie Fellowships for Early Stage Research Training (EST) (Contract number MEST-CT-2005 - 020542).

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