Chapter 6

The hedgehog and the snail

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Protostome animals, such as insects, snails and worms, seem to be organized up-side down when compared to deuterostomes, such as vertebrates. If this is true, then their respective ventrally (belly-side) and dorsally (back-side) located nervous system are homologous (inherited from a common ancestor, Arendt and Nübler-Jung 1994). Although it has been argued that aspects of nervous system development are similar between protostomes and deuterostomes, there appears to be a lack of similarity in genes involved in development of the midline of the nervous system between these animals (Arendt and Nübler-Jung 1999). Here we show that an ortholog of the deuterostome midline patterning gene hedgehog is expressed in the ventral midline during early development of the snail Patella vulgata, thereby presenting the first evidence that midline patterning is conserved between deuterostomes and protostomes.

In protostomes as well as deuterostomes, the midline is an embryonic region that functions in patterning the adjacent nervous tissue (Sasai and De Robertis 1997; Menne et al. 1997). The ventral midline in insects (protostomes) is the region from which cells detach to form the ventrally located nerve cords. In vertebrates (deuterostomes) the midline is originally located dorsally. During neurulation it folds inwards and becomes the ventral part of the dorsally located neural tube and is then called ventral midline or floor plate.

The dorsoventral axis inversion theory holds that protostomes and deuterostomes have an inverted dorsoventral organization (Arendt and Nübler-Jung 1994). It was originally based on anatomical observations (Geoffroy St. Hilaire 1822) and has recently received support from molecular data (De Robertis and Sasai 1996; Arendt and Nübler-Jung 1997). The theory predicts that the protostome and deuterostome nervous systems are homologous (Arendt and Nübler-Jung 1999), as well as their respective midline region. It would thus be likely that similar genes are involved in the embryonic patterning of the midline region, but this has so far not been found.

An important gene involved in midline patterning in vertebrates is sonic hedgehog (shh). Sonic hedgehog is expressed in the midline of the early embryo, but most prominently in the ventral midline (floor plate) of the developing nervous system. It plays a role in differentiation of ventral neural tube structures and dorsoventral patterning of adjacent tissues (Sasai and De Robertis 1997). Contrary to this situation, the
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Figure 1: Hedgehog expression in the ventral midline of a protostome (see colour figure on page 136)

The Patella hedgehog gene is expressed in the ventral midline of the so-called trochophore larva at 24 hrs of development. **(A)** Alignment of part of the deduced hedgehog protein of the gastropod mollusc Patella vulgata with the hedgehog proteins of the fruit fly (Drosophila), the human (Homo), a cephalochordate (Branchiostoma) and a sea urchin (Lytechinus). Note that humans have three different hedgehog genes: sonic hedgehog (shh), indian hedgehog (ihh), and desert hedgehog (dhh). A dash indicates an amino acid residue that is identical to the residue at the same position in the Patella hedgehog protein. A dot indicates a gap introduced to optimise the alignment. The sequence of the Patella hedgehog gene has been deposited in GenBank under accession number AF435840. **(B, C)** Whole-mount embryos in situ hybridised for the Patella hedgehog gene seen from the ventral side (B) and from the right lateral side (C). **(D, E)** Scanning electron micrographs, ventral view (D) and right lateral view (E) of larva of the same age. The dashed line in (D) shows the ventral midline running from the future mouth (stomodaeum), over the foot, towards a ciliated structure below the foot (telotroch). Expression is also seen in the region around the anterior sensory structure of this larva (apical tuft). Anterior is up in all pictures. Abbreviations: a - apical tuft, s - developing shell, st - stomodaeum, p - prototroch, f - foot, t - telotroch.
hedgehog gene of the protostome insect Drosophila is not expressed in the ventral midline. Instead, its most prominent function in this animal is setting up the border between segments (Lawrence and Struhl 1996). This argues against homology of the deuterostome and protostome midline.

Interestingly, the hedgehog homologue of the snail Patella vulgata is expressed in the ventral midline of the 24 hours old snail larva (see figure). Our data on hedgehog in Patella is the first example where an ortholog of a deuterostome midline gene is expressed in the ventral midline of a protostome larva. Significant further indications for similarities in nervous system development between protostomes and deuterostomes can thus be established by broadening our view and looking at a less-studied organism, such as the snail Patella vulgata. The striking correspondence in midline signalling between vertebrates and molluscs (snails) is indicative of an ancient role of the hedgehog gene in early patterning of the nervous system.