Current Biology *18*, 116–123, January 22, 2008 ©2008 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2007.12.028

Report

Developmental Regulation of Central Spindle Assembly and Cytokinesis during Vertebrate Embryogenesis

Esther K. Kieserman,[1] Michael Glotzer,[2] and John B. Wallingford[1,*]
[1]Section of Molecular Cell and Developmental Biology
Institute for Cellular and Molecular Biology
University of Texas
Austin, Texas 78712
[2]Department of Molecular Genetics and Cell Biology
University of Chicago
Chicago, Illinois 60637

Summary

Mitosis and cytokinesis not only ensure the proper segregation of genetic information but also contribute importantly to morphogenesis in embryos [1–4]. Cytokinesis is controlled by the central spindle, a microtubule-based structure containing numerous microtubule motors and microtubule-binding proteins, including PRC1 [4–8]. We show here that central spindle assembly and function differ dramatically between two related populations of epithelial cells in developing vertebrate embryos examined in vivo. Compared to epidermal cells, early neural epithelial cells undergo exaggerated anaphase chromosome separation, rapid furrowing, and a marked reduction of microtubule density in the spindle midzone. Cytokinesis in normal early neural epithelial cells thus resembles that in cultured vertebrate cells experimentally depleted of PRC1 [5, 6, 9, 10]. We find that PRC1 mRNA and protein expression is surprisingly dynamic in early vertebrate embryos and that neural-plate cells contain less PRC1 than do epidermal cells. Expression of excess PRC1 ameliorates both the exaggerated anaphase and reduced midzone microtubule density observed in early neural epithelial cells. These PRC1-mediated modifications to the cytokinetic mechanism may be related to the specialization of the midbody in neural cells [11–15]. These data suggest that PRC1 is a dose-dependent regulator of the central spindle in vertebrate embryos and demonstrate unexpected plasticity to fundamental mechanisms of cell division.

Results and Discussion

Changes in cell behaviors often accompany the tightly regulated changes in gene expression in developing embryos. For example, patterned expression of cytoskeletal regulators controls coordinated cell shape changes during morphogenesis of the vertebrate neural tube [16]. More fundamental aspects of cell behavior, such as cytokinesis, also vary during development, and such variation could have developmental consequences. Cell division has been studied extensively in vertebrate cultured cells and in very early cleavage-stage embryos, but little is known about the basic mechanisms of cytokinesis in more mature vertebrate embryos.

Because of their external development and very large cell size, the embryos of the frog *Xenopus* provide exceptional access to cell behaviors associated with vertebrate embryogenesis [17]. We chose to exploit these attributes to compare cytokinesis in two ectoderm-derived, superficial epithelial cell populations that are each engaged in extensive proliferation. First, we chose to examine the early neural plate, which will eventually roll up to form the hollow central nervous system characteristic of vertebrate animals [18–20]. The second population of cells chosen was the tailbud epidermis [21]. The superficial position of these tissues allows comparison by high-resolution confocal imaging (Figure S1, available online). The mechanism of cytokinesis is highly conserved among metazoans, and given that both tissues are ectodermally derived, we were surprised to find striking differences in cytokinetic mechanisms between these two cell populations.

In vivo time-lapse imaging of intact *Xenopus* embryos revealed that cells in the neural plate exhibit a marked exaggeration of anaphase spindle elongation (anaphase B). Chromosomes separated in these cells until the chromatin closely apposed the cell cortex (Figure 1A, bracket; Figure 1C; Movie S1; Figure S2B). In contrast, in the tailbud epidermis, anaphase chromosomes stopped in roughly the geometric center of the new daughter cells (Figure 1B, bracket; Figure 1C; Movie S2; Figure S2B), a pattern similar to that of many vertebrate cells dividing in culture.

This change in the extent of anaphase was accompanied by a shift in the timing of cytokinetic furrowing (Movies S1 and S2). The early neural and later epidermal cells are of similar size and required roughly similar time periods to complete cytokinesis (Figure S2A; Figures 1A and 1B). However, cytokinesis in epidermal cells began roughly 4 min after anaphase onset, but the onset of furrowing was delayed in neural cells, beginning roughly 6 min after anaphase onset (Figures 1A and 1B, arrowheads; Figure 1D). A short burst of accelerated ingression of the cleavage furrow in neural cells compensated for the delayed start of cytokinesis, allowing both populations to complete furrowing by about 12 min after anaphase onset (Figure 1E).

Chromosome separation and cytokinesis are controlled in large part by the mitotic spindle [4], so we examined spindle dynamics in neural-plate and tailbud epidermal cells. Using tau-GFP to visualize microtubules [22] in tail epidermal cells, we observed a typical sequence of events: An obvious microtubule-rich spindle formed and spanned the midzone throughout anaphase and telophase (Figure 2A, Movie S3). Later in telophase, the microtubules at the midzone were compressed into a prominent, microtubule-rich midbody by the ingressing cytokinetic furrow (Figure 2E, arrow). Throughout anaphase and telophase, tau-GFP revealed an obvious microtubule-rich spindle that was consistently observed spanning the midzone.

By contrast, imaging of tau-GFP in neural-plate cell divisions revealed a dramatic decrease in microtubule density in the spindle midzone during late anaphase and early telophase (Figure 2B, Movie S4). Midzone microtubules of early neural cells were almost entirely disassembled prior to the onset of cytokinesis, leaving a microtubule-poor zone between the ingressing cleavage furrows (Figures 2B and 2I, arrow). As cytokinesis progressed, microtubules were concentrated at or

*Correspondence: wallingford@mail.utexas.edu

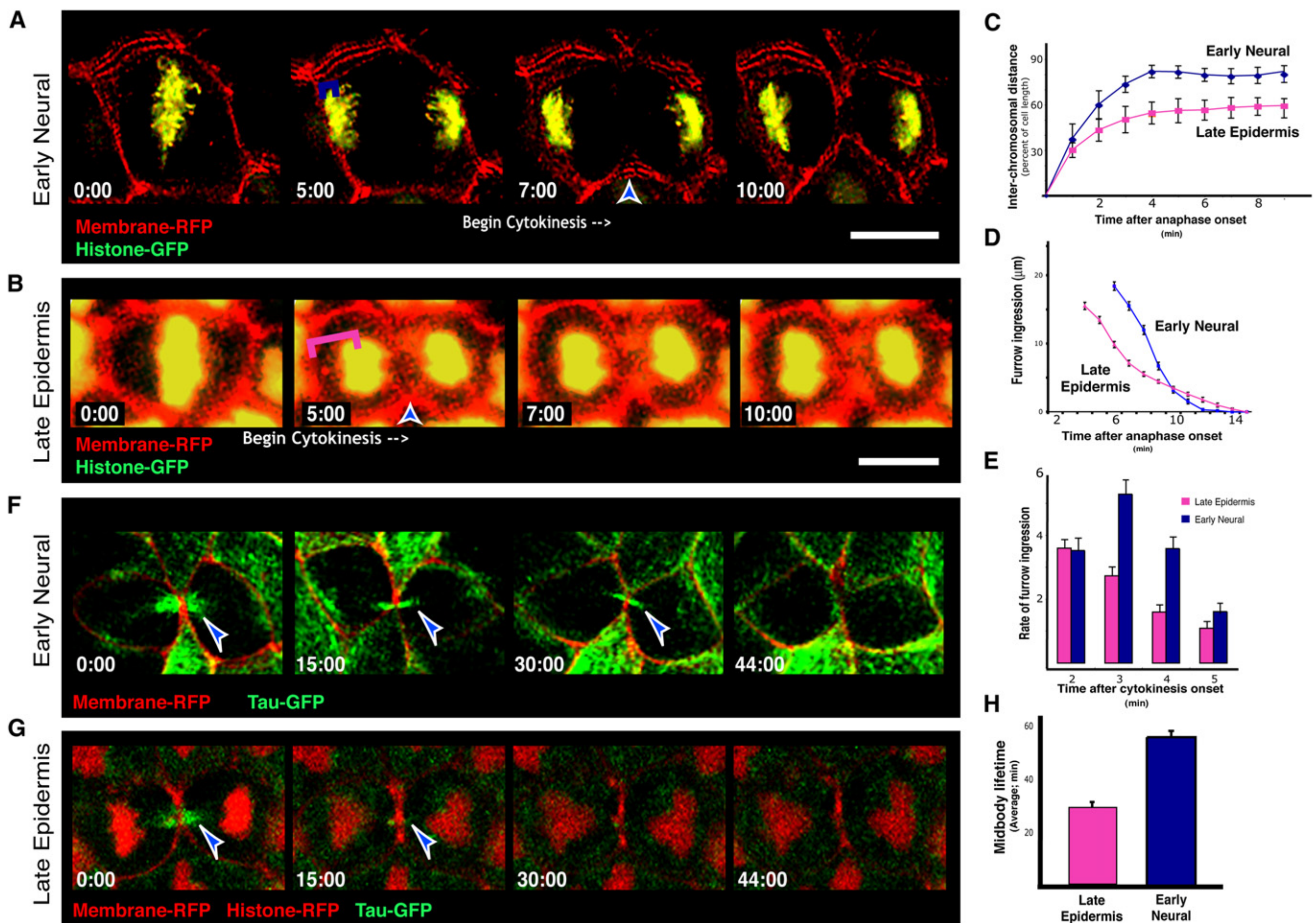

Figure 1. Novel Modifications to Cytokinetic Mechanisms in Early Neural Epithelial Cells In Vivo

(A and B) Still frames taken from movies of cell division in later tail epidermal cells (A) or in earlier neural epithelial cells (B). Membranes are labeled with RFP, and chromosomes are labeled with H2B-GFP. Bracket indicates distance between chromosomes and cell cortex; arrowhead indicates onset of cytokinesis; time is indicated as min:s in each panel; scale bars represent 10 μm.

(C) Graph illustrating chromosome separation during anaphase in early neural (blue) and later epidermal (pink) cells expressed as percentage of cell length (n = 20 cells from three different embryos). Cell sizes are comparable between these two populations (see Figure S2).

(D) Graph showing progress of cytokinetic furrow ingression over time for early neural (blue) and late epidermal (pink) cells (n = 20 cells from three different embryos).

(E) Graph showing instantaneous rate of cytokinetic furrow ingression at time points during mid-cytokinesis for early neural (pink) and late epidermal (blue) cells (n = 20 cells from three different embryos).

(F and G) Still frames taken from movies showing the lifetime of the midbody in the late epidermis (G) and the early neural tissue (F). Microtubules are labeled with Tau-GFP, and membranes are labeled with membrane-RFP. Arrowheads indicate midbodies.

(H) Graph showing average midbody lifetime for late epidermal (pink) and early neural (blue) cells (n = 14 neural cells from three different embryos; n = 11 epidermal cells from two different embryos).

near the ingressing furrow. These microtubules accumulated around the cytokinetic furrow as it ingressed, and only in late telophase did these microtubules bundle to form a midbody (Figures 2B and 2J, arrowhead).

These differences in midzone microtubule density were confirmed by immunostaining for α-tubulin (Figures 2C–2J). To quantify this difference, we used identical imaging parameters and measured pixel intensity along the length of the spindle in immunostained cells, as indicated in Figure 3 (dashed lines in panels A and B). We averaged pixel intensity across the spindles of several cells, and the compiled data are shown in Figure 3F. In tailbud epidermal cells, we observed a consistently high density of α-tubulin signal across the length of the spindle, except in the center of the midbody, which is inaccessible to tubulin antibodies [23] (Figure 3F, pink line). By contrast, average microtubule density in neural-plate cells was significantly lower across most of the midzone, though density at the spindle poles was comparable to that in epidermal cells (Figure 3F, dark blue line).

Our data thus indicate that *Xenopus* embryonic epidermal epithelial cells divide with kinetics and morphologies reminiscent of human cells in culture. By contrast, neural-plate cells display rapid and exaggerated anaphase chromosome separation, rapid cytokinetic furrowing, and reduced microtubule density in the spindle midzone. This spectrum of modifications to the pattern of cytokinesis is similar to that observed in cells experimentally depleted of the microtubule-bundling protein PRC1 [5–10]. These findings suggested the possibility that the differences we identified in cell-division mechanisms between neural and epidermal cells may be governed by PRC1.

We therefore examined the spatiotemporal pattern of PRC1 transcription by using in situ hybridization. Because PRC1 is

Download English Version:

https://daneshyari.com/en/article/2043734

Download Persian Version:

https://daneshyari.com/article/2043734

Daneshyari.com