Review

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Cytokinesis Special Focus

Concurrent cues for cytokinetic furrow induction in animal cells

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Animal cells are deformable, yet live together bound into tissues. Consequently, physical perturbations imposed by neighbors threaten to disrupt the spatial coordination of cell cleavage with chromosome segregation during mitosis. Emerging evidence demonstrates that animal cells integrate multiple positional cues during cleavage-furrow induction, perhaps to facilitate error correction. Classical work indicated that the asters provide the stimulus for furrow induction, but recent results implicate the central spindle at least as much. Similarly, although classical work concluded that the stimulus occurs at the cell equator, new evidence shows that asters modulate cortical contractility outside the equator as well. Meanwhile, a newly revealed distinction between stable and dynamic astral microtubules suggests that these subsets might have complementary effects on furrow induction.

The animal condition demands an error-tolerant cytokinetic mechanism

Animal cells use the position of the mitotic apparatus as a spatial cue to determine where to assemble a cortical actomyosin ring that, by constricting around the cell equator, creates the cleavage furrow and physically partitions daughter cells. If the cleavage furrow failed to pass between the nascent daughter nuclei, it would render pointless the delicate care with which the mitotic apparatus sorts chromosomes. This would be a disaster for most cells, so perhaps it seems odd that animal cells have no apparent clue where to cleave until nearly the end of mitosis. Many eukaryotes do it differently. Plant cells know the division plane even before prophase [1,2]. In diatoms, the spindle migrates to the furrow site and cytokinesis proceeds even when migration is prevented [3].

Animal cells, however, are bound by their surfaces into deformable sheets. In even the most orderly animal embryos, cells shove each other around as they divide. By the time a sea urchin embryo hatches and swims away, not yet more than a hollow ball of cells, several thousand cytokineses must have (mostly) gone right. Cells must be deformable to enable morphogenesis and must divide during morphogenesis to enable embryology as we know it. Unicells do not have this problem, and plants avoid it by restricting cells to expansion within regular files. Perhaps, then, one of the ancient adaptations that make animals possible is that animal cells approach the end of mitosis with the cell surface still a blank slate. Animal cells must be able to revise or entirely re-induce the cleavage furrow even once it is underway to enable correction for errors or perturbations.

To this we owe a remarkable proof that the position of the mitotic apparatus determines the cleavage plane: if the spindle is physically displaced by micromanipulation during cleavage in urchin eggs, the existing furrow regresses and a new one develops above the new spindle midplane [4,5]. This experiment not only shows that the mitotic apparatus determines the cleavage plane, it shows also that the entire cortex remains responsive long after the initial cue has been sent. It also shows that the furrow, once formed, needs ongoing support from something that departs with the spindle. Finally, whatever feature of the mitotic apparatus signals to the cortex, it remains well into telophase. During this time, the mitotic apparatus changes dramatically: astral microtubules elongate, chromosomes decondense, nuclei reassemble and centrosomes can even separate (Box 1). Therefore, ideas about furrow induction that depend finely on the precise geometry of a transient stage in mitosis are intrinsically doubtful. For example, it is tempting to ascribe to the aligned chromosomes a role in furrow induction (perhaps there is a diffusible 'scent of chromosome') but in anaphase they move, then become sequestered in nuclei, and thus seem unlikely to account for a persistent furrowing stimulus.

The previous paragraph exemplifies certain rhetorical habits, which blight literature on cytokinesis. The first is to assume that the mitotic apparatus emits some instructive cue. Direct evidence for this remains elusive. The second is to imply that a single condition must account exclusively for all aspects of furrow induction. Although Rappaport acknowledged that cells might not know about the 'custom of accepting as most likely the simplest hypothesis', he nevertheless continued that 'hybrid theories' – which do not assume a single, continuous stimulus – are 'interesting examples of ingenuity' from an era 'before the laws of parsimony became ingrained' [6]. Perhaps the lineage of cells that survived to modern times valued error correction more than parsimony; it now seems likely that many cues contribute to furrow induction or persistence without being absolutely essential. If chromosomes offer any positional cue, even transiently (e.g. see Ref. [7]), it would be surprising if cells were to ignore it entirely. It might help to adopt the perspective of the cortex – an active medium, which

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Box 1. Transformations of the mitotic apparatus

Because of the central role that microtubules of the mitotic apparatus are believed to have in positioning the division plane in animal cells, it is helpful to clarify the terms used here to refer to various subpopulations of microtubules. In Figure Ia, key stages of division are traced from purple urchin zygotes: metaphase, late anaphase, early and late telophase. For micrographs on which these drawings are based, see Figure 2 in Ref. [11]. Figure Ib shows a schematic plot of proliferation, differentiation and disappearance of functionally distinct subsets of microtubules (MTs), denoted by color, arising from one centrosome within the mitotic apparatus. Time runs vertically; width approximates the number of MTs; the left edge corresponds to those microtubules that point to the cell pole and the right edge corresponds to those that point most centrally (i.e. toward chromosomes). Nearly all microtubules within the mitotic apparatus start out as centrosomal MTs (yellow). Before nuclear envelope breakdown, the two centrosomes have moved to opposite sides of the nucleus, pushed apart by overlapping, antiparallel microtubules that prefigure interpolar MTs (cyan) of the spindle. Microtubules invade the nucleus and attach to condensed chromosomes at their kinetochores; kinetochore MTs (blue) and interpolar MTs constitute the mitotic spindle proper. Some kinetochore MTs (indicated by the violet thorn) probably originate at the kinetochore rather than the centrosome [65,66]. As chromosomes align during metaphase, highly dynamic microtubules radiate in all directions from both centrosomes; these are the asters (yellow to red). During anaphase, kinetochore MTs disassemble as chromosomes move poleward; interpolar MTs remain and antiparallel sliding among these, the founders of the central spindle (green), elongates the anaphase spindle. (Note: in urchin embryos, centrosomes duplicate in anaphase.) Meanwhile, the asters enlarge and astral MTs (red) reach the cortex almost everywhere. As the cleavage furrow ingresses during telophase, it collects some equatorially directed astral MTs into the central spindle; the central spindle therefore incorporates both astral and interpolar MTs. Once the furrow has ingressed fully, the remnant of the central spindle – the 'midbody' – remains in the canal between the two daughter cells, disappearing after abscission. At the extreme right, the gray figure signifies the rise and fall of microtubule nucleation with each mitosis.

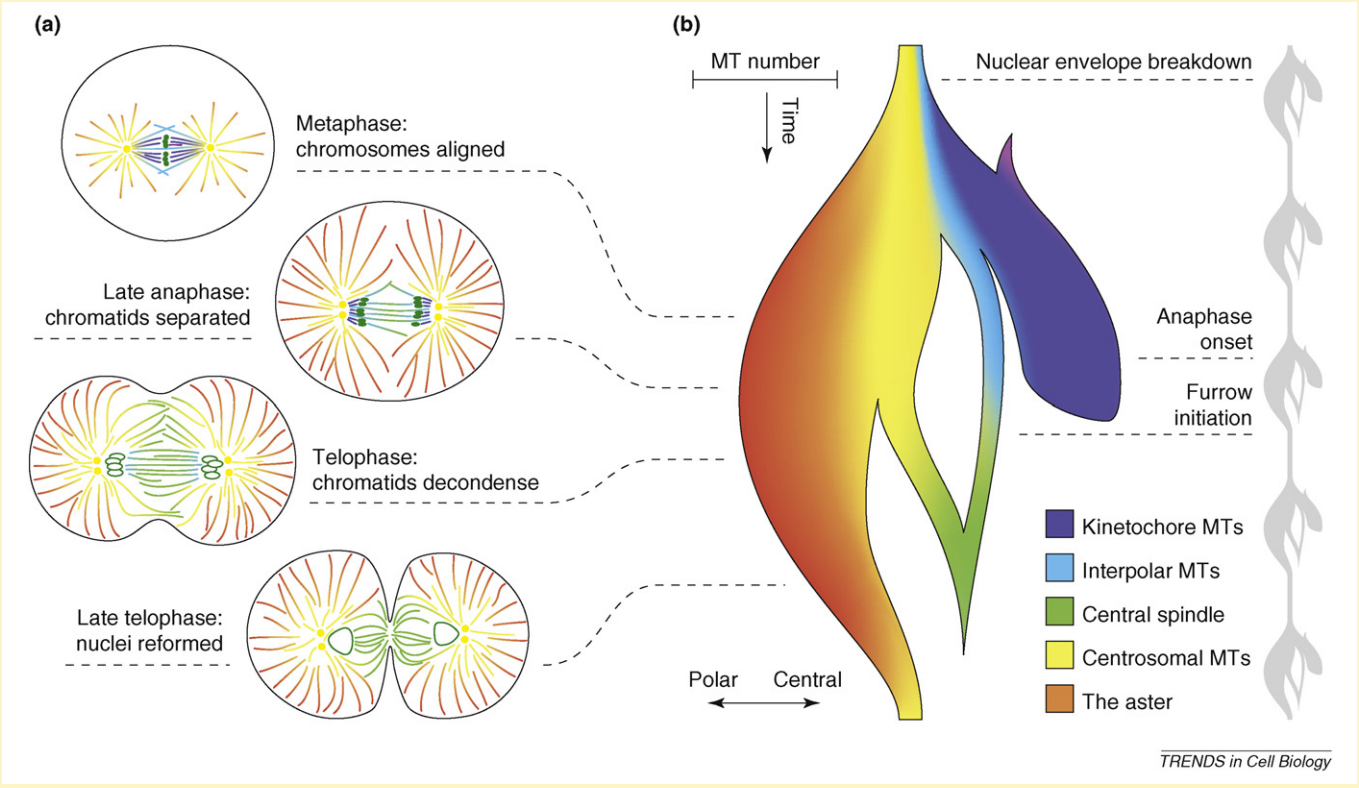

Figure I. The taxonomy of microtubules during animal cytokinesis. **(a)** Geography of microtubule arrays during key stages of mitosis, traced from purple urchin zygotes. **(b)** Phylogeny of microtubules of the mitotic apparatus, depicting the differentiation, proliferation, and disappearance of each major taxon, indicated by the same color code as in (a). Note that the central spindle descends lineally from two distinct populations.

develops a pattern based on diverse cues – instead of hunting the single message from the spindle.

Here, I review this general sketch: patterned activation of the small GTPase Rho links mitotic apparatus geometry to cortical actomyosin assembly. The entire cell cortex becomes competent for Rho-dependent actomyosin assembly – 'C-phase' [8] – during late anaphase. Something about dynamic astral microtubules locally inhibits actomyosin recruitment, thereby focusing otherwise-global myosin activation to the equator. Not all astral microtubules are equivalent; a stable population arises during anaphase. To amplify the initially weak equatorial zone of Rho activity, and hence promote local myosin recruitment, these microtubules could direct delivery of Rho regulators, possibly coming from the central spindle, without a supply of which the initial furrow zone is unlikely to survive. Competition for such survival factors among local cortical patches selects among possible furrows for the candidate best aimed toward the central spindle. Either the initial 'astral' cue or the central spindle signal usually suffices to elicit furrowing, but efficient ingression to completion requires both.

The classical dialectic in furrow specification is between polar relaxation models – the mitotic apparatus prompts the polar cortex to relax, leaving an annulus of contractile material around the equator – and hypotheses for equatorial

stimulation – some condition promotes contractility at the cell equator [6,9]. Compared with this simple dichotomy, the scenario sketched earlier hardly seems parsimonious. Was it designed by a committee? Ignoring the classical dialectic, however, it seems elegant: it amounts to a timed global change in cortical state, which is spatially patterned by one piece of information, which spatial bias is then ratified by another coincident piece of information.

Myosin recruitment to the cell equator

Recent work greatly clarifies the origin of the cytokinetic apparatus. I focus on myosin recruitment in this article because the dynamics of actin filaments in embryonic cells remain poorly described. Phosphorylation of Ser19 on the regulatory light chain of myosin II, the motor of the contractile ring, promotes assembly into active bipolar oligomers. Although there must exist subpopulations of phosphorylated myosin that have not yet formed productive complexes, and dephosphorylated myosins that have not yet disassembled, antibodies specific for Ser19-phosphomyosin provide an approximate readout of myosin recruitment.

Using this approach, Foe described the pattern and timing of myosin activation in urchin zygotes [11]. The cortex labels uniformly for phosphomyosin during interphase and prophase. This superficially suggests the premise of the polar relaxation hypothesis, in which asters trigger local disassembly of an initially uniform contractile network. However, as the cell proceeds through mitosis, phosphomyosin disappears. By anaphase onset, there is no phosphomyosin on the cortex anywhere. So, in urchin zygotes, there is no initially uniform cortical network to locally relax. Because phosphomyosin disappears from the cortex on time in cells arrested in metaphase by nocodazole, this transition is independent of microtubules or mitotic progress. After clearing myosin from the cortex, the cell re-deploys it in an equatorial band no sooner than late anaphase. The cleavage furrow in normal urchin embryos soon becomes far richer in phosphomyosin than the interphase cortex [11].

The fact that active myosin II seems to arrive at the equator *de novo* might seem trivial. However, this speaks against not only polar relaxation in the original sense (but see later) but also – for urchin eggs – any scenario that requires a global cortical contraction (e.g. see Ref. [12]). This is actually rather puzzling. Many cells round up before division, becoming measurably stiffer [13,14] as if they contract uniformly, hence the 'global contraction–polar relaxation' hypothesis. (Having injected >10^5 urchin eggs, I can attest that they stiffen during anaphase.) However, this is precisely when they have the least contractile material on the cortex. Therefore, something other than cortical actomyosin must account for this phenomenon (a companion review in this issue discusses the phenomenon of cell rounding [10]).

In urchin embryos, there is no evidence that actomyosin accumulates at the equator via convection of assembled material along the cortex (cortical flow). There is no visible phosphomyosin elsewhere on the cortex to flow into the furrow and, furthermore, phosphomyosin arrives normally at the equator in cells lacking detectable actin filaments [11]. Similar observations have been reported in cultured cells [15]. This rules out, for these cells, models involving a self-amplifying contraction of a cortical actomyosin network [16]. Indeed, the cortex is remarkably stationary in healthy urchin embryos. Not so in some other cells. Timelapse confocal microscopy of myosin fused to green fluorescent protein (GFP–myosin) reveals dramatic cortical actomyosin flow in *Caenorhabditis elegans* zygotes during both polarization and cytokinesis [17]. Even here, however, negligible flow occurs during the initial phase of cytokinetic myosin recruitment; in metaphase, myosin declines throughout the cortex and then, in anaphase, abruptly reappears as a flock of irregular blotches (Figure 1a; see also Ref. [18]). These coalesce somewhat but, in normal embryos, their initial appearance is unambiguously equatorial. Only as furrow ingression commences does flow bring myosin toward the equator (Figure 1b).

Curiously, second cleavage in *C. elegans* seems different from first. The posterior daughter resembles the zygote but, in the anterior daughter (AB), cortical myosin–GFP is uniformly bright until mid-anaphase, at which point the poles dim (Figure 1a). The equatorial myosin zone in AB thus seems to be carved from a uniform domain but the early furrow zone is still a flock of blotches that appear in place. These three accounts – urchin eggs, worm zygotes and the worm AB cell – seem different but all involve global M-phase reduction in cortical myosin activity, upon which is superimposed a spatially patterned C-phase recruitment, with variation in how much these phases overlap in time.

Rho activity links the shape of the mitotic apparatus and the pattern of actomyosin assembly

Myosin behavior during cytokinesis implies a localized signal, which recent studies equate with localized Rho activity (for a review, see Ref. [19]). Like most small GTPases, Rho cycles between GTP-bound (active) and GDP-bound (inactive) states through intrinsic but slow hydrolysis and exchange, which is accelerated by GTPase-activating proteins (GAPs) and guanine-nucleotide-exchange factors (GEFs). Active Rho promotes myosin recruitment through Rho kinase and assembly of unbranched actin through formins. Although Rho-family GTPases were originally described as 'cell state switches' whose activity selected between different cytoskeletal regimes, live imaging often reveals local zones of activity for one or another GTPase, sometimes concurrently. The most remarkable example is wound healing in *Xenopus* oocytes, when concentric zones of Rho and Cdc42 activity direct assembly of a contractile ring-like structure [20]. Clearly, small GTPases can differentiate even very close zones within the cell cortex.

Rho was first implicated in cytokinesis because injection of C3 transferase, a specific Rho inhibitor, stops furrowing in urchin and frog embryos [21,22]. Interest in Rho as a cytokinetic signal escalated once genetic studies in *C. elegans* and *Drosophila* revealed a striking association between the central spindle, a plus-end-directed microtubule motor and both a Rho-family GAP and GEF [23–25]. The GAP (CYK-4 in worms, Tumbleweed in flies,

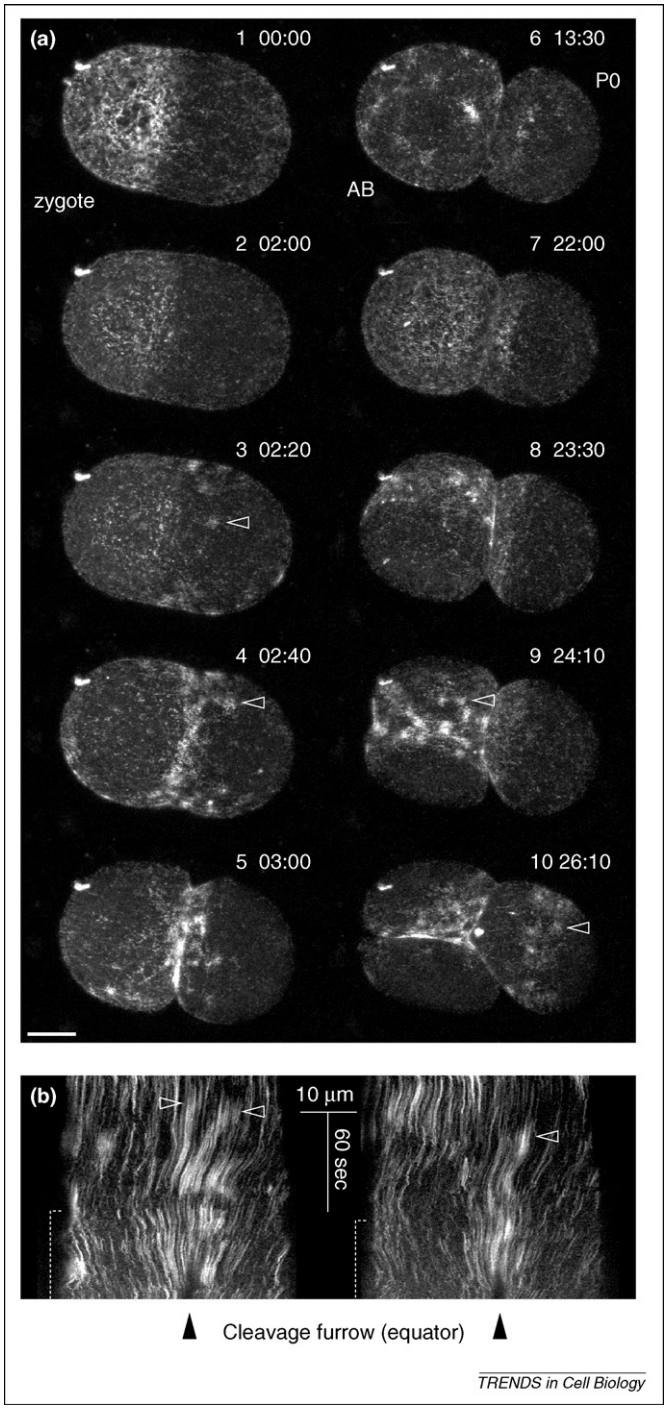

Figure 1. Myosin recruitment during embryonic cytokinesis in *C. elegans*. **(a)** Each image is a maximum intensity projection of five 1-μm confocal sections taken at 10 s intervals (see supplementary movie online) in a slightly compressed embryo expressing GFP– non-muscle myosin heavy chain (NMY2) [17]. Anterior is to the left; scale, 10 μm. The polarized zygote has a myosin-rich anterior cap, which fades at the metaphase–anaphase transition (frames 1 and 2). Myosin blotches appear rapidly at the equator (arrowhead, frames 3 and 4) and, in flattened embryos, along the margins to the anterior and posterior (probably because these margins are further from the asters). Early in the second cell cycle, the AB cell exhibits a rapidly contractile actomyosin network (frame 6) which, during mitosis, is replaced by a uniform and stationary layer (frame 7). This layer, like the anterior cap in the zygote, diminishes as the cell enters anaphase and as myosin blotches appear in the equator (frames 8 and 9). Note that, in frame 7, the spindle is oriented perpendicular to the focal plane; in frame 8 it is oblique because the slightly compressed cell rotates the spindle to the long axis; in frame 9 it is parallel to the focal plane. The spindle is not directly visible in these images but the rotation is apparent in the supplementary movie online. In P1, the myosin blotches appear in an otherwise empty cortex (arrowhead, frame 10). **(b)** Kymographs reveal coherent motion by plotting a single spatial axis versus time; a particle moving in the spatial axis of the kymograph appears as a diagonal streak; its slope is its velocity. The two kymographs here exhibit nearby medial strips, 1 μm wide and 5 μm apart, from the same sequence of a zygote expressing GFP–NMY2 filmed at one frame every half second. These cover the first appearance of equatorial myosin blotches (hollow arrowheads) to the ingression of the furrow (solid arrowheads). Blotches appear when the streaks in the kymograph are still predominantly vertical, indicating no motion in the kymograph axis; streaks begin to converge toward the equator approximately 1 min later (dashed line). In the left panel, convergent flow is more apparent; in the right panel, a myosin blotch (arrowhead) appears in an almost myosin-free region. Horizontal scale, 10 μm; vertical scale, 1 min.

MgcRacGAP elsewhere) forms a heterotetramer with the motor (ZEN-4/Pavarotti/MKLP1), which localizes to the central spindle midzone during anaphase and telophase. This complex – centralspindlin – binds and activates the GEF (LET-21/Pebble/Ect-2; I will use the *C. elegans* names here). These findings suggest how microtubule geometry could localize a signal for cortical actomyosin assembly [24,26].

Thus, it was gratifying, if not entirely surprising, to find that a narrow zone of Rho activity prefigures and remains associated with the cleavage furrow. Using a GFP-based probe for active Rho, we documented furrow-associated Rho activity zones in embryos of several urchins, starfish and frogs [27]. Others have documented similar Rho localization zones in mammalian cells and *C. elegans* embryos [25,28,29]. Active Rho is likely to directly regulate actomyosin assembly into the contractile ring. Injection of C3 into sand dollar eggs abolishes cortical myosin recruitment during cytokinesis [11]. Because formins are required for cytokinetic apparatus assembly in many cells [15,30,31], and because formins are Rho-dependent (for a review, see Refs [32,33]), it is likely that Rho also promotes assembly of unbranched actin at the equator.

Rho activity behaves like a cortical read-out of mitotic apparatus geometry. First, the width of the Rho zone scales linearly with cell diameter in normal urchin and frog embryos [27]. Using micromanipulation, we showed that the Rho zone narrows when the spindle is brought closer to the cortex, and broadens (and dilutes) if the spindle is further away or if the extent of the asters is diminished by drug treatment [27]. Second, the entire cortex remains competent to activate Rho through at least the first half of furrow ingression: when the spindle is displaced after the furrow has started, the old furrow loses Rho activity and regresses; then a new Rho zone and furrow develops over the spindle midplane [27]. In urchin embryos, an established Rho zone can be erased within tens of seconds, implying rapid Rho turnover within the furrow. Likewise, a patch of cortex pushed near the spindle midzone rapidly acquires active Rho [27]. Therefore, Rho activity is not a static pattern, but a dynamically maintained response within the cortex to mitotic apparatus position.

It is not yet clear how to reconcile such observations in large embryonic cells with available information about known cytokinetic Rho regulators. Consider an urchin zygote: the spindle is small relative to cell diameter, meaning the equatorial cortex is not much closer to the presumed signal source than is the polar cortex. It seems like a much better spatial cue in small cells, in which the distance from spindle midzone to equatorial cortex is much smaller than the distance to the poles. And if Rho activity is primarily modulated by the arrival of central spindle-localized factors, then how do we explain

numerous experiments conducted in diverse cells [34–37] that demonstrate induction of a furrow between two asters unconnected by a spindle?

Asters inhibit cortical myosin recruitment

The vast array of experimental results summarized by Rappaport in his monumental review would seem to have put polar relaxation to rest [6]. However, a series of recent papers resurrect the idea that the first spatial bias imposed on the cortex is some kind of inhibition associated with astral microtubules. Genetic, pharmacological or physical disruption of the normal conjunction between the astral midplane and the central spindle show that both the asters and the central spindle provide genetically and spatially separable cues, which coincide in normal cells (Figure 2).

In *C. elegans* zygotes, centralspindlin is normally required only for furrow completion, not initiation [38–40]. However, if the spindle poles fail to be stretched apart during anaphase, the centralspindlin components become essential for furrow initiation [41] (Figure 2b,c). Dechant and Glotzer [41] explained this effect by showing that the furrow initiation site is distinguished by a local minimum of microtubule density, which is absent if the spindle poles do not move apart. This explanation is controversial because at least two other studies in *C. elegans* failed to detect a local minimum [28,42]. Nevertheless, the demonstration of synergy between the central spindle and the distance between asters is clear.

Subsequent studies accomplished spatial separation of these normally coincident effects. Bringmann and Hyman [43] cut one spindle pole away from the rest of the mitotic apparatus, thus achieving a slight spatial displacement between the midplane between the two asters and the midzone of the central spindle, which are normally coincident. The initial furrow appeared at the astral midplane, but a second furrow appeared over the central spindle and, in wild-type cells, the 'astral' furrow abdicated in favor of the one which, by crossing the central spindle, correctly apportioned chromosomes (Figure 2d,e). By conducting, in *C. elegans* zygotes and early blastomeres, Rappaport's classic torus experiment [35], we confirmed the spatial independence of an astral cue from a dominant, central spindle-associated cue [34] (Figure 2g). Although we found it challenging to repeat Bringmann's spindle-severing experiment, our attempts yielded another informative geometry: cells that inherit only a centrosome and no nucleus, and hence build no spindle, nevertheless manifest a deeply ingressing furrow (Figure 2j). The asters alone, therefore, suffice to induce a furrow but so does the central spindle, and the latter overrides the former.

Two furrows appear, in tellingly different ways, during cleavage in zygotes whose spindle is posteriorized either by nocodazole treatment or in various mutants [18]. The cortex around the posteriorized spindle remains myosin-depleted during anaphase, but myosin recruits to the anterior cortex (i.e. away from the spindle). A furrow ingresses at the margin between the myosin-rich and myosin-poor zones in the cortex, while cortical flow develops toward the anterior. The second furrow bisects the spindle and requires centralspindlin (Figure 2k,l). Practically all the cortex that is not penetrated by astral microtubules displays myosin blotches characteristic of the equator in normal cells, which require Rho and LET-21 but not centralspindlin. Thus, the aster and central spindle cues are kinematically and genetically distinct as well as spatially separable.

In urchin zygotes, the existence of a local minimum of microtubule density at the equator was demonstrated long ago in a painstaking ultrastructural study [44] and our recent immunofluorescence analysis confirms it [11]. Foe used timed nocodazole treatments to assess myosin recruitment after microtubule depolymerization. If cells arrest before sister chromatid separation, myosin remains inactive [11]. Accordingly, the cortex cannot respond to the mitotic apparatus before anaphase [37]. However, if cells separate sister chromatids, then, in the absence of dynamic microtubules, cortical myosin recruitment takes place globally (in agreement with Canman's experiments in cultured cells [8]). Strikingly, as cells are allowed more time in anaphase with astral microtubules, myosin recruitment becomes progressively more confined to the equator. Recent work in cultured cells, using Rho localization rather than myosin recruitment to assess the effect of dynamic microtubules also demonstrates inhibition by asters [45]. Likewise, a recent paper demonstrates local relaxation of cortical actin associated with the asters [46]. Like the Rho zone, inhibition of cortical contractility by asters emerges as a common theme among diverse animal cells.

Is polar relaxation back from the dead? Not quite: in urchin and worm zygotes there is nothing to relax at the poles in early anaphase; in *C. elegans* the shape of the egg means the area adjacent to the equator, not the poles, is actually closest to the aster centers. Numerous physical experiments discourage attention to the polar cortex itself and undermine inhibition-only mechanisms (e.g. see Ref. [47]; for an extensive review see Ref. [6]). These do not, however, rule out that some of the action takes place just peripheral to the equator. Let us consider a milder hypothesis, 'tropical depression' – partial inhibition of actomyosin recruitment in the regions neighboring the equator – as a compromise. And is it really the astral microtubules that mediate inhibition of contractility? The compelling organization of microtubules and their potential as roads evokes speculation, but there is still little direct evidence linking this effect to microtubules themselves or their ends.

Not all the microtubules are the same

Ever since Hiramoto sucked out the spindle from an urchin egg at late anaphase and found that the cleavage furrow still ingressed [48,49], conventional wisdom has been that microtubules have basically done whatever they do to induce furrowing once the furrow appears. Drug studies seemingly confirm this because treating urchin eggs with microtubule poisons after furrow onset does not stop furrowing in progress [50,51]. But people rarely check whether the microtubules actually go away. This is important because imaging of cultured mammalian cells implicates stable microtubule ends in furrow induction. Canman proposed that these microtubules, perhaps specially graced by having passed near chromosomes, promoted furrowing [52]. Another study showed that small

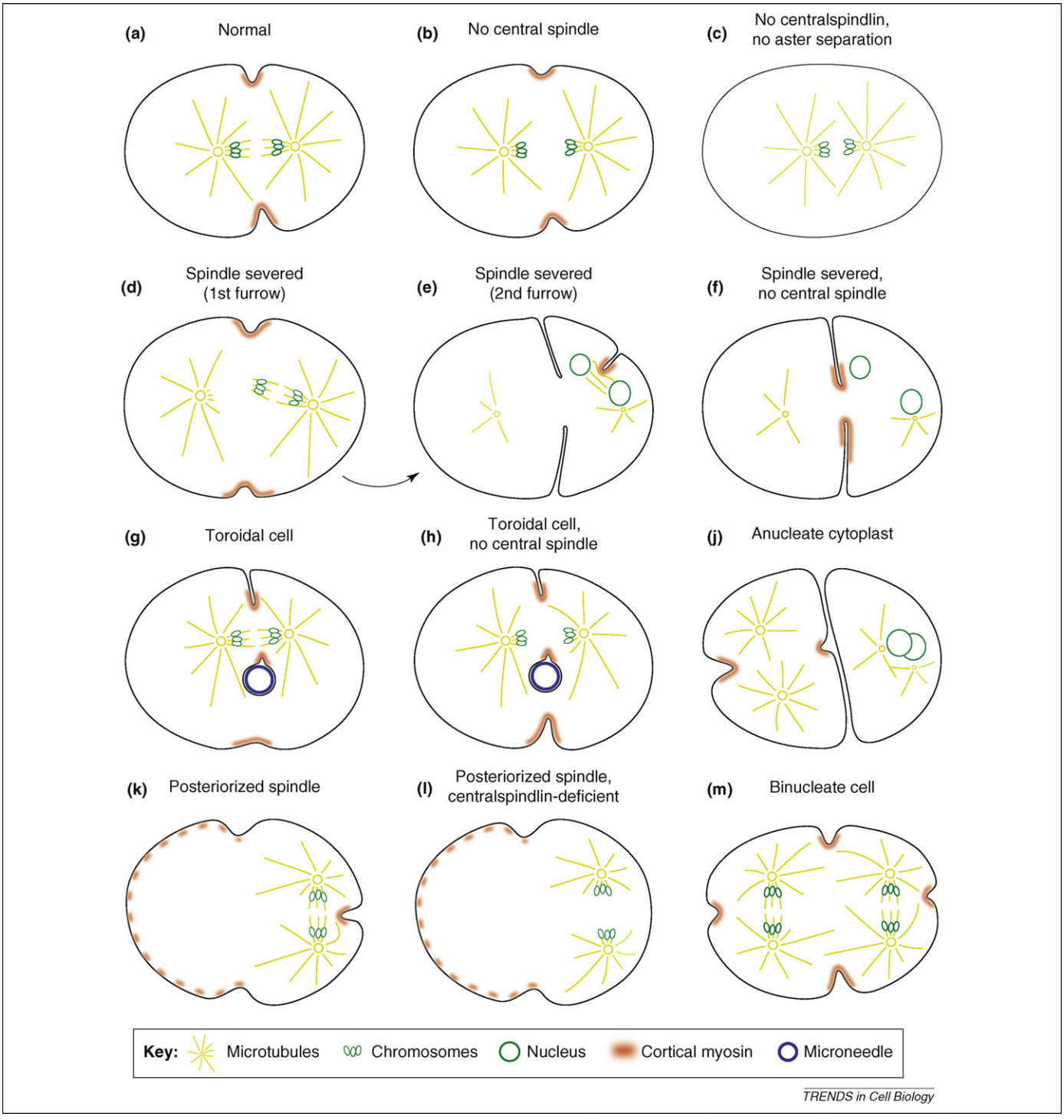

Figure 2. Geometrical conditions supporting cleavage-furrow formation in *C. elegans* zygotes. Microtubules are yellow, chromosomes or nuclei are green, and orange indicates an actively ingressing furrow and/or myosin-rich cortex; blue circles in G and H represent a glass microprobe perforating the cell. See main text for citations. **(a)** Normal zygote. **(b)** Zygote lacking a central spindle owing to depletion of ZEN-4, CYK-4, KLP-7 or SPD-1: no central spindle, poles further apart than normal; furrow regresses in ZEN-4- or CYK-4-deficient cells, but completes in the absence of SPD-1. **(c)** Zygote lacking both centralspindlin and GPR1/2: no central spindle, no separation of spindle poles, no furrow. **(d)** The initial furrow after cutting away the anterior spindle pole appears midway between the asters, not over the spindle midzone, but **(e)** a second furrow forms above the midzone, and the initial furrow regresses. **(f)** In the absence of the central spindle (but with centralspindlin still present; SPD-1 or KLP-7 depleted), the initial furrow completes and no secondary furrow forms. **(g)** In cells perforated between the spindle midzone and the cortex, furrows form both on the spindle side and the far side, but the far-side furrow is tentative and usually fails to ingress deeply. **(h)** In perforated embryos lacking SPD-1, the far-side furrow is much more persistent and can even complete. **(j)** Anucleate cytoplasts containing only two asters mount deeply ingressing furrows (which can, but do not usually, abscise); this situation arises if a zygote fails to form a secondary furrow [as in (e)] after spindle severing [as in (d)], yielding one binucleate and one anucleate daughter. **(k)** In zygotes with posteriorized spindles, two furrows form, one away from the asters, the other over the central spindle; in this experiment, no posterior furrow forms in the absence of centralspindlin **(l)**. **(m)** Binucleate cells with parallel spindles divide from one to four; note that all furrows in this geometry usually complete, perhaps because the 'Rappaport furrow', which forms between two asters not connected by a spindle, will eventually come into proximity with the spindle midzones as they are gathered toward the center.

'furrowlets' form over even single taxol-stabilized microtubules [53].

Foe examined nocodazole-treated urchin eggs at successive stages of mitosis and found that, after anaphase onset, a subset of the astral microtubules becomes stable [11]. These stable ones aim predominantly toward the equator and, furthermore, co-localize with the earliest flecks of myosin recruited to the equator. The correlation between stable microtubules and the nascent furrow is especially striking because centralspindlin includes a plus-end-directed motor that stabilizes or bundles microtubules *in vitro*. Whether they appear before or after the Rho zone, or in a pattern or not, stable microtubules associate with the ingressing furrow and remain until abscission.

What good are stable microtubules? Computer simulations show that dynamic microtubules are poor roads for motor-driven advection because when the road disappears from under the cargo before it gets where it is going, the cargo has to diffuse around and find a new track [54]. Indeed, an aster consisting solely of dynamic microtubules seems more likely to disperse than concentrate plus-end-directed cargos, by dropping motors off at random distances from the centrosome. By contrast, stable microtubules make effective concentrators, especially if the motor is highly processive and sticks for a moment when it reaches the end [54]. Whether ZEN-4 has these qualities is unknown, but the mechanism is plausible, makes testable predictions, and seems robust to cell size and microtubule number.

These findings have two consequences. First, we can forget the notion that microtubules are dispensable after furrow onset because proofs thereof relied on drugs (to which stable microtubules are, well, stable) or physical extirpation (which cannot possibly remove all microtubules). Second, it might resolve how midzone-associated centralspindlin could provide a spatial cue in large cells. It is entirely unknown why some microtubules become stable and why the equatorial bias. Our results admit the possibility of positive feedback from Rho [11], but a better idea is that centralspindlin or chromosome passenger proteins, Ran, or some other scent of chromosome wafts off from the spindle midzone as chromosomes separate, and blesses the first microtubules it alights upon (e.g. see Ref. [7]). The stable microtubule array would thereby reflect the position of the central spindle while reaching all the way to the cortex.

Once again, however, furrowing cannot absolutely require any particular microtubule geometry. Cells cleave even when the mitotic apparatus is continually stirred by a rotating needle [55], which surely does not help the microtubule array, stable or not, deliver anything anywhere. A recent study agrees that no specific microtubule population or property is truly essential for furrowing [56].

Redundancy? No; separation of powers

How can so much be going on, yet so little of it seem to be important? The long history of experimental results certainly indicates that animal cell cleavage is robust to perturbations. There is a big difference between asking what is necessary, what is sufficient, and what is actually participating. The possibility of redundancy means that the set of 'necessary' things could be empty even though 'sufficient' and 'involved' cannot possibly be so. *C. elegans* zygotes apparently use at least two spatial cues, which seem redundant for furrow positioning. However, considering the possibility of errors, these are not truly redundant mechanisms that accomplish the same task: one proposes and the other endorses. The 'astral' and 'midzone' signals occur consecutively and the latter is dominant, either ratifying or vetoing the initial specification [34,43].

In toroidal *C. elegans* zygotes and AB cells, the aster-positioned furrow is rather tentative, rarely getting as bright with GFP–myosin or ingressing as much as the furrow crossing the central spindle [34]. In Bringmann's experiments, the aster-positioned furrow never completes unless the egg lacks either central spindle components SPD-1 or KLP-7, or the myosin phosphatase [43]. Assuming that the spindle-positioned furrow depends on centralspindlin, then the furrow that forms in ZEN-4- or CYK-4-depleted eggs is aster-positioned and that too fails to complete. So too does the far-side furrow usually regress when we perforate cells, except occasionally in SPD-1-deficient cells [34]. Likewise for cells that lack a nucleus or spindle, a furrow develops between two asters and then (usually) regresses [34]; likewise for the anterior furrow in cells with a posteriorized spindle [18]. Thus, the aster-associated signal can initiate but not sustain furrowing while the spindle-associated signal sustains furrowing to completion.

SPD-1 is required for stabilizing the central spindle but not for cytokinesis [57] (Figure 2b). In the absence of SPD-1, Bringmann found that, without a central spindle, no spindle-crossing furrow forms but the mis-aimed aster-positioned furrow completes [43] (Figure 2f). In ZEN-4- or CYK-4-deficient embryos, which also lack a central spindle, this would not happen. Similarly, we found that in toroidal cells lacking SPD-1, both spindle-crossing and far-side furrows ingress deeply and can complete [34] (Figure 2h). Apparently, the central spindle inhibits the aster-positioned furrow if it is going the wrong way. Without the central spindle, the cell cannot tell whether the furrow passes between the daughter nuclei. It is as if furrows compete for something that they need to survive (centralspindlin is one obvious candidate), and the central spindle normally hoards this stuff, doling it out to the furrow which, by aiming correctly, approaches the midzone. During normal cytokinesis, a suggestion made to the cortex by the asters is ratified or vetoed by the central spindle.

A couple of the challenges: scaling and turnover

This tripartite mechanism – in which anaphase entry enables the cortex to develop contractility, the cortex reads the position of the asters, and then the furrow itself sniffs out the central spindle – seems appropriate to large embryonic cells in which the mitotic apparatus lies deep beneath the cell surface. Available space precludes discussion of comparable results from cultured cells (however, see Refs [15,45,46]), but it deserves mention that small cells face different challenges. The relevant spatial cues become better defined the smaller cells become because the spindle

occupies a much greater fraction of cell volume and the distances across which signals must travel are much smaller. Conversely, a mitotic HeLa cell fits nicely into the cleavage furrow of the urchin zygote: in small cells, the spatial confinement of signals or actomyosin assembly within a narrow cortical zone seems much harder in the face of diffusion.

One might wonder to what extent large and small cells even use the same mechanism. It could be that the relative weight given to one or another activity changes as cells decline in volume. This might be why cell perforation experiments yield different results in different cell types. In Rappaport's famous toroidal sand dollar egg, a furrow formed when two asters were closely juxtaposed [35]. By contrast, Cao and Wang [58] perforated adherent mammalian cells and found that, when given a choice between a zone in which astral microtubules overlap or a zone adjacent to the central spindle, the cortex develops contractility only near the central spindle. Perforation of *C. elegans* zygotes or early blastomeres (which are intermediate in size) yields an intermediate result [34] (Figure 2g). Cell size is not everything – similar-sized cells in the same embryo can differ markedly in their reliance on the central spindle [59] – but direct examination of size scaling within the same animal would be valuable.

That small cells could extract spatial cues from chromosome position itself is obvious because Aurora B, after leaving kinetochores at anaphase onset, arrives at the equator in a microtubule-dependent way [60,61]. That small cells can develop furrows that do not match chromosome position or the central spindle is equally clear from accounts of cleavage from one to four in binucleate mammalian cells with parallel, unconnected spindles [36]. If cells (or cell fragments) lack any other positional cues, then the cortex can aim a furrow toward the nearest oriented bundle of microtubules [62]. With so many positional cues available, maybe the problem is not which is essential, but rather how the cortex integrates the available information, normally, into a single decision.

This issue awaits resolution of a major problem. If Rho activity indeed constitutes the key paint job declaring 'cut along the dotted line', not only is it still unclear how centralspindlin might create this zone, it is even less apparent how this zone can be so rapidly snuffed out. The intrinsic Rho cycle seems far too slow to account for rapid revision of the furrow zone after perturbation [19]. Even in unperturbed cells, the total area of the Rho zone shrinks during furrowing because its linear width along the polar axis stays roughly constant as the furrow circumference vanishes. Trivially, some ubiquitous Rho GAP might shut off Rho everywhere but the equator. Yet only CYK-4, which is a poor GAP for Rho if it is one at all [23,63], consistently volunteers as a candidate for Rho inactivation during cytokinesis [64]. Something is missing.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tcb.2009.01.008.

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