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The basal position of nuclei is one pre-requisite for asymmetric cell divisions in the early mouse embryo

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ABSTRACT

The early mouse embryo undertakes two types of cell division: symmetric that gives rise to the trophectoderm and then placenta or asymmetric that gives rise to inner cells that generate the embryo proper. Although cell division orientation is important, the mechanism regulating it has remained unclear. Here, we identify the relationship between the plane of cell division and the position of the nucleus and go towards identifying the mechanism behind it. We first find that as the 8-cell embryo progresses through the cell cycle, the nuclei of most – but not all – cells move from apical to more basal positions, in a microtubule- and kinesin-dependent manner. We then find that all asymmetric divisions happen when nuclei are located basally and, in contrast, all cells, in which nuclei remain apical, divide symmetrically. To understand the potential mechanism behind this, we determine the effects of modulating expression of Cdx2, a transcription factor key for trophectoderm formation and cell polarity. We find that increased expression of Cdx2 leads to an increase in a number of apical nuclei, whereas down-regulation of Cdx2 leads to more nuclei moving basally, which explains a previously identified relationship between Cdx2 and cell division orientation. Finally, we show that down-regulation of aPKC, involved in cell polarity, decreases the number of apical nuclei and doubles the number of asymmetric divisions. These results suggest a model in which the mutual interdependence of Cdx2 and cell polarity affects the cytoskeleton-dependent positioning of nuclei and, in consequence, the plane of cell division in the early mouse embryo.

Introduction

In the mouse embryo, division from the 8 to 16 cell stage leads to segregation of progenitors for two distinct lineages: outer cells that give rise to the extra-embryonic trophectoderm contributing to the placenta, and inner cells that give rise to the inner cell mass (ICM) contributing to the embryo proper. As the blastocyst forms, inner cells are wholly surrounded by neighbouring cells, whereas outer cells contact other cells only on one side. One view, the 'inside–outside' hypothesis, suggests that this cell–cell apposition and specific inside microenvironment results in a distinct cell fate (Tarkowski and Wroblewska, 1967; Yamanaka et al., 2006). The alternative model suggests that cell fate is determined by cell polarity that is established along the apical–basal axis (Johnson and Ziomek, 1981b). Cell polarity is manifested by both cell morphology, for example formation of microvilli in the apical domain (Reeve and Ziomek, 1981), and on the molecular level, for example by apical accumulation of aPKC, Par6, Par3 proteins (Plusa et al., 2005; Vinot et al., 2005) and transcripts of Cdx2 (Skamagki et al., 2013). Consequently, a symmetric division (parallel to the apical–basal axis) gives rise to outer, polarized cells and an asymmetric division (orthogonal to the apical–basal axis) produces an outer, polarized cell and an inner, unpolarized cell (Johnson and Ziomek, 1981a; Zernicka-Goetz et al., 2009). Early observations showed that indeed 8–16 cell divisions are either parallel or orthogonal in respect to the apical–basal axis resulting in either two polar or polar and apolar daughter cells, but never in two apolar couplets (Johnson and Ziomek, 1981a; Sutherland et al., 1990). More recently, molecular markers predestining cells to asymmetric or symmetric divisions have been identified. A tendency of cells to undergo symmetric or asymmetric divisions has been correlated with expression of Cdx2, key for trophectoderm formation (Jedrusik et al., 2008, 2010; McDole and Zheng, 2012; Niwa et al., 2005; Ralston and Rossant, 2008; Strumpf et al., 2005). When the level of Cdx2 expression is high, cells tend to divide

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symmetrically and give rise to trophectoderm, while, in contrast, Cdx2 depletion leads to preferentially asymmetric divisions and formation of the ICM (Jedrusik et al., 2008). Asymmetric divisions are also facilitated by the expression of methylotransferase Carm1, which inhibits expression of Cdx2 (Parfitt and Zernicka-Goetz, 2010). Carm1 and Cdx2 levels correlate also with the expression of polarity markers such as aPKC or Par3: high expression of Cdx2 (low expression of Carm1) enhances apical accumulation of aPKC and Par3 (Jedrusik et al., 2008; Parfitt and Zernicka-Goetz, 2010). Although plane of cell division has a crucial meaning for the fate of the progeny of the dividing cell, the mechanism regulating the division orientation in mammalian embryos remains largely unknown.

Results and discussion

Position of the nucleus and the plane of cell division in 8-cell embryos

In order to gain insight into the mechanism regulating the plane of cell division in the early mouse embryo, we first wished to determine the events preceding symmetric vs. asymmetric divisions at the 8-cell stage. One of the most striking reorganizations occurring at this developmental stage is relocation of nuclei. While at the early 8-cell stage, all nuclei are located apically towards the end of the cell cycle, the nuclei of some, but not all, cells become repositioned towards the baso-central part of the cell (Reeve and Kelly, 1983). The biological meaning behind this has remained however unknown. To address this, it was important to first confirm this observation and examine its dynamics and mechanism. To this end, we determined the distance between the nucleus and apical domain at early (pre-compacted) and late (compacted) 8-cell stage, 8 h apart. We found that the apical-to-nucleus distance increases during this period >1.7 fold (5.1 ± 2.4 μm ($\pm$ SD) vs. 8.8 ± 3.3 μm ($\pm$ SD), $p < 0.0001$) reflecting translocation of nuclei from an apical to a baso-central position (Fig. 1A). As a result the proportion of cells with an apical localization of the nucleus decreases significantly as 8-cell embryo undergoes compaction (69% in pre-compacted embryo vs. 19% in compacted embryos; total of 32 nuclei from 8 pre-compacted and 32 nuclei from 8 compacted embryos analysed, Fig. 1B–D).

To ensure that this relocation does not reflect an artefact of fixation, we analysed the exact behaviour of the nuclei in time-lapse studies. To this end, we injected synthetic mRNA for Gap43-RFP, as a membrane marker, into one blastomere of a 2-cell embryos expressing histone H2B-GFP, as a marker of nuclei. We filmed their development from the mid-8-cell stage, using fluorescent wide-field or spinning-disc confocal microscopy collecting images every 10–15 min on 12–15 focal planes for each time point, as described previously (Morris et al., 2010). The analyses of these movies revealed that 30 min before nuclear envelope break-down (NEBD) at the 8–16-cell transition only 14% of nuclei (9/63, 21 embryos) were located apically (Fig. 1C). Embryos were heterogeneous with respect to the number of apical and baso-central nuclei prior to the division. In most cases, the apical nuclei constitute 0–50% of the analysed blastomeres in individual embryos, on average 16% ($\pm$29%, SD). Nuclei localized baso-centrally constitute 67–100% of the analysed nuclei, on average 84% ($\pm$29%, SD). Importantly, time-lapse studies allowed us to determine the division planes at the 8–16-cell stage and relate it with the position of the nucleus. This revealed that in all cases asymmetric divisions occur when nuclei become re-positioned baso-centrally (14/14), while symmetric divisions occur when the nucleus is positioned either apically (18%, 9/49) or baso-centrally (82%, 40/49), (Fig. 1E and F) suggesting that for a cell to divide asymmetrically, its nucleus moves basally.

Spatiotemporal pattern of the nuclear movement

In order to better understand the developmental significance of the nuclear translocation, we followed the dynamics of the nuclear movement throughout the 8-cell stage. To this end we injected 2-cell embryos with synthetic mRNA for Gap43-RFP, as a membrane marker. H2B-GFP, a nuclear marker, was either expressed endogenously by the embryos or introduced by mRNA injection at 2-cell stage. The embryos ($n=32$) were recorded from late 4-cell or pre-compacted 8-cell stages until the 8–16-cell transition. In agreement with our previous observations, the majority of nuclei in 8-cell blastomeres were initially localized apically. As the cell cycle progresses, most of them move basally (73%, 27/37), but some maintain an apical position (27%, 10/37). On the other hand, all nuclei that start the 8-cell stage in a baso-central position maintain it: they either show no net movement (52%, 22/42) or move basally (36%, 15/42), and only few (12%, 5/42), move apically, staying however in the baso-central regions of the cell (Fig. 2A–C). In all cases the nuclear translocation was gradual, and usually occurred in the first half of the cell cycle (Fig. 2B and C). Its precise timing varied between blastomeres, even in the same embryo.

As blastomeres with nuclei located baso-centrally prior to the NEBD may divide either asymmetrically or symmetrically, we examined whether plane of the division is determined by the pattern of the nuclear movement. As we show in Fig. 2D, nuclei that moved to the baso-central location from an initial apical position and nuclei that maintained their baso-central position throughout the 8-cell stage were able to divide either symmetrically or asymmetrically. Only 2 analysed cells had nuclei that retained the baso-central position regardless of the net apical movement they displayed. Interestingly, they divided symmetrically, but due to the small number it is difficult to draw any conclusion from this observation. Together, our analysis shows that although nuclei display different dynamics and direction of the movement, the exact pattern does not seem to be important for the plane of 8–16-cell division.

Re-positioning of the nucleus is microtubule- and kinesin-dependent

To determine the mechanism of nuclei translocation, we examined its dependence on cytoskeletal components. To depolymerize microtubules, we used nocodazole (5 μg/ml) as previously (Ajduk et al., 2011; Gong et al., 2010). We found that in nocodazole-treated embryos, 28% of nuclei (20/72, 11 embryos) maintained their apical position, whereas in control embryos only 9% of nuclei (18/207, 32 embryos) failed to leave the apical domain ($p < 0.0001$) (Fig. 3A). Thus, upon nocodozale treatment the mean distance between the nucleus and the apical membrane was significantly lower in comparison to the control cells (14.7 ± 5.1 μm ($\pm$ SD) vs. 16.8 ± 4.2 μm ($\pm$ SD), $p < 0.001$) (Fig. 3B and C). To depolymerize actin filaments, we used cytochalasin D (2 μg/ml) (Ajduk et al., 2011). We found that this treatment, although blocked the embryo compaction, had no effect upon the behaviour of nuclei: nuclei moved baso-centrally with a frequency similar to that observed in control, untreated embryos (88%, 14/16, 5 embryos vs. 91%, 189/207, 32 embryos, respectively) (Fig. 3A). There were also no significant differences between nuclear apical distances achieved in cytochalasin D-treated and control embryos (Fig. 3B and C).

Since these results suggest that translocation of the nuclei depends on microtubules, we investigated which motor proteins might be involved. Motor proteins responsible for the transport of organelles along microtubules form two families: the kinesin family, most members of which mediate transport towards the plus-end, and dyneins which support the minus-end transport (Hirokawa, 1998). Both types of motor proteins can associate with nuclear envelope either through the SUN–KASH complex (Crisp et al.,

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