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Distinct modes of mitotic spindle orientation align cells in the dorsal midline of ascidian embryos

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ABSTRACT

The orientation of cell division can have important consequences on the choice of cell fates adopted by each daughter cell as well as on the architecture of the tissue within which the dividing cell resides. We have studied in detail the oriented cell divisions that take place in the dorsal midline of the ascidian embryo. The dorsal midline cells of the ascidian embryo emerge following an asymmetric cell division oriented along the animal–vegetal (A–V) axis. This division generates the NN (Notochord–Neural) cell at the margin and the E (Endoderm) cell more vegetally. Deviating from the default mode of cell division, these sister cells divide again along the A–V axis to generate a column of four cells. We describe these cell divisions in detail. We show that the NN cell mitotic spindle rotates 90° to align along the A–V axis while the E cell spindle forms directly along the axis following the asymmetric migration of its centrosomes. We combine live imaging, embryo manipulations and pharmacological modulation of cytoskeletal elements to address the mechanisms underlying these distinct subcellular behaviours. Our evidence suggests that, in E cells, aster asymmetry together with the E cell shape contribute to the asymmetric centrosome migration. In NN cells, an intrinsic cytoplasmic polarisation of the cell results in the accumulation of dynein to the animal pole side. Our data support a model in which a dynein-dependent directional cytoplasmic pulling force may be responsible for the NN cell spindle rotation.

1. Introduction

The orientation of the cell division axis has important consequences for tissue architecture and cell fate. The cell division axis is defined by the orientation of the mitotic spindle, with the cleavage furrow always forming at right angles to the spindle (reviewed in Conrad and Rappaport, 1981). According to Sachs's rule, which was originally proposed based on his studies on plant cells, each new cell division plane intersects the preceding plane at a right angle so that the "default" orientation of a spindle would be 90° to the previous spindle (reviewed in Wilson, 1925; Strome, 1993). The 90° default orientation can be explained by the movements of the duplicated centrosomes, which migrate 90° in opposite directions around the nucleus and serve as the asters of the next mitotic spindle. Hertwig formulated another important rule stating that spindles align with longest axis of the cell, such that spindle orientation can be controlled by cell shape. Minc et al. (2011) further refined Hertwig's rule by proposing a computational model that fully predicts the orientation of a mitotic spindle of sea urchin eggs imposed into various shapes. The key element of this computational model is the assumption that a cytoplasmic pulling force generated on the spindle pole by each microtubule is proportional to its length, as initially proposed by Hamaguchi and Hiramoto (1986). However, *in vivo* evidence for the presence of such a pulling force in the context of cell shape-dependent spindle orientation is lacking. Most of our current knowledge on the molecular control of spindle orientation in metazoans is based on asymmetric cell divisions of highly polarised cells such as the *Drosophila* neuroblasts (NBs) and sensory organ precursors (SOPs) and the *C. elegans* zygote (reviewed in Morin and Bellaïche, 2011). In these systems, distinct polarising cues converge on cortical NuMA proteins. The NuMA family of proteins regulate the activity of the dynein/dynactin motor complex to exert a pulling force on astral microtubules from the cell cortex (Morin and Bellaïche, 2011). Importantly, the cortical NuMA-based mechanism is likely to be evolutionary-conserved since it also operates in chick neuroepithelium (Peyre et al., 2011), mouse embryonic skin progenitors (Lechler and Fuchs, 2005) and mammalian cell culture systems (Zheng et al., 2010; Kiyomitsu and Cheeseman, 2012). However, it is not yet clear whether the NuMA-based system is applicable to all oriented cell divisions.

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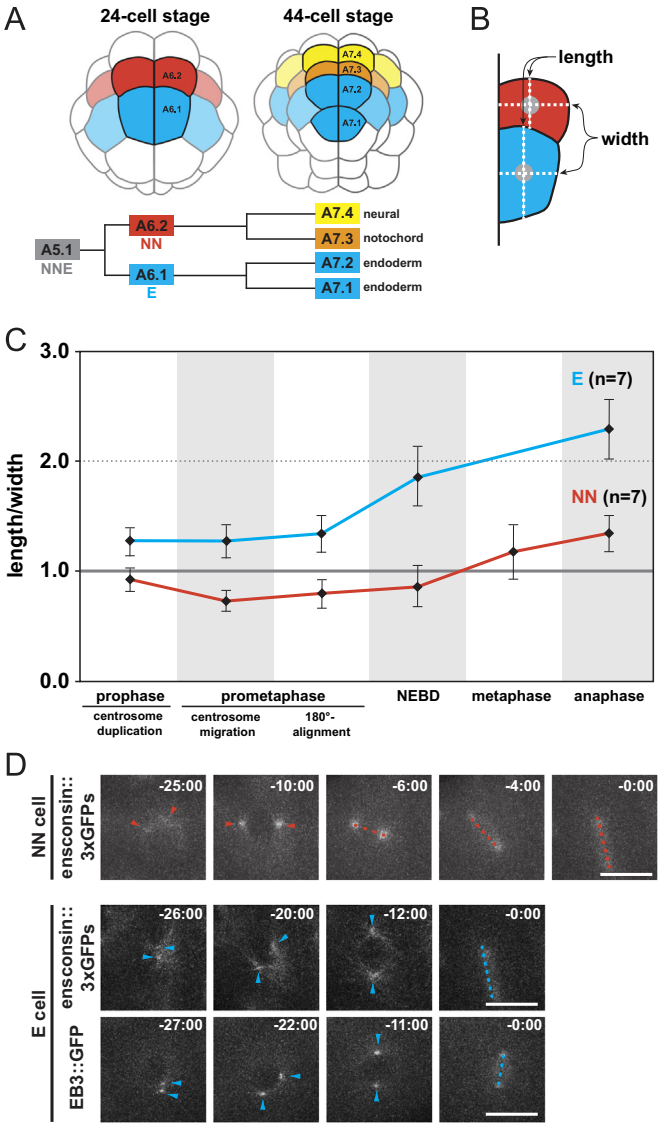

Fig. 1. Oriented cell division of the medial NN and E cells. (A) Drawings of the 24- and 44-cell stage embryos highlighting the cell lineage of the medial pair of NNE cells (A5.1 cells). Embryos are in vegetal pole view with dorsal side up. NNE cell divides into one Notochord/Neural mother cell (NN cell/A6.2, in red) and one Endodermal precursor (E cell/A6.1, in blue) at 24-cell stage. The division axis is parallel to the "meridian" line connecting the animal and vegetal poles. For simplicity, this geodesic is referred to as A–V axis in the text. These two daughter cells then divide again along the A–V axis to generate one neural precursor (A7.4, in yellow), one notochord precursor (A7.3, in orange) and two endoderm precursors (A7.2 and A7.1, in blue) at the 44-cell stage. (B) Schematic drawing representing how the length and width of NN and E cells were measured (dashed lines). Grey circles and black vertical line represent nuclei and the embryonic midline, respectively. Animal pole side is at the top and embryo midline on the left. (C) Measurement of the length/width ratio of NN and E cells during their cell cycle. The temporal changes to the length/width measurements of NN cells are represented by a red line and E cells by a blue line. "180°-alignment" indicates the time point at which the centrosomes become aligned 180° around the nucleus in both cells. Error bars indicate the standard deviation. (D) Centrosome and spindle dynamics in NN and E cells. Upper panel: selected frames from a confocal 4D image set of an NN cell in an ensconsin::3xGFP-expressing embryo. Bottom panel: selected frames from confocal 4D image sets of E cells in (upper) ensconsin::3xGFP- and (lower) EB3::GFP-expressing embryos. Each image is a max intensity projection of selected z-sections. Arrowheads indicate centrosomes and dotted lines correspond to the spindle axis, in red for NN and blue for E cells. Animal pole side is up and embryo midline on the right. Time is indicated as minutes:seconds with 0:00 corresponding to the onset of anaphase. Scale bars are 20 μm.

The embryogenesis of many invertebrates proceeds with an invariant cell division pattern (Wilson, 1925; Freeman, 1983). An invariant cell division pattern implies a precise control of cell division orientation and timing. In many animal groups, the mechanisms controlling these precise cell division patterns have not been addressed. In this study, we use ascidian embryos, which develop with an invariant bilateral holoblastic cleavage pattern (Conklin, 1905), as a model to explore oriented cell divisions. Ascidians are invertebrate chordates of the phylum Tunicata, a sister group of the vertebrates (Delsuc et al., 2006; Vienne and Pantarotti, 2006; Satoh et al. 2014). The blastula fate maps of ascidians and vertebrates reveal a high degree of topological similarity and ultimately give rise to tadpole larvae with a central notochord and dorsal hollow nervous system (Lemaire et al., 2008). In this study, we focus on two oriented cell divisions that take place in the dorsal midline of ascidian blastula stage embryos (Fig. 1A). These oriented cell divisions occur in a sister cell pair, named NN (Notochord/Neural) and E (Endoderm) cells. Their mother cell, the NNE cell (Notochord/Neural/Endoderm), divides along the animal–vegetal (A–V) axis to generate one NN cell and one E cell. Contrary to Sachs's rule, these sister cells then divide again along the A–V axis to form a column of four cells on the dorsal side of the 44-cell "blastula" stage embryo (Fig. 1A). The molecular mechanisms that determine the differential fates of daughter cells following each of these asymmetric divisions are well documented (Hudson et al., 2013; Takatori et al., 2010; Kim et al., 2007; Minokawa et al., 2001; Picco et al., 2007). Here we provide a detailed description of the oriented cell division of NN and E cells along the A–V axis and present our evidence showing that distinct mechanisms govern spindle orientation in these two cell types.

2. Results

2.1. Oriented cell divisions of the medial NN and E cells

All experiments described in this study were conducted using the ascidian, *Phallusia mammillata*. *Phallusia* embryos are transparent and injected mRNA is efficiently translated in eggs, thus enabling the visualisation of tagged proteins and subcellular structures in living eggs and embryos (Prodon et al., 2010). Importantly, all cell divisions up to the gastrula stage take place within the planar plane. As described above, contrary to Sachs's rule, NN and E cells both divide along the A–V axis as their mother cell (NNE) did. We addressed whether Hertwig's rule could instead be implicated in the NN and E cell divisions. To do this, we conducted live-imaging of embryos in which microtubules and cell membranes were labelled with ensconsin fused with three GFPs in tandem (ensconsin::3xGFP) (von Dassow et al., 2009; Negishi et al., 2013) and FM4-64 (Prodon et al., 2010), respectively. NN and E cells are quadrilateral (=tetragon) with one side corresponding to the embryonic midline (Fig. 1B). To describe length, we measured the line that is parallel to the embryonic midline and crosses the centre of the nucleus or of the spindle (Fig. 1B). For the width, the line that is perpendicular to the length and crosses the centre of the nucleus or spindle was measured (Fig. 1B). During prophase and prometaphase, the length/width ratio of NN cells remains below 1.0, but increases during metaphase to reach 1.3 ± 0.16 by anaphase ($n=7$ cells) (Fig. 1C, red line). The E cell on the other hand is elongated throughout the entire cell cycle (Fig. 1C, blue line). Thus, for both NN and E cells, the cell division axis corresponds to the final long axis of the cell.

While both NN and E cells divide along the long axis of the cell, our observations of centrosome and spindle dynamics revealed that their spindles align via distinct mechanisms (Fig. 1D; Movie 1). In the NN cell, the duplicated centrosomes are located on the