INDUCTION OF CLEAVAGE FURROWS IN MITOTICALLY-ARRESTED *ECHINARACHNIUS PARMA* EMBRYOS

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Following fertilization, embryonic cells undergo a rapid series of synchronous cell divisions driven by the cyclic activation and inactivation of the cyclin-dependent kinase $p34^{cdc2}$. This kinase is thought to not only orchestrate the condensation of chromatin but also the radical reorganization of the cytoskeleton upon entry into mitosis. Conversely, the rapid decline of MPF (Maturation Promoting Factor; the complex formed by $p34^{cdc2}$ and cyclin B) activity upon anaphase onset is thought to play a critical role in regulation of the timing of cytokinesis. Based on observations that $p34^{cdc2}$ can phosphorylate the regulatory light chain of myosin II on residues known to inhibit myosin contractility (Satterwhite et al., *J Cell Biol*, 124: 129-137; Yamakita et al., *J Cell Biol*, 118: 595-605), it has been proposed that MPF activity inhibits myosin-based contractility up until anaphase onset, where this suppression would presumably be lifted. However, there are many examples (such as amphibian and mollusk embryos) where surface contractility is uncoupled from the events of mitosis, suggesting that there are additional parameters that play critical roles in the timing of cytokinesis.

Based on observations by Rappaport (*Dev Biol*, 158: 265-273), and our own mapping of sea urchin myosin light chain phosphorylation *in vivo*, we asked whether cleavage furrows could be induced in cells arrested in mitosis, where the cortex is presumably in an "inactivated" state. Towards these ends, we expressed a truncated form of cyclin B from *Arbacia punctulata* for which the domain targeting cyclin B for ubiquitin-mediated destruction has been deleted (Δ90 cyclin; Murray et al., *Nature*, 339: 281-286). When expressed in sea urchin eggs or tissue culture cells, cells undergo anaphase-like chromosome separation, but the chromatin remains condensed, and the cells do not undergo cytokinesis (Wheatley et al., *J Cell Biol*, 138:385-393; Hinchcliffe et al., *J Cell Biol*, 140: 1417-1426). The recombinant protein was purified from bacterial extracts, and assayed for its ability to convert an interphase-arrested *Xenopus laevis* extract to a mitotic state as determined by histone H1 kinase activity. Δ90 cyclin was then concentrated and injected into one blastomere of a two-cell *Echinarachnius parma* embryo prior to the second cell division. Injection volumes varied between 0.5 and 4%, resulting in an intracellular concentration of approximately 1 μM Δ90 cyclin. ninety-one percent of cells injected with buffer alone underwent normal division (n=34). In contrast, only 18% of Δ90-injected cells divided (n=45). Examination of injected cells reveals that while injected blastomeres fail to divide, microtubules of the mitotic apparatus are still visible and spindle poles undergo an anaphase B-like separation as previously reported (Wheatley et al., *J Cell Biol*, 138:385-393; Hinchcliffe et al., *J Cell Biol*, 140: 1417-1426). Hoescht staining of injected blastomeres reveals that the chromatin remains condensed, suggesting that the cell has arrested in mitosis. If cells were injected close to the time of the metaphase-anaphase transition, the injected blastomeres went on to cleave normally, only to arrest during the following mitosis (n=8). Additionally, we observed spindle pole splitting, resulting in three to four asters per cell. Thus, while Δ90-injected cells proceed into mitosis and undergo anaphase chromosome segregation, the cellular clock remains arrested in a metaphase-like state, and the cells do not divide.

One hallmark of cell division in early echinoderm embryos is the extensive elaboration of astral microtubules that accompanies anaphase and the fall in $p34^{cdc2}$ activity. However, no such astral microtubule elongation was observed in Δ90-arrested blastomeres by bright field microscopy. Thus, the failure of Δ90-arrested cells to undergo cytokinesis could be attributed to either myosin inactivation by extended $p34^{cdc2}$ activity or a loss of cortical stimulation by the mitotic apparatus. To discriminate between these possibilities, we physically displaced the position of the spindle poles such that the aster centers were placed in close proximity to the cell surface. As illustrated in Figure 1, displacement of the aster centers of a Δ90-arrested cell to a position

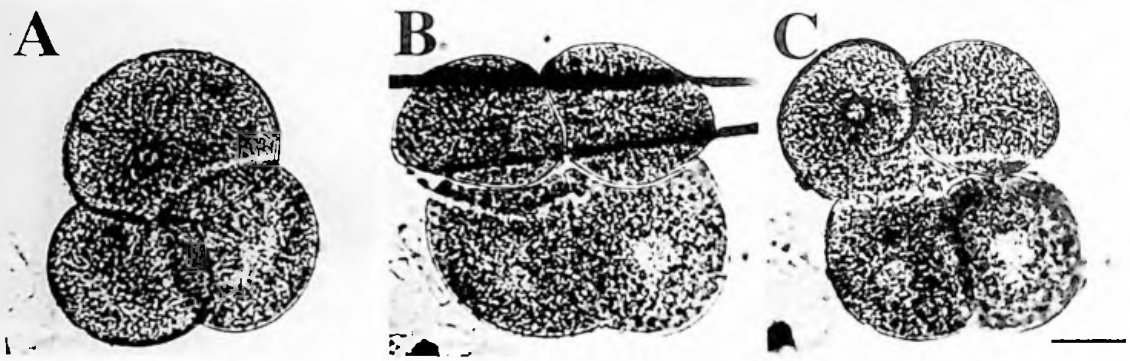

Figure 1. Cleavage furrow induction in a Δ90 cyclin-arrested cell.
One cell of a two cell embryo was injected with Δ90-cyclin B (marked with an oil droplet), and upon appearance of prophase spindles in control blastomeres, needles were placed down upon the surface of the arrested blastomere to isolate the spindle poles within a confined area. A. Before needle displacement. B. Seven minutes Post-displacement. C. Ten minutes post-displacement. Bar 25 μm.

adjacent to the surface results in the formation of a unilateral furrow. In cases where the furrow went to completion once the needles were lifted, the resultant blastomeres remained arrested whereas the uninjected blastomeres continued to divide. In cases where spindle poles were aligned normal to the plane of the coverslip, or the spindle poles had separated beyond their capacity to induce a furrow, a furrow failed to form. As an additional measure of cortical contractility in Δ90-arrested cells, arrested blastomeres were drawn into a fire-polished pipet resulting in a cylindrical cell and a reduced distance between the aster centers and the cell surface. Furrowing was also detected in cylindrical Δ90-arrested cells, although these furrows did not complete cytokinesis, possibly due to physical constraints placed on the blastomere.

Results of these studies invoke an alternative model for the $p34^{cdc2}$-mediated timing of cytokinesis whereby the induction of cleavage furrows is not regulated by the acquisition of contractile competence upon anaphase onset, but rather a regulation of the geometric relationship between the mitotic apparatus and the cell cortex through the modulation of microtubule dynamics. This work was supported by a New Investigator Award to D.R.B. and a NRSA fellowship (GM 18823) to C.B.S.