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A function for the midbody remnant in embryonic patterning

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Asymmetric cell divisions combine cell division with fate specification and one general model of how this is achieved was proposed already decades ago[1,2]: During interphase, the cell polarity axis is specified, followed by orientation of the spindle along the polarity axis and segregation of fate determinants along the polarity axis during mitosis. In most cells, the polarity axis and the spindle will usually align with the long axis that the cell had before division, also called Hertwig's rule[3–6]. In the C. *elegans* embryo, the first polarity axis also forms along the long axis of the embryo by enrichment of myosin in the anterior[7] and formation of mutually exclusive anterior and posterior cortical polarity domains, mediated through directional cortical contractile flow[8–10]. The directionality of this flow is determined by an extrinsic cue, the entry of the sperm, which inhibits Rho-dependent myosin activation at the future posterior pole by bringing with it the Rho GTPase activating protein CYK-4[11,12]. Moreover, since there is no previous division 'history' before the first cleavage, mechanisms have to ensure that the nucleus-centrosome complex undergoes a 90 degree rotation so that the spindle can subsequently elongate along the long axis[13–15]. Additional mechanisms ensure that the site of cleavage is perpendicular to the long axis[16,17]. Hence, tight coupling of an extrinsic cue to intrinsic polarity formation and spindle elongation enables alignment of the division orientation with the long axis of the organism and successful segregation of fate determinants.

The key event in dorsoventral axis formation during the next division in the C. *elegans* embryo is a similar 90 degree rotation of the nucleus-centrosome complex during prophase in the germline blastomere onto the anteroposterior axis.[14] In contrast to the first division, this rotation is an alignment along the short axis of the cell, a violation of Hertwig's rule (**Fig. 1A**). Our recent work[18] suggests that a combination of two previously proposed models might best explain the interplay between extrinsic and intrinsic polarity cues necessary for this violation: The 'cortical-site model' suggested the existence of a special landmark at the cell membrane, the midbody remnant of the first division, which combined with the spatial constraints of the oblong egg, directs nucleus-centrosome complex rotation.[19-22] The midbody remnant is an organelle that forms at the end of cytoplasmic division when the fully constricted actomyosin furrow embraces the condensed material of the spindle midzone.[23] The role of the midbody remnant has been questioned by a differing model, in which the cortical protein LET-99, by its unique lateral localization pattern, reduces cortical pulling forces in two cortical domains along the anteroposterior axis, thereby leading to spindle orientation violating the geometric rule.[24-26] Our findings unify these two models by showing that although LET-99 will bias spindle rotation onto the anteroposterior axis, the spindle needs to be tethered to the midbody remnant in order to become skewed ventrally during elongation (**Fig. 1B**). We find that tethering of the spindle to the midbody remnant requires formation of a transient, cortical, actin-rich structure at the site of the midbody remnant which depends on the germline blastomere having posterior

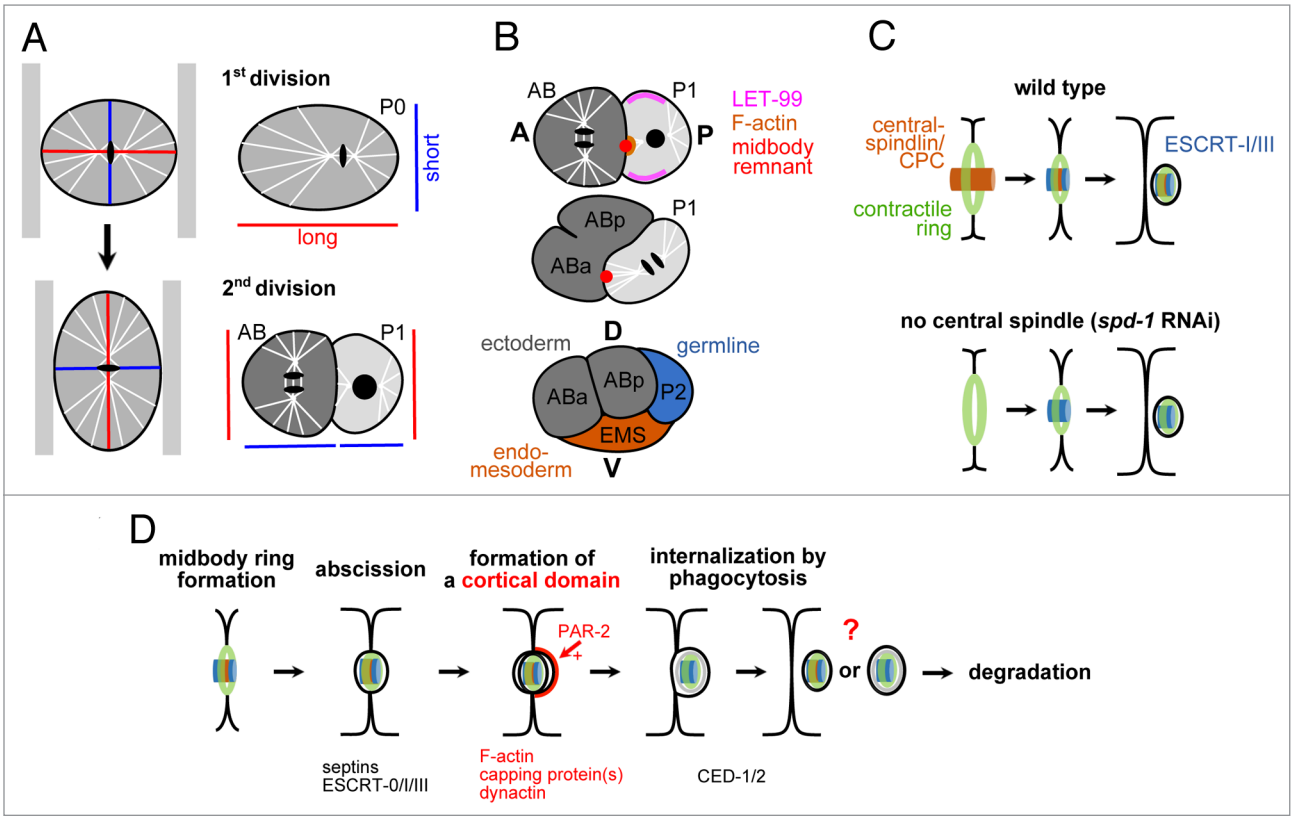

Figure 1. A. Hertwig's rule and its violation during the division of the P1 blastomere in *C. elegans*. Left: Exerting a compressive force along the long axis of a cell can transform this axis into the short axis and the spindle will re-orient along the new long axis. Right: Long and short cell axes during the first two cleavages in the *C. elegans* embryo. Note that all cells except P1 obey Hertwig's rule. **B.** Function of the midbody remnant during dorsoventral axis formation in *C. elegans*. See text for details. A = anterior; *P* = posterior; D = dorsal; V = ventral. **C.** Midbody remnant internalization does not require the central spindle-derived part of the midbody. Schematic adapted from ref. 27. CPC = chromosomal passenger complex; ESCRT = endosomal sorting complex required for transport. **D.** Hypothetical scheme for a midbody remnant 'pathway' in *C. elegans* early embryogenesis. Bottom: Protein factors required for the respective step. See text for details.

polarity. This tethering function of the midbody remnant does not require the core of the midbody remnant (the condensed 'remainder' of the central spindle), neither does it require abscission or midbody remnant internalization. Interestingly, it has been shown that the midbody ring component of the midbody (the outer 'shell' derived from the actomyosin ring) is sufficient to recruit the molecular machinery that will mediate cytokinetic abscission[27] (**Fig. 1C**). Additionally, a recent report shows that midbody remnant internalization during *C. elegans* embryogenesis is not required for patterning, morphogenesis or development[28] and most remnants seem to simply represent 'junk' after abscission since they become cleared by the phagocytosis/engulfment machinery that usually takes care of cell corpses.[28,29]

Taking together these findings[27,28] and our observations that midbody remnants are internalized by neighboring cells with low cortical tension that did not participate in the division that gave rise to the respective midbody remnant,[18] allows us to propose the following sequence of processes for the *C. elegans* embryo (**Fig. 1D**): (1) depending on cell polarity, nascent midbody remnants can organize asymmetric cortical domains; (2) abscission seems to occur on both sides of the midbody remnant and only requires the midbody ring component; (3) remnants are internalized by cells in a stereotyped fashion and are subsequently degraded; (3) the internalization mechanism is phagocytosis/engulfment; (4) the stereotypy is probably explained by the fact that endocytosis and phagocytosis is facilitated in cells with low cortical tension.[30]

Although a common picture for midbody remnant inheritance seems to emerge from these recent studies of *C. elegans* embryogenesis, several mechanisms have been discussed in other systems.[31-33] Recent studies in *Drosophila* seem to reconcile apparent conflicts by suggesting that inheritance regulation and developmental functions of midbody remnants seem to depend on tissue and cell type, cell polarity, regulation of centrosome duplication and inheritance, as well as on the extracellular environment.[34-37] These findings very strongly disfavor a uniform inheritance mechanism for midbody remnants and probably also a uniform function in development. One main reason for this is that cytokinesis itself has dramatically different outcomes depending on the cellular and developmental context, e.g., instead of undergoing abscission, cells can also stabilize and remodel their midbodies to form long-lasting intercellular bridges. Thus, although there is ample

evidence for developmental functions of cytokinesis, these functions have not been systematically analyzed and linked to alternative fates and functions of the midbody remnant. It will therefore be necessary to investigate how developmental programs differentially regulate cytokinesis and modulate specific processes such as trafficking to the midbody, abscission, phagocytosis, and regulation of cortical dynamics to obtain a clearer picture of midbody remnant functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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