Review

Coordinating cell polarity with cell division in space and time

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Decisions of when and where to divide are crucial for cell survival and fate, and for tissue organization and homeostasis. The temporal coordination of mitotic events during cell division is essential to ensure that each daughter cell receives one copy of the genome. The spatial coordination of these events is also crucial because the cytokinetic furrow must be aligned with the axis of chromosome segregation and, in asymmetrically dividing cells, the polarity axis. Several recent papers describe how cell shape and polarity are coordinated with cell division in single cells and tissues and begin to unravel the underlying molecular mechanisms, revealing common principles and molecular players. Here, we discuss how cells regulate the spatial and temporal coordination of cell polarity with cell division.

Cell division: getting space and time right

To achieve successful cell division, cellular events must be tightly regulated both spatially and temporally. The genome must be fully replicated before the cell enters mitosis and chromosomes properly attached to the mitotic spindle before segregation begins. Cytokinesis cannot start unless the chromosomes have separated, and it must occur perpendicular to the axis of chromosome segregation. These events are coordinated with cell growth, cell polarity and, in multicellular organisms, tissue architecture. Disruption can result in aneuploidy, cell death, abnormal proliferation and developmental disorders [1–3]. To ensure successful coordination, cells have multiple, often redundant, strategies: (i) coupling of events such that a later event does not occur until the previous one is completed, (ii) checkpoint surveillance mechanisms that sense whether an event has been completed properly, and (iii) feedback mechanisms in which the output of an event can either be reinforced (positive feedback) or inhibited (negative feedback).

Although our knowledge on cell cycle regulation has progressed enormously, we still know little about how cell shape and polarity influence cell division. In recent years, work in various model systems has started to uncover the molecular mechanisms behind the crosstalk between these processes.

Here, we describe how cell shape and polarity influence the position of the spindle and, therefore, the site of cell division in unicellular and multicellular organisms. We then discuss how shape and polarity exert temporal control over cell division and, conversely, how mitotic kinases control cell polarity. The emerging data point to regulation via feedback mechanisms to ensure tight coordination of these processes.

Spatial regulation of cell division

In non-polarized cells, proper cleavage-plane orientation ensures that the cytoplasm is accurately partitioned and that cells develop in the correct environment (Figure 1a). In polarized cells, cleavage-plane orientation also determines whether the division will be symmetric, producing identical daughters, or asymmetric, producing daughters with different fates (Figure 1b). Below we discuss how cells ensure spatial coordination of their division axis.

Symmetrically dividing cells

Typically, the position of the mitotic spindle determines the site of cytokinesis, and physically moving a spindle can change the cytokinesis plane [4,5]. Both astral and midzone microtubules are required to determine the cleavage-plane site [6]. In the absence of polarity cues, cells tend to divide perpendicularly to their longest axis, however, this is not a universal rule. In a recent study, individual sea urchin eggs, which are non-adherent and divide symmetrically, were placed into microfabricated wells with defined shapes to study how geometry influences the position of the cleavage plane [7]. The nucleus positioned in the centre of the cell in a microtubule-dependent manner and stretched along a precise axis; after nuclear envelope breakdown the spindle elongated along the same axis, dictating the position of the cleavage furrow. For most shapes, the axis of spindle elongation corresponded to the long axis of the cell, but for particular shapes this rule did not apply, suggesting that shape alone can determine the plane of division, at least in embryonic cells (Figure 2a).

Molecular insight into how cells might sense their shape and find their middle comes from studies in the fission yeast *Schizosaccharomyces pombe,* rod-shaped cells that grow by tip extension and divide in the middle. After cell division, daughter cells elongate only at the old end but start growing at the new end after DNA replication. The position of the cleavage plane is regulated by a positive signal emanating from the nucleus and an inhibitory signal emanating from the cell tips [8–11]. Both signals regulate the localization of the anillin-like protein Mid1 (Figure 2b) [12,13]. In interphase, Mid1 localizes in the nucleus and to medial cortical patches (termed cortical nodes) [13,14]. This latter localization depends on the SAD kinase Cdr2

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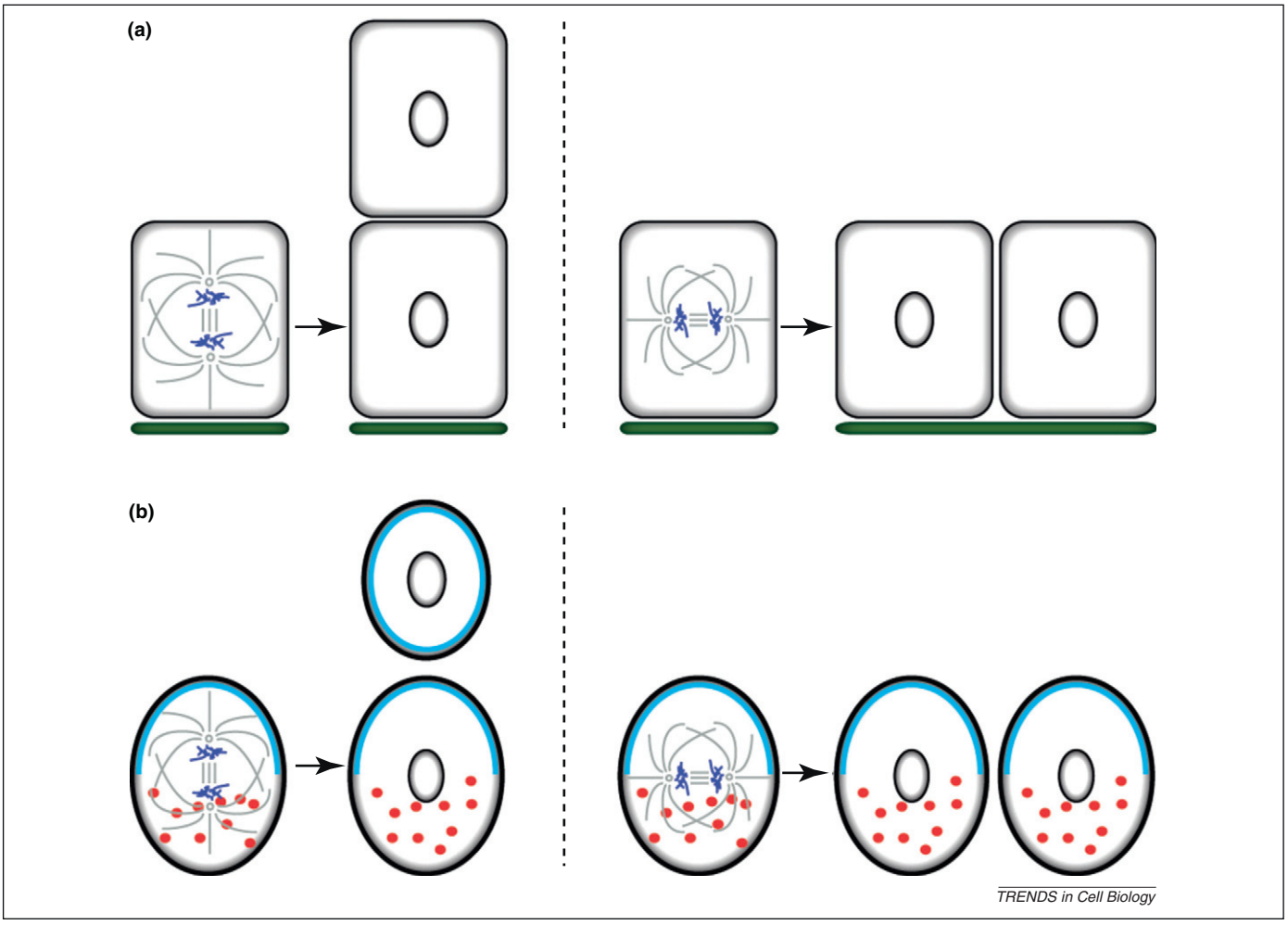

Figure 1. Spindle positioning in nonpolarized and polarized dividing cells. **(a)** A non-polarized, symmetrically dividing cell determines the fate of its daughters by orienting the mitotic spindle. Depending on the orientation, daughter cells will assume different positions in the tissue after division. **(b)** In polarized cells (red dots: cytoplasmic determinant; blue line: cortical determinant), the orientation of cell division can either lead to an asymmetric division whereby the two daughter cells are different in fate (left), or to the birth of two identical, but polarized cells (right).

[15,16]. The DYRK family kinase Pom1 prevents Mid1 from spreading towards the non-growing end. This depends on the ability of Pom1 to phosphorylate Cdr2 (below and Figure 2b). An additional unknown signal acts from the growing end [10,11,17,18] (Figure 2b). In G2/M, phosphorylation of nuclear Mid1 by the mitotic kinase Polo-like kinase (Plk1) triggers Mid1 nuclear export and its ability to recruit contractile ring components to the middle of the cell, temporally coordinating mitosis with cytokinesis [19]. Therefore, microtubule-dependent positioning of the nucleus [20] and inhibitory signals at the cell tips dictate the position of the division plane (reviewed in [21]).

Both sea urchin and fission yeast regulate cleavage-plane positioning based only on cell shape, independently of any external signal. By contrast, symmetrically dividing animal cells establish cell matrix adhesions or contacts with neighboring cells and, *in vivo*, coordinate their division plane with tissue architecture. *In vitro*, adhesion to the extracellular matrix (ECM) plays a major role in orienting the division plane [22]. HeLa cells grown on coverslips with patterned shapes of fibronectin (e.g. rectangular, discoid, triangular) assumed the corresponding shapes during interphase. In mitosis, the spindle oriented parallel to the substrate and according to the interphase shape despite cell rounding: along the long axis in the majority of rectangular cells, random in the discoid cells, and parallel to the hypotenuse in triangular cells. Additional experiments suggested that shape is not the only factor influencing spindle orientation. Cells grown on different ECM patterns that all led to a squared shape had mitotic spindle orientations based on ECM distribution, not shape (Figure 2c). The ECM orients the mitotic spindle by influencing the distribution of cortical cues. These cues originate in interphase cells and are maintained during mitosis where they associate with retraction fibers, membrane tubes that maintain cell connections with the ECM at cell rounding. The forces applied by retraction fibers provide a cortical polarizing signal that is essential to orient the spindle [23].

Is ECM-dependent spindle orientation relevant *in vivo*, where orientation of the division plane is crucial to develop and maintain tissue architecture? *Drosophila* cells vary their cleavage-plane position in response to the shape of neighboring cells to maintain the geometric regularity of epithelia [24]. This might result from the distribution of cell–cell contacts during interphase, similar to the behavior of cultured cells. Integrins – cell adhesion receptors – are required for spindle orientation in several systems [25,26]. In the mammalian epidermis, integrins control

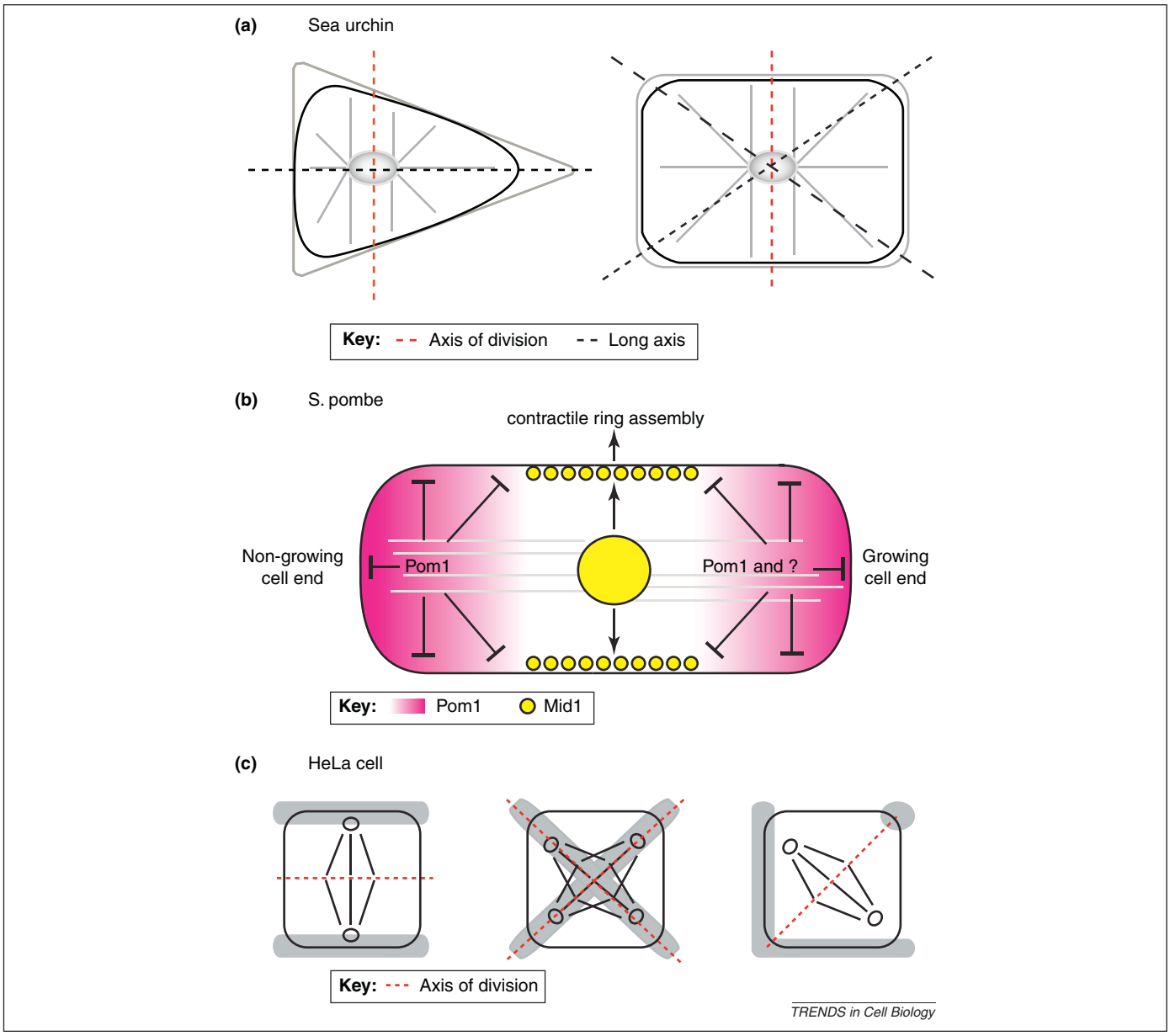

Figure 2. Cell geometry regulates the position of the cytokinesis furrow. (a) Sea urchin one-cell embryos (black lines) are placed in chambers of different shapes (grey lines). Microtubule-generated forces position the nucleus centrally and promote cleavage-plane positioning. The 'long-axis rule' predicts that cells divide perpendicular to the longest axis in the cell. The left-hand cell follows this rule, however, the right-hand cell does not (longest cell axis, black dotted line; axis of cell division, red dotted line). **(b)** In *S. pombe*, two regulatory signals, both acting on the anillin-like protein Mid1 (yellow circles), position the cytokinesis furrow. A positive signal comes from the nucleus (large yellow circle), which is centrally located in a microtubule-dependent fashion (grey lines). A second Pom1-dependent negative signal from the poles (pink gradient) inhibits lateral spreading of Mid1. From the growing end an additional unknown signal participates in this inhibition because in *pom1* mutants Mid1 only spreads to the nongrowing end. **(c)** HeLa cells (black lines) constrained on twin bars, cross, or L-dot fibronectin micropatterns (grey shapes) all assume a square shape but have different spatial distribution of attachment sites to the ECM and elongate the spindle (black rhomboid) according to these attachment sites (axis of division, red dotted line).

asymmetric apical localization of polarity proteins and LGN (Box 1). This correctly aligns the spindle and promotes skin stratification and proper differentiation [27]. Although these are asymmetrically dividing cells, it is likely that adhesion to the ECM regulates the distribution and possibly activity of LGN and NUMA, major spindle-positioning regulators, in all cells.

Therefore, shape alone has the ability to control the position of the cleavage plane. Cells in tissues can override this, however, because they must coordinate their site of division with neighboring cells or with the axis of the tissue to maintain proper architecture.

Asymmetrically dividing cells

In asymmetrically dividing cells, polarity orients the division axis and overcomes shape or cell adhesion information [28]. Here, we summarize two polarized systems in which spindle positioning has been extensively characterized, revealing common principles: the *Drosophila* neuroblast and the *Caenorhabditis elegans* embryo (Box 1, Figure 3; [29,30] for review).

In *Drosophila* neuroblasts, apico-basal polarity regulates spindle orientation via a set of conserved proteins (Box 1, Figure 3a). Neuroblasts have a second pathway, however: the telophase rescue pathway. In Inscuteable

Box 1. *C. elegans* embryos and *Drosophila* neuroblasts divide asymmetrically

Cell polarity and asymmetric cell division are essential features of many, if not all, animal cells. Together they ensure the proper inheritance of localized molecules essential for cell diversity. A common set of molecules regulate polarity and spindle positioning in *C. elegans* embryos and *Drosophila* neuroblasts.

Sperm entry defines the first axis of polarity, the anterior–posterior (A–P) axis, in *C. elegans* embryos. Subsequent to sperm entry, six PAR proteins (for 'partitioning-defective'), as well as the atypical protein kinase C (PKC-3), localize at the cortex along the A–P axis. The PAR-3–PAR-6–PKC-3 complex localizes at the anterior and PAR-2 and PAR-1 at the posterior. As mitosis progresses, the mitotic spindle initially forms in the center of the embryo and is then displaced towards the posterior pole by cortical pulling forces exerted on astral microtubules under the regulation of PAR polarity. Two Gα subunits of heterotrimeric G proteins, their receptor-independent activators GPR-1/2 and the coiled-coil protein LIN-5, control cortical pulling forces. GPR-1/2 and LIN-5 become enriched at the posterior cortex after nuclear envelope breakdown (Figure 3b). This enrichment directs dynein-mediated forces that act on the posterior pole, resulting in asymmetric spindle positioning. The first embryonic division segregates the anterior PAR complex to the anterior larger cell, whereas PAR-2 and PAR-1 are restricted to the smaller posterior cell.

Drosophila neuroblasts become polarized during early prophase. Bazooka (PAR-3), Par6 and the atypical kinase aPKC (PKC-3) together with the adaptor protein Inscuteable (Insc) localize at the apical plasma membrane. Subsequently, the Notch-Delta inhibitor Numb localizes to the basal cortex. After apico-basal polarity is established, the mitotic spindle is set up along the polarity axis. Insc recruits and links the apical PAR complex to Gα and its activator PINS (Partner of Inscuteable, GPR-1/2 in *C. elegans*), which in turn ensures correct orientation of the mitotic spindle via the microtubule-associated dynein-binding protein MUD (functional homolog of LIN-5). The spindle is asymmetric and cytokinesis generates a larger apical cell that remains a neuroblast and a smaller basal ganglion mother cell (GMC). In mammalian cells, heterotrimeric G proteins together with LGN (PINS/GPR-1/2) and Numa (Mud/LIN-5) also regulate spindle orientation.

mutants (Box 1) the mitotic spindle can induce a polarity axis. This rescue occurs in late mitosis, is mediated by interaction of the mitotic spindle with the cortex and requires PINS, the tumor suppressor Discs Large (a PSD95 family member) and Kh63 (a kinesin). PINS and Gα become localized in a crescent close to a spindle pole resulting in polarization of the cell cortex relative to the mitotic spindle, and this allows asymmetric cell division [31]. Whether both poles can polarize the cortex is unknown.

A similar rescue mechanism was proposed in *C. elegans* [32] and was recently described. Similarly to *Drosophila* neuroblasts, spindle positioning is regulated by anterior–posterior polarity in the one-cell embryo (Box 1, Figure 3b). Conserved anterior and posterior PAR proteins occupy about half of the embryonic cortex. PAR domain length is altered before cytokinesis in particular mutants (too short or too long) but it is corrected during furrow ingression [33,34]. Abnormally small PAR domains, whether anterior or posterior, expand to reach the site of cell division. PAR domain correction depends on Gα, GPR-1/2 and LIN-5, and occurs possibly via Gα control of microtubule–cortex interactions. These interactions drive actomyosin-based cortical reorganization, which repositions the PAR boundary. Thus, in *C. elegans* early embryos, polarity controls the position of the mitotic spindle, which determines the site of furrow ingression. In turn, the cytokinesis furrow contributes to positioning the polarity boundary, regulating proper segregation of PAR proteins (Figure 3b).

These rescue mechanisms provide a robust feedback pathway that helps ensure the success of asymmetric cell divisions. An interesting question is whether these cells delay mitosis to correct polarity defects, suggesting a polarity checkpoint (see below).

The plane of cytokinesis can also be determined in a spindle-independent but polarity-dependent manner. Myosin forms a furrow on the basal cortex of *Drosophila* neuroblasts even in the absence of a mitotic spindle [35]. In *mud* mutants (Box 1), where the spindle is orthogonal to the normal polarity axis, myosin is still enriched at the basal cortex. These cells initiate two furrows: one basal constriction that often results in an anucleated basal 'polar body', and a spindle-driven furrow. Basal asymmetric enrichment of myosin is dependent on PINS revealing a polarity-dependent and spindle-independent mechanism for furrow ingression (Figure 3c). A similar mechanism exists in *C. elegans* neuroblasts [36]. In these cells the mitotic spindle is initially centred but elongates asymmetrically to generate a smaller anterior cell. Myosin is asymmetrically enriched at the anterior, and inactivation of myosin leads to a more symmetric division. Myosin asymmetry depends on polarity, as in *Drosophila* neuroblasts. The authors propose that asymmetric cortical tension driven by myosin, rather than spindle positioning, defines the cleavage furrow.

Common concepts arise from the examples in this section: shape and polarity control spindle positioning, which in turn regulates cleavage-plane position in many cells. In addition, reciprocal interaction between cell polarity and furrow positioning increases the robustness of the system by ensuring the coordination of these two processes (Figure 3c).

Temporal regulation of cell division: linking cell polarity to cell cycle progression

The decision of when to divide is as important as where to divide. The timing of division determines the position of the cell in a developing organism. In addition, polarized cells only divide after polarity is fully established, in order that polarized factors are correctly segregated to daughter cells (Figure 1b). Here, we discuss findings in yeast demonstrating that the timing of cell division is coupled to cell size and morphology. We then review a metazoan polarized system where cell polarity regulates cell division timing. Finally, we review how mitotic kinases are implicated in cell polarity regulation.

Regulation of cell division timing by cell geometry in yeast

Yeast cells enter mitosis only after reaching the proper cell size. What is the correct size and how is it measured? Importantly, how is cell growth timed with cell division? In *S. Pombe*, Cdk1/Cdc2 is maintained in an inactive state during G2 by Wee1-dependent phosphorylation. Dephosphorylation of Cdk1/Cdc2 by the Cdc25 phosphatase triggers entry into mitosis [37]. In *wee1* mutants, cells enter

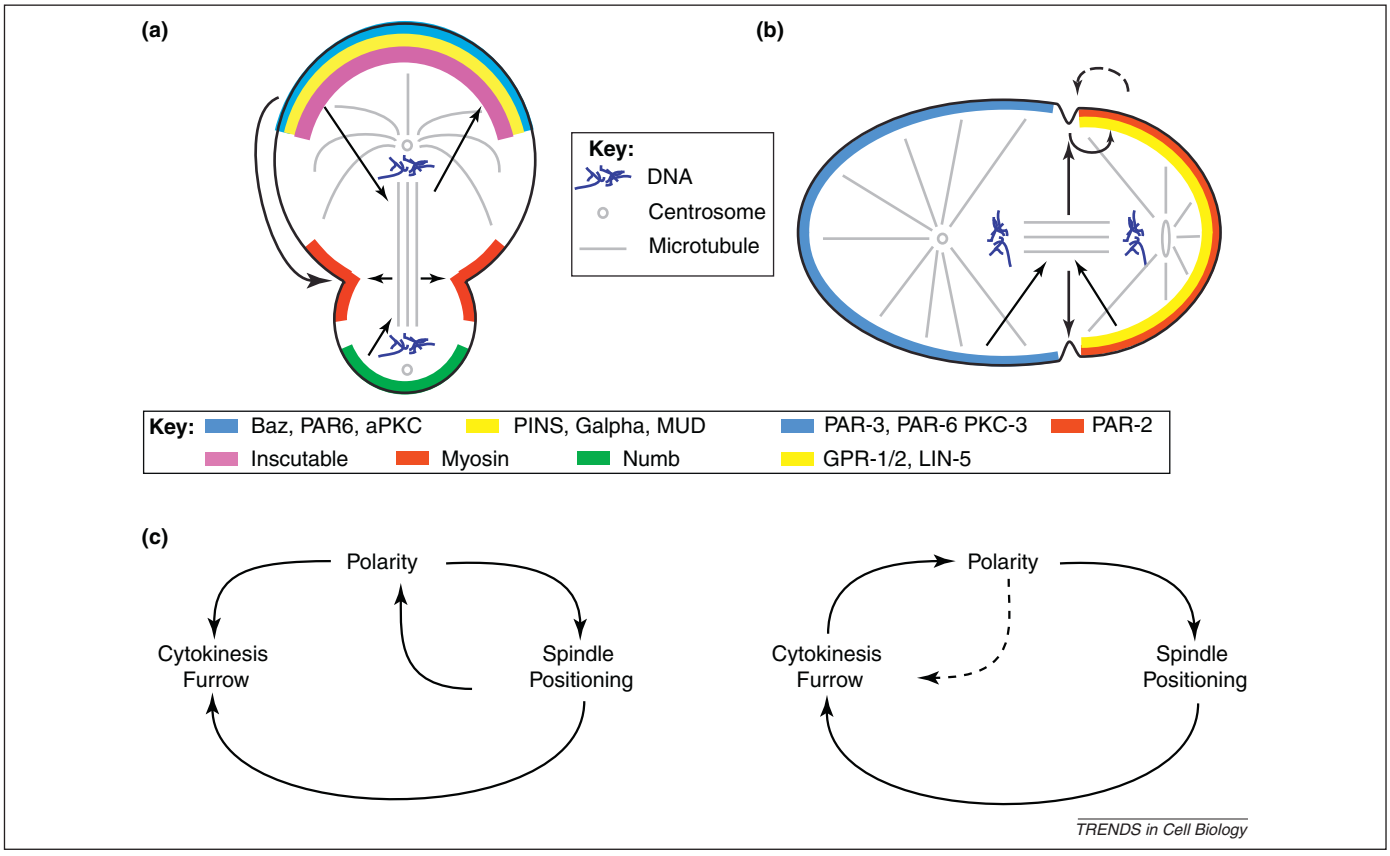

Figure 3. **Crosstalk between cell polarity, spindle positioning and cytokinesis furrow ingression**. **(a)** *Drosophila* neuroblasts are polarized, with Baz, PAR6 and aPKC complex (blue), PINS, Gα and MUD (yellow), and Inscuteable (pink) at the apical cortex and Numb (green) at the basal cortex. Cortical polarity controls the position of the mitotic spindle via PINS (arrow from the apical crescent to the spindle), which in turn dictates the position of furrow ingression. In the absence of a mitotic spindle, PINS can asymmetrically position myosin (red) for asymmetric cell division (arrow from the apical crescent to myosin at the furrow ingression). If an axis of polarity is not properly established, interaction of the mitotic spindle with the cortex allows the correct localization of PINS and Gα in a mechanism referred to as telophase rescue (arrow from the spindle to the apical crescent). **(b)** The one-cell *C. elegans* embryo is polarized, with PAR-3, PAR-6 and PKC-3 at the anterior (blue) and PAR-2 (red) at the posterior. PAR polarity controls the asymmetric enrichment of GPR-1/2 and LIN-5 (yellow) at the posterior. Gα, GPR-1/2 and LIN-5 position the spindle, which determines the site of cytokinesis furrow ingression (arrows from the spindle to furrow ingression). In cells where the PAR domain length is altered, the cytokinesis furrow, via Gα, can reposition the PAR domain to match the site of cell division (arrow from furrow to polarity). In *C. elegans*, neuroblast polarity asymmetrically positions myosin for polarized division. Whether this is true in the embryo is unknown (dotted line). **(c)** Schematic representation of the crosstalk between polarity, spindle positioning and furrow positioning.

mitosis precociously, indicating a role for Wee1 in the coordination between size and division [38]. Cdk1 and Wee1 use a Pom1 gradient as a spatial cue for sensing cell size [16,18]. Wee1 localizes at medial cortical nodes with its inhibitor Cdr2. By contrast, Pom1 forms a gradient emanating from the tips. In short cells the Pom1 gradient overlaps with the medial cortical regions allowing Pom1 to inhibit Cdr2. As a result, Wee1 is active and entry into mitosis is delayed. As the cell elongates, Cdr2 is released from the inhibitory influence of Pom1 and inactivates Wee1, allowing entry into mitosis (Figure 4). Pom1 is also important for controlling division-plane positioning, as described above. Using the same molecules to regulate the timing and position of division provides a robust and efficient means of coordinating these events. Additional mechanisms are involved, however, because *S. pombe* cells depleted of Pom1 are only 10% shorter than wild-type cells.

Pom1 gradient formation occurs through dynamic membrane binding of Pom1. Pom1 autophosphorylates and phosphorylated Pom1 is unable to bind to the membrane. The landmark protein Tea4, which localizes to the tips [39], recruits Pom1 and the protein phosphatase Dis2 [40], which locally dephosphorylates Pom1 [41]. This allows binding of Pom1 to membranes at the cell tips. Lateral diffusion of Pom1 away from the Tea4 domain results in autophosphorylation and membrane detachment [41]. Intracellular gradients such as this are crucial for the regulation of mitotic events in many cells [42].

S. cerevisiae has also evolved a mechanism to coordinate the timing of cell division with cell geometry. After a period of isotropic growth, mother cells initiate budding at a defined cortical site. To prevent formation of a binucleated cell, *S. cerevisiae* delays entry into mitosis until a bud has formed in a mechanism called the morphogenesis checkpoint [43], which relies on the SAD-related kinase Hsl1. Hsl1 localizes at the bud neck where it binds septins, small GTP-binding proteins essential for cytokinesis. Once the bud forms, Hsl1 recruits the Wee1-homolog Swe1 and regulates its degradation, allowing mitotic entry. In cells where a bud fails to form, or where the actin cytoskeleton is perturbed, Hsl1 localizes to the bud neck, as in wild-type cells, but remains inactive. As a result Swe1 is not degraded, leading to a delay in entry into mitosis (Figure 4) [44]. What triggers Hsl1 activation is not known but it has been proposed that the local geometry of septins plays a role [43,45]. Although the morphogenesis checkpoint does not appear to use a gradient-based signal for geometry sensing, both fission and budding yeast use a SAD kinase

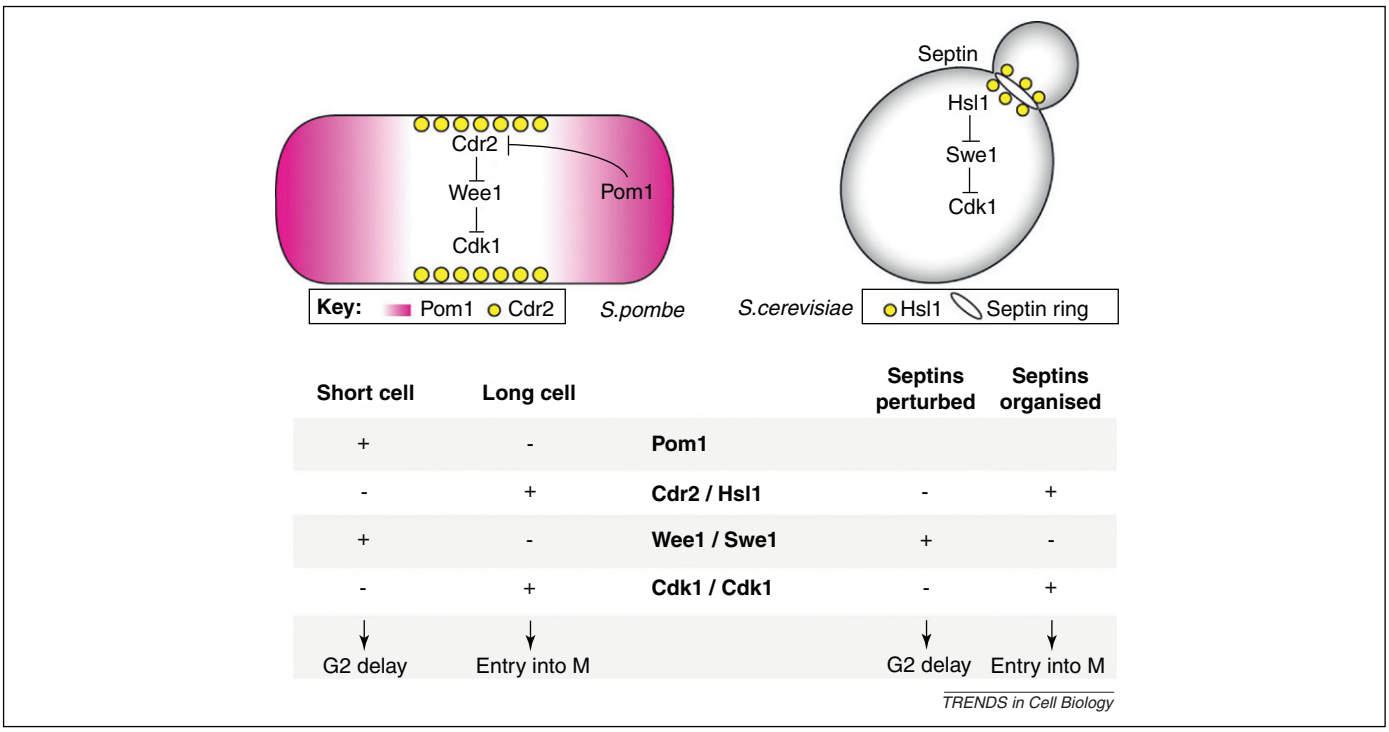

Figure 4. Coordination of cell geometry and cell division in yeast. In *S. pombe* Pom1 (pink) forms a gradient emanating from both poles, whereas the Wee1 inhibitor, the SAD kinase Cdr2 (yellow circles), is localized to a medial cortical region. In short cells, Pom1 inactivates Cdr2. Wee1 phosphorylates and inhibits Cdk1, leading to a G2 delay. In long cells, Cdr2 inhibition by Pom1 is relieved, allowing inhibition of Wee1, activation of Cdk1 and mitotic entry. In *S. cerevisiae* the SAD kinase Hsl1 (yellow circles) is recruited to the bud neck by septins. Upon bud formation, Hsl1 recruits and regulates the degradation of Swe1, thereby promoting entry into mitosis. In cells where septin organization is perturbed, Hsl1 is recruited to the bud neck, where it 'senses' this disorganization. Hsl1 remains inactive leading to a G2 delay.

(Cdr2 and Hsl1) in response to a spatial cue to regulate cell cycle progression.

By contrast, cell cycle progression can also regulate shape/morphogenesis. In *S. cerevisiae*, DNA replication checkpoint mutants have aberrant bud morphogenesis [46]. Consistent with this, the replication stress checkpoint protein Rad53 physically interacts with septins and localizes at the bud neck [47], suggesting a role in coordinating cell morphology with DNA replication. Similarly, completion of DNA replication and polarized growth in *S. pombe* cells are also linked via a recently described mechanism that requires the checkpoint proteins Chk2/Rad53 and Rad3 (ATR) [48]. Why polarized growth is linked to cell cycle progression at the DNA replication level is unknown. Biased segregation of DNA strands has been shown in many cells including *S. pombe* [49]. We speculate that coordination of cell polarity and DNA replication might be essential for such asymmetric segregation of DNA strands.

Regulation of cell division timing by cell polarity in metazoan cells

It is likely that, as in yeast cells, polarity also regulates cell cycle progression in metazoan cells. The mechanisms coupling polarity and cell cycle progression remain elusive, however. Such coordination could be achieved by polarity proteins regulating the activity or localization of mitotic kinases (e.g. Cdk1 regulation by Pom1) or by polarity checkpoints, similar to morphogenetic checkpoints.

C. elegans one-cell embryos divide asymmetrically to form a larger anterior cell (AB) and a smaller posterior cell (P1). Cortical PAR polarity controls enrichment of the cytoplasmic mitotic kinase Polo-like kinase (PLK-1) at the anterior of the one-cell embryo [50–52]. As a result, AB inherits higher levels of PLK-1 at division. The higher PLK-1 levels drive AB into mitosis earlier than its posterior sister P1 [51,52]. *par-3* mutants, which have low PLK-1 levels in both AB and P1, have a synchronous cell cycle at the two-cell stage, resembling the slower timing of the P1 cell. Conversely, *par-2* mutants, which have high PLK-1 levels in both AB and P1, have a synchronous but fast cell cycle, as do AB cells [51,52]. In *C. elegans* embryos, therefore, polarity controls PLK-1 distribution, which in turn regulates asymmetric entry into the cell cycle of AB and P1. These findings demonstrate that PAR polarity can regulate differential cell cycle entry at the two-cell stage. However, whether polarity controls mitotic entry via PLK-1 in one-cell embryos is not known.

Regulation of cell polarity by mitotic kinases in metazoan cells

Several studies highlight a role for mitotic kinases in polarity control. The mitotic kinase Aurora A is highly conserved and implicated in centrosome maturation, spindle assembly and mitotic entry [53]. In Aurora A mutant *Drosophila* neuroblasts, the cell fate determinant Numb fails to localize asymmetrically to the basal cortex and is segregated into both daughter cells [54–56]. Numb localization depends on Aurora A phosphorylation of Par-6, which is found in a complex with the polarity protein Lethal giant larvae (Lgl) and aPKC. Phosphorylation of Par-6 triggers the release of Lgl allowing Bazooka to bind to the Par-6-aPKC complex. aPKC then phosphorylates Numb to regulate its basal localization (Figure 5a) [57].

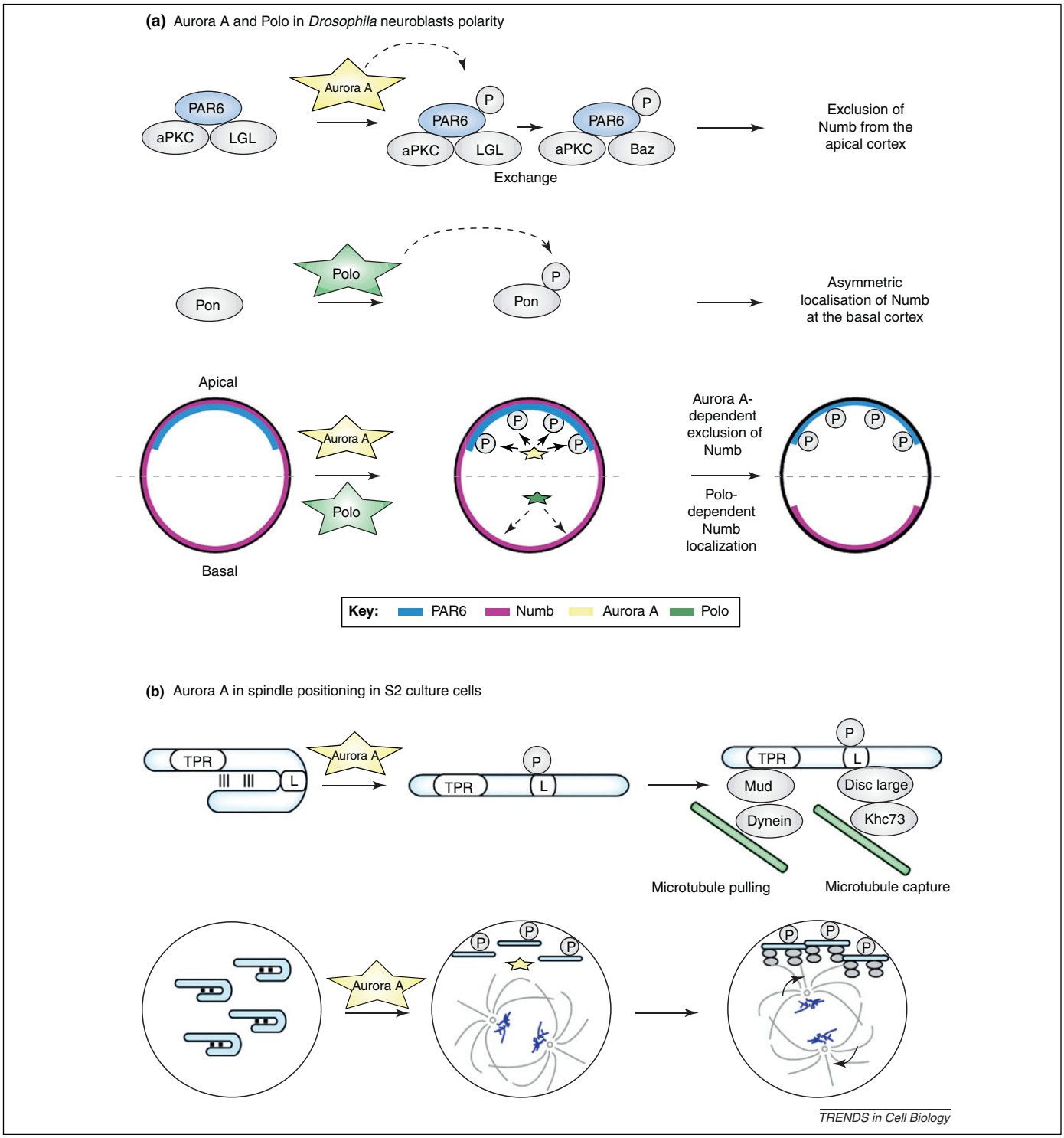

Figure 5. **Polarity and spindle-position regulation by Aurora A and Polo**. **(a)** In *Drosophila* neuroblasts polarity is controlled by Aurora A and Polo. Aurora A (yellow) phosphorylates PAR6 (blue) in the PAR6/aPKC/LGL complex at the apical cortex triggering the exchange of LGL for Baz. aPKC can then phosphorylate and exclude Numb (pink) from the apical cortex. Concomitantly, Polo (green) phosphorylates Pon, which in turn localizes Numb to the basal cortex. **(b)** Aurora A regulates spindle positioning in S2 culture cells. Aurora A phosphorylates PINS (blue) on the linker domain (L). Once phosphorylated, PINS mediates microtubule (green) capture via Discs Large and the kinesin Khc73. Complete PINS function requires its TPR domain, which binds Mud for dynein-dependent microtubule pulling.

Similarly to Aurora A mutants, aPKC and Numb are mislocalized in mutants of the mitotic kinase Polo (*plk1*). Polo phosphorylates Pon (partner of Numb), which localizes Numb asymmetrically to the basal cortex of neuroblasts [58]. Therefore, Numb localization is controlled by two mechanisms: Aurora A- and aPKC-dependent exclusion from the apical cortex, and Polo- and Pon-dependent localization to the basal cortex (Figure 5a).

In *Drosophila*, Aurora A is also important for another aspect of asymmetric cell division: spindle positioning. Using an elegant S2 cell culture system designed to study asymmetric cell divisions, it was shown that spindle

positioning depends upon two domains of PINS [59]. One, the PINS linker, is phosphorylated by Aurora A. After phosphorylation it promotes partial spindle positioning by binding to the kinesin Khc73 via Discs large. The partial function is probably due to the fact that Khc73 is a plus end kinesin and therefore unable to produce pulling forces on centrosomes. Full function requires the TPR domains of PINS, which bind Mud (Box 1). This suggests a model in which the PINS linker captures astral microtubules, while the TPR domains, by binding to Mud, promote dynein-dependent pulling (Figure 5b). The linker domain, which is phosphorylated by Aurora A, would also provide coordination between cell cycle progression and spindle positioning [59].

In *C. elegans* embryos, downregulation of the Aurora A homolog, AIR-1, causes cell polarity defects [60,61]. PAR-2, normally found at the posterior of one-cell embryos, is found at both poles in *air-1(RNAi)* embryos, whereas PAR-6 is found in a central band [60]. It is not known whether AIR-1 phosphorylates PAR-6 or any other PAR protein in *C. elegans*.

PLK-1 is required for asynchronous cell cycle entry of the anterior and posterior cells in two-cell embryos. However, depletion of *plk-1* also results in defects in the localization of PAR proteins in one-cell embryos. Furthermore, depletion of *plk-1* can partially rescue the lethality and polarity defects of *par-2* temperature-sensitive mutants, suggesting that PLK-1 is either a positive regulator of the anterior PAR proteins or a negative regulator of PAR-2 [60]. Whether PAR proteins themselves are substrates of PLK-1 is unknown.

The Cdc2 homolog CDK-1 controls the timing of spindle positioning in *C. elegans*. This ensures that the spindle is fully assembled and functional when it is displaced [62]. The target(s) of CDK-1 in this process has not been identified but proteins required for spindle positioning are likely substrates.

Therefore, mitotic kinases can regulate polarity, providing a means to link cell cycle progression to cell polarity. In some cases, however, the role of these kinases in polarity regulation might be independent from their cell cycle role [63], but this requires further study.

Concluding remarks

Cell division offers a beautiful example of spatiotemporal coordination. In asymmetrically dividing cells, or in cells in tissues, cell division must be coordinated with the polarity axis or with the architecture of the tissue. A challenge for the future is to identify the molecular mechanisms behind the coordination between cell shape/polarity and cell division. Mitotic kinases regulate polarity; however, whether and how such regulation constitutes a coupling between cell division and cell polarity, or instead represents a mitotic-independent role for these kinases, is not always clear. Furthermore, how polarity regulates cell division in metazoan cells remains elusive. Are these processes coupled such that completion of cell polarity triggers entry into mitosis? Or do surveillance mechanisms (polarity checkpoints) arrest cell cycle progression if polarity is not normal? Studies in *C. elegans* and *Drosophila* will pave the way for studies in other organisms. Indeed, these processes are highly conserved at the molecular level, conceptual level, or both.

Spatiotemporal coordination during cell division is essential to ensure the proper balance between symmetric and asymmetric divisions, to properly position cells in tissues and to control the balance between proliferation and differentiation. Disruptions in this coordination might result in tumorigenesis or developmental disorders, underscoring the importance of studying the crosstalk and coordination between these fundamental cell biological processes.

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