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The role of MAPK signaling in patterning and establishing axial symmetry in the gastropod *Haliotis asinina*

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Abstract

Gastropods are members of the Spiralia, a diverse group of invertebrates that share a common early developmental program, which includes spiral cleavage and a larval trochophore stage. The spiral cleavage program results in the division of the embryo into four quadrants. Specification of the dorsal (D) quadrant is intimately linked with body plan organization and in equally cleaving gastropods occurs when one of the vegetal macromeres makes contact with overlying micromeres and receives an inductive signal that activates a MAPK signaling cascade. Following the induction of the 3D macromere, the embryo begins to gastrulate and assumes a bilateral cleavage pattern. Here we inhibit MAPK activation in 3D with U0126 and examine its effect on the formation and patterning of the trochophore, using a suite of territory-specific markers. The head (pretrochal) region appears to maintain quadri-radial symmetry in U0126-treated embryos, supporting a role for MAPK signaling in 3D in establishing dorsoventral polarity in this region. Posterior (posttrochal) structures – larval musculature, shell and foot – fail to develop in MAPK inhibited trochophores. Inhibition of 3D specification by an alternative method – monensin treatment – yields similar abnormal trochophores. However, genes that are normally expressed in the ectodermal structures (shell and foot) are detected in U0126- and monensin-perturbed larvae in patterns that suggest that this region has latent dorsoventral polarity that is manifested even in the absence of D quadrant specification.

Keywords: Gastropod; MAPK; Organizer; Mesoderm; Dorsoventral axis; D-quadrant; U0126 gastrulation

Introduction

Spiralia comprises a number of invertebrate phyla, including annelids, molluscs and sipunculans, who share a common cleavage pattern and an early larval stage known as the trochophore. The conserved cleavage pattern of spiralians often enables the identification of homologous blastomeres and the elucidation of their cell fate (Verdonk and van den Biggelaar, 1983). Some of these cell lineages appear to be autonomously specified during the early cleavage (Cather and Verdonk, 1979; Clement, 1976), as isolated blastomeres can form tissues (e.g. Wilson, 1904). However, blastomere deletion experiments have demonstrated that inductive interactions are also required in spiralian development (Cather and Verdonk, 1979; Clement, 1976; van den Biggelaar and Guerrier, 1979, 1983).

Spiralian cleavage, following the first two divisions, involves the vegetal macromeres generating successive tiers of animal micromeres. This spiral cleavage pattern results in an embryo that is divided into quadrants of tiered blastomeres designated A, B, C and D, which form the left, ventral, right and dorsal regions of the embryo, respectively (Fig. 1A) (Verdonk and van den Biggelaar, 1983). Spiralians undergo either equal or unequal cleavage. In some unequal cleavers an extrusion of cytoplasm, a polar lobe, is formed and inherited by one of the daughter macromeres, which results in it being larger than the others. In contrast, in equally cleaving spiralians the four embryonic quadrants maintain equal cleavage patterns until the 32-cell stage (Gonzales et al., 2007; van den Biggelaar, 1977; van den Biggelaar and Guerrier, 1979). Equal cleavage has been

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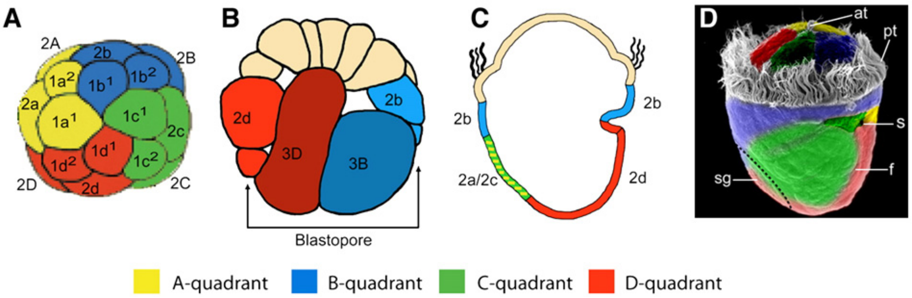

Fig. 1. Spiral cleavage and fate map in equally cleaving gastropods based on and adapted from the *Patella* fate map by Dictus and Damen (1997). (A) Animal view; (B–D) lateral views with dorsal left and animal pole up. (A) 16-cell embryo showing the quadri-radial symmetry and nomenclature of blastomeres. (B) Blastula section showing 3D making contact with overlying micromeres. The arrows indicate the margins of the future blastopore. (C) Section of a trochophore showing the posttrochal location of 2nd quartet ectodermal derivatives at the end of gastrulation. (D) *H. asinina* trochophore larva coloured to show the distribution of micromere lineages following gastrulation. The margin of the shell gland (sg) is indicated with a dashed line; 1st quartet micromeres are tan in panels B and C; at, apical tuft in the pretrochal region; pt, prototroch; s, stomodeum; f, foot in the posttrochal region.

proposed to represent the ancestral condition for both molluscs and spiralians (Freeman and Lundelius, 1992; Henry, 2002; van den Biggelaar and Haszprunar, 1996; but also see Dohle, 1999).

The establishment of the dorsoventral axis and the organization of the embryo are closely linked. Determining the dorsoventral axis in spiralians involves the specification of one blastomere quadrant to form the dorsal, D-quadrant (Arnolds et al., 1983; Cather and Verdonk, 1979; Clement, 1962; Gonzales et al., 2007; Henry, 2002; Henry and Martindale, 1987; van den Biggelaar and Guerrier, 1979). D-quadrant specification differs between equal and unequal cleaving molluscs (see Henry et al., 2006 and references therein). In some unequally cleaving gastropods, such as *Ilyanassa obsoleta*, the large macromere inheriting the polar lobe becomes specified to form the D-quadrant (Clement, 1976; van den Biggelaar and Guerrier, 1983; Verdonk and van den Biggelaar, 1983). Deletion of the polar lobe results in a larva that does not develop a dorsoventral axis and lacks many larval structures including foot, eyes, heart and an external shell (Clement, 1952, 1962). At the 32-cell stage of equally cleaving gastropods, such as *Patella*, *Tectura* and *Haliotis*, one of the cross-furrow macromeres assumes a centralized position, filling the blastocoel and making prolonged contact with the overlying first quartet micromeres (Fig. 1B; van den Biggelaar and Guerrier, 1979, 1983; van den Biggelaar, 1977; Verdonk and van den Biggelaar, 1983). This is the first break from the radial organization of the blastomeres and the cell becomes induced to form the D-quadrant macromere, 3D (Boring, 1989; Damen and Dictus, 1996; Gonzales et al., 2007; Kühtreiber et al., 1986; Martindale et al., 1985; van den Biggelaar, 1977). Following 3D specification, the embryo gastrulates with the invagination of the macromeres and epibolic movement by the micromeres. Extensive movements of the 2nd quartet micromeres in later gastrulation results in the blastopore forming the stomodeum on the ventral side below the prototroch, the 2d lineage extending along the ventral and posterior midline and the 2b lineage being pushed into a more dorsal and lateral position (Figs. 1C–D; van den Biggelaar and Dictus, 2004).

The specification and induction of the 3D cell in gastropods has been inhibited by a number of techniques including cell ablation, preventing micromere–macromere contact and inhibiting MAPK activation (Gonzales et al., 2007; Henry et al., 2006; Damen and Dictus, 1996; Kühtreiber et al., 1988; Lambert and Nagy, 2001, 2003; Lartillot et al., 2002; Martindale, 1986; Martindale et al., 1985). Ablation of the 3D macromere results in larvae that are normal but largely lack mesodermal structures (Martindale et al., 1985). Similarly, embryos treated with a MAPK inhibitor lack larval retractor muscles (Lambert and Nagy, 2003). However, ablation of the presumptive 3D cell, before it has attained its central position, as well as chemically preventing the presumptive 3D cell from obtaining this position, generally results in gastropod larvae that lack a distinguishable dorsoventral axis and fail to develop posttrochal structures such as a foot and shell (Gonzales et al., 2007; Damen and Dictus, 1996; Martindale, 1986; Martindale et al., 1985). However, there are cases where quadri-radial organization has been observed in the posttrocnal region in perturbed embryos (Gonzales et al., 2007; Kühtreiber et al., 1988). In these experiments, the pretrochal region is partially or fully radialized, with the maintenance of a quadri-radial cleavage pattern (Damen and Dictus, 1996; Martindale et al., 1985). Taken together, these results suggest that the D-quadrant acts as an organizer of molluscan development and is crucial for the correct specification of the pretrochal region as well as the establishment of posttrochal structures (Henry et al., 2006). In *Ilyanassa*, deletion of D-quadrant macromeres prior to the 32-cell stage results in larvae that resemble those from polar lobe deletions, while deletion of the 3D macromere results in increasingly mild abnormalities the later these deletions are performed (Clement, 1952, 1962). It seems therefore, that in both equal and unequal cleavers the period between the fifth and sixth cleavage is when the inductive activity of the D-quadrant occurs.

To assess the organizing role of the D-quadrant, most studies to date have focused on the effect on embryonic cleavage patterns and morphological landmarks that are known to be derived from certain micromere lineages. While these studies have provided considerable insight into the role of 3D in patterning the embryo, interpretation of the precise effect of 3D signaling on cell specification and morphogenesis has been difficult, particularly in relation to the formation of posttrochal