ORIGINAL ARTICLE

Characterization of *twist* and *snail* gene expression during mesoderm and nervous system development in the polychaete annelid *Capitella* sp. I

Kariena K. Dill · Katrin Thamm · Elaine C. Seaver

Abstract To investigate the evolutionary history of mesoderm in the bilaterian lineage, we are studying mesoderm development in the polychaete annelid, *Capitella* sp. I, a representative lophotrochozoan. In this study, we focus on the Twist and Snail families as candidate mesodermal patterning genes and report the isolation and in situ expression patterns of two *twist* homologs (*CapI-twt1* and *CapI-twt2*) and two *snail* homologs (*CapI-sna1* and *CapI-sna2*) in *Capitella* sp. I. *CapI-twt1* is expressed in a subset of mesoderm derivatives during larval development, while *CapI-twt2* shows more general mesoderm expression at the same stages. Neither *twist* gene is detected before the completion of gastrulation. The two *snail* genes have very distinct expression patterns. At cleavage and early gastrula stages, *CapI-sna1* is broadly expressed in precursors of all three germ layers and becomes restricted to cells around the closing blastopore during late gastrulation; *CapI-sna2* expression is not detected at these stages. After gastrulation, both *snail* genes are expressed in the developing central nervous system (CNS) at stages when neural precursor cells are internalized, and *CapI-sna1* is also expressed laterally within the segmental mesoderm. Based on the expression patterns in this study, we suggest a putative function for *Capitella* sp. I *twist* genes in mesoderm differentiation and for *snail* genes in regulating CNS development and general cell migration during gastrulation.

Keywords *Capitella* sp. I · *twist* · *snail* · Spiralian mesoderm · Polychaete

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Introduction

Mesoderm evolved near the base of the bilaterian lineage and is one of the defining characteristics that unite bilaterians. It is often speculated that the invention of this third germ layer allowed for the elaboration and diversification of body plan morphologies in the Bilateria (Perez-Pomares and Munoz-Chapuli 2002). To reconstruct the ancestral origins of mesoderm and discover how the mesoderm developmental pathway was modified in the lineages leading to extant bilaterian phyla, it is important to analyze mesoderm development in diverse taxa representing all three of the major bilaterian groups: Ecdysozoa, Lophotrochozoa, and Deuterostomia. Molecular and genetic studies from model organisms (mainly *Drosophila* and various vertebrates) have identified genes that show a conserved role in mesoderm development between vertebrates and *Drosophila*. These genes have been used as candidates in broader investigations of mesoderm development in a variety of species. In this study, we focus on the transcription factors *twist* and *snail*, which are two of the best-characterized regulators of mesoderm development in the bilaterian lineage (Technau and Scholz 2003).

Twist encodes a basic helix–loop–helix (bHLH) transcription factor and was first identified as a zygotic gene required for gastrulation and mesoderm specification in the long germ-band insect, *Drosophila* (Simpson 1983; Thisse et al. 1988). Further studies demonstrated that *twist* plays a dual role in mesoderm development in *Drosophila*. First, Twist is expressed on the ventral side of the blastula where it specifies the presumptive mesoderm and activates a number of genes required for mesoderm development, including *snail* (Leptin 1991). Second, after gastrulation, *Drosophila twist* is expressed in a subset of mesoderm derivatives and is required for proper muscle development (Baylies and Bate 1996; Castanon and Baylies 2002; Cripps et al. 1998). Studies in the short germ-band insect, *Tribolium castaneum*, demonstrate that *Tribolium twist* regulates both mesoderm specification and mesoderm differentiation (Handel et al. 2005; Sommer and Tautz 1994). Homologs of *twist* have been studied in numerous other species representing diverse phyla including vertebrates (Hopwood et al. 1989; Tavares et al. 2001; Wang et al. 1997; Wolf et al. 1991), a lancelet (Yasui et al. 1998), a nematode (Harfe et al. 1998), and one lophotrochozoan, the limpet *Patella vulgata* (Nederbragt et al. 2002). In most species, *twist* genes are expressed in a subset of the mesoderm after this tissue is specified where they regulate mesoderm patterning and muscle differentiation (see references above). The prevailing theme that emerges from these studies is that the function of *twist* in mesoderm specification may be unique to insects, while a function in mesoderm differentiation represents an ancestral bilaterian trait.

The *snail* genes encode zinc-finger transcriptional repressors that regulate a variety of cell behaviors, including cell movement, cell adhesion, cell division, and cell survival (reviewed in Barrallo-Gimeno and Nieto 2005). The first Snail family member was identified as an embryonic patterning gene in *Drosophila* (Grau et al. 1984; Nusslein-Volhard et al. 1984) and was later shown to be essential for mesoderm development (Alberga et al. 1991; Leptin 1991). Snail family members have also been described as regulators of mesoderm development in a variety of other species, including *Tribolium* (Sommer and Tautz 1994), ascidians (Wada and Saiga 1999), amphioxus (Langeland et al. 1998), *Xenopus* (Mayor et al. 1993; Sargent and Bennett 1990), zebrafish (Thisse et al. 1995; Thisse et al. 1993), mouse (Nieto et al. 1992; Smith et al. 1992), and chick (Nieto et al. 1994). The *snail* genes are key regulators of cell motility, and careful molecular and genetic analyses demonstrate that the conserved function for *snail* genes in mesoderm development is in inducing the movement of mesodermal cells during gastrulation rather than in mesoderm specification (Barrallo-Gimeno and Nieto 2005; Nieto 2002). In many contexts, Snail proteins

regulate cell movement by inducing epithelial to mesenchymal transitions (EMT) via direct repression of E-cadherin (Batlle et al. 2000; Cano et al. 2000). However, *snail* genes also regulate cell motility in EMT-independent processes where cells move while maintaining contact with their neighbors in processes such as mesoderm migration during gastrulation in *Drosophila*, *Xenopus*, and zebrafish (reviewed in Barrallo-Gimeno and Nieto 2005).

Expression patterns of *snail* genes (or their proteins) have been examined in two lophotrochozoans, the leech annelid *Helobdella robusta* (Goldstein et al. 2001) and the gastropod mollusk *P. vulgata* (Lespinet et al. 2002). In *H. robusta*, labeling with an anti-Snail antibody reveals broad expression in the early embryo including precursors of ectoderm and mesoderm (Goldstein et al. 2001). In *P. vulgata*, the expression of *snail* genes was examined by whole-mount in situ hybridization and transcripts were detected almost exclusively in ectodermal derivatives (Lespinet et al. 2002). Thus, in the two lophotrochozoan species studied to date, *snail* genes are not exclusively expressed in the mesoderm or presumptive mesoderm; based on these data, it was hypothesized that *snail* genes regulate cell motility but not mesoderm development in the lophotrochozoan lineage (Lespinet et al. 2002).

As described above, characterization of *twist* and *snail* genes in lophotrochozoans is limited to just a few published studies, and for the *snail* genes, there is variation in expression patterns between species; thus, it is difficult to draw conclusions about conserved functions for *twist* and *snail* genes in this lineage. To further investigate mesoderm development in the Lophotrochozoa, we are analyzing the Twist and Snail families in the polychaete annelid *Capitella* sp. I. In this paper, we report the isolation of two *twist* homologs (*CapI-twt1* and *CapI-twt2*) and two *snail* homologs (*CapI-sna1* and *CapI-sna2*) in *Capitella* sp. I, and characterize the in situ expression patterns for all four genes during embryonic and larval development. Our findings do not indicate a function for *Capitella* sp. I *twist* or *snail* genes in mesoderm specification. Instead, expression for *CapI-twt1* and *CapI-twt2* correlates with later stages of mesoderm differentiation while expression of the *snail* genes correlates with cell movements during gastrulation and/or central nervous system (CNS) development.

Materials and methods

Animal husbandry

A *Capitella* sp. I colony was maintained in the laboratory using published culture methods (Grassle and Grassle 1976). Adults were kept at 19°C in bowls of 0.2-μM filtered seawater (FSW) with organically enriched ocean

mud as a food source and brood tubes were recovered by filtering bowl contents through a fine sieve as previously described (Seaver et al. 2005). Embryos and larvae were dissected from brood tubes, rinsed in FSW to remove mud and other debris, and fixed for in situ hybridization or phallacidin staining (see methods below).

Gene isolation

Fragments of *CapI-twt1* and *CapI-sna1* were amplified by degenerate polymerase chain reaction (PCR) using genomic DNA as template. A ~200-bp fragment of *CapI-twt1* was isolated using a nested PCR strategy with degenerate primers designed to correspond to the highly conserved bHLH domain of Twist proteins. A 67-bp fragment of *CapI-sna1* was isolated after a nested PCR using degenerate primers designed to correspond to highly conserved portions of the second and third zinc-finger domains. Degenerate PCR fragments were ligated into the pGEM-T-easy vector (Promega) and sequenced at Macrogen (South Korea).

The *CapI-twt1* fragment was extended using a mixed stage complementary DNA (cDNA) library as template. Nested PCR was performed in the 5 and 3′ directions using vector primers in combination with primers specific to the *CapI-twt1* fragment. The *CapI-sna1* sequence was extended in the 5 and 3′ directions using the BD SMART rapid amplification of cDNA ends (RACE) cDNA amplification kit (BD Biosciences Clontech). RACE cDNA template was generated from mixed stage (5–9) *Capitella* sp. I larvae. RACE PCR was performed according to the BD SMART RACE protocol using primers specific to the *CapI-sna1* fragment. RACE fragments were ligated into a pGEM T-easy vector (Promega) and sequenced (Macrogen). Sequences obtained from the original degenerate PCR products were conceptually spliced together with the 5 and 3′ RACE products; the resulting 968-bp composite transcript for *CapI-twt1* and 2,171-bp composite transcript for *CapI-sna1*, which predict complete open reading frames for both genes, were submitted to GenBank (*CapI-twt1* EF192048 and *CapI-sna1* EF192044). An additional 3′ RACE fragment representing a unique *snail* sequence was also isolated; this gene was named *CapI-sna2*.

CapI-twt1 and *CapI-sna1* sequences were used to search sequences of *Capitella* sp. I expressed sequence tag (EST) libraries. Searches identified one EST representing *CapI-sna2* and three ESTs representing a second *twist* gene, which we named *CapI-twt2*. A pBluescript SK phagemid (Stratagene) containing the 1,195-bp *CapI-twt2* insert was recovered from glycerol stocks and then sequenced for verification (Macrogen). Sequence of the *CapI-sna2* EST was used to design primers to amplify additional sequence of *CapI-sna2* from mixed stage cDNA template. The resulting 1,269-bp PCR fragment was ligated into pGEM T-easy vector (Promega) and sequenced at Macrogen. *Capitella* sp. I ESTs identified in our searches were sequenced by the Joint Genome Institute (Department of Energy, Walnut Creek, CA, USA) as part of the *Capitella* sp. I 8× genome sequencing project. EST sequences used in this study have been submitted to GenBank (*CapI-twt2* EF192049 and *CapI-sna2* EF192045). Degenerate and gene-specific primer sequences are available upon request (seaver@hawaii.edu).

Gene orthology analyses

Phylogenetic analyses of the Twist and Snail families were performed to determine gene orthology. *Capitella* sp. I genes were used as query sequences in BlastX searches of the GenBank database (NCBI) to identify related genes for orthology analyses. Alignments of amino acid sequences were made with the ClustalW program included in the MacVector package and corrected by hand for obvious alignment errors. MrBayes (Huelsenbeck and Ronquist 2001) was used to perform Bayesian phylogenetic analyses with the "wag" amino acid model and 1,000,000 generations sampled every 100 generations with four chains. For both gene families, a consensus tree was produced with PAUP*4.01b10 (Swofford 2000) using 9,500 trees representing 950,000 stable generations. A second independent method for determining gene orthology, maximum likelihood (ML), was performed with PhyML (Guindon and Gascuel 2003) using the model of evolution selected via ProtTest (JTT+G for Twist and Dayhoff I+G for Snail; Abascal et al. 2005) and 1,000 bootstrap replicates. The nexus alignment files are available upon request (seaver@hawaii.edu), and accession numbers for sequences used in the alignments are in the supplementary material ESM 1.

Whole mount in situ hybridization

Embryos and larvae were fixed and processed for whole mount in situ hybridization according to published protocols (Seaver and Kaneshige 2006; Seaver et al. 2001). Digoxigenin-labeled riboprobes were generated with the Megascript kit (Ambion), and each probe was diluted to a working concentration of 1 ng/μl. Riboprobes for *CapI-twt2* and *CapI-sna2* include the complete sequences for these genes as submitted to GenBank. The *CapI-twt1* riboprobe is a 458-bp 3′ RACE fragment corresponding to bp 510–968 of the *twt1* GenBank sequence, and the *CapI-sna1* riboprobe is a 1,204-bp 5′ RACE fragment corresponding to bp 1–1,204 of the *sna1* GenBank sequence.

Phallacidin-staining

Capitella sp. I larvae were stained with BODIPY FL-phallacidin (Molecular Probes) as described previously (Seaver et al. 2005).

Results

Molecular characterization of twist and snail orthologs from *Capitella* sp. I

We isolated two putative *twist* orthologs and two putative *snail* orthologs from *Capitella* sp. I (see "Materials and methods"). Each of the *Capitella* sp. I *twist* genes encode proteins that contain bHLH domains and WR motifs typical of the Twist family (Fig. S1a). To assess the orthology of *CapI-twt1* and *CapI-twt2*, we performed phylogenetic analyses using an alignment of the bHLH domains from various Twist proteins as well as more distantly related bHLH proteins (Paraxis and Hairy). Results indicate that the *Capitella* sp. I Twist proteins group within the Twist family (Fig. S2).

CapI-sna1 and *CapI-sna2* encode proteins that each contains five Snail-type zinc-fingers, and in addition, CapI-Sna1 contains a SNAG domain at the N terminus (Fig. S1b). The Snail superfamily is subdivided into two subfamilies, Snail and Scratch, which are thought to have originated from a duplication in an ancestral *snail/scratch* gene early in the metazoan lineage (reviewed in Nieto 2002). We also identified two *scratch* genes in *Capitella* sp. I, which to our knowledge represent the first report of the presence of *scratch* genes in lophotrochozoans (Fig. S3). To assign orthology to the *Capitella* sp. I *snail* and *scratch* genes, we performed phylogenetic analyses using an amino acid alignment of the zinc-finger regions of Snail and Scratch sequences from *Capitella* sp. I and a diverse representation of other metazoan species. Figure S3 shows a Bayesian inference consensus tree, which clearly indicates that *CapI-sna1* and *CapI-sna2* are members of the Snail family.

Capitella sp. I development

The development of *Capitella* sp. I has been described previously (Bhup and Marsden 1981; Eisig 1899; Seaver et al. 2005; Werbrock et al. 2001). Figure 1 provides an overview of embryonic and larval development (Fig. 1a), highlighting features relevant for interpreting the expression patterns in this study (Fig. 1b–h). Early embryogenesis follows a stereotypic pattern of unequal spiral cleavage. After cleavage and gastrulation, *Capitella* sp. I develops through a trochophore larval phase, which is characterized by anterior (prototroch) and posterior (telotroch) cilliary

bands separated by the mid-body, where the segments and ventral nerve cord (VNC) form (Fig. 1b,g, and h). The larval phase is brief and nonfeeding, and metamorphosis involves only minor morphological modifications; the prototroch and telotroch are lost but most other structures are maintained in the juvenile and adult.

Mesoderm development in *Capitella* sp. I, including embryonic origins and larval development, was described morphologically more than 100 years ago (Eisig 1899). Mesoderm arises from three distinct blastomeres in the early cleavage stage embryo: third quartet micromeres, 3c and 3d, and fourth quartet micromere, 4d (Eisig 1899). After blastopore closure, two large mesodermal stem cells located posteriorly divide asymmetrically producing bilateral rows of mesoderm cells (mesodermal bands) that extend towards the anterior of the larva; these mesodermal bands proliferate and give rise to segmental mesoderm (Anderson 1973; Eisig 1899; E. Seaver, unpublished observations). An analysis of cell division patterns in *Capitella* sp. I indicates that the segmental tissue arises from two separate zones of cell proliferation (Seaver et al. 2005): lateral growth zones generate ectoderm and mesoderm of the first ten segments, and a posterior growth zone generates ectoderm and mesoderm of the posterior larval and juvenile segments (Fig. 1d,e). The mesodermal bands described by Eisig likely represent the mesodermal component of the lateral growth zones. Tissue originating from lateral growth zones expands circumferentially around the larvae (Seaver et al. 2005), and by stage 6, a morphological distinction between the ectoderm and mesoderm is apparent (Fig. 1b,c).

Expression of twist genes in *Capitella* sp. I

To determine the spatiotemporal expression of *CapI-twt1*, we performed whole mount in situ hybridization using a probe encompassing a 458-bp fragment of this transcript. No expression is detected in embryos during cleavage, blastula, or gastrula stages (data not shown). Soon after blastopore closure (late stage 3), *CapI-twt1* transcript is detected in a small patch of cells at the anterior midline in the head and in two longitudinal bands in the mid-body (Fig. 2a). Expression in both of these domains persists and expands throughout larval development. Expression associated with the foregut appears at stage 5 (Fig. 2c,d), and expression around the hindgut appears at stage 7 (Fig. 2i,j).

The longitudinal bands of *CapI-twt1* expression in the mid-body at stages 3 and 4 (Fig. 2a,b) correspond to the approximate position of dividing cells in the lateral growth zones at these stages (Seaver et al. 2005 and summarized in Fig. 1d). Analysis with differential interference contrast (DIC) microscopy reveals that these *CapI-twt1* expressing cells are located between the surface ectodermal cells and

the yolk rich endoderm; we therefore judge the expression to be restricted to the mesodermal portion of the lateral growth zones (mesodermal bands). At stage 5, when the lateral growth zone tissue has expanded dorsally and ventrally around the larva (Seaver et al. 2005), longitudinal bands of *CapI-twt1* expression encompass only a subset of the segmental mesoderm (Fig. 2c,d). Each 'band' consists of two non-overlapping stripes of expression occupying different dorsal-ventral positions; a stripe in the anterior ~two thirds of the mid-body is displaced towards the dorsal side of the larva while a stripe in the posterior ~one third of

the mid-body is positioned closer to the ventral midline (Fig. 2d). The difference in the position of *CapI-twt1* positive cells in the anterior vs posterior of the mid-body is less dramatic at stage 6 when the anterior stripe, lying closer to the ventral midline, is only slightly dorsal of the posterior stripe (Fig. 2h). At stage 7, *CapI-twt1* is expressed in segmentally iterated patches of cells arranged in three longitudinal rows on either side of the mid-body: a mid-lateral row spanning the entire mid-body region and dorsal-lateral and ventral-lateral rows extending only through the anterior-most ~7–8 segments (Fig. 2k, arrowheads). The
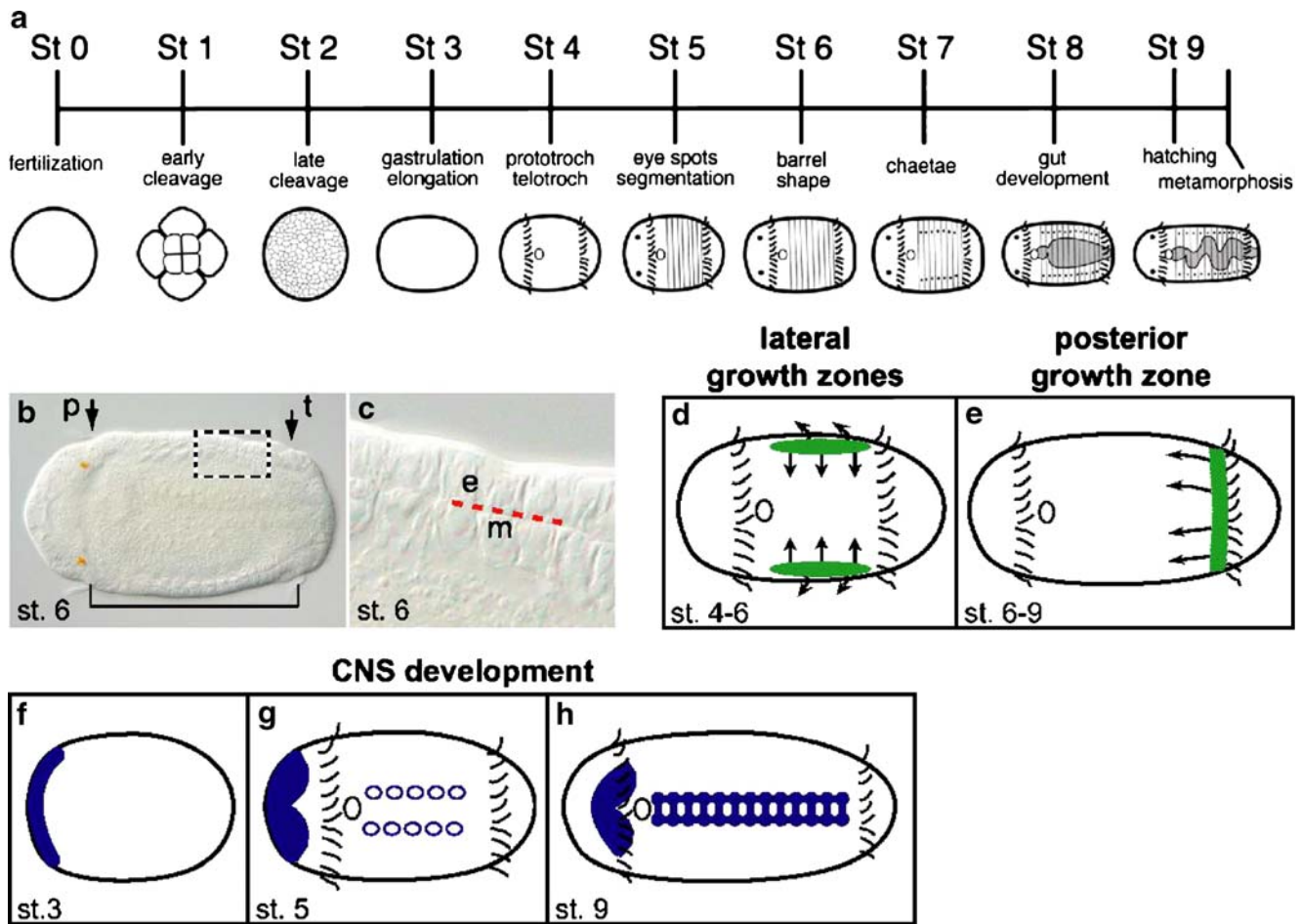

Fig. 1 Summary of *Capitella* sp. I embryonic and larval development. Anterior is towards the *left* in each image or diagram. **a** Schematic of embryonic and larval stages. Each stage is approximately 24 h in length at 19°C; the transition from embryonic to larval life (hatching) occurs at stage 4 when the cilliary bands of the prototroch (*p*) and telotroch (*t*) penetrate the egg membrane. Segmentation begins at stage 5, and 13 segments form during larval development. **b** DIC image of a stage-6 larva in ventral view. The mid-body is shown with a *bracket*. **c** Magnification of the *boxed region* in **b** showing a clear morphological distinction between ectodermal (*e*) and mesodermal (*m*) tissue layers (*red dashed line*). **d** In post-gastrula embryos (late stage 4), the lateral growth zones, which appear as longitudinal bands of dividing cells located in ventral lateral positions, expand circumferentially around the larvae giving rise to ectodermal and mesodermal

tissue of the first ten segments (Seaver et al. 2005). **e** During stage 6, the region of dividing cells transitions to a posterior position just anterior to the telotroch and represents a more classically described posterior growth zone, which gives rise to the posterior larval and juvenile segments (Seaver et al. 2005). **f** Brain development begins at stage 3 and is morphologically apparent as a thickening of the anterior neural ectoderm (Bhup and Marsden 1981; Frobius and Seaver 2006). **g** Two bilateral cerebral ganglia have formed by stage 5 (N. Meyer, personal communication). The VNC forms in the segmental region of the body; ventral ectodermal cells on either side of the midline give rise to the ganglia of the VNC (*open circles* in **g**; Eisig 1899; Seaver et al. 2005). **h** The brain is fully internalized by stage 9, and the VNC has fused at the ventral midline to form one ganglion in each segment (Eisig 1899 and N. Meyer, personal communication)

posterior boundary of the dorsal-lateral and ventral-lateral rows corresponds to the approximate position of the thoracic/abdominal boundary. In a ventral view at stage 7, the morphological distinction between mesoderm and ectoderm is clearly visible; *CapI-twt1* transcript is detected in the mesodermal layer and is excluded from the ectoderm (Fig. 2i). The transition in the *CapI-twt1* mid-body expression

between stages 6 and 7 is abrupt, and there are no apparent morphological features that correlate with either of these patterns. Expression in the mid-body diminishes during stage 8, and at stage 9, *CapI-twt1* transcript is detected in the mesoderm of the posterior-most segments and within the mesoderm in the posterior growth zone (Fig. 2m, arrowhead).
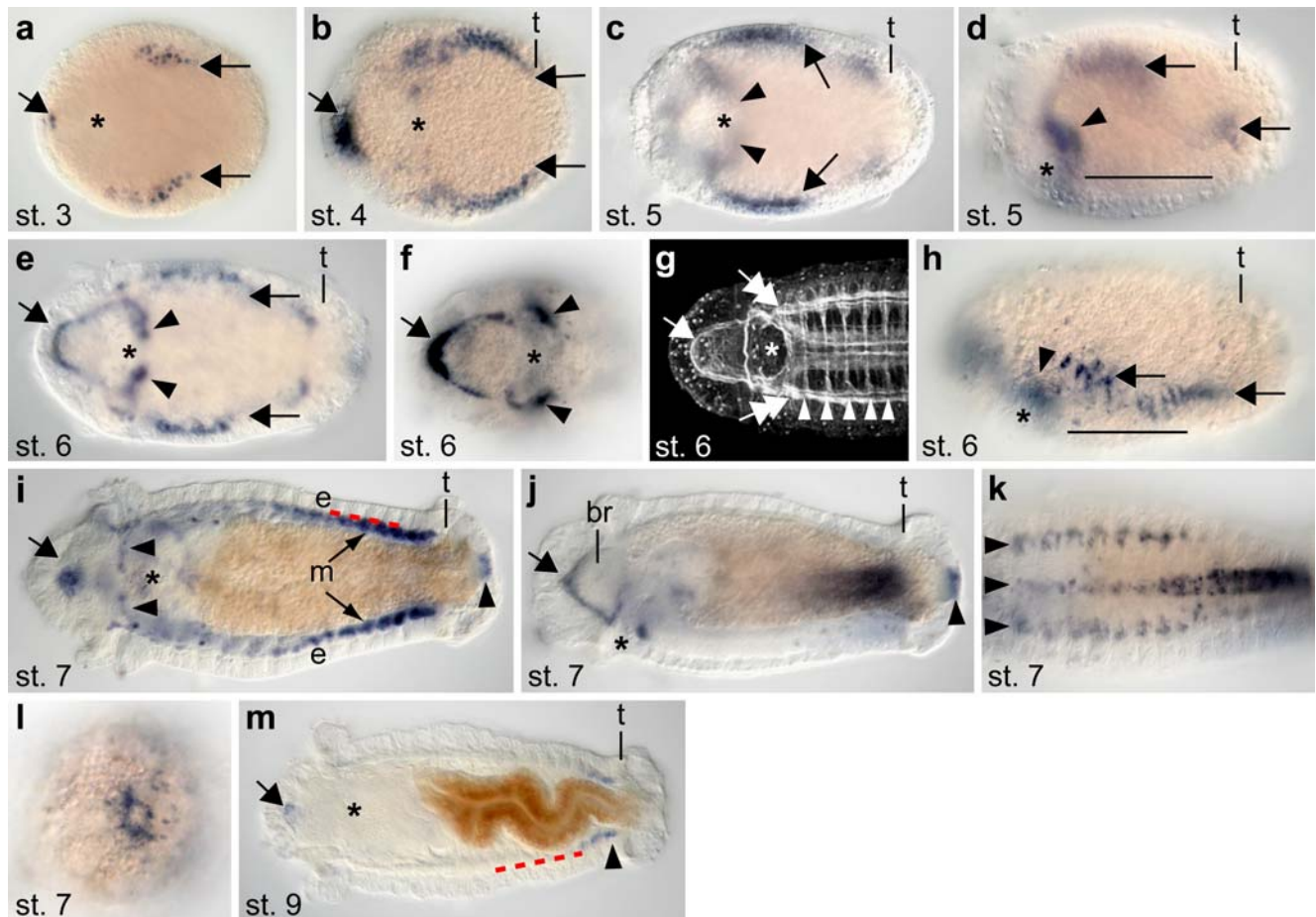

Fig. 2 The *CapI-twt1* transcript is detected after gastrulation in a subset of mesodermal derivatives. Subpanels **a**, **b**, **c**, **e**, **g**, **i**, and **m** are ventral views, anterior to the *left*; **d**, **h**, **j**, and **k** are lateral views with ventral *down* and anterior to the *left*. Indicated in each subpanel: *st.* stage; *asterisk* stomodeum; *t* telotroch; *br* brain. **a** *CapI-twt1* is expressed in a small patch of cells in the head (*diagonal arrow*) and in longitudinal bands in left and right ventro-lateral positions (*horizontal arrows*). **b** Expression is more prominent at stage 4 with a larger patch of *CapI-twt1* positive cells in the head (*diagonal arrow*) and longitudinal bands spanning the entire mid-body region (*horizontal arrows*). **c** Longitudinal bands of *CapI-twt1* expression are maintained at stage 5 (*arrows*). **d** A lateral view at stage 5 shows that the *CapI-twt1* positive cells are off-set towards the dorsal side of the larva in the anterior ~two thirds of the mid-body and are positioned closer to the ventral midline (*solid line*) in the posterior ~third (compare *horizontal arrows* in **d**). A third domain of expression at stage 5 is associated with the developing foregut (*arrowheads* in **c** and **d**). **e**, **f** At stage 6, expression is detected on either side of the stomodeum in the region of the developing foregut (*arrowheads*) and in the mid-body mesoderm

(*horizontal arrows* in **e**). There is also an arc of expression in the head (*diagonal arrow*), which co-localizes with muscle fibers (*arrow* in **g**). **g** Phallicidin stains F-actin filaments in the longitudinal (*double-headed arrows*) and circumferential (*arrowheads*) muscle fibers in the mid-body and the anterior extension of the longitudinal muscle fibers forming an arc in the head (*arrow*). The ventral nerve cord is also visible as longitudinal stripes on either side of the ventral midline. **h** At stage 6, *CapI-twt1* positive cells in the anterior portion of the mid-body are closer to the ventral midline than at stage 5 (*solid line*, compare to **d**). **i** *CapI-twt1* expression is confined to the mesoderm of the larval segments. **j** A deep focal plane showing expression associated with the foregut (staining on the anterior and posterior sides of the stomodeum), in the hindgut (*arrowhead*), and in the head muscle (*arrow*). **k** Close-up of **j** in a surface focal plane. *CapI-twt1* expression is detected in three longitudinal stripes (*arrowheads*) in the mid-body. **l** Posterior view showing expression in a ring of cells around the posterior end of the hindgut. **m** At stage 9, expression is limited to the mesoderm of the posterior growth zone (*arrowhead*) and a small patch in the head (*arrow*)

Expression of *CapI-twt1* in the head is initiated at stage 3 in a small cluster of subepidermal cells at the anterior midline (Fig. 2a) and expands to additional cells at stage 4 (Fig. 2b). There is a transient downregulation of expression in the head at stage 5 (Fig. 2c,d). At stage 6, *CapI-twt1* is detected prominently in a thin layer of cells forming an arc in the head (Fig. 2e,f, arrows), a pattern that corresponds to the shape and position of muscle fibers in the head at the same stage (Fig. 2g, arrow). This expression is maintained, and in lateral view, it is apparent that the *CapI-twt1* expression domain is positioned beneath the surface ectoderm in an arc of cells along the anterior edge of the

brain (Fig. 2j, arrow). Thus, in the head, *CapI-twt1* is expressed in the mesoderm, likely in a developing muscle fiber, which lies between the brain and the anterior ectodermal epithelia. Head expression is downregulated during stage 8 and is only detected in a small domain at the anterior midline at stage 9 (Fig. 2m, arrow).

CapI-twt1 is also expressed in association with the developing foregut and hindgut. Initially, expression in the foregut is weak (stages 4 and 5; Fig. 2b–d, arrowheads) and becomes more pronounced at stage 6 (Fig. 2e,f, arrowheads). *CapI-twt1* is maintained at lower levels in the anterior foregut at stage 7 (Fig. 4i,j) but is not detected in
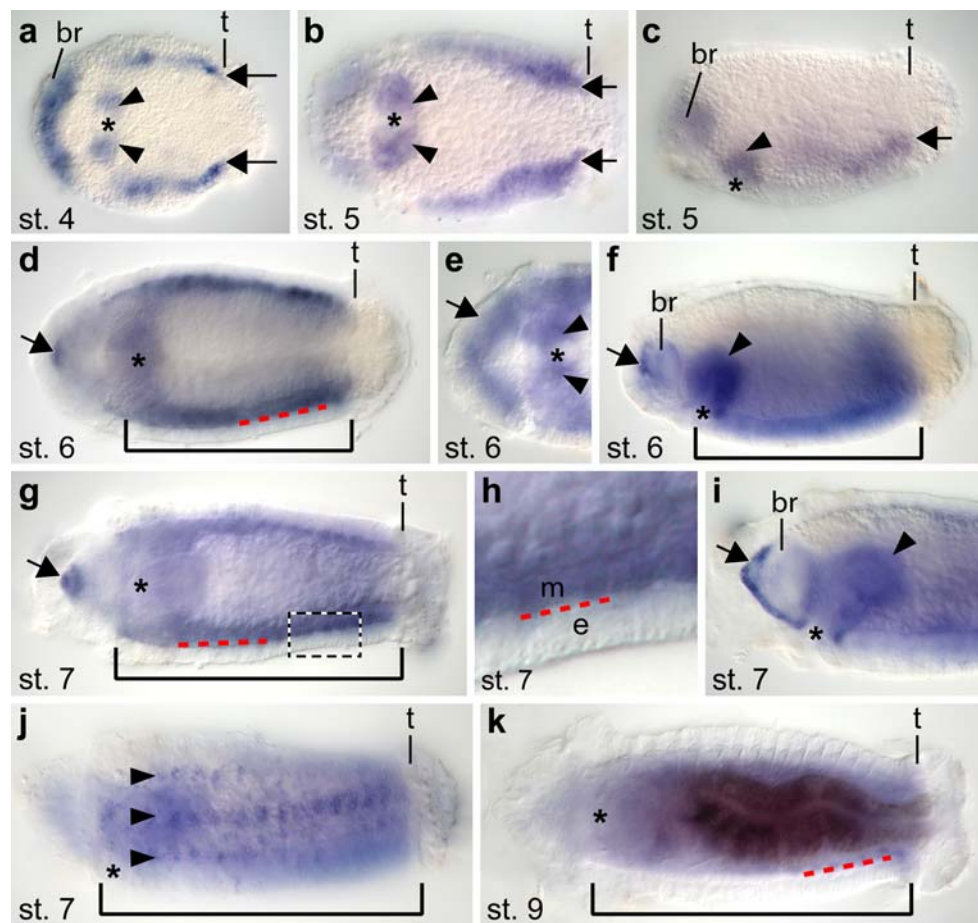
Fig. 3 *CapI-twt2* is broadly expressed in the developing mesoderm and is most prominent in tissues that also express *CapI-twt1*. Subpanels **a**, **b**, **d**, **e**, **g**, **h**, and **k** are ventral views with anterior to the *left*; **c**, **f**, **i**, and **j** are lateral views with ventral *down* and anterior to the *left*. Indicated in each subpanel: *st.* stage; *asterisk* stomodeum; *t* telotroch; *br* brain. *Red dashed lines* (**d**, **g**, and **k**) mark the boundary between ectoderm (*e*) and mesoderm (*m*) and brackets (**d**, **f**, **g**, **j**, and **k**) mark the mid-body. **a** *CapI-twt2* expression is first detected at stage 4 in longitudinal bands in the mid-body (*arrows*), in the region of the developing foregut (*arrowheads*), and in the region of the developing brain. **b**, **c** At stage 5, expression expands in the region of the foregut (*arrowheads*) and in the mid-body (*arrows*) while expression in the brain appears more faint. **d–f** At stage 6, *CapI-twt2*

is expressed throughout the entire mid-body mesoderm (see *red dashed line* in **d**); expression is also prominent in the developing foregut (*arrowheads* in **e** and **f**) and in a broad domain forming an arc in the head (*arrows*). **g**, **h** Expression in the mid-body mesoderm persists into stage 7, and transcript is still excluded from the ectodermal layer (see *red dashed lines*, **h** is a magnification of the boxed region in **g**). **i** Lateral view of a deep focal plane; expression in the developing foregut (*arrowhead*) is maintained at stage 7, while expression in the head (*arrow*) is now localized between the brain and surface ectoderm. **j** Same larva as **i**, surface focal plane showing dorsal-, ventral-, and mid-lateral stripes of *CapI-twt2* expression (*arrowheads*). **k** At stage 9, *CapI-twt2* is expressed at low levels throughout head and mid-body mesoderm

this tissue at subsequent larval stages. Beginning at stage 7, CapI-twt1 transcript is detected in a ring at the position of the developing hindgut (Fig. 2l and vertical arrowheads in i and j). Hindgut expression is downregulated during stage 8 (data not shown) and no longer detected at stage 9. The foregut and hindgut are likely derived from ectoderm, but are also associated with muscles used for feeding and excretion (Saulnier-Michel 1992). CapI-twt1 expression in the foregut is external to the epithelium in what appear to be muscles around the stomodeum (Fig. 2f,g).

In comparison to CapI-twt1, CapI-twt2 exhibits broader expression throughout most of the larval mesoderm. CapI-twt2 transcript is first detected at stage 4 (Fig. 3a) in two longitudinal bands located ventro-laterally in the mid-body, in the foregut (arrowheads), and in the brain. Expression is maintained through stage 9, and although it overlaps with CapI-twt1 expression in several domains, there are distinct characteristics to the CapI-twt2 pattern. We elaborate on each CapI-twt2 expression domain below.

CapI-twt2 expression in the longitudinal bands (Fig. 3a) is similar to CapI-twt1 expression in this domain at stage 4; thus, it appears that CapI-twt2 may also be expressed in the mesodermal bands at this stage. CapI-twt2 expression in the lateral mid-body continues at stage 5 in a subset of the segmental mesoderm (Fig. 3c). The anterior portion of this expression pattern is not displaced dorsally, as CapI-twt1 is at this stage (Fig. 3c compare to Fig. 2d). At stages 6 and 7, CapI-twt2 expression expands to encompass all of the segmental mesoderm (Fig. 3d, f–j). A lateral view at stage 7 also shows broad expression of CapI-twt2 in the mid-body; however, more pronounced expression is apparent within dorsal-lateral, ventral-lateral, and mid-lateral rows (Fig. 3j, arrowheads). These rows are very similar in position to the three rows of CapI-twt1 expression at this stage (Fig. 2k). At stage 9, CapI-twt2 expression is diffuse throughout the segmental mesoderm and contrasts with the more restricted expression of CapI-twt1 at this stage (compare Fig. 3k to Fig. 2m).

CapI-twt2 is initially expressed more broadly in the head than CapI-twt1 and is detected throughout the developing brain at stages 4 and 5 (Fig. 3a,c). Expression is reduced in the brain at stage 6 and is instead concentrated in tissue anterior to the brain (Fig. 3d–f, arrows). At stage 7, CapI-twt2 is expressed in head mesoderm in a thin arc of cells located between the brain and the anterior ectoderm (Fig. 3i, arrow). Thus, at early larval stages, CapI-twt2 head expression correlates with brain development and is quite distinct from the CapI-twt1 head expression. However, at stage 7, both transcripts are expressed in association with a muscle in the head.

CapI-twt2 is also expressed in the developing foregut from stage 4 to 7 (Fig. 3a,b,c,e,f, and i, arrowheads). In contrast to the CapI-twt1 foregut expression, which appears to be in foregut-associated muscles, CapI-twt2 is expressed within the ectodermally derived foregut epithelium.

Expression of snail genes in Capitella sp. I

Expression of CapI-sna1 transcript was examined from early cleavage stages through the end of larval development (stage 9). CapI-sna1 is detected in all four blastomeres in four-cell stage embryos (Fig. 4a). At later cleavage stages, transcript is detected in a subset of cells, and although we did not determine the identity of each CapI-sna1 positive cell, it is clear from the overall pattern that the transcript is not restricted to precursors of a particular germ layer or embryonic quadrant (data not shown). Towards the end of gastrulation, CapI-sna1 expression is downregulated in most of the embryo, and transcript is only detected in surface cells around the closing blastopore (Fig. 4b). Expression in this region continues through the end of gastrulation. After blastopore closure, CapI-sna1 positive cells are located deep within the embryo and coincide with the position of endodermal cells (Fig. 4c).

After gastrulation at stage 4, CapI-sna1 is detected in bilateral stripes that extend through the mid-body and correlate with the position of the lateral growth zones (Fig. 4d, arrows); these stripes are located below the surface ectoderm and likely represent segmental mesoderm. CapI-sna1 expression persists in the lateral mesoderm through the end of stage 6 (Fig. 4f–i, arrows) and is downregulated in this domain during stage 7. Expression of CapI-sna1 in the lateral mesoderm (stages 4–6) partially overlaps with the longitudinal bands of CapI-twt1 and CapI-twt2, but the patterns are not identical. At stages 8 and 9, CapI-sna1 is expressed in both the mesoderm and ectoderm of the posterior growth zone and at lower levels in the mid-body and head mesoderm (Fig. 4j–m).

After gastrulation, the most prominent expression of CapI-sna1 is in the developing CNS. CNS development in Capitella sp. I has been described previously and is referenced in Fig. 1. The brain develops as paired cerebral ganglia in the head and is derived from neural precursors in the anterior ectoderm (Fig. 1f–h). The VNC develops in the segmental mid-body; ganglia of the VNC are derived from neural precursors located on either side of the ventral midline (Fig. 1f–h). CapI-sna1 transcript is detected in the brain very early in its development, before significant thickening of the neuroectoderm (Fig. 4c). Over time, the maturation of the pattern in the anterior neural ectoderm mimics the progression of brain development (Fig. 4e,g,h,i). At stage 4, there is increased thickening of the anterior neural ectoderm, and CapI-sna1 expression in this region likewise expands (Fig. 4e). At stage 6, internalization of neural precursor cells is complete, and the brain is now segregated from the non-neural ectoderm (see Fig. 3h).
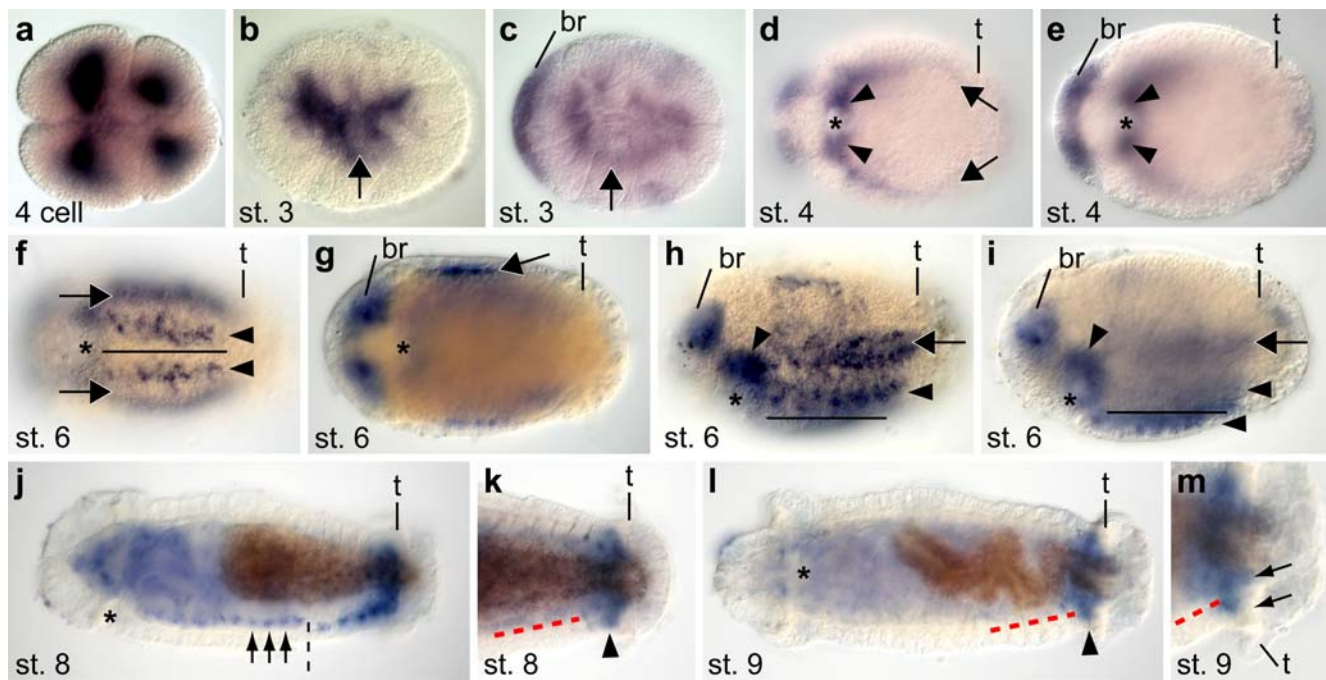
Fig. 4 *CapI-sna1* has a dynamic expression pattern through cleavage, embryonic, and larval stages. Subpanel **a** is an animal view, **b–g**, **k**, **l**, and **m** are ventral views with anterior to the *left*; **h**, **i**, and **j** are lateral views with ventral *down* and anterior to the *left*. Indicated in each subpanel: *st.* stage; *asterisk* stomodeum; *t* telotroch; *br* brain. *Solid lines* (**f**, **h**, and **i**) mark the ventral midline, and *red dashed lines* (**k**, **l**, and **m**) mark the boundary between the ectoderm (*e*) and mesoderm (*m*). Images **d** and **e** show the same larva at surface (**d**) and deep (**e**) focal planes; this same scheme follows for **f/g** and **h/i**. **a** The *CapI-sna1* transcript is detected in all four blastomeres at the four-cell stage. During stage 3, *CapI-sna1* is expressed along the blastopore (**b**, *arrow*) and after gastrulation is complete (**c**), in the developing brain and deep within the center of the embryo (*arrow*). **d**, **e** At stage 4, *CapI-sna1* transcript is detected in the developing foregut (*arrow-*

heads), laterally in stripes that extend along the entire mid-body (**d**, *arrows*), and in the brain (**e**). **f–i** At stage 6, *CapI-sna1* expression persists in the brain (**g–i**), foregut (*arrowheads* in **h** and **i**), and lateral mesodermal stripes (*arrows* in **f**, **g**, and **h**). Additionally, expression in the ventral nerve cord is detected flanking the ventral midline (*arrowheads* in **f**, **h**, and **i**). **j** At stage 8, *CapI-sna1* exhibits general mesoderm expression and is also expressed in the ganglia of the VNC (*arrows* point to three of the ganglia), the position of which shifts ventrally at the thoracic/abdominal boundary (*dashed line*). *CapI-sna1* is expressed in the posterior growth zone (*arrowhead*) at stage 8 (**k**) and stage 9 (**l**). Subpanel **m** is a close-up view of the posterior end of the larva shown in **l** illustrating expression in both mesoderm and ectoderm of the posterior growth zone (*arrows*)

CapI-sna1 brain expression persists at stage 6 and is notably absent from the overlying non-neural ectoderm (Fig. 4g–i). Also at stage 6, *CapI-sna1* expression is detected on either side of the midline in the VNC (Fig. 4f,h, and i, arrowheads). At stage 8, expression in the VNC has matured into discrete patches within each segment that correlate with the position of the VNC ganglia (Fig. 4j).

CapI-sna1 also has an expression domain in the foregut. Expression in the foregut is first detected at stage 4, lateral to the stomodeum (Fig. 4d,e, arrowheads) and persists through stage 6 (Fig. 4h,i, arrowheads). The *CapI-sna1* foregut expression is present within the foregut epithelium.

CapI-sna2 expression was also examined and is detected almost exclusively in the CNS; the only exception is a brief period of expression in the foregut epithelium at stages 6 and 7 (Fig. 5f,g, arrowheads). In contrast to *CapI-sna1*, the *CapI-sna2* transcript is not detected before larval stages. Expression in the brain is initially detected at stage 4 (Fig. 5b).

At stage 5, brain expression diminishes (Fig. 5c,d); a second transient wave of *CapI-sna2* expression in the brain is detected at stage 6 (Fig. 5e,f). Brain expression is again downregulated by the beginning of stage 7 (Fig. 5g).

CapI-sna2 is also expressed in the developing VNC. At stage 4, *CapI-sna2* is expressed in several patches of cells arranged in two rows flanking the ventral midline in the anterior mid-body (Fig. 5a, arrows). Based on the position and timing of this expression, we interpret it to be in precursors of the VNC (see Fig. 1f–h for details and references). During stage 5, expression in the VNC expands posteriorly ~three fourths of the way to the telotroch (Fig. 5c, d, arrows). Expression in the VNC extends all the way to the telotroch at stage 6 and is downregulated in the anterior segments (Fig. 5e,f, arrows). By stage 7, when the axon tracts of the VNC are well formed, *CapI-sna2* is only detected in the posterior VNC (Fig. 5g,h, arrows). *CapI-sna2* expression is reduced after stage 7, and by the end of larval development (stage 9), transcript is only detected in
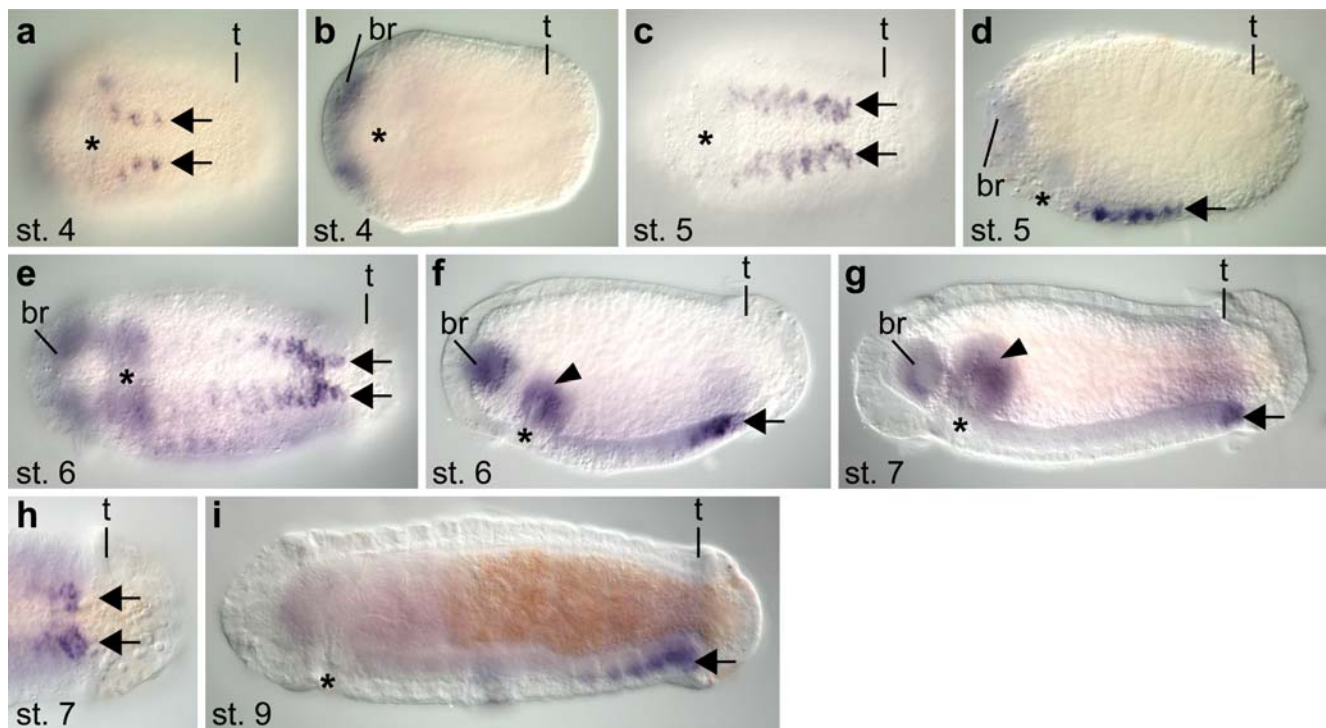
Fig. 5 *CapI-sna2* is expressed in the CNS and in the foregut. **a–c**, **e**, and **h** are ventral views with anterior to the *left*; **d**, **f**, **g**, and **i** are lateral views with ventral *down* and anterior to the *left*. Indicated in each subpanel: *st.* stage; *asterisk* stomodeum; *t* telotroch; *br* brain. *CapI-sna2* transcript is first detected at stage 4 in two to three bilateral patches flanking the ventral midline (**a**) and at the anterior edge of the larva in the developing brain (**b**). Subpanel **b** is a deeper focal plane of the same larva shown in **a**. **c**, **d** At stage 5, *CapI-sna2* is expressed in the developing VNC in segmentally iterated patches of cells that flank the ventral midline and extend ~three quarters of the way to the telotroch. **e**, **f** At stage 6, expression in the VNC extends posteriorly to the telotroch and is diminished at the anterior end (*arrows*). Expression is also detected in the brain and the developing foregut (**f**, *arrowhead*). Similar domains of expression are detected at stage 7 (**g**); however, the ventral nerve cord expression is limited to a small patch just anterior to the telotroch (**g** and **h**, *arrows*). **i** At stage 9, *CapI-sna2* expression is maintained only at the posterior end of the ventral nerve cord (*arrows*)

the VNC ganglia of the posterior most ~4–5 segments (Fig. 5i).

Discussion

Twist genes and mesoderm development in *Capitella* sp. I

In accordance with previously described ancestral functions for *twist* genes in the Bilateria (Technau and Scholz 2003), our data point towards a role for the *Capitella* sp. I *twist* genes in regulating mesoderm differentiation. Within the segmental region, *CapI-twt1* is expressed in a discrete subset of mesoderm cells: bilateral bands of cells at stages 3–6 and three lateral-longitudinal rows of cells along each side of the larva at stage 7 (Fig. 2). *CapI-twt2* is expressed broadly throughout the segmental mesoderm, and highest levels of expression are detected in the same subset of cells that also express *CapI-twt1* (Fig. 3). We suggest that *CapI-twt2* may have a specific function in these high-expressing cells in addition to a general function throughout the

segmental mesoderm. There are no obvious morphological features within the segments that correspond to these *twist* expression domains, and we predict that the expression patterns mark precursors to a specific subset of mesoderm derivatives. Major derivatives of polychaete segmental mesoderm include muscle, coelomic mesoderm, nephridia, and gonads (Ruppert and Barnes 1994). In *Capitella* sp. I, nephridia and gonads are limited to a subset of mid-trunk segments (Eckelbarger et al. 1984; and E. Seaver, unpublished observations). Because the *CapI-twt1* and *CapI-twt2* expression patterns are segmentally iterated along the entire A/P-axis, these genes are unlikely to regulate nephridia or gonad development. Thus, we postulate that *CapI-twt1* and *CapI-twt2* may influence the development of the coelomic mesoderm or late-forming larval muscle (Seaver et al. 2005) or both. Expression domains for *CapI-twt1* and *CapI-twt2* in the head implicate these genes in the development of the muscle anterior to the brain.

CapI-twt1 and *CapI-twt2* are not expressed during cleavage stages when mesoderm precursors are specified, indicating that these genes do not regulate the initial

specification of mesoderm cell lineages. This feature distinguishes mesoderm development in *Capitella* sp. I from the mesoderm development in *Drosophila* and *Tribolium* where, in addition to regulating mesoderm differentiation after gastrulation, *twist* genes are required for specifying the mesoderm precursor territory during blastula stages (Handel et al. 2005; Leptin 1991; Simpson 1983; Sommer and Tautz 1994; Thisse et al. 1988). This molecular difference may be associated with the different cellular mechanisms of early development between *Capitella* sp. I and insects. In insects, mesoderm is defined by positional cues in the syncytial blastula before cellularization. In contrast, *Capitella* sp. I (Eisig 1899), the amphipod *Parhyale hawaiensis* (Gerberding et al. 2002), and many other crustaceans (Dohle et al. 2004) develop via stereotypic holoblastic cell cleavage programs whereby mesoderm precursors can be identified based on stereotyped cell lineages. Recently, a study in *Parhyale* found *ph-twist* expression in a subset of segmental mesoderm during mesoderm differentiation but not during early cleavage stages when mesoderm cell lineages first arise (Price and Patel 2007). The authors relate the difference in the timing of *twist* expression in *Parhyale* vs *Drosophila* to the distinct mechanisms of mesoderm development in these two groups and suggest that conserved mesoderm regulatory genes may be deployed with different timing in animals with different mechanisms of mesoderm cell fate specification (Price and Patel 2007). In this regard, we might find more similarities between the genetic regulation of mesoderm development in *Capitella* sp. I and animals that develop with stereotyped cell lineages than between *Capitella* sp. I and *Drosophila*.

Mesoderm development and the expression of *Capitella* sp. I snail genes

In the context of mesoderm development in bilaterians, the function of *snail* genes has been attributed to regulating morphogenetic movements of mesoderm precursors during gastrulation (reviewed in Nieto 2002). However, there are currently no data indicating that *snail* genes have a comparable function specific to the mesoderm during gastrulation in lophotrochozoans. In the mollusk, *P. vulgata*, *snail* genes are expressed almost exclusively in ectoderm (Lespinet et al. 2002). Among annelids, *snail* genes are expressed before and during gastrulation, but expression is not limited to mesodermal lineages. Snail proteins in *H. robusta* (Goldstein et al. 2001) are detected in the precursors of multiple tissue layers before gastrulation, and in this study, we show *CapI-sna1* expression in multiple tissue layers before gastrulation and in the cells around the closing blastopore at late gastrula stages (Fig. 4). Thus, among the lophotrochozoans studied to date, it appears that *snail* genes might regulate cell movements during gastrulation, but this function is not likely exclusive to the mesoderm. After gastrulation, *H. robusta* Snail protein and *CapI-sna1* messenger RNA transcripts are detected in a subset of segmental mesoderm, indicating a putative function for annelid *snail* genes in mesoderm differentiation. However, this putative late mesodermal function for annelid *snail* genes contrasts to the function for *snail* genes in the mesoderm during gastrulation in ecdysozoans and deuterostomes.

Expression of *Capitella* sp. I snail genes in the developing CNS

Both of the *Capitella* sp. I *snail* genes are expressed prominently in the CNS during early stages of neurogenesis at stages when neural precursors are internalized from the epithelial ectoderm (Figs. 4 and 5). Although CNS development in *Capitella* sp. I is not yet described in terms of individual cell behaviors, any process whereby cells leave an epithelium and become internalized must involve cell migration, changes in cell adhesive properties, and/or a regulated asymmetric cell division (e.g., delamination), all of which are cell behaviors known to be regulated by *snail* genes (reviewed in Barrallo-Gimeno and Nieto 2005). Among arthropods, *snail* genes are expressed in and required for the internalization of neuroblasts in *Drosophila* (Ashraf et al. 1999; Ashraf and Ip 2001; Cai et al. 2001), and expression patterns for Snail family genes in *Glomeris marginata* (myriapod; Pioro and Stollewerk 2006) and *Cupiennius salei* (chelicerate; Weller and Tautz 2003) indicate that this function is conserved among diverse arthropod groups. Our data suggest a similar function for *snail* genes in a lophotrochozoan, but it is not clear whether this represents an evolutionarily conserved trait between these groups. In *P. vulgata*, *Pv-sna2* is expressed in a sensory structure associated with the apical tuft implying involvement in the neural differentiation of this structure (Lespinet et al. 2002). However, Snail protein expression in *H. robusta* does not support a role for *Hro-snail* in leech neurogenesis (Goldstein et al. 2001). Studies in additional representative lophotrochozoans are needed to determine whether or not the function of *snail* genes in CNS development is a shared trait between arthropods and lophotrochozoans.

References

Abascal F, Zardoya R, Posada D (2005) ProtTest: selection of best-fit models of protein evolution. Bioinformatics 21:2104–2105

Alberga A, Boulay JL, Kempe E, Dennefeld C, Haenlin M (1991) The *snail* gene required for mesoderm formation in *Drosophila* is expressed dynamically in derivatives of all three germ layers. Development 111:983–992

Anderson DT (1973) Embryology and phylogeny in Annelids and Arthropods. Pergamon, Oxford

Ashraf SI, Ip YT (2001) The Snail protein family regulates neuroblast expression of *inscuteable* and *string*, genes involved in asymmetry and cell division in *Drosophila*. Development 128:4757–4767

Ashraf SI, Hu X, Roote J, Ip YT (1999) The mesoderm determinant *snail* collaborates with related zinc-finger proteins to control *Drosophila* neurogenesis. EMBO J 18:6426–6438

Barrallo-Gimeno A, Nieto MA (2005) The Snail genes as inducers of cell movement and survival: implications in development and cancer. Development 132:3151–3161

Batlle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, Garcia De Herreros A (2000) The transcription factor snail is a repressor of *E-cadherin* gene expression in epithelial tumour cells. Nat Cell Biol 2:84–89

Baylies MK, Bate M (1996) *twist*: a myogenic switch in *Drosophila*. Science 272:1481–1484

Bhup R, Marsden JR (1981) The development of the central nervous system in *Capitella capitata* (Polychaeta, Annelida). Can J Zool 60:2284–2295

Cai Y, Chia W, Yang X (2001) A family of snail-related zinc finger proteins regulates two distinct and parallel mechanisms that mediate *Drosophila* neuroblast asymmetric divisions. EMBO J 20:1704–1714

Cano A, Perez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA (2000) The transcription factor snail controls epithelial-mesenchymal transitions by repressing *E-cadherin* expression. Nat Cell Biol 2:76–83

Castanon I, Baylies MK (2002) A Twist in fate: evolutionary comparison of Twist structure and function. Gene 287:11–22

Cripps RM, Black BL, Zhao B, Lien CL, Schulz RA, Olson EN (1998) The myogenic regulatory gene *Mef2* is a direct target for transcriptional activation by Twist during *Drosophila* myogenesis. Genes Dev 12:422–434

Dohle W, Gerberding M, Hejnol A, Scholtz G (2004) Cell lineage, segment differentiation, and gene expression in crustaceans. In: Scholtz G (ed) Evolutionary developmental biology of Crustacea. Sweets and Zeitlinger, Lisse, The Netherlands, pp 95–133

Eckelbarger KJ, Linley PA, Grassle JP (1984) Role of ovarian follicle cells in vitellogenesis and oocyte resorption in *Capitella* sp. I (Polychaeta). Mar Biol 79:133–144

Eisig H (1899) Zur Entwicklungsgeschichte der Capitelliden. Mittheilungen Aus der Zoologischen Station Zu Neapel 13:5–39

Frobius AC, Seaver EC (2006) *Capitella* sp. I *homeobrain-like*, the first lophotrochozoan member of a novel paired-like homeobox gene family. Gene Expr Patterns 6:985–991

Gerberding M, Browne WE, Patel NH (2002) Cell lineage analysis of the amphipod crustacean *Parhyale hawaiensis* reveals an early restriction of cell fates. Development 129:5789–5801

Goldstein B, Leviten MW, Weisblat DA (2001) *Dorsal* and *snail* homologs in leech development. Dev Genes Evol 211:329–337

Grassle JP, Grassle JF (1976) Sibling species in the marine polution indicator *Capitella* (Polychaeta). Science 192:567–569

Grau Y, Carteret C, Simpson P (1984) Mutations and chromosomal rearrangements affecting the expression of *snail*, a gene involved in embryonic patternin in *Drosophila melanogaster*. Genetics 108:347–360

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52:696–704

Handel K, Basal A, Fan X, Roth S (2005) *Tribolium castaneum twist*: gastrulation and mesoderm formation in a short-germ beetle. Dev Genes Evol 215:13–31

Harfe BD, Vaz Gomes A, Kenyon C, Liu J, Krause M, Fire A (1998) Analysis of a *Caenorhabditis elegans Twist* homolog identifies conserved and divergent aspects of mesodermal patterning. Genes Dev 12:2623–2635

Hopwood ND, Pluck A, Gurdon JB (1989) A *Xenopus* mRNA related to *Drosophila twist* is expressed in response to induction in the mesoderm and the neural crest. Cell 59:893–903

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Langeland JA, Tomsa JM, Jackman WR Jr, Kimmel CB (1998) An amphioxus *snail* gene: expression in paraxial mesoderm and neural plate suggests a conserved role in patterning the chordate embryo. Dev Genes Evol 208:569–577

Leptin M (1991) *twist* and *snail* as positive and negative regulators during Drosophila mesoderm development. Genes Dev 5:1568–1576

Lespinet O, Nederbragt AJ, Cassan M, Dictus WJ, van Loon AE, Adoutte A (2002) Characterisation of two *snail* genes in the gastropod mollusc *Patella vulgata*. Implications for understanding the ancestral function of the snail-related genes in Bilateria. Dev Genes Evol 212:186–195

Mayor R, Essex LJ, Bennett MF, Sargent MG (1993) Distinct elements of the *xsna* promoter are required for mesodermal and ectodermal expression. Development 119:661–671

Nederbragt AJ, Lespinet O, van Wageningen S, van Loon AE, Adoutte A, Dictus WJ (2002) A lophotrochozoan *twist* gene is expressed in the ectomesoderm of the gastropod mollusk *Patella vulgata*. Evol Dev 4:334–343

Nieto MA (2002) The *snail* superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol 3:155–166

Nieto MA, Bennett MF, Sargent MG, Wilkinson DG (1992) Cloning and developmental expression of *Sna,* a murine homologue of the *Drosophila snail* gene. Development 116:227–237

Nieto MA, Sargent MG, Wilkinson DG, Cooke J (1994) Control of cell behavior during vertebrate development by *Slug*, a zinc finger gene. Science 264:835–839

Nusslein-Volhard C, Weischaus E, Kluding H (1984) Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I. Zygotic loci on the second chromosome. Wilhem Roux Arch Dev Biol 193:267–282

Perez-Pomares JM, Munoz-Chapuli R (2002) Epithelial-mesenchymal transitions: a mesodermal cell strategy for evolutive innovation in Metazoans. Anat Rec 268:343–351

Pioro HL, Stollewerk A (2006) The expression pattern of genes involved in early neurogenesis suggests distinct and conserved functions in the diplopod *Glomeris marginata*. Dev Genes Evol 216:417–430

Price AL, Patel NH (2007) Investigating divergent mechanisms of mesoderm development in arthropods: the expression of *Ph-twist* and *Ph-mef2* in *Parhyale hawaiensis*. J Exp Zoolog B Mol Dev Evol (in press)

Ruppert E, Barnes R (1994) Invertebrate zoology. Thomson learning, Belmont

Sargent MG, Bennett MF (1990) Identification in *Xenopus* of a structural homologue of the *Drosophila* gene *snail*. Development 109:967–973

Saulnier-Michel C (1992) Polychaeta: Digestive system. Wiley-Liss, New York

Seaver EC, Kaneshige LM (2006) Expression of 'segmentation' genes during larval and juvenile development in the polychaetes *Capitella sp. I* and *H. elegans*. Dev Biol 289:179–194

Seaver EC, Paulson DA, Irvine SQ, Martindale MQ (2001) The spatial and temporal expression of *Ch-en*, the *engrailed* gene in the polychaete *Chaetopterus*, does not support a role in body axis segmentation. Dev Biol 236:195–209

Seaver EC, Thamm K, Hill SD (2005) Growth patterns during segmentation in the two polychaete annelids, *Capitella sp. I* and *Hydroides elegans*: comparisons at distinct life history stages. Evol Dev 7:312–326

Simpson P (1983) Maternal-zygotic gene interactions during formation of the dorsoventral pattern in *Drosophila* embryos. Genetics 105:615–632

Smith DE, Franco del Amo F, Gridley T (1992) Isolation of *Sna*, a mouse gene homologous to the *Drosophila* genes *snail* and *escargot*: its expression pattern suggests multiple roles during postimplantation development. Development 116:1033–1039

Sommer RJ, Tautz D (1994) Expression patterns of *twist* and *snail* in *Tribolium* (Coleoptera) suggest a homologous formation of mesoderm in long and short germ band insects. Dev Genet 15:32–37

Swofford DL (2000) PAUP*: Phylogenetic analysis using parsimony (*and other methods). Sinauer, Sunderland, MA

Tavares AT, Izpisuja-Belmonte JC, Rodriguez-Leon J (2001) Developmental expression of chick *twist* and its regulation during limb patterning. Int J Dev Biol 45:707–713

Technau U, Scholz CB (2003) Origin and evolution of endoderm and mesoderm. Int J Dev Biol 47:531–539

Thisse B, Stoetzel C, Gorostiza-Thisse C, Perrin-Schmitt F (1988) Sequence of the *twist* gene and nuclear localization of its protein in endomesodermal cells of early *Drosophila* embryos. EMBO J 7:2175–2183

Thisse C, Thisse B, Schilling TF, Postlethwait JH (1993) Structure of the zebrafish *snail1* gene and its expression in wild-type, *spadetail* and *no tail* mutant embryos. Development 119:1203–1215

Thisse C, Thisse B, Postlethwait JH (1995) Expression of *snail2*, a second member of the zebrafish snail family, in cephalic mesendoderm and presumptive neural crest of wild-type and *spadetail* mutant embryos. Dev Biol 172:86–99

Wada S, Saiga H (1999) Cloning and embryonic expression of *Hrsna*, a *snail* family gene of the ascidian *Halocynthia roretzi*: implication in the origins of mechanisms for mesoderm specification and body axis formation in chordates. Dev Growth Differ 41:9–18

Wang SM, Coljee VW, Pignolo RJ, Rotenberg MO, Cristofalo VJ, Sierra F (1997) Cloning of the human *twist* gene: its expression is retained in adult mesodermally derived tissues. Gene 187:83–92

Weller M, Tautz D (2003) Prospero and Snail expression during spider neurogenesis. Dev Genes Evol 213:554–566

Werbrock AH, Meiklejohn DA, Sainz A, Iwasa JH, Savage RM (2001) A polychaete *hunchback* ortholog. Dev Biol 235:476–488

Wolf C, Thisse C, Stoetzel C, Thisse B, Gerlinger P, Perrin-Schmitt F (1991) The *M-twist* gene of Mus is expressed in subsets of mesodermal cells and is closely related to the *Xenopus X-twi* and the *Drosophila twist* genes. Dev Biol 143:363–373

Yasui K, Zhang SC, Uemura M, Aizawa S, Ueki T (1998) Expression of a twist-related gene, *Bbtwist*, during the development of a lancelet species and its relation to cephalochordate anterior structures. Dev Biol 195:49–59