MICROTUBULE STABILITY SPECIFIES THE TIMING OF CYTOKINESIS IN *ECHINARACHNIUS PARMA* BLASTOMERES

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The partitioning of cytoplasm by cytokinesis is coordinated in time and space with the process of nuclear division or karyokinesis. Entry into mitosis in eggs is mediated by the cyclic activation of the protein kinase $p34^{cdc2}$, which along with its noncatalytic subunit, cyclin B, is known as maturation promoting factor (MPF). The onset of anaphase is marked by the destruction of cyclin B (and thus inactivation of MPF activity) and the initiation of chromosome segregation. In echinoderm embryos, there is also a concomitant elaboration and elongation of astral microtubules, which in turn specify the position of the cleavage furrow. Cleavage furrows are not normally observed until after the onset on anaphase, and it has been proposed that this is due to an inhibition of myosin II activity by MPF activity (Satterwhite, et al, *J Cell Biol*, 124: 129-137, 1992). However, previous studies indicated that the cortical actin cytoskeleton is capable of responding to signals from the mitotic apparatus over a wide window of time (Rappaport and Rappaport, *Dev Biol* 158: 265-273, 1993). Additionally, we demonstrated recently that blastomeres arrested in mitosis with nondegradable forms of cyclin B were capable of forming cleavage furrows if the asters were placed in close proximity to the cortex (Shuster and Burgess, *J Cell Biol* 146: 981-992; 1999). Together, these results demonstrate that the timing of cytokinesis is not accomplished by regulating the actomyosin-based cytoskeleton.

Minimally, cytokinesis requires an intact contractile apparatus (the cortical actin cytoskeleton), the cleavage stimulus (unknown), and the stimulus delivery system (astral microtubules). The induction of cleavage furrows in mitotically-arrested cells indicates that even in the presence of elevated MPF levels, the cortical actin cytoskeleton is responsive to the cleavage stimulus throughout mitosis. However, it was not clear from these experiments that the cleavage stimulus or the mitotic apparatus were capable of stimulating precocious furrows in normal cells. To assess the relationship between the mitotic cycle and the initiation of cleavage furrow formation in eggs of the sand dollar *Echinarachnius parma*, we measured the timing between the onset of anaphase and the induction of cleavage furrows in normal and geometrically altered cells. The fluorescent chromatin stain, Hoescht 33342 was used to monitor chromosome condensation, and astral microtubule elongation and furrow formation was monitored using Nomarski optics. In two cell embryos cultured at 17-18°C, the distance from the midpoint between the spindle poles and surface was approximately 50 μm, and furrowing could be detected 7 ± 1.1 minutes (n=26) following the initiation of chromosome separation. To reduce the distance between the spindle poles and the cell surface, blastomeres were drawn into capillary pipettes of a predetermined internal diameter following nuclear envelope breakdown, and the timing between anaphase onset and furrow formation was monitored. We found that reducing the distance from the spindle poles to the surface similarly decreased timing between anaphase onset and furrowing until a spindle pole-to-surface distance of 22-25 μm was achieved. Below 25 μm, the timing of furrowing could not be

induced earlier than 4 minutes past anaphase onset. Earlier measurements in sand dollar embryos revealed that the time required for the asters to specify the furrow position in the cortex was approximately 1 minute, with furrowing initiated approximately 2.5 minutes later (Rappaport and Ebstein, *J Exp Zool*, 158: 373-382). Therefore, our observations that cleavage could not be detected before 4 minutes post anaphase onset is in agreement with Rappaport's measurements, that predict the timing from furrow specification to the initiation of cleavage to be approximately 3.5-4 minutes.

These measurements suggested that in normal cells, furrows could not be induced prior to anaphase onset. Since our earlier experiments had demonstrated that the cortex was capable of responding to signals from the asters throughout the mitotic cycle, it is possible that either the cleavage stimulus or the asters were not competent to induce a furrow prior to the fall of MPF activity at anaphase. Similar experiments using needles to alter the spindle pole-to-surface distance indicated that the stimulus was active prior to anaphase onset (Rappaport and Rappaport, *Dev Biol* 158: 265-273). Additionally, our earlier experiments in cells arrested in mitosis suggested that cells cannot induce cleavage furrows prior to anaphase because pre-anaphase astral microtubules are either not long enough or dense enough to induce furrows (Shuster and Burgess, *J Cell Biol* 146: 981-992; 1999). To determine whether astral microtubule density affects the timing of cytokinesis, two cell embryos were resuspended in 0.8% hexylene glycol (which stabilizes microtubule arrays) following nuclear envelope breakdown, and blastomeres were then drawn into capillary pipettes, and the timing between anaphase onset and chromosome separation was measured. Preliminary results demonstrated that furrows could be detected in cells within 1 minute of anaphase onset, suggesting that the cleavage stimulus was active just prior to the metaphase-anaphase transition. Future permutations of these experiments will determine whether the cleavage stimulus is active earlier in the mitotic cycle.

Together, these experiments implicate the regulation of the cleavage stimulus delivery system (astral microtubules) as an important parameter specifying the timing of cytokinesis. Indeed, experiments in other systems have identified modulators of microtubule nucleation and turnover as substrates of $p34^{cdc2}$. Efforts are underway to identify those factors in echinoderm eggs, and to address their roles in the temporal regulation of cytokinesis. Supported by NIH GM 58231.