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## ***snail* expression during embryonic development of the coral *Acropora*: blurring the diploblast/triploblast divide?**

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**Abstract** Although corals are nominally diploblastic, the early development of *Acropora millepora* involves a process that clearly resembles gastrulation in higher metazoans. This similarity at the morphological level led us to search for the *Acropora* equivalents of genes whose key roles in gastrulation are conserved across the higher Metazoa. We here report the characterisation of one such gene, *snail*, which in both *Drosophila* and the mouse is expressed in cells undergoing an epithelial-mesenchyme transition and/or morphogenetic movements. In addition to an N-terminal SNAG domain, the *Acropora* snail protein contains four zinc fingers with sequences diagnostic for members of the snail protein subfamily. In situ hybridisation reveals expression in epithelial tissue in the central portion of one side of the flattened pre-gastrulation embryo, which continues to express *snail* as it is engulfed by its opposite layer. Comparison to *snail* expression during gastrulation in bilaterians such as *Drosophila* reveals striking similarities and suggests mechanistic, and possibly evolutionary, links between the processes of mesoderm formation in bilaterians and endoderm formation in the Cnidaria.

**Keywords** Snail · Gastrulation · Coral · *Acropora* · Epithelial-mesenchymal transition

The animal kingdom is traditionally divided into diploblasts (the non-bilaterian lower Metazoa including the Cnidaria) and triploblasts (the bilaterian higher Metazoa). As the names indicate, this distinction is based on possession of two body layers, the ectoderm and endoderm, or three, the ectoderm, endoderm and mesoderm. The third layer, the mesoderm, is created during gastrulation, which is commonly defined as “the morphogenetic movements of the early embryo that lead to the generation of the third embryonic layer” (Nieto 2002).

The split between diploblasts and triploblasts is not, however, absolute with only some members of the Class Hydrozoa being diploblasts in the strictest sense of the word (Hyman 1940). In all other “diploblasts” the distinction is open to question. Indeed, some jellyfish have a massive middle layer, or mesoglea, although it is not regarded as mesoderm on the grounds that it is acellular. However, in some diploblasts, the mesoglea is invaded by cells, making the diploblast/triploblast distinction somewhat arbitrary (summarised in Willmer 1990).

During its embryonic development the branching reef coral, *Acropora millepora*, undergoes a process that appears similar to gastrulation. This observation led us to search for the *Acropora* orthologs of genes whose key roles in gastrulation are conserved across the higher Metazoa. One such gene is *snail*, which in diverse animals is expressed in tissues undergoing morphogenetic movements or epithelial-mesenchyme transitions.

A 519-bp snail EST (Kortschak et al. 2003) was used to probe a pre-settlement cDNA library. The longest of six clones was chosen for sequencing and found to contain an insert of 1,329 nucleotide residues, including a complete open reading frame encoding a predicted protein of 264 amino acids. The nucleotide sequence of the *Acropora snail* (*snail-Am*) cDNA is available from GenBank (accession number AY532062).

Snail proteins are characterised by the presence of a SNAG domain at the amino terminus, which binds to the E box of target genes, and by four or five zinc fingers (Fig. 1A–C). The *Acropora* protein contains an N-terminal SNAG domain that is identical to those of hu-

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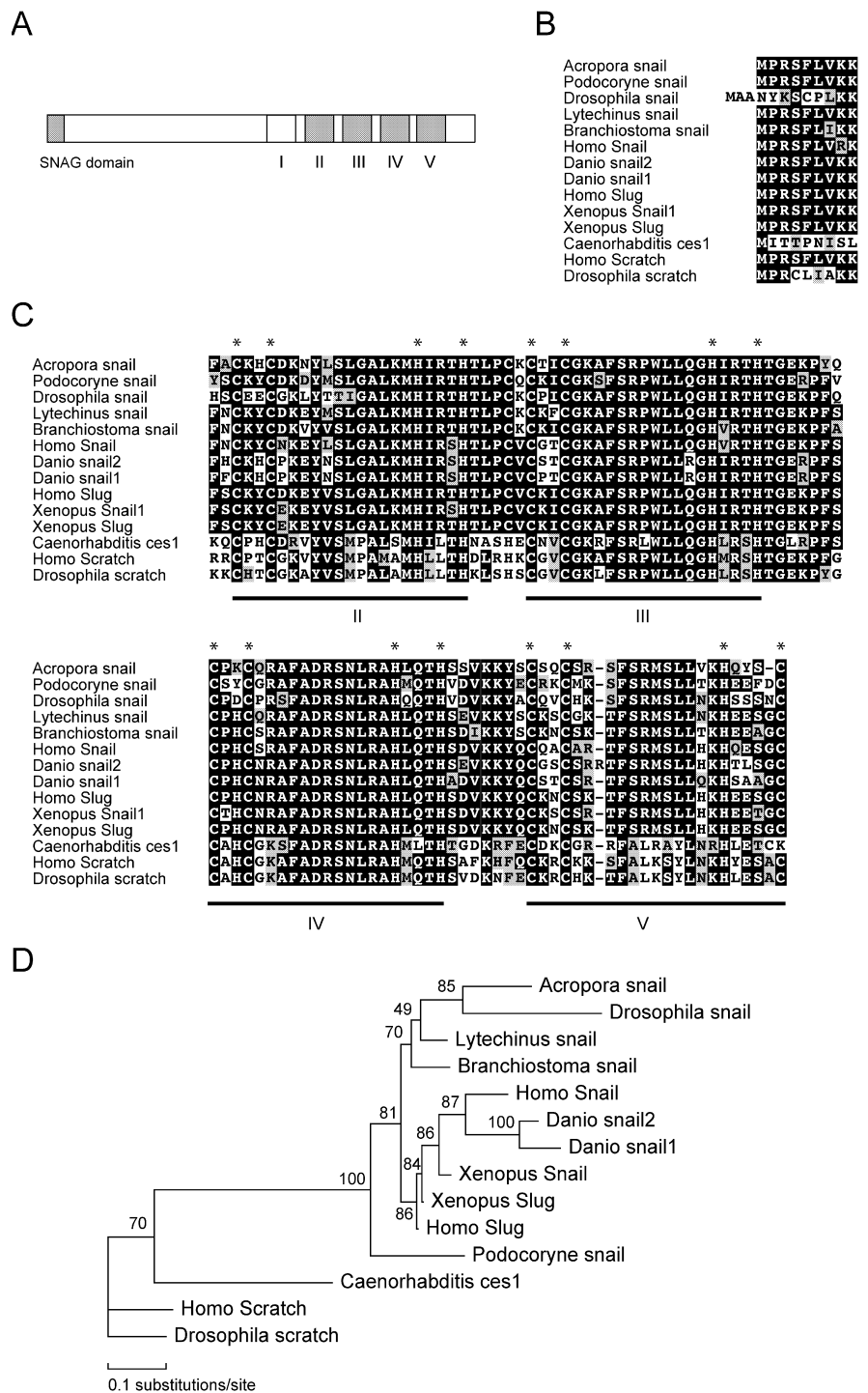
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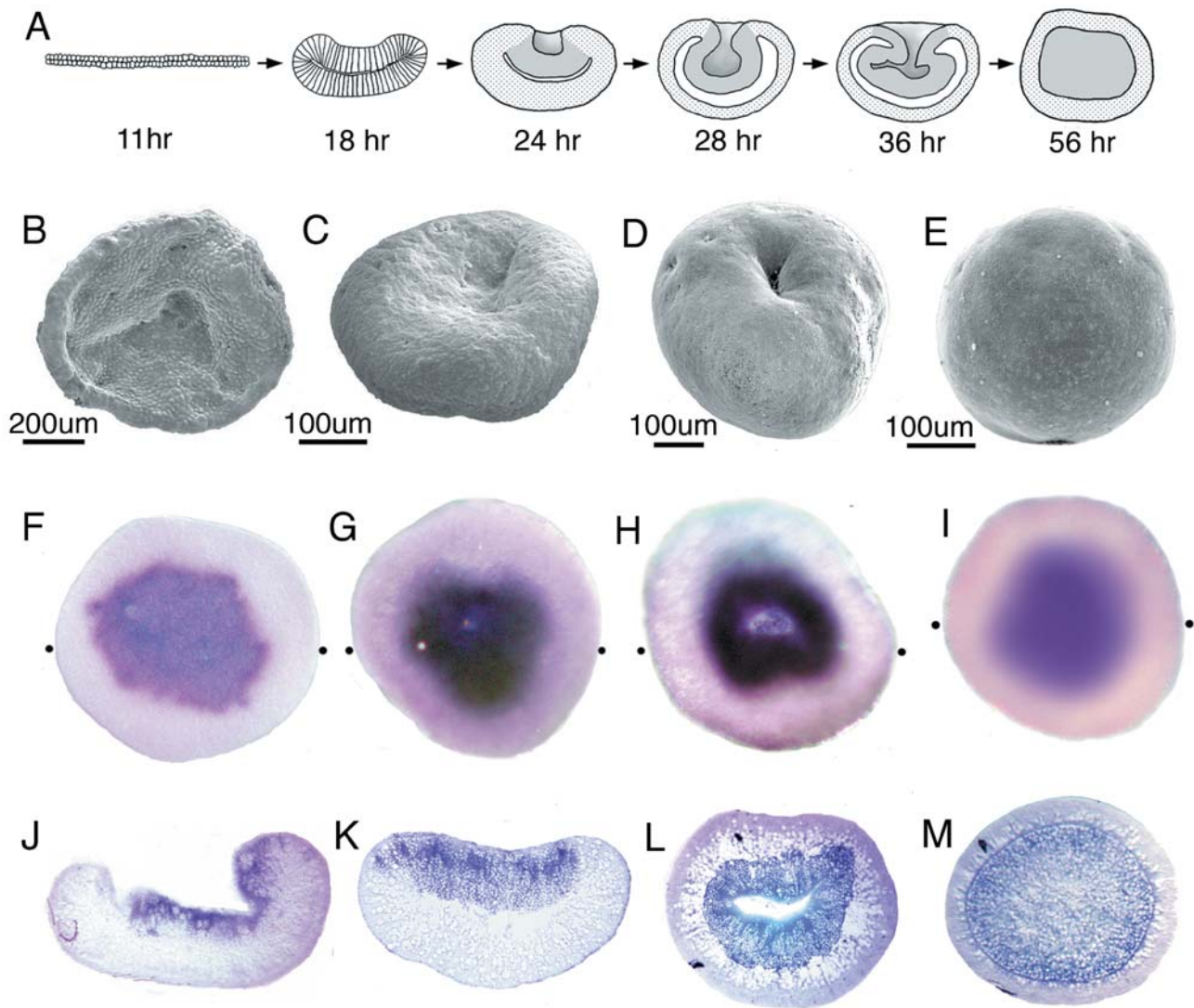
**Fig. 1A–D** Sequence comparisons of *Acropora* snail and related proteins. **A** Diagrammatic view of a typical snail protein showing the diagnostic features: an N-terminal SNAG domain plus four or five zinc fingers. **B** The *Acropora* SNAG domain fits the consensus and is identical to those of *Podocoryne* snail and human Slug and Scratch. **C** The zinc finger region of the *Acropora* protein compared to related proteins in other organisms. The positions of zinc fingers II–V are shown and the conserved cysteines and histidines are indicated with asterisks. Note that some organisms, including human and *Acropora*, have only four zinc fingers, while others have five. **D** The zinc finger II–V regions of snail proteins compared in **C** were aligned manually prior to Maximum Likelihood phylogenetic analyses in MolPhy version 2.3 (Adachi and Hasegawa 1996) using the Dayhoff model of protein evolution and local rearrangement of the NJ trees (1,000 bootstraps). In these analyses, *Drosophila* scratch (AAD38602) served as out-group. The other sequences were retrieved from the databases for inclusion in the analyses: *Podocoryne* snail (CAD 21523), *Drosophila* snail (P08044), *Lytechinus* snail (AAB67715), *Branchiostoma* snail (AAC35351), human Snail (CAB52414), *Danio* snail2 (AAA87196), *Danio* snail1 (NP 571141), human Slug (O43623), *Xenopus* Snail (P19382), *Xenopus* Slug (Q91924), *Caenorhabditis* ces1 (NP 492338) and human Scratch (AAK01467)



man Slug and Scratch (Fig. 1A, B), and zinc fingers corresponding to those numbered ZFII to ZFV under the standard nomenclature (Manzanares et al. 2001; Fig. 1A, C). The ZFII and V sequences clearly identify the *Acropora* protein as a snail, rather than scratch, subfamily member; note that ZFI is absent from *Acropora* snail as well as from a number of other snail proteins.

Phylogenetic analyses (Fig. 1D) based on the four zinc fingers using maximum likelihood methods are broadly

consistent with published studies and clearly group *Acropora* snail with other invertebrate snail sequences. In agreement with previous analyses (Manzanares et al. 2001; Lespinet et al. 2002) the relationships between invertebrate snail proteins appear complex, and have not been unequivocally established. Nevertheless, these analyses strongly support a distant relationship between the *Acropora* and *Podocoryne* snail sequences.



**Fig. 2A–M** Expression of *snail-Am* during the embryonic development of the coral *Acropora*, as established using in situ hybridisation. **A** Diagrammatic summary of the morphological changes in the *Acropora* embryo during gastrulation. The *times* are typical values for the conditions under which the embryos develop in the field. **B–E** Scanning electron micrographs showing the external morphology of embryos similar to those stained in **F–I** and **J–M**. **B** An embryo with the concavity just starting to form at 10–15 h. **C** The embryo has shrunk in diameter and thickened relative to **B** at about 18 h. In **D** the embryo is almost spherical and the pore is closing at 36–48 h. **E** By 56 h the pore has closed and the embryo is spherical. **F–I** In situ hybridisations of whole-mount embryos of successively older ages corresponding approximately to the SEMs shown in **B–E**. **J–M** To visualise the internal organisation of *snail-*

expressing tissues whole-mount in situ preparations were embedded in soft Araldite and sectioned, with section thickness adjusted as necessary to allow visualisation of the stain. Vertical sections of preparations of a similar age to those shown in **F–H**. The plane of section is at 90° to the page on a line joining the *black dots* in **F–I**. **J** Cells lining the centre of the concavity are expressing *snail-Am*. **K** At this stage the cells comprising the embryo have become longer and thinner as revealed by the expressing cells (the staining zone is the width of one cell). **L** This embryo is at approximately the stage shown in **A** 36 h and **H**. The section is lateral to the pore but clearly shows how the expressing tissue is now being engulfed. **M** At this stage the blastopore has closed, creating a distinct endoderm which continues to express *snail-Am*, and ectoderm, as shown diagrammatically in **A** 56 h

The embryonic development of *Acropora* for the time interval discussed in this paper is diagrammed in Fig. 2A. During this interval the *Acropora* embryo develops from a histologically uniform bilayer of cells packed with lipid granules (Fig. 2A 11 h) into a sphere, with morphologically distinct cell types internally and externally (Fig. 2A 56 h, **M**; for a more detailed description of this process see Hayward et al. 2002). However, the mechanisms by which this change occurs have yet to be established,

especially with regard to the relative contributions of cell movement and changes in cell shape and the mechanisms by which former ectodermal cells reorganise to form an apparently uniform endoderm.

*snail* expression was studied by northern blotting and in situ hybridisation using previously described methods (Hayward et al. 2001). Northern blots (not shown) revealed that the *Acropora snail* mRNA is present in all stages tested after 15 h post fertilisation. Expression of

*snail-Am* is first detected as the disc-shaped *Acropora* embryo starts to shrink in circumference and the edges start to thicken, creating a concavity (Fig. 2A 18 h, B, C). At this stage, *snail-Am* expression is restricted to the cells on the concave side (Fig. 2F, J). Later, as the concavity deepens and begins to close on itself, the ectodermal *snail-Am*-expressing cells become elongated (Fig. 2A 18–24 h, K) and eventually become internalised (Fig. 2A 18–56 h, K–M). As the blastopore closes, the internalised cells lose their epithelial character (Fig. 2A 56 h, M). Thus, during the gastrulation-like stage of *Acropora* development, *snail-Am* is expressed in a pattern that is strikingly reminiscent of those of the corresponding *Drosophila* (e.g. Leptin et al. 1992; Nieto 2002) and vertebrate genes (e.g. Sefton et al. 1998).

That an *Acropora snail* ortholog should be expressed in such a strikingly *Drosophila*-like pattern during a process that is strongly reminiscent of gastrulation contradicts expectations based upon the classical distinction between diploblastic and triploblastic animals and indicates that *snail* expression was already associated with creation of a new internal tissue layer in the common ancestor of the two groups.

Gastrulation is one specific manifestation of an epithelial-mesenchyme transition in amniotes and, in *Acropora*, *snail*-expressing tissue appears to undergo such a transition. Thus, while at a cell biological level the ancestral function of *snail* may have been to mediate cell motility by, for example, repressing cadherin expression, the association of *snail* expression with presumptive endoderm in *Acropora* may point to an early evolutionary link between *snail* and the origin of differentiated tissue layers in the Metazoa.

The expression of *snail* in the hydroid *Podocoryne*, and the behaviour of cells containing it, have both interesting similarities and differences when compared to the situation in *Acropora*. During *Podocoryne* embryonic development *snail* mRNA, which is provided maternally, is present in all developmental stages (Spring et al. 2002), in contrast to the situation in *Acropora*, where maternally provided *snail*, if present, is at levels too low to be detected. Endodermal expression is seen transiently in the *Podocoryne* planula larva, but was not noted during gastrulation. However, in the *Podocoryne* medusa, a life stage lacking in *Acropora*, *snail* and several other molecular markers commonly associated with mesoderm are found in the entocodon, a tissue with early embryonic origins, which is located between the ectoderm and endoderm and which is the source of striated muscle (Boero et al. 1998; Spring 2000, 2002; Müller et al. 2003). Thus, just as in the case of the long-standing distinction between bilaterally and radially symmetrical animals (Hayward et al. 2002), the molecular evidence from the studies on both *Acropora* and *Podocoryne snail* indicates just how unclear the boundary is between “simple” diploblastic animals such as cnidarians and “higher” triploblasts.

**Note added in proof** While this manuscript was in review a paper appeared which provides an excellent discussion of some of the issues touched on here concerning the possible evolutionary relationship between endoderm and mesoderm. Technau U, Scholz CB (2003) Origin and evolution of endoderm and mesoderm. *Int J Dev Biol* 47:531–539.

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