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Chapter 14
Symmetry Does not Come for Free: Cellular Mechanisms to Achieve a Symmetric Cell Division

Damian Dudka and Patrick Meraldi

Abstract During mitosis cells can divide symmetrically to proliferate or asymmetrically to generate tissue diversity. While the mechanisms that ensure asymmetric cell division have been extensively studied, it is often assumed that a symmetric cell division is the default outcome of mitosis. Recent studies, however, imply that the symmetric nature of cell division is actively controlled, as they reveal numerous mechanisms that ensure the formation of equal-sized daughter cells as cells progress through cell division. Here we review our current knowledge of these mechanisms and highlight possible key questions in the field.

14.1 Introduction

To build an organism, cells have to divide both asymmetrically, to increase diversity of the tissue, and symmetrically, to increase specific cell populations (Morin and Bellaiche 2011). The equilibrium between these two types of divisions shapes tissue architecture, and any imbalance may lead to developmental abnormalities or favour cancer formation in adult tissues (reviewed in Noatynska et al. 2012; Yamashita et al. 2010; Bajaj et al. 2015; Pease and Tirnauer 2011). A symmetric cell division is defined by the equal inheritance of cell-fate determinants and the equal size of the daughter cells (Knoblich 2008; Tzur et al. 2009; Sung et al. 2013), since cell size influences the behaviour and survival of daughter cells. Prescott observed already over 50 years ago that the products of an amoeba cell division will only divide again after reaching the size of their parental cell; an asymmetric cell division will thus lead to daughter cells that differ in their cell cycle timing (Prescott 1955, 1956). A more recent study on *Drosophila melanogaster* stem cells demonstrated that abnormally small daughter neuroblasts become

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dormant faster, resulting in developmental abnormalities due to insufficient number of neuronal progenitors (Kitajima et al. 2010). Similarly, in human tissue culture cells the bigger cell arising from an unequal cell division will enter the subsequent mitosis faster, while the smaller cell will undergo apoptosis more often (Kiyomitsu and Cheeseman 2013). Differences in daughter cell volumes may also lead to the unequal distribution of various organelles, since they are fragmented and uniformly partitioned within the cytoplasm as cells enter mitosis (reviewed in Jongsma et al. 2015). Even though it has been long assumed that symmetric cell divisions are a default state and that asymmetric divisions arise by breaking the symmetry of the spindle apparatus, recent studies uncovered cellular mechanisms that correct potential asymmetries in spindle or chromosome position and ensure symmetric cell division (Kiyomitsu and Cheeseman 2013; Tan et al. 2015). This implies that the symmetry of the cell division is not a default state, but rather that it needs to be actively established. Once symmetry is set, cells can either divide symmetrically or break the symmetry to obtain daughter cells of different cell size. Consistent with this hypothesis, the sizes of daughter cells in symmetric divisions do not differ by more than 15%, indicating a low variability that might be the result of mechanisms that favour symmetric cell division (Tzur et al. 2009; Sung et al. 2013). Here, we review these mechanisms, in order of their time of action, and explore key future questions.

14.2 The Bipolar Spindle Determines the Position of the Cytokinetic Furrow

The size of daughter cells is determined by the position of the cleavage furrow during cytokinesis. This position is in turn controlled by several elements of the mitotic spindle in the preceding mitosis. Rappaport first demonstrated in 1961 with his groundbreaking sand dollar egg experiments that the location of the two centrosomes at spindle poles plays a key role in positioning the cleavage furrow (Rappaport 1961). When he confined sand dollar eggs with a glass bead to obtain a toroid-shaped cell, he obtained a binucleate horseshoe-like cell after the first cell division. As this cell divided a second time, it yielded two spindles but four cleavage furrows—two between opposite spindle poles within a spindle and two between spindle poles of neighbouring spindles. Later experiments performed again in sand dollar eggs (Shuster and Burgess 2002), *C. elegans* (Baruni et al. 2008) and mammalian somatic cells (Rieder et al. 1997) confirmed that the cleavage furrow builds up in between adjacent centrosomes of two separate spindles. More recent studies demonstrated that the position of the cytokinetic furrow is, beyond centrosomes, also influenced by the position of the spindle mid-zone and of chromosomes (see below and Tan et al. 2015; Canman et al. 2003; Bringmann and Hyman 2005). The key role of centrosomes and of the bipolar spindle in positioning the cleavage furrow is reflected by the behaviour of cells possessing more than two

centrosomes, which tend to form multipolar spindles. If cells with multipolar spindles proceed to anaphase, it will lead to the mispositioning of the furrow and to aberrant asymmetric cell divisions (Ganem et al. 2009) (Fig. 14.1). Such multipolar divisions are rare and most of the time lethal; instead, cells often cluster their centrosomes into two poles using the activity of microtubule motors such as dynein and HSET or more rarely inactivate some of their centrosomes (Ganem et al. 2009; Basto et al. 2008; Sabino et al. 2015; Quintyne et al. 2005; Kwon et al. 2008; Leber et al. 2010). While centrosome clustering allows the formation of a bipolar spindle, it nevertheless frequently causes chromosome segregation errors (Ganem et al. 2009). Furthermore in *Drosophila* neuroblasts, which normally divide in an asymmetric manner, supernumerary centrosome clustering leads to spindle orientation defects (see below) and the appearance of symmetric cell divisions that favour tumour formation (Basto et al. 2008). Overall this indicates that the formation and organization of the bipolar spindle is a key determinant for the (a)symmetric nature of cell division.

14.3 Cell Shape Influences the Symmetry of Cell Division

A first factor that influences the bipolar spindle and the outcome of cell division is cell shape. The rounding of mitotic cells is a key feature that is conserved in virtually all metazoans, pointing to an evolutionary important role (McConnell 1930; Harris 1973; Cramer and Mitchison 1997; Kunda et al. 2008; Luxenburg et al. 2011; Cadart et al. 2014). Cell rounding favours the formation of a bipolar spindle, as shown by experiments in which it was prevented genetically or by mechanical constraints (Fig. 14.2; Lancaster et al. 2013). A failure in cell rounding leads to spindle pole splitting, which may cause asymmetric cell divisions via the formation of multipolar spindles. The symmetry of cell division is also influenced by the cell shape in the preceding interphase, as shown by experimental manipulations and in silico modelling (Fink et al. 2011; Gibson et al. 2011; Minc et al. 2011). By placing urchin eggs in micro-chambers of different shapes, Minc and colleagues proved that the nucleus is positioned in the centre of the cell mass and is able to "perceive" cell shape. Similarly placing human culture cells in micro-channels resulted in frequent asymmetric cell division due to the displacement of nuclei. This displacement led to a spindle mispositioning that could not be corrected most likely because the cell cortex was beyond the reach of the astral microtubules (Cadart et al. 2014; Lancaster et al. 2013; Fig. 14.2). Computational modelling showed that nuclear positioning depends on microtubules that stretch and position the nucleus along the axis of the future mitotic spindle and thus determine the position of the cleavage furrow (Gibson et al. 2011). These studies indicate that cells normally sense their shape with the aim to position their nucleus in the centre of the cells, thus favouring a future symmetric position of the cytokinetic furrow.

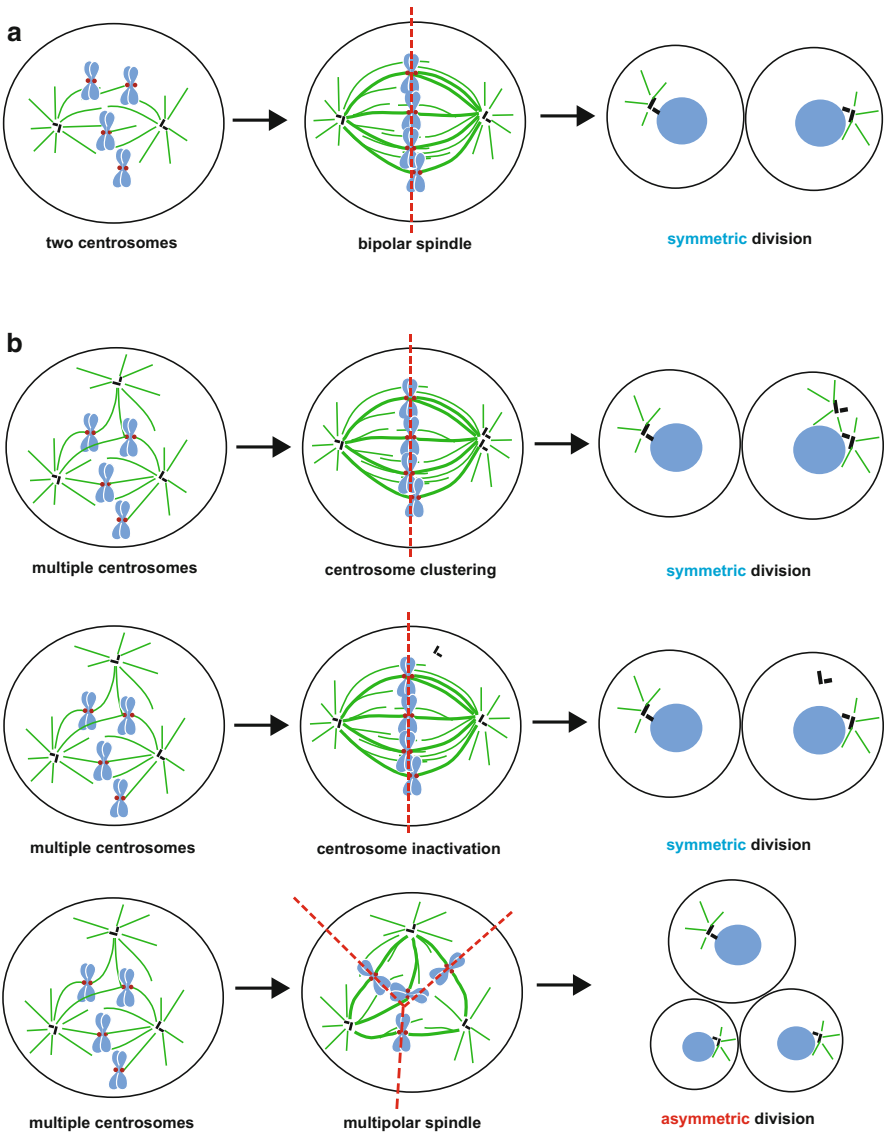

Fig. 14.1 Bipolar spindle assembly is a prerequisite of the symmetric cell division. (**a**) Cells with two centrosomes build a symmetric bipolar spindle and divide into two equal-sized daughter cells. Centrosomes are depicted in *black*, spindle microtubules in *green*, chromosomes in *blue* and kinetochores in *red*. (**b**) Cells with multiple centrosomes can also form a symmetric bipolar spindle by either centrosome clustering (*top panel*) or centrosome inactivation (*middle panel*) and divide symmetrically, or alternatively they can build an asymmetric, multipolar spindle (*lower panel*) and give rise to progeny of different size

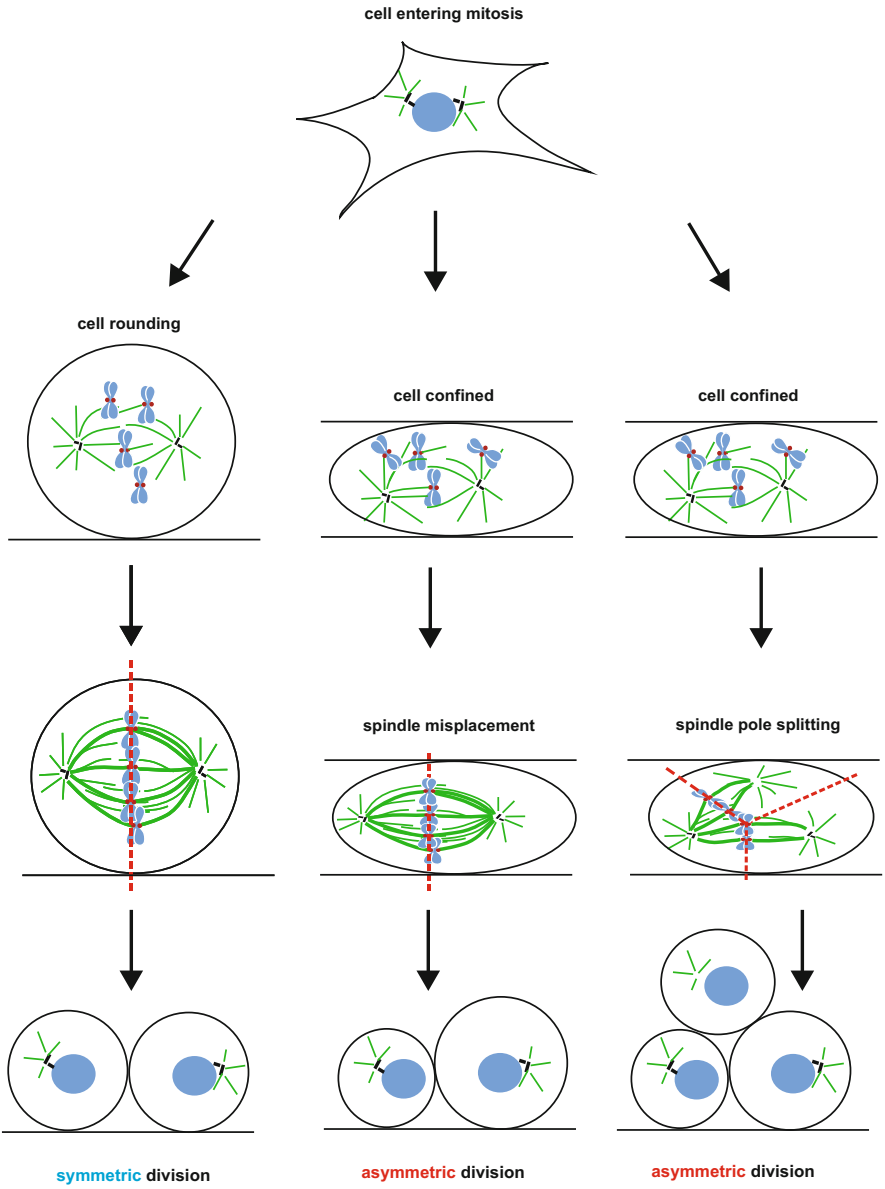

Fig. 14.2 Cell rounding combined with a central position of the nucleus at mitotic entry ensures a symmetric cell division. In a normal cell division (*left panel*), the nucleus has a central position, and mitotic rounding allows the formation of a bipolar spindle formation in the middle of the cell. In case mitotic rounding is prevented, cells might displace the nucleus (*middle panel*) or split the spindle poles leading to a multipolar spindles (*right panel*), two conditions that are each associated with asymmetric cell divisions

14.4 Controlling Spindle Positioning in Metaphase

Once a bipolar spindle is formed, it has to maintain a central positioning, as any displacement of the mitotic spindle might influence the plane of division, as shown almost 100 years ago by Conklin, who observed asymmetric division of centrifuged eggs of *Crepidula* in which the spindle had been displaced (Conklin 1917). Observation made 50 years later revealed that the mitotic spindle does not occupy a fix position within the cell, but instead is constantly moving around the centre of the cells; the mechanisms controlling this behaviour, however, remained unclear (Hughes 1952; Sato 1974). These movements, which consist of rapid back and forth displacements along the spindle axis, are called "spindle rocking". They are part of a mechanism that senses and actively corrects spindle positioning in metaphase. O'Connell and Wang first proposed this concept, after showing that cell deformations caused by micromanipulations lead to spindle movements and the repositioning of the spindle. This correction movement was blocked by injection of the anti-dynein antibodies, pointing to a central role of this microtubule motor protein in spindle positioning (O'Connell and Wang 2000). More recent work confirmed that spindle positioning and orientation is a readout of dynein-dependent pulling forces that emanate from the cell cortex via astral microtubules. Cytoplasmic dynein is recruited at the cell cortex by a tripartite complex comprised of Gα, LGN and NuMA (Kiyomitsu and Cheeseman 2013, 2012; Nguyen-Ngoc et al. 2007; Kotak et al. 2013). The cortical forces and spindle positioning are fine-tuned by a number of microtubule-associated proteins, such as EB1, LIS1, CLIP170 or NuDE, which interact with dynein complex on spindle microtubules (reviewed in Kardon and Vale 2009). Spindle rocking and dynein localization at the cell cortex is under the control of Polo-like kinase 1 (PLK1), a protein kinase that accumulates on centrosomes: PLK1 disrupts the interaction between dynein and the NuMA–LGN complex through phosphorylation and releases it from the cortex when the centrosome is in close proximity (Fig. 14.3a). This results in an asymmetric localization of dynein that creates a force imbalance, which pulls the mitotic spindle back towards the cell centre when it is positioned in an off-centred manner. As a result, the spindle undergoes the stereotypical rocking movements, which overall centre the position of the spindle during metaphase and favour symmetric cell divisions.

14.5 Spindle Positioning in Acentrosomal Cells

Spindle positioning also determines the (a)symmetry of acentrosomal cell divisions. Meiotic mouse oocytes are a classical example of how an asymmetric spindle position leads to an asymmetric cell division yielding a big oocyte and a small polar body (reviewed in Chaigne et al. 2012). The first mitotic division of the mouse embryo, however, is symmetric. In mice, the early embryos do not have centrioles;

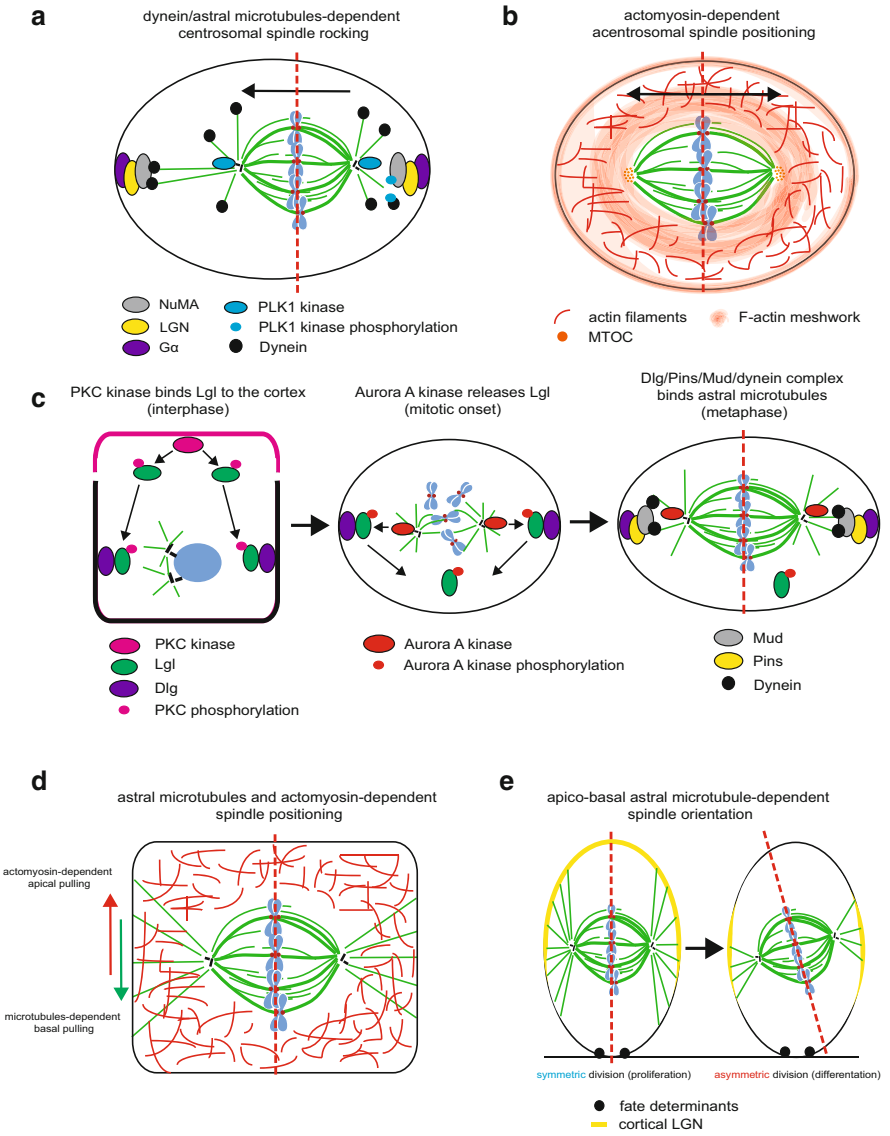

Fig. 14.3 Precise spindle positioning is essential for symmetric cell division in multiple contexts. (**a**) Spindle rocking in metaphase depends on the centrosomal kinase PLK1, which disrupts the interaction between dynein and the NuMA/LGN complex at the proximal cell cortex. This negative regulatory loop creates a differential dynein pulling gradient that centres the mitotic spindle. (**b**) Spindle positioning in acentrosomal mouse zygote depends on tension created by actomyosin cytoskeleton and F-actin cage surrounding the spindle, thus guiding it to the central position of the cell and ensuring symmetric cell division. In both meiosis and mitosis, myosin X mediates spindle orientation by directly linking cortical actin and astral microtubules. (**c**) To define the mitotic spindle axis, polarized epithelial cells break the PKC kinase-dependent polarity at the mitotic entry as Aurora A kinase phosphorylates Dlg protein to release it from Lgl. This allows the formation of the Dlg/Pins/Mud complex at the cell cortex, which will position the spindle in the centre of a cell via dynein-dependent microtubule pulling. (**d**) During gastrulation of frog embryos,

hence, the spindle poles lack centrosomes and astral microtubules. Instead, spindle poles form by clustering of microtubule-organizing centres (MTOCs; Schuh and Ellenberg 2007; Courtois et al. 2012). Symmetric division of the mouse zygote relays on two processes that both depend on the F-actin cytoskeleton: pronuclei meeting at the centre of the cell and the centralization of the mitotic spindle itself (Fig. 14.3b). Indeed, F-actin depolymerisation with Cytochalasin D results in asymmetric cell division and yields two blastomeres of unequal size (Chew et al. 2012). Moreover, Cytochalasin D-treated cells cannot restore the central position of the spindle, if it has been moved towards the cell cortex by a micromanipulation needle. At the molecular level, it was found that a F-actin/Myosin Vb-mediated cytoplasmic flow first drives a centred pronuclei meeting (Almonacid et al. 2015). In the second step, a spindle pole-localized pool of myosin II exerts tension on an F-actin cage surrounding the spindle and thus ensures the precise position of the spindle in the centre of a cell. The organization of the F-actin cage requires the presence of the Subcortical Maternal Complex (SCMC), which is composed of Mater, Filia, Floped and Tle6 (human NLRP5, KHDC3L, OOEP and TLE6) and is present only in oocytes and early embryos. Embryonic cells with a disrupted SCMC divide asymmetrically and die at the cleavage stage (Yu et al. 2014). Once centred, the symmetric position of the spindle does not depend on F-actin anymore as instead it relies on passive forces such as friction and the viscosity of the cytosol (Chaigne et al. 2016).

14.6 Controlling Spindle Orientation

In polarized tissues, symmetric cell divisions do not only rely on the central positioning of the spindle but also on its proper orientation in three dimensions. Polarized cells will divide in an asymmetric manner if the spindle axis and the polarity axis coincide, as fate determinants will be selectively inherited by one daughter cell; however, if these two axes are perpendicular to each other, a symmetric cell division will ensue (Haydar et al. 2003; Kosodo et al. 2004). Spindle orientation in polarized epithelial cells depends on cell-to-cell adhesion and cortical polarity proteins, which control astral microtubules and the spindle position via microtubule associated proteins (Reinsch and Karsenti 1994; Lu et al. 2001; den Elzen et al. 2009; Lu and Johnston 2013). Misorientation of the spindle changes the axis of division, which may cause developmental defects or tissue deformations, as shown in neuronal stem cells (Godin et al. 2010) and in skeletal muscle stem cells

Fig. 14.3 (continued) actomyosin cytoskeleton and astral microtubules pull the spindle in opposite sites, and this interplay leads to precise spindle positioning, to symmetric cell division and ultimately to the tissue thickening. (**e**) Neuronal progenitors relay on proper spindle orientation as a specific pool of astral microtubules stabilizes the spindle perpendicularly to the basal lamina, which secures the equal partitioning of the fate determinants and therefore symmetric cell division

(Dumont et al. 2015), which can all divide asymmetrically or symmetrically. Such cells must strictly regulate spindle orientation, in particular in the z-axis, to divide symmetrically, as any alteration in spindle orientation may result in asymmetric cell division.

Cells have developed various strategies to control the orientation of the spindle in the z-axis. A first mechanism relies on altering cell polarity at mitotic onset. Studies in flies have shown that the symmetric division in polarized epithelial cells is regulated via the localization of the Lgl protein (LLGL1 in human), which in polarized interphase cells is bound to the cortical Dlg protein (hDLG in human) (Guilgur et al. 2012). At mitotic onset, the aPKC kinase clears the apical cortex from Lgl protein and the Aurora A kinase relocates it to the cytoplasm (Fig. 14.3c). This alters the polarization of the cell and allows Pins protein (LGN in human) to bind to the cortical protein Dlg, which is no longer occupied by Lgl. In turn, Pins binds to spindle pole protein Mud (related to NuMA in human; Merdes et al. 1996), which itself recruits dynein. Cortical dynein then pulls onto the astral microtubules to stabilize the spindle in the planar position (Bell et al. 2015; Carvalho et al. 2015). Such a mechanism of phosphorylation-driven polarity breakage is not specific to flies, as it has been also seen in mammals (Jaffe et al. 2008; Hao et al. 2010; Durgan et al. 2011).

Another model of control over symmetric cell division in polarized cells was found in frog embryos. During *Xenopus laevis* early embryo gastrulation the polarized epithelial cells divide symmetrically to spread the epithelial tissue. An uncontrolled positioning of the spindle in these cells would lead to random changes in the division axis, impairing efficient tissue spreading and instead promoting tissue thickening. The mitotic spindle of these epithelial cells display dynamic movements along the x and y axis, allowing spreading of the epithelium; however the z-axis is fixed by pushing forces emanating from astral microtubules at the apical membrane and from the actomyosin cytoskeleton at the basal membrane to avoid tissue thickening (Fig. 14.3d; Woolner and Papalopulu 2012). Cooperation of astral microtubules and actin cytoskeleton is mediated by myosin X, which is a motor protein that links microtubules to actin and promote the bipolar spindle assembly in meiosis (Weber et al. 2004). Myosin X was also showed to help position the mitotic spindle in polarized and non-polarized cells by directly coupling centrosome-anchored astral microtubules to the subcortical actin clouds and retraction fibres (Kwon et al. 2015; Toyoshima and Nishida 2007; Liu et al. 2012).

Finally, during mammalian neurogenesis there is a pool of neuroepithelial cells, which proliferate and expand via symmetric division or differentiate into neurons upon asymmetric divisions (reviewed in Gotz and Huttner 2005; Martynoga et al. 2012). Several studies suggested that a subtle misorientation of the mitotic spindle may decide about the symmetry of the division during neurogenesis (Haydar et al. 2003; Kosodo et al. 2004), implying that cells had to develop mechanisms to keep the spindle orientation in check in order to preserve homeostasis of the tissue (Fig. 14.3e). These results should, however, be interpreted with caution, since other studies failed to find a correlation between spindle misorientation and tissue malformation (Konno et al. 2008; Noctor et al. 2008; Insolera et al. 2014). Spindle

pole proteins such as ASPM (Fish et al. 2006) and Huntingtin (Godin et al. 2010), as well as cortical proteins LGN (Konno et al. 2008) and Inscutable (INSC in human; Postiglione et al. 2011) control spindle orientation during neurogenesis. Recently, using fixed and live cell imaging Mora-Bermudez et al. distinguished a specific subset of astral microtubules reaching the apical and basal part of the cell cortex in mouse neuronal progenitors. These microtubules anchor the spindle in a planar position, and their numbers correlate with abundance of LGN at the cell cortex. Consistently, neuronal progenitors of mice lacking LGN protein or overexpressing a dominant-negative form of LGN (which prevents microtubule binding to the cortex) had fewer apico-basal microtubules, while the number of central astral microtubules was not changed. Stabilization or destabilization of these microtubules by pico-molar concentrations of taxol or nocodazole resulted in asymmetric cell division, suggesting that the spindle orientation controls via cortical LGN and apico-basal microtubules an essential regulatory mechanism to ensure symmetric cell divisions (Mora-Bermudez et al. 2014).

14.7 Controlling Chromosome Positioning

Although centrosomes play a key role in determining the position of the cytokinetic furrow, other elements of the spindle, such as the chromosomes themselves, exert an influence (Fig. 14.4a). In Ptk2 cells that were forced into anaphase with monopolar spindles, chromosome-derived signals influenced cleavage furrow positioning by fortifying adjacent microtubules that reached the cell cortex (Fig. 14.4b; Canman et al. 2003). A more recent study in human cells confirmed that position of chromosomes in bipolar spindles could also influence the symmetry of the cell division (Tan et al. 2015). A partial impairment of centrosome duplication led to bipolar spindles with an unequal number of centrioles at each spindle pole, resulting in an asymmetric position of the chromosomes within the spindle. This asymmetry was normally corrected prior to anaphase via changes in microtubule dynamics. However, if cells with an asymmetric chromosome position were forced to enter anaphase, e.g. after inactivation of the spindle assembly checkpoint, an asymmetric cell division would ensue even if the mitotic spindle itself was positioned symmetrically. This indicated that the symmetry of the chromosome position provides an important cue for symmetric cell divisions (Fig. 14.4c). This work also postulated that the initial symmetry of the chromosome position and therefore the symmetry of the two half-spindles may provide a reference point, from which cells develop an asymmetry to develop an asymmetric cell division. While this hypothesis remains to be proven, there is anecdotal evidence supporting it. In *Drosophila melanogaster*, the asymmetric cell division of neuroblasts is driven by the asymmetry of the bipolar spindle. The symmetry is broken in anaphase as one of the half-spindles elongates faster than the other; this displaces the cleavage furrow from the equator of the cell and leads to an asymmetric cell division (Kaltschmidt et al. 2000). Furthermore, Ren and colleagues demonstrated that the first asymmetric

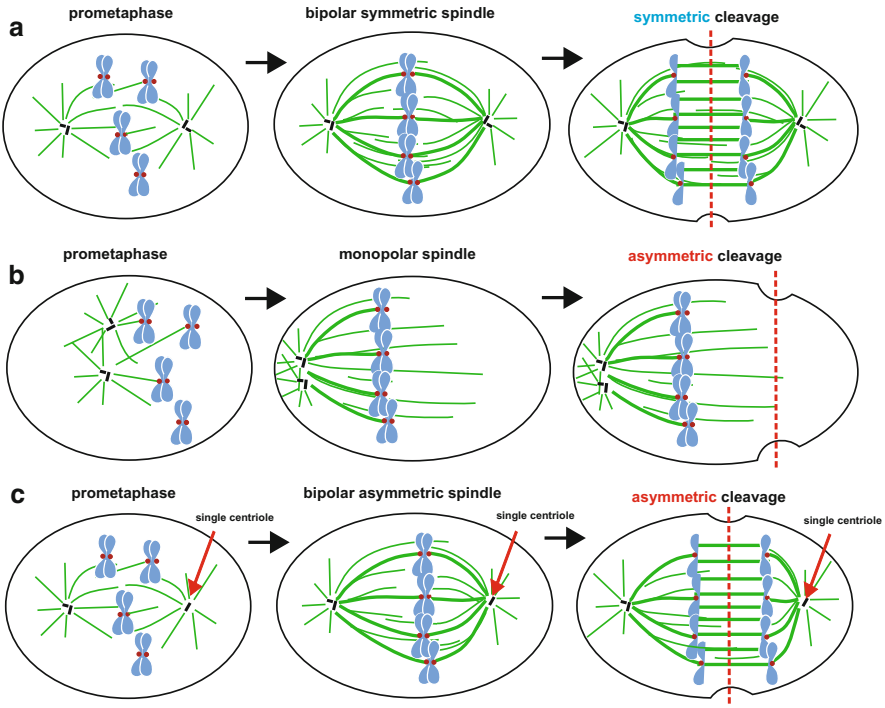

Fig. 14.4 Chromosome position within the mitotic spindle influences the symmetry of the cell division. (**a**) In normal conditions, mitotic cells put the metaphase plate in the middle of the mitotic spindle. (**b** and **c**) In cases cells are allowed to enter anaphase with (**b**) a monopolar spindle due to inhibition of Eg5 kinesin (Canman et al. 2003) or (**c**) with an asymmetrically positioned metaphase plate, which may arise if the centriole numbers in each spindle pole is not the same (Tan et al. 2015), chromosome position will modify the positioning of the cleavage furrow resulting in an asymmetric cell division

division in leech *Helobdella robusta* relies on breaking the symmetry of the mitotic spindle in metaphase, by downregulating the levels of γ-tubulin on one of the two spindle poles (Ren and Weisblat 2006). This downregulation results in unequal lengths of two half-spindles leading to the asymmetric cell division. Such asymmetry in half-spindle lengths can also occur in neural precursors of the mouse neural cortex, which divide asymmetrically yielding one progenitor and one differentiated cell (Delaunay et al. 2014). Neurons preferentially originated from cells formed from larger half-spindles, which raises the possibility that spindle asymmetry may direct the fate of daughter cells. This asymmetry depends on moesin, which is affected by the Wnt/planar cell polarity pathway, as moesin depletion enhances spindle asymmetry and altered the fate of daughter cells. Based on these observations, we conclude that the symmetry of the mitotic spindle provides an additional level of the control over the symmetry of the division.

14.8 Spindle Positioning in Anaphase

While cells have a dynein-dependent spindle-centring mechanism in metaphase, they do not monitor the position of the spindle before anaphase. Therefore excessive spindle motion in metaphase may lead to improper spindle positioning at anaphase onset (Kiyomitsu and Cheeseman 2013; Collins et al. 2012). The spindle asymmetry can also be corrected in anaphase in a dynein-dependent manner, as this correction was abolished when cells were microinjected with anti-dynein antibodies or treated with low doses of nocodazole (Collins et al. 2012). Several mechanisms have been proposed to control this dynein-dependent movements: first it has been proposed that dynein pulls harder on longer astral microtubules attached to the distal part of the cell, as those long astral microtubules would recruit more motor proteins (Collins et al. 2012; Tolic-Norrelykke 2010); second dynein is recruited to the cell cortex through LGN-independent pathways specifically in anaphase. This includes a pathway that requires the 4.1 cortical protein and a pathway that depends on the phospholipid PIP2, which recruits NuMA to the cell cortex once CDK1 kinase (the main mitotic-entry regulator) activity drops allowing the PP2A phosphatase to dephosphorylate NuMA (Fig. 14.5a; Kiyomitsu and Cheeseman 2013; Kotak et al. 2013). Therefore, even after sister chromatids started to segregate to the prospective future daughter cells, dynein-mediated mechanisms monitor and reinforce the symmetry of the dividing cell.

14.9 Balancing Cortical Forces in Late Mitosis

Assembly of the cytokinetic furrow depends on the position of the centrosomes, the spindle mid-zone and chromosomes. However, the cortical forces also contribute to the furrow positioning. Symmetrically dividing cells depleted of Anillin, an actomyosin cytoskeleton protein involved in blebs formation (Charras et al. 2006), fail to keep the furrow at the equatorial position of the cell due to improper polar contractions (Piekny and Glotzer 2008). More recent findings show that tension imbalance at the polar cortex in cytokinetic cells may lead to abnormal cell shape oscillations resulting in the furrow displacement and possibly to aneuploidy (Sedzinski et al. 2011). This study observed minor shape oscillations caused by cytoplasmic flow in early cytokinesis in mammalian cells that could be enhanced by local actin depolymerization or laser ablation of the cortex. These results implied that enhanced polar contractility is responsible for shape oscillations and that to prevent them, cells balance the tension on the poles during cytokinesis. Previous observations suggested that bleb formation at the poles of the dividing cell could serve as a release valve for polar tension (Charras et al. 2005; Tinevez et al. 2009). Consistent with this hypothesis, induction of blebs by laser ablation was sufficient to counterbalance polar contractions; moreover, inhibition of blebbing led to major shape oscillations (Sedzinski et al. 2011). Overall, this indicates that bleb formation

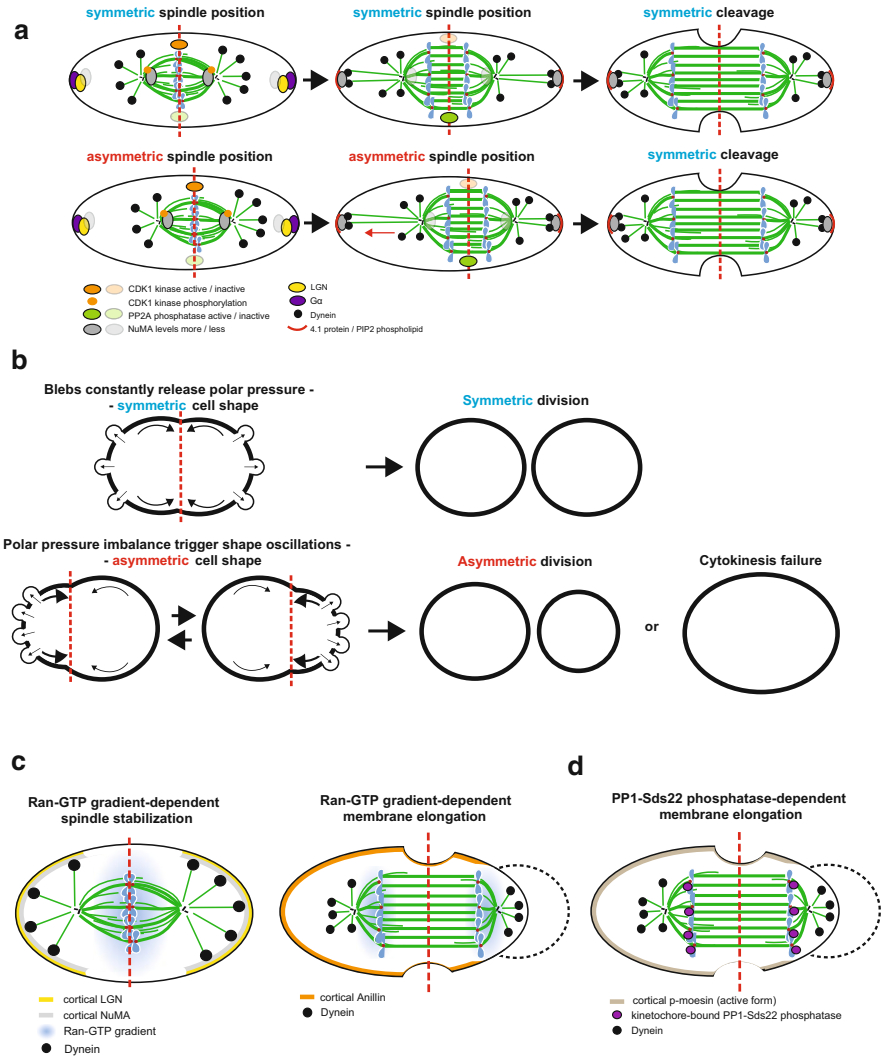

Fig. 14.5 Anaphase mechanisms correct asymmetries in spindle positioning to ensure symmetric cell division. (**a**) In anaphase, the dephosphorylation of CDK1 sites on NuMA and the binding to the 4.1 proteins allow an enhanced recruitment to the cell cortex generating dynein-dependent pulling forces that re-centre the mitotic spindle. (**b**) Cortical blebbing works as a polar pressure-releasing mechanism that stabilizes cell size oscillations and enables symmetric cell division. (**c**) Chromosome-associated Ran-GTP gradient stabilizes the spindle in metaphase by clearing LGN and NuMA from the equatorial cortex; later in anaphase, it causes membrane elongation by clearing Anilin from the lateral cortex in a proximity-dependent manner. (**d**) Finally, we hypothesize that similar to Ran-GTP gradient, the kinetochore-associated PP1-Sds22 phosphatase may also enable membrane elongation by dephosphorylating cortical moesin at the mitotic exit and thus provide a final mechanism for symmetric cell division

at the cell poles allows cell to compensate for shape oscillations during cytokinesis to ensure a stable position of the cytokinetic furrow and the symmetry of cell division (Fig. 14.5b).

14.10 Membrane Elongation in Anaphase

A final mechanism that ensures the symmetry of cell division is the chromatin-bound Ran gradient, which links the position of the mitotic spindle to the regulation of the cell cortex. Ran is a small GTPase, which forms a gradient around the chromatin in mitotic cells, due to the binding of its guanine exchange factor, RCC1, on chromosomes. The Ran-GTP gradient orchestrates bipolar spindle formation in acentrosomal mitoses, as originally found in *Xenopus* egg extracts (Carazo-Salas et al. 1999; Kalab et al. 1999; Ohba et al. 1999; Wilde and Zheng 1999; Zhang et al. 1999) and may contribute to spindle formation in somatic cells (Gruss et al. 2002; Moore et al. 2002). Ran-GTP releases spindle-supporting proteins from α- and β-importin to promote spindle formation (Gruss et al. 2001; Nachury et al. 2001; Wiese et al. 2001). The Ran-GTP gradient is also thought to polarize actin in mouse oocytes to ensure the asymmetric meiotic division by regulating localization of the cortical actomyosin proteins, such as myosin II (Deng et al. 2007; Yi et al. 2011; Dehapiot et al. 2013), Arp2/3 complex and the Cdc42 GTPase. It further controls spindle orientation by regulating the cortical localization of Pins and Mud in *Drosophila* neural stem cells (Wee et al. 2011; Bird et al. 2013) and their orthologs LGN and NuMA in human cells (Bird et al. 2013). In metaphase the Ran-GTP gradient specifically restricts the localization of LGN and NuMA to the lateral cortex by clearing them from the apical and basal membrane, which helps to maintain a correct axis of division (Fig. 14.5c; Kiyomitsu and Cheeseman 2012). Finally, the Ran-GTP gradient plays a role in anaphase if the chromosome masses are closer to one cell membrane, as it will reduce the cortical levels of Anilin (Silverman-Gavrila et al. 2008). This will cause a membrane relaxation and elongation that will compensate for the asymmetry of the anaphase spindle and yield equal-sized daughter cells (Kiyomitsu and Cheeseman 2013).

14.11 Conclusions and Outlook

The presence of numerous cellular mechanisms at the level of the mitotic spindle, chromosomes and the cell cortex ensuring the formation of equal-sized daughter cells indicates that cells actively impose symmetric cell divisions. Many of these mechanisms involve negative spatial regulatory systems, reminiscent of the Min system controlling the position of the FtsZ cytokinesis ring in prokaryotes, pointing to common strategies (Adams and Errington 2009; Kiekebusch and Thanbichler 2014; Haeusser and Margolin 2016). The principles of other mechanisms, such as

the forces that ensure the symmetric position of the chromosomes within the spindle, remain to be precisely defined (Dumont and Mitchison 2009). Beyond these detailed mechanistic aspects, there are a number of general questions to address in the future. First, we postulate that the study of mechanisms ensuring symmetric cell division is also relevant to understand asymmetric cell division, as cells may use symmetricity as an initial reference point to break symmetry and thereafter impose a controlled asymmetry. Strikingly, many asymmetric cell divisions emerge from an initially symmetric configuration, be at the level of the spindle, such as *D. melanogaster* neuroblasts, or at the level of spindle positioning, such as *C. elegans* embryos. Such a hypothesis needs, however, to be proven; moreover, it will be essential to identify the mechanistic switches that break the symmetry during cell division.

A second key point will be to study how known mechanisms might work in other contexts. For example, it might be worthwhile to explore how F-actin cytoskeleton-mediated cortical tension, which is essential in acentrosomal cells, influences symmetric cell divisions in centrosomal cells. Since myosin X helps to position the spindle by linking actin and microtubules in centrosomal cells, it is possible that the actomyosin cytoskeleton provides redundancy to the astral microtubule/dynein-driven spindle positioning. Indeed, *Drosophila* embryos devoid of centrioles have only mild developmental defects probably due to the lack of cilia (Basto et al. 2008). One could speculate that the F-actin cytoskeleton provides centrosome-free flies sufficient compensatory mechanics to ensure symmetric cell divisions and develop a whole organism (N.B. these flies nevertheless fail at the asymmetric neuroblast divisions). Such an approach might be particularly relevant, since centrosomes have been found to also nucleate actin filaments (Farina et al. 2016). Another example could be membrane blebbing, which so far has been only studied in the context of non-polarized cells. It is tempting to hypothesize that this mechanism could also contribute to regulate the symmetric cell division in polarized cells. Therefore, it would be interesting to explore if the cortical tension release by blebs also contributes to cytokinetic furrow positioning in symmetrically dividing epithelial cells, where spindle position plays a major role in defining the size of daughter cells (Bell et al. 2015; Carvalho et al. 2015; Woolner and Papalopulu 2012).

Finally, it is likely that new mechanisms controlling the symmetricity of cell division might emerge. For example, it was recently found that segregating chromosomes induce polar relaxation during late anaphase (Rodrigues et al. 2015). This relaxation was known to be caused by the clearing of actin and phosphorylated ezrin/radixin/moesin (ERM) complex from the poles before cleavage furrow formation (Hickson et al. 2006). This recent study demonstrated that the proximity to chromatin in late anaphase is the key signal for relaxation and membrane blebbing at the poles. This pathway depends on the regulatory PP1 phosphatase subunit Sds22, which specifically binds to kinetochores until late anaphase. Depletion of Sds22 delays anaphase cell elongation and causes cytokinesis defects (Kunda et al. 2012). Even though PP1-Sds22-dependent polar relaxation has not been linked to symmetric cell division, it would be exciting to test whether it contributes to

symmetric cell divisions in cases where the anaphase spindle is positioned asymmetrically. Such a mechanism would again link spindle positioning to the control of the cortical organization, providing an additional feedback loop to ensure symmetric cell division at every step of mitotic progression.

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