Coupling changes in cell shape to chromosome segregation

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Abstract

Animal cells undergo dramatic changes in shape, mechanics and polarity as they progress through the different stages of cell division. These changes begin at mitotic entry, with cell-substrate adhesion remodelling, assembly of a cortical actomyosin network and osmotic swelling, which together enable cells to adopt a near spherical form even when growing in a crowded tissue environment. These shape changes, which likely aid spindle assembly and positioning, are then reversed at mitotic exit to restore the interphase cell morphology. Here we discuss the dynamics, regulation and function of these processes, and how cell shape changes and sister chromatid segregation are coupled to ensure that the daughter cells generated through division receive their fair inheritance. [Edits OK?]

Introduction

During cell division, the entire set of cellular components must be segregated before being partitioned into two daughter cells. This includes cellular constituents that are present in relatively large numbers, like ribosomes, which are partitioned by stochastic processes at division; cellular components that are present in smaller numbers, like mitochondria and the Golgi, which tend to be fragmented and actively transported to opposing cell poles to facilitate their fair inheritance[1]; and structures that must be segregated with high precision because their function is dependent on exact copy number, like centrioles and chromosomes. Therefore, although the basic goal of division is the same across all kingdoms of life[2, 3], it is especially challenging for eukaryotic cells, in which segregation of genetic material must be coordinated in space and time with organelle segregation and cytokinesis. The problem is further compounded for cells in multicellular tissues like epithelia, where cells are highly polarized in shape and structure and physically connected to one another. In the context of a tissue, cell divisions must also be resistant to the potentially adverse influence of extrinsic forces, while responding to cues that determine the axis of cell division to facilitate the maintenance of polarity and tissue relaxation[4, 5].

For animal cells all these processes are facilitated by "mitotic rounding". This is the process by which cells adopt a relatively simple, symmetric and spherical shape as they enter mitosis. Although this is not a universal phenomenon[6,7], most eukaryotic cells that lack a rigid cell wall[8], and therefore have a flexible form, begin to round up in this way as they enter mitosis. Although the precise function of mitotic rounding in different systems remains to be determined, the spherical shape is likely to provide a suitable environment for the spindle to assemble, while also delimiting the space in which astral microtubules [G] have to search to capture mitotic chromosomes[9]. In addition, by simplifying cell geometry, mitotic rounding might contribute to the accurate partitioning of cellular contents into the two daughter cells. In a similar manner, the apical movement of cell mass accompanying mitotic rounding in cells dividing in columnar epithelia, such as neuroepithelia[10], facilitates the preservation of apical polarity cues during a symmetrical division. Rounding also helps to set the stage for the dramatic redistribution of cell mass that is associated with anaphase elongation and cytokinesis, which is important for the restoration of normal cell packing in a tissue that has been subject to mechanical stretch[11].

To aid division, changes in cell shape during mitotic entry and exit are coupled to the cell cycle clock by the activity of the master mitotic kinase Cdk1—CyclinB. Cellular architecture begins to be remodeled during prophase, as CDK1—CyclinB levels rise, causing cells to adopt a typical, spherical mitotic cell shape[12] while the centrosomes separate and begin to nucleate microtubules to capture and align chromosomes. These changes in cell shape are then reversed following the drop in CDK1—CyclinB levels at anaphase. Then, as sister chromatids separate, cells leaving mitosis elongate to make space for the extending spindle, while simultaneously assembling the contractile ring machinery to generate two

daughter cells. The two processes must be highly coordinated in space and time to ensure the equal inheritance of genetic material in the daughter cells.

In this review we discuss the dynamics and the contribution of cell shape changes to mitosis in cell culture and in the context of an epithelial tissue. Finally, we explore the importance of crosstalk between the cell cortex and the mitotic spindle for precise cell division.

Entry into mitosis

For many animal cells, entry into mitosis begins with a dramatic series of changes in cell shape, movement and polarity[10, 13, 14] (Fig 1A and B). Although these changes have not been investigated in much detail, they are some of the first signs of a cell approaching mitosis. These are triggered by rising levels of Cdk1—CyclinB[15], along with rising activities of a cohort of mitotic kinases (most notably Aurora A, Aurora B and Polo like kinase1). This sets up a positive feedback loop that culminates in full kinase activation and a global shift in the phosphorylation of the proteome[16-18]. As a result, within minutes of mitotic entry, cell shape and structure are rapidly altered[12].

Adhesion remodeling during mitotic entry
One of the factors contributing to this rapid change in cell shape is the loss of attachment to the underlying substrate. This is suggested by the observation that the loss of integrin-based adhesions is sufficient to cause interphase cells in culture to adopt a near-spherical shape - the minimum energy conformation for a cell with high cortical tension (as for a given volume, the sphere has the least surface area). At a molecular level, this rapid change in adhesion is likely triggered by activation of CDK1—CyclinB[19-22]. Although CDK1—CyclinB directly phosphorylates a number of adhesion complex components[23], including integrins[24], focal adhesion kinase and paxillin[25], the major molecular event responsible for the loss of adhesions in many cell types appears to be Rap1 inactivation[12]. This is supported by the fact that many cultured cells expressing a constitutively activated form of the Rap1 GTPase retain adhesions and remain flat as they enter mitosis despite extensive CDK1—CyclinB-mediated phosphorylation of adhesion proteins[12]. In addition, this experiment demonstrates the importance of adhesion remodelling for normal mitotic cell rounding.

Although the loss of cell-substrate adhesions during entry into mitosis is rapid, most animal cells maintain physical connections with their environment throughout mitosis (Fig.2) as a result of incomplete mitotic disassembly. For animal cells in culture, the actin-based structures that remain are called "retraction fibers" [26] (Fig 2). Although these lack myosin II and do not provide robust cell-substrate adhesion (enabling mitotic cells to be dislodged by a physical blow to the cell culture flask), they have been proposed to act like tent cables[5] - keeping rounded cells tethered to the extracellular matrix. Importantly, for cells in culture with a isotropic interphase shape, these retraction fibers have also been suggested to provide cells with a physical memory of the pattern of interphase cell-substrate adhesions, which then guide mitotic spindle orientation[27] (Box 1), and the re-establishment of these adhesions when cells re-spread at mitotic exit[28,29]. Similar structures have been observed in mitotic cells within an epithelium, which retain long basal "retraction fibers" as they round up during mitosis. These maintain physical contact between cells and their underlying basal extracellular matrix and appear to aid the restoration of apical basal polarity upon mitotic exit[30, 31]. Importantly, epithelial cells also retain their E-cadherin-based adherens junctions and tight junctions during the process of division to preserve tissue integrity[32, 33].

Assembling the mitotic cortical actin network

The adhesion remodeling that takes place as cells enter mitosis is accompanied by profound changes in the organization of cortical actin cytoskeleton (Fig. 2A), which culminate in the assembly of a dense cortical actin network that is characteristic of mitotic cells[34]. The loss of basal actin cables (also known as "stress fibers") is likely to be a direct consequence of the disassembly of cell-substrate adhesions[13]. There is also a shift in the dominance of different cortical actin nucleators at the onset of mitosis, from the Arp2/3 complex to formins[14, 35, 36]. For cells entering mitosis in culture, this is apparent from the loss of lamellopodia, which are actin-based membrane protrusions that are generated by branched actin filament nucleation downstream of Rac, the SCAR/WAVE complex[37-39], and the Arp2/3 complex. Although the extent to which residual Arp2/3 activity contributes to the generation of mitotic structures is currently unclear[35, 36,14], this loss of lamellipodial actin is accompanied by the activation of the formin-based actin nucleator Diaphanous (Dia)[14], which drives the assembly of a cortical network of actin filaments. Importantly, once activated, through physical association with Rho family GTPases, Dia generates non-branched actin filaments. These provide an ideal substrate for non-muscle myosin II minifilaments[40], which walk along antiparallel actin filaments in a step-wise fashion to generate cortical tension. In human and fly cells, assembly of this Dia-dependent cortical actin mesh appears to be regulated by Ect2 (called Pbl in Drosophila) [13, 14] - a potent Rho family GTPase activating protein[41]. Ect2 is released from the nucleus as levels of nuclear CDK1/CyclinB increase at the onset of mitosis[13]. Once in the cytoplasm, Ect2 activates Rho family GTPases at the plasma membrane (Fig 2). These include RhoA[42, 43] which, when bound to GTP, activates Dia and Rok (Rho-kinase), which in turn activates non-muscle myosin II[44]. In addition, Ect2 has been suggested to associate with the polarity protein complex aPKC-Par6-Cdc42, leading to the activation of the Rho family GTPase Cdc42[14, 45]. In this way, Ect2 may direct a shift in polarized actin organization, enabling epithelial cells to assemble an isotropic cortical actomyosin network as they round up to divide[14]. Interestingly, the same set of proteins drive both the assembly and polarization of a cortical contractile actomyosin network prior to division of the *C. elegans* zygote[46].

Many other changes in the dynamics and organization of actin filaments contribute to the assembly and remodeling of mitotic cortical actomyosin network. For example, phosphorylation-induced changes in the activities of proteins that negatively regulate actin filament formation, including LIM kinase, cofilin and Wdr1, may contribute to an increase in the actin filament turnover rate upon entry into mitosis[35, 47,48-50]. Furthermore, the activation of actin filament-binding proteins, such as ERM (Ezrin/Radixin/Moesin) proteins brought about by the Slik kinase[51, 52] leads to the physical crosslinking of the actin filaments to proteins embedded in the plasma membrane [52, 53]. These changes in actin organization are likely to be aided by a shift from the phospholipid PIP3 towards PIP2 in the plasma membrane[54], which has been shown to inhibit protrusion formation[55] and to activate ERM binding[56]. Together, these changes in actin filament nucleation, turnover and dynamics lead to the assembly of a thin but

dense network of actin filaments and non-muscle myosin II motors underneath the plasma membrane[34].

Although this reorganization of the cytoskeleton begins in prophase, cytoskeletal remodelling accelerates following the loss of the nuclear-cytoplasmic compartment barrier (a process known as nuclear envelope breakdown (NEB))[57]. One might speculate that this is due to the release of proteins previously sequestered in the nucleus. In addition to residual nuclear Ect2, this includes Ran-GTP, which catalyzes the release of proteins from importins [G] to alter microtubule dynamics and cortical actomyosin[58]. Simultaneously, the mixing of cytoplasm and nucleoplasm induced by the loss of the nuclear compartment is likely to induce a sudden drop in the concentration of proteins localized to only one of the two compartments - inducing profound changes in cellular biochemistry and cyto-architecture. This is augmented by osmotic swelling[59] and a sudden influx of water, leading to a significant (>10%) increase in cell volume that is independent of the actin cytoskeleton[60, 61], and a dramatic increase in cell height[59, 62, 63]. In combination, changes in cell architecture, actin remodeling, cell-substrate adhesion and osmotic swelling, cause cells to assume a spherical shape by the time they have assembled a metaphase plate (Fig 3A).

Changes in mechanical properties

Although spherical mitotic cells resemble non-adherent interphase cells, their mechanical properties are profoundly different[13, 59, 63-65]. In fact, entry into mitosis is accompanied by a rapid, nearly ~10-fold decrease in cell compliance (equivalent to an increase in cell stiffness). This is dependent on both osmotic swelling, which can be inhibited by the Na+/H+ anti-porter poison (EIPA)[59], and the cortical actomyosin network[13, 52]. These forces are sufficient to deform neighbouring cells within a crowded epithelial tissue[11, 66](Fig 3B)**,** can drive the apical movement of cell mass in a columnar epithelium[63], and are strong enough to induce morphogenetic tissue buckling in developing fly and lumen growth in zebrafish epithelial tissues[66,67]. Thus the stable, rounded shape that is characteristic of metaphase cells reflects a balance of forces (Fig. 3): the mitotic cortical actomyosin network, which is crosslinked to the plasma membrane by ERM proteins[52], acts like the elastic skin of a balloon to counter internal pressure established by the activity of osmotically active ion pumps[59, 60].

Mitotic exit

The duration of mitosis depends both on mitotic timers and on satisfaction of the spindle assembly checkpoint [G][68-71]. When a minimal time [not sure that 'sufficient time' is clear. Sufficient for what specifically?] has elapsed and the last pair of chromosomes has been aligned at the metaphase plate (reviewed in [72]), the APC [G] (anaphase promoting complex-reviewed in [73]) is activated. This induces cleavage of cohesin, the protein complex that holds sister chromatids together, enabling the movement of sister chromatids to opposing poles of the elongating anaphase spindle[74] (Fig 4A). At the same time, APC-mediated protein degradation reverses many of the regulatory changes that accompany entry into mitosis, triggering the rapid degradation of CyclinB and the loss of mitotic kinase activity

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(reviewed in [75]). This drives cells out of mitosis – a process that culminates in cell division (reviewed in [76]).

Assembly and positioning of the actomyosin ring

Though many of the cell biological changes induced upon entry into mitosis are reversed during mitotic exit (for example, reassembly of the nuclear compartment (reviewed in [77])) the same is not true for actin remodelling. In fact, the mitotic cortical actin network must not be disassembled at mitotic exit. This is because the machinery used to generate the actomyosin network that establishes the characteristic spherical shape of the metaphase cortex also controls the assembly of the acto-myosin contractile ring [G] that is used to drive cytokinesis. This includes Ect2, RhoA, Dia, actin, non-muscle Myosin II and Anillin[78]; all of which appear to be further activated by the drop in CDK1-CyclinB activity at mitotic exit[79, 80]. In this regard, mitotic rounding can be seen as a prelude to division. Although the activity of this set of actin regulators is required for the assembly of the actomyosin network necessary for both rounding and cytokinesis, the assembly of an actomyosin ring likely involves additional factors, for example, the local loss of active ERM proteins[52, 81], and the recruitment of septins[82-84] and Bar-domain proteins, which aid membrane deformation during cytokinesis across eukaryotes[85, 86].

Structural changes at mitotic exit begin with elongation of the spherical mitotic cells, which now adopt a more elliptical shape. This is followed by cleavage furrow formation, which finally divides the cell into two daughter cells. The cortical remodelling triggered at mitotic exit to facilitate these shape changes, cannot be subject to autonomous regulation; that is an actomyosin ring cannot be simply assembled and constricted at a fixed time and place following the activation of the APC. If each daughter cell is to inherit a single copy of the nuclear genome, cells must ensure that the actomyosin ring bisects the plane of the elongating anaphase spindle. Given the speed at which cells change their shape (elongation and cleavage furrow formation) during mitotic exit, this requires active crosstalk between the spindle and the cortex (see [87] for information about the duration of these signals). This contrasts with the situation during mitotic rounding, when changes in cell shape and actin organization appear to be regulated relatively independently of microtubules and microtubule-based structures[13] (an exception being a potential role for the cortex in centrosome separation at mitotic entry[9, 88, 89]). Similarly, spindle assembly appears to be largely independent of the presence of actin filaments both *in vivo*[9] and *in vitro*[90]. This difference implies that mitotic exit must be accompanied by profound changes in spindle-cortex communication - as has been long appreciated (we suggest reading Rappaport's book for an in depth review of the early literature[87]). Insights into the mechanism came from Rappaport, who carried out a series of seminal experiments showing how overlapping antiparallel astral microtubules can direct the local formation of an ectopic furrow during mitotic exit[87]. Although ectopic furrows do not form in analogous experiments in all systems[91], in most animal cells tested, the overlapping microtubules that form between the poles of the anaphase spindle[92] direct the formation and position of the actomyosin furrow to ensure that the

division plane bisects the spindle. At a molecular level this is achieved through recruitment of "centralspindlin complex" – a set of proteins that orchestrate the ensuing molecular events[92]. In brief, overlapping antiparallel microtubules of the anaphase spindle recruit, and are further organized by, a set of kinesin motors (reviewed in detail in [76]). Following the APC-induced fall in CDK1-cyclinB activity, these in turn recruit a scaffolding protein, RacGAP1/MgRacGAP [Are these two alternative names?]together with the RhoGEF Ect2 to the spindle midzone. This shift in Ect2 localization leads to the repolarization of cortical actomyosin network and associated proteins such as Anillin[93] through the local activation of RhoA[94, 95]. RacGAP1 together with Ect2 promotes the local activation of RhoA[96]. Further, RacGAP1 may itself inactivate RacGTP at the midzone[97] to help establish a gradient of Rac and Arp2/3 activity, low at the midzone and high at the poles, aiding anaphase elongation, which may be augmented by the PIP2/PIP3 gradient found in many anaphase cells[98] (PIP2 high at the furrow, facilitating Rho activation, and PIP3 high at the poles, facilitating Rac activation). Finally, this process is promoted by the local action of the mitotic kinases Plk and Aurora B[94, 99] (a component of the chromosomal passenger complex (CPC)), which persist at the midzone.

Until recently, it was thought that this same set of cues emanating from the spindle positions the furrow in the same way across all animal cell divisions. However, spindle-cortex crosstalk is flexible. Thus, asymmetries in the cortex can bias the site of furrow formation[100-102]. In addition, pre-existing cortical asymmetries can modify the rate of anaphase elongation[101] to alter the size of daughter cells. Although the instances identified so far are not as extreme as in budding yeast, where the spindle is moved so that it aligns with the position of the future furrow prior to anaphase[103], it is clear from these few instances that the actin-based cortex can contribute to the positioning of the division plane.

Polar relaxation

Physical models of cytokinesis suggest that the essential factor required for a successful division is the establishment of a gradient of cortical contractility across the cell[76], whereby the cortex is more contractile at the center and relatively compliant at cell poles. Thus, the assembly and constriction of a central actomyosin ring may not be sufficient to drive cytokinesis in cells with a rigid mitotic cortex[52, 104]. In line with this, division involves both the assembly of an actomyosin ring, induced by overlapping microtubules that are positioned between spindle poles, and concomitant softening of the cortical actomyosin network at cell poles[105, 106] – a process we term polar relaxation. Depending on the relative timing of this local polar loss of pERM proteins, actin and non-muscle myosin II, cell elongation can be a smooth and gradual process or can be accompanied by furious rounds of blebbing – as physical connections between the plasma membrane and the underlying cortical actin network are repeatedly lost and re-established[105]. Moreover, failure to induce timely relaxation of the polar cortex can lead to catastrophic division failure[105].

Three signals have been identified that may contribute to relaxation of the cortex at opposing cell poles (Fig. 4B). First, polar relaxation will likely occur as an

indirect consequence of the movement of the actomyosin network from cell poles towards the central region of the furrow at anaphase[105]. It is interesting to note that anaphase cell elongation has been shown to depend on ROK and non-muscle myosin II activity in fly cells[69, 107], both of which are also required for furrow formation, indicating an association between the two processes. In addition, polar relaxation may be induced by additional signals that emanate from the anaphase spindle that are independent of overlapping microtubules and centralspindlin. In previous decades, this type of activity was often attributed to the ability of the growing, plus ends of astral microtubules to induce cortical softening at cell poles[108]. Although the complexity of microtubule-based signals are yet to be resolved (e.g. furrow components accumulate at the tips of microtubules in the absence of microtubule overlap in cells exiting mitosis with a monopolar spindle[109, 110]), it is now clear that the anaphase spindle[106, 111-113] also signals to the cortex via microtubule-independent signals. In support of this, polar relaxation still occurs near anaphase chromatin in cells that lack spindle poles and/or microtubules[106].

Recent work suggests that one or more of the signals may be chromatin-based. In one study in human cells in culture, this was attributed to the action of RCC1, a Ran GEF[112]. It was postulated that the association of RCC1 with mitotic chromatin establishes a local Ran-GTP gradient in anaphase that is high at cell poles and low at the cell centre. Ran-GTP then acts via importin-β to influence the activity and/or localization of a wide variety of actin regulators that carry an NLS (nuclear localization signal)[111, 114]. These include Anillin[115], which is lost from the polar cortex following the approach of chromosomes[112], leading to weakening of the mitotic cortical actomyosin network[116] (as is the case during meiosis[116]). This Ran-GTP pathway, it has been argued, aids spindle positioning by negatively regulating local recruitment of NuMA-LGN[111] and Anilllin[112] when anaphase chromatin in an off-center spindle approaches one cell pole more closely than the other, to ensure equal partitioning of cell mass during symmetric divisions.

In a separate study it was proposed that a kinetochore-localized phosphatase[117], PP1, functions together with a regulatory subunit Sds22 to promote the loss of cortical actomyosin network from the poles of cells during mid anaphase – helping to trigger polar relaxation. In support of this idea, the approach of kinetochores carrying PP1 and Sds22[106] to cell poles is accompanied by PP1-Sds22 dependent dephosphorylation of ERM proteins[51, 52, 81] and the loss of polar actomyosin[118]; prior to the accumulation of actomyosin at the cell midzone. Since PP1 promotes the large-scale reversal of mitotic phosphorylation at mitotic exit[119], aiding inactivation of the spindle checkpoint[120], the rise in PP1 activity at anaphase effectively couples cortical mechanics (that lead to the shape changes) to the change in mitotic state. In addition, since the kinases that act in opposition to PP1 (notably AuroraB and Polo kinase) remain at the centre of the spindle during anaphase, where they localize to regions of microtubule overlap (reviewed in [121, 122]), the anaphase spindle may set up a phosphorylation gradient across the cell – with high levels of kinase-mediated phosphorylation near the spindle midzone and high levels of PP1-Sds22 mediated de-phosphorylation towards the cell poles. In principal, such a phosphorylation gradient could bridge the physical gap between the spindle, the cytoplasm and the cortex; helping to coordinate

events across the cell and to promote polarization of the cortical actomyosin prior to cytokinesis.

Completion of division

At this stage, the contraction of the centrally positioned actomyosin ring transforms the cell into a dumbbell shape. Although this ring usually closes in a symmetrical fashion in cells in culture (Fig 5A), the ring moves apically as it constricts in many epithelia[83, 84, 123] (Fig. 5B). In some fly epithelia, this basal to apical movement of the ring may be a consequence of the lack of actomyosin at the apical surface of mitotic cells[14]. In addition, it has been suggested that this polarized geometry is a simple consequence of tissue mechanics, as the contractile forces that neighbouring cells exert on one another through apically positioned cell-cell junctions resist ring closure in fly epithelia[83, 84, 124]. The opposing forces of ring closure and tissue tension have other consequences. In fly epithelia, they drive the physical separation of the actomyosin ring and the associated plasma membrane from the existing junction (Fig 5B), leading to the formation of a straight, new planar interface between the two daughter cells[123]. The formation of this new interface may be aided by the reactivation of the Arp2/3 complex at the cortex close to the midbody[123], which may promote the delivery of membrane from vesicular stores[125] and may generate pushing forces on opposing membranes aiding the formation of a new cell-cell interface[126]. At the same time, these forces may help trigger abscission, since a loss of tension across the division site has been shown to be a pre-requisite for the physical separation of animal cells in culture[127].

The completion of the division process also requires disassembly of the actomyosin ring and the microtubule-rich midbody [G]. This is associated with the binding of RacGAP1 to the membrane, the recruitment of FIP3 in place of Ect2[128], Ect2 degradation[129], and the loss of PIP2[130], Rho and actin from the midzone[98, 131]. Following disassembly of the microtubule-based midbody, which is regulated by centrosomes[132], mitotic kinases and Spastin[133, 134] ESCRTIII is recruited to the thin membrane tube that connects daughter cells, where it triggers abscission as cells enter G1 (reviewed in [10, 134]). Importantly, an abscission checkpoint has been proposed to act as an additional layer of control to coordinate division with chromosome segregation, as lagging chromosomes act through Aurora B to a delay ESCRTIII-mediated abscission[135, 136].

Finally, as cells return to an interphase state, the nuclear-cytoplasmic compartment boundary is re-established through the re-assembly of nuclear pores and ESCRTIII-mediated remodelling of the nuclear envelope[137]. In addition to its other effects[138], this restoration of the nuclear compartment boundary is likely to insulate the cortex from the direct influence of chromatin-based signals to prevent further structural changes in the cell. Further, proteins containing NLS like Anillin, RacGAP1 and Ect2 that drive actin-dependent changes in mitotic cell shape can then return to the nucleus to re-establish interphase cell organization. Simultaneously, an increase in the activity of Rap1 is thought to promote the assembly of cell-substrate adhesions[12]. This involves Aurora B-mediated

phosphorylation of targets including FHOD1, a formin, and the microtubule binding protein EB3[139], which promote cell re-spreading.

Conclusions and perspectives

Cell division necessitates a rapid, near complete remodeling of cell shape, structure and polarity, which functions to partition the entire suite of cellular components into two daughter cells. This is an incredibly complex physical process, especially for animal cells dividing in the context of a multicellular tissue, and must be precisely regulated. During entry into mitosis, events such as adhesion remodelling, assembly of a cortical actomyosin network, microtubule remodelling and spindle morphogenesis are coordinated by entrainment to the same clock — that is, increasing levels of mitotic kinase activity — as well as by the dramatic biochemical changes that accompany the sudden loss of the compartment boundary that separates the cytoplasm and nucleus at the transition from prophase into prometaphase. Thus, for most purposes, the microtubule and actin-based structures that are essential for chromosome segregation and cell division, respectively, are constructed in parallel. Nevertheless, a successful division requires spatial coordination of the cortex and the spindle, necessitating crosstalk between these two systems. This communication is essential to ensure that the metaphase spindle is properly oriented with respect to interphase and mitotic cell shape, cell-cell junctions and tissue tension; and that the cytokinesis ring is positioned so that it bisects the anaphase spindle. Finally, the return to interphase organization also requires both temporal (cyclin degradation, the loss of mitotic kinase activity and the increasing dominance of mitotic phosphatases) and spatial control (gradients of activity, and re-establishment of compartment boundaries). As an example of this, the nuclear envelope seals from the poles to the centre[138]; enabling lagging chromosomes to be gathered up into daughter nuclei to prevent chromothripsis[140].

Whilst some of the events of cell division are now relatively well understood, much remains to be discovered. The precise mechanisms underlying spindle-cortex crosstalk remains an interesting and unsolved problem. In addition, little is known about the mechanisms used to ensure that mitosis and cell division are mechanically robust[62], the precise segregation of various organelles during division[141-143], and the mechanisms used to bias these processes to achieve the same precision during asymmetric divisions. Finally, very little is known about the evolution of eukaryotic cell division, which appears to have its origins in archaea[2, 144]. Because progression through mitosis is accompanied by rapid, sequential and wholesale changes in cell structure, the study of cell division provides researchers with an ideal window into the dynamic regulation of cell architecture. The bulk of these events are driven by changes in the activities of a small set of kinases and phosphatases making it relatively easy to identify the switches and molecules that underpin these changes. Moreover, this type of research is being aided by the advent of live super-resolution microscopy, which allows changes in cell architecture to be imaged at an unprecedented level, together with CRISPR/Cas9-mediated genome editing and optogenetics, which provide new tools to test hypotheses and reveal the underlying molecular mechanisms. Therefore, although the mass of cell division literature (some of which we have been able to summarize

here) can seem overwhelming and the field saturated, there are rich untapped seams waiting to be mined and the perfect tools with which to do it.

Display items:

BOX1 Spindle orientation guided by cortical cues
Components of the molecular machinery used to guide the orientation of the mitotic spindle in response to cues in the cortex were first identified in genetic screens. These include a small set of highly conserved proteins, Galphai, LGN/Pins and NuMA/Mud. Following nuclear envelope breakdown, these proteins localize to the cell cortex where they recruit the minus-end directed motor dynein/dynactin[145]. As dynein walks along astral microtubules, towards the minus ends and the spindle pole, it generates the forces required to reposition the spindle[146].

Although many actin regulators (for example afadin, myosin X, LIM kinase) have been implicated in spindle positioning, it is not clear whether individual actin regulators relay specific information to guide polarization of the cortex, as is often suggested, or function collectively in construction of the mitotic cortical actin network. The latter is important, since the loss of cortical actin leads to the accumulation of cortical polarity cues together with dynein at centrosomes[147]. In this case, it is clear that actin is not needed for dynein to generate pulling forces but to provide a physical platform on the plasma membrane upon which polarity cues can be stably bound and read. This situation is further complicated by the fact that spindle assembly depends on mitotic cell geometry - another actin-dependent process.

Figures:

Figure 1: Cell shape changes during mitosis
As animal cells become committed to mitosis their internal architecture and physical connections to the environment are rapidly remodeled, causing cells to assume a typical, spherical mitotic cell shape. These structural changes are reversed during mitotic exit as cells divide and revert to their initial interphase morphology. These structural changes are coupled to the cell cycle clock, and many are directed by changes in protein phosphorylation driven by the rise and fall of Cdk1/cyclinB activity.

A. As the levels of Cdk1/CyclinB rise (red line), the cell begins the assembly of its cortical acto-myosin network (blue line), both of which are accelerated by loss of the nuclear-cytoplasmic compartment boundary. The cortical actin network that is assembled upon entry into mitosis is largely independent of the activity of the actin nucleator Arp2/3 (purple line), which is inhibited at the onset of prophase. At anaphase, this mitotic acto-myosin network is remodeled to generate a contractile ring (green line). In an epithelium, this, along with the reactivation of Arp2/3, generates two daughter cells separated by a straight new cell-cell interface.

B. At the onset of mitosis, cells stop moving and reduce their adhesions to the substrate with the rising levels of Cdk1/Cyclin B activity. Centrosomes separate (green), cells begin to assemble the cortical actomyosin network and increase in height to round up. Rounding is expedited by nuclear envelope permeabilization. Chromosomes (blue) then align at the metaphase plate. Once all the chromosomes are attached to the spindle and the spindle assembly checkpoint has been satisfied, the activation of the APC triggers the cleavage of cohesin, allowing sister chromatids to move to the opposite poles, and the degradation of cyclinB leading to a collapse in Cdk1 activity. This is accompanied by the relaxation of the cortical acto-myosin network at cell poles (red) and by the concentration of acto-myosin at the cell centre, to form a contractile ring. As cells divide, chromatids begin to decondense, and the nuclear envelope and lamina reform.

Figure 2: Cortical actomyosin network reorganization during mitotic rounding

A. Isolated cells in culture: Several factors contribute to the changes in the actomyosin network that aid in mitotic cell rounding. The activity of the actin nucleator Arp2/3 is reduced, while that of negative regulators of actin turnover increased. The increasing levels of Cdk1-CyclinB activity leads to progressive loss of integrin based cell-substrate adhesion. The rise in nuclear Cdk1-CyclinB also leads to the export of Ect2 from the nucleus, activating RhoA and its targets, Dia, Rok and Myosin (red-blue), leading to the formation of a contractile actomyosin network. Following its mitotic activation, the Slik kinase phosphorylates and activates ERM proteins (Moesin in flies), tethering this actomyosin network to the plasma membrane (yellow). The cells retain actin rich retraction fibers, which both direct spindle orientation and help in cell re-spreading following division.

Figure 3: Forces experienced by the cells during mitotic rounding

An isolated cell dividing in culture (A) and a cell dividing in an epithelium (B) experience a plethora of forces (indicated by arrows). At metaphase, these are balanced to give cells their characteristic stable rounded shape. Cells retain retraction fibers, which tether them in place. Osmotic swelling causes an increase in the internal pressure (blue), which is counteracted by a contractile acto-myosin network. The actomyosin network is tethered to the cortex by active ERM proteins. Cortically localized dynein motors then pull on astral microtubules to align the spindle and to determine the plane of cell division. (B) In comparison to cells in culture, cells in an epithelium have additional complexities, as they are surrounded by neighbouring cells that constantly push back on the dividing cell. In addition, they retain their adherens junctions during cell division.

Figure 4: Polar relaxation and coupling of chromosome segregation to cytokinesis

A. As the sister chromatids begin to separate and Cdk1-cyclin B activity declines at the onset of anaphase, the CPC (a complex containing active Aurora B) relocalizes from the kinetochores to the spindle midzone, a process that is dependent on Mklp2, a component of centralspindlin. Centralspindlin also

recruits Ect2 to the spindle midzone. By contrast, the phosphatase, PP1/Sds22, remains on the kinetochores.

B. As the sister chromatids begin to approach the poles, the cell elongates. Anaphase cell elongation is likely regulated by a combination of forces and signals – many of which emanate from the anaphase spindle. Following chromosome separation, the activity gradients of the CPC and PP1 separate to oppose one another – leading to an opposing kinase-phosphatase gradient with PP1 dominating at the poles and the CPC at the midzone. This, together with the local Ran-GTP gradient generated by the association of the Ran GEF RCC1 with mitotic chromatin, likely facilitates the local dephosphorylation of ERM proteins and the loss of Anillin at cell poles, establishing a gradient of acto-myosin across the cell that helps to drive cell division.

Figure 5: Structural changes at mitotic exit

A. During mitotic exit, the actomyosin network used to drive mitotic rounding is remodeled to form an acto-myosin ring that is used to drive cytokinesis. As cells exit from mitosis, the nuclear lamina beings to reform from the poles thus allowing time for lagging chromosomes to become incorporated into the nascent nucleus. ESCRTIII mediated abscission leads to the formation of two daughter cells, which form new cell-substrate adhesions and respread following abscission.

B. In cells dividing in an epithelium the acto-myosin ring moves from basal to apical as it contracts. Additionally, these cells need to form a new cell-cell interface. It has been proposed that this is driven by reactivation of Arp2/3, which pushes adjacent cell membranes together. Finally abscission mediated by ESCRTIII leads to the formation of two daughter cells.

[Au: Please write a one-sentence explanation, under each highlighted reference, to very briefly explain what the main finding of the work is]

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Key points

- Animal cells undergo dramatic changes in their shape as they progress through mitosis and division. This process begins with rounding soon after cells enter mitosis.

- Mitotic rounding is an active process that depends on a combination of de-adhesion, actomyosin-based contraction and osmotic swelling.

- Adhesion remodelling is essential for normal cell rounding during mitosis and is triggered by inactivation of the small GTPase Rap1.

- Entry into mitosis triggers a dramatic change in actin organization and dynamics, leading to the assembly of a formin-based actomyosin network that is tethered to the overlying membrane via activated ERM proteins.

- The changes in actin organization and cell shape that accompany mitotic exit are driven by a combination of actomyosin ring assembly together with polar relaxation, which can be induced by chromatin based signals.

- While remodelling of the actin and microtubule cytoskeletons appears to be relatively independent during mitotic entry, spindle elongation and cytokinesis must be tightly coupled in space and time to ensure precise cell division.

Competing interests statement

The authors declare no competing interests.

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biological form. For many years this research has focused on the control of cell shape through the cell cycle in both normal and cancer cells.

Glossary

Astral microtubulues
A subpopulation of dynamic microtubules, which originate from the centrosomes but do not attach to the chromosomes. They are present only during mitosis.

Retraction fibers
Actin rich fibers retained by cells as they round up as the enter mitosis. These hold the cells in place and are thought to guide spindle orientation to determine the division axis.

Non-muscle Myosin II
An ATP-dependent motor protein that forms large bipolar "minifilaments". In non-muscle cells, these both crosslink actin filaments and walk along actin filaments to drive network contraction.

Adherens junctions
Protein complex consisting of cadherins and catenin, involved in cell-cell junctions in epithelial and endothelial cells.

Tight junctions
Cell-cell junctions, consisting of occludins, claudins and associated proteins, that bring membranes of adjacent cells into close proximity to form a permeability barrier. They are positioned apical to the adherens junctions in vertebrates and (as septate junctions) basal to adherens junctions in most invertebrate epithelia.

Arp2/3 complex
Protein complex consisting of seven polypeptides (including actin-related protein2 - arp2 and actin-related protein 3 - arp3), which regulates the nucleation of branched actin filament networks from the filament minus end.

Formins
Proteins defined by the presence of a formin homology 2 (FH2) domain, nucleating actin filaments from growing (+) ends to generate parallel or antiparallel filaments that are a good substrate for non-muscle myosin II.

Importin
A protein complex that transports proteins containing nuclear localization sequence (NLS) into the nucleus. The complex consists of importin-α and importin-β.

Spindle assembly check point
The checkpoint that delays anaphase onset until chromosomes are properly attached to the metaphase spindle.

Anaphase promoting complex

A E3 ubiquitin ligase which targets specific cell cycle proteins for degradation by the 26S proteasome upon satisfaction of the spindle assembly checkpoint to trigger the separation of sister chromatids and the transition to anaphase.

Acto-myosin contractile ring
A contractile structure composed of actin and myosin filaments that deforms the plasma membrane to drive cytokinesis.

Centraspindilin complex
Protein complex which localizes to overlapping antiparallel microtubules in anaphase to recruit proteins that drive local actomyosin ring formation, so that closure of the ring bisects the spindle.

The Chromosome Passenger Complex
Protein complex consisting of kinase Aurora B and its regulatory subunits Borealin, INCENP and Survivin, which relocalize from kinetochores to overlapping antiparallel microtubules at the midzone of cells in anaphase, to regulate actomyosin ring formation and, later, abscission.

Midbody
Also called Flemming body, is a transient structure that connects the two daughter cells towards the end of cytokinesis. It functions to control abscission, a process which leads to the physical separation of the two daughter cells.