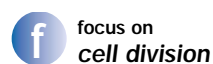


# The spindle cycle in budding yeast



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The mitotic spindle of the budding yeast *Saccharomyces cerevisiae* will probably be the first such organelle to be understood in molecular detail. Here we describe the mitotic spindle cycle of budding yeast using electron-microscope-derived structures and dynamic live-cell imaging. Recent work has revealed that many general aspects of mitosis are conserved, making budding yeast an excellent model for the study of mitosis.

The microtubule cytoskeleton of *S. cerevisiae* was first viewed using the electron microscope in 1966, and was described as a 'fibre apparatus' that is present in the nucleus<sup>1</sup>. Since then, advances in microscopy have led to increased analysis of this apparatus. For example, in 1974–75 important ultrastructural observations of yeast mitosis came from describing cell-cycle events and analysing the first cell-division cycle (*cdc*) mutants<sup>2,3</sup> (Fig 1). In 1976 Peterson and Ris<sup>4</sup> used high-voltage electron microscopy (HVEM) to analyse the yeast spindle in thick sections, revealing the three-dimensional features of the organelle. Light-microscopic analysis became possible upon the development of antibodies that recognize yeast tubulin, and this led to the description of the cytoskeleton in 1984 by Adams and Kilmartin<sup>5</sup>. Recent studies have brought several sophisticated and sensitive cytological techniques to bear on the question of how mitotic spindles are formed and how they function. Light-microscopic analysis has been advanced by live-cell imaging of green fluorescent protein (GFP)-tagged chromosomes or spindle components<sup>6–8</sup>, fluorescent-speckle microscopy (FSM) of microtubules<sup>9</sup>, fluorescence recovery after photobleaching (FRAP)<sup>9</sup>, and *in situ* hybridization of specific chromosomal loci<sup>10,11</sup>. All of these techniques have benefited from confocal or deconvolution microscopy. Electron-microscopic studies have allowed the development of higher-resolution immunolocalization protocols<sup>12,13</sup>, three-dimensional reconstruction from serial electron micrographs<sup>14,15</sup>, and the use of electron tomography<sup>16,17</sup>. Although this work has expanded our understanding of yeast spindle cycle immensely, several new questions have come to light and some old and challenging ones remain. Most importantly, this work has shown that several mitotic features in yeast are conserved<sup>18</sup>.

Unlike vertebrate cells, yeast cells do not break down the nuclear envelope during mitosis (so-called 'closed mitosis'). All the microtubules in the cell are nucleated by the spindle pole body (SPB), the equivalent in yeast of the centrosome, which is embedded in the nuclear envelope and has faces in both the nucleus and the cytoplasm. Microtubules are responsible for chromosome movement in two ways. First, cytoplasmic microtubules function in nuclear positioning, for example by moving the nucleus to the bud neck during mitosis<sup>19</sup>, and second, chromosomes attach directly to the nuclear microtubules of the mitotic spindle. When and where these microtubules are formed, how they are organized, and when they carry out their function relative to other cell-cycle events have been investigated by in-depth cytological analyses of yeast. This work, which is reviewed here has revealed that many features of mitosis, such as metaphase and anaphase A and B, are conserved in these cells.

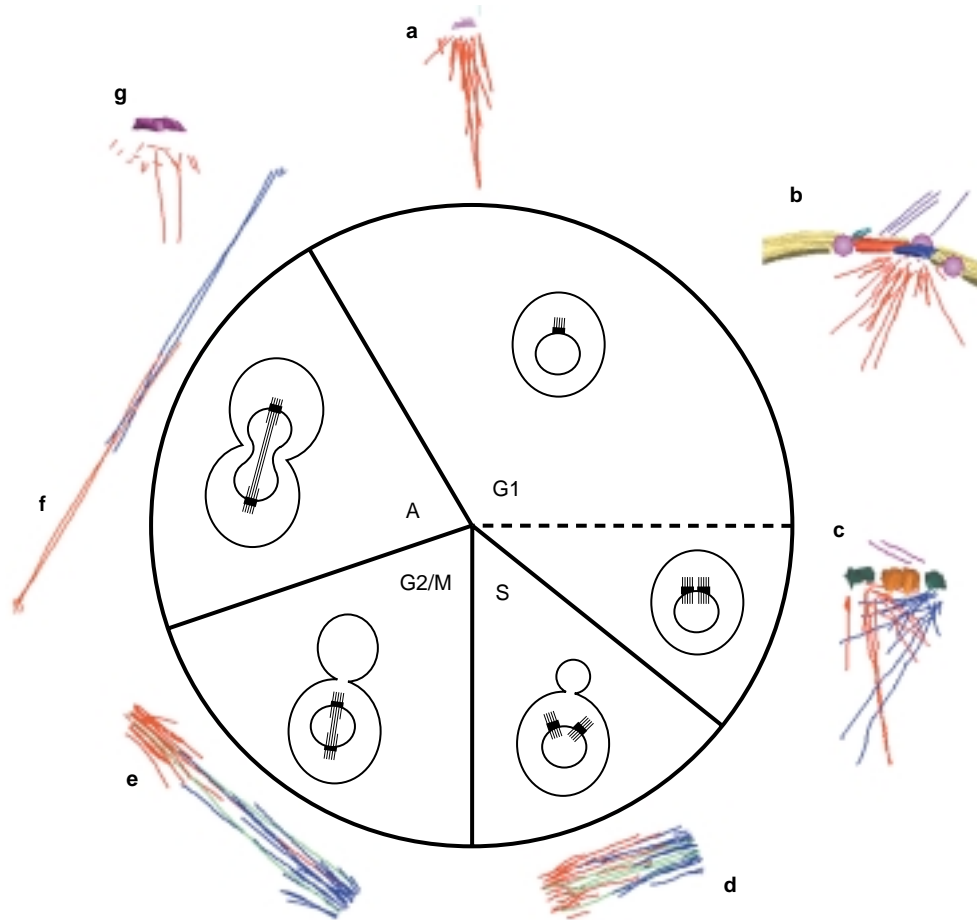
## SPB duplication

The spindle cycle begins with the duplication of the SPB late in G1 phase or possibly in early S phase (reviewed in ref. 20), and all spindles are organized from two poles that arise from this duplication event. In yeast, the SPB is a layered organelle, wherein the layers are

called 'plaques'. These plaques have been the subject of sophisticated structural analyses, including electron tomography both in vitreous ice<sup>16</sup> and *in situ*<sup>17</sup>. These studies have shown that the SPB is a striated structure organized around a paracrystalline array of the protein Spc42p. Almost 20 other components of the SPB have been identified by a variety of techniques, including mass-spectrometric identification of proteins in enriched SPBs<sup>21</sup>. Electron tomography has been used to show that all of the microtubule ends at SPBs *in situ* are capped<sup>17</sup>, indicating that these are the minus ends of the microtubules, which is consistent with the ability of SPBs to nucleate microtubule formation *in vitro*<sup>22,23</sup>.

SPB duplication is completed after START, the point of commitment to the cell cycle. However, cells arrested at START (using mating factor, *cdc28* mutations, or *CLN* depletion) contain SPBs with a 'satellite' structure that is a precursor to a new SPB<sup>3</sup>. This observation reveals that the spindle cycle begins before START. This satellite-bearing SPB can adopt one of two fates at START, one of which is in the mating pathway in which nuclear fusion is initiated at the SPB, specifically at the satellite<sup>3</sup>. The other fate is accompanied by progression into the cell cycle, wherein the satellite gives rise to new SPBs<sup>3,24</sup>. It has been proposed that a licensing event that allows SPBs to become competent to duplicate occurs in late anaphase under the control of the mitotic-exit network<sup>25</sup>. Recently, reduplication of SPBs in budding yeast has been demonstrated, and the licensing event necessary to allow reduplication seems to correlate with mitotic exit and the removal of mitotic cyclin (*CLB1-4*) activity<sup>26</sup>.

The mechanism of SPB duplication is thought to be a conservative event, that is, the existing SPB is maintained and a new SPB is assembled, setting up an asymmetry in the mitotic spindle. Detailed analysis, using synchronized cells, of the events that occur between satellite formation and completion of SPB duplication has recently shown that the satellite, which is on the cytoplasmic face of the elongated bridge, expands into a 'duplication plaque'<sup>24</sup>. This structure has the layered appearance of a mature SPB<sup>24</sup> and has been observed by electron tomography<sup>17</sup> (Figs 1b and 2; see Supplementary Information). The satellite and duplication plaque both contain several SPB components<sup>24</sup>. The duplication plaque is inserted into a pore that is opened in the nuclear envelope, to insert the organelle into the membrane<sup>24</sup>. The pore is opened specifically at the end of the half-bridge structure, and it has been proposed that retraction of the half-bridge contributes to formation of the pore<sup>24</sup>. After the insertion of the duplication plaque, the nuclear face of the newly formed SPB is matured by addition of Spc110p<sup>24</sup>, which connects the central plaque to the  $\gamma$ -tubulin complex in the nucleus<sup>27,28</sup>. The completion of SPB duplication results in duplicated side-by-side SPBs connected by the bridge, each with a complete set of nuclear microtubules (Fig. 1c). Several genes with products that function in this process have been identified (reviewed in ref. 20); many of these gene products, as well as SPB components, are widely conserved. Adams and Kilmartin<sup>20</sup> argue that key regulators and features of SPB duplication may be conserved in centrosome duplication.



**Figure 1 Schematic diagram of the yeast cell cycle.** The budding and spindle cycle, on the basis of Byers and Goetsch<sup>3</sup>; phases of the cycle are G1, S (DNA synthesis), G2/M (metaphase) and A (anaphase). **a**, A single SPB (pink) in G1 phase has nuclear (red) and cytoplasmic (green) microtubules. **b**, A duplicating SPB later in G1 phase has nuclear microtubules (red) and some cytoplasmic microtubules (purple). The existing SPB is shown in dark blue and the growing new SPB (duplication plaque, see Fig 2) is shown in green with the connecting bridge structure shown in red. This model also includes the nuclear envelope (yellow) and three nuclear-pore complexes (pink). **c**, Duplicated side-by-side SPBs (black) are connected by the bridge (red) after duplication in late G1 phase or in S phase. Each SPB has nuclear microtubules (red and blue) and the structure has cytoplasmic microtubules (purple). **d-f**, After SPB separation, bipolar spindles are formed from microtubules from one SPB (red) or the other (blue). The SPBs in these models are not shown. Short **d** and medial **e** spindles contain microtubules (green) with ends so close to each SPB that their polarity

cannot be determined<sup>15</sup>. Short spindles (**d**) are present before metaphase (see text); medial spindles (**e**) are likely to be metaphase spindles. Note that the short, presumptive kinetochore microtubules do not meet at the spindle midzone but have a gap between their ends. Long spindles (**f**) are in the midst of anaphase B and contain few, but highly organized, long microtubules; these spindles also have a few residual kinetochore microtubules. **(g)** Tomography of SPBs from such late-anaphase spindles reveals several very short microtubules (medial length 50 nm, red) that are presumed to be kinetochore microtubules<sup>17</sup>. In this model (**g**), the SPB is shown magenta and the two longest microtubules that form the central spindle are truncated. Electron tomography<sup>17</sup> was used for (**a-d**), whereas (**e-g**) are reconstructions from micrographs of serial thin sections<sup>15</sup>. Spindle models are reprinted from *J. Cell Biol.* (**129**, 1601–1615; 1995) with permission from Rockefeller Univ. Press; *Mol. Biol. Cell* (**10**, 2017–2031; 1999) with permission from the American Society of Cell Biology; and *Biol. Cell* (**91**, 305–312; 1999) with permission from Elsevier.

**Spindle assembly**

SPBs separate after duplication to form the mitotic spindle. This is one of the least well understood phases of the spindle cycle. A key event is the severing, by an unknown mechanism, of the bridge structure that connects the two SPBs. Separation of the SPBs allows formation of bipolar spindles, but also regenerates the half-bridge structure on which the satellite will form in the next cell cycle, and is therefore required for SPB duplication in the next cycle. The genetic requirements for SPB separation include *CLB* kinase activity<sup>26,29</sup> and the activity of either of the kinesin-like motor proteins encoded by *CIN8* and *KIP1*<sup>30</sup>. Cells that lack these activities arrest after DNA synthesis, and exhibit large buds and duplicated side-by-side SPBs. Electron tomography of duplicated side-by-side SPBs has revealed the presence of a large number (10–14) of relatively short nuclear microtubules (~130 nm) from each SPB that interdigitate near the bridge<sup>17</sup> (Fig. 1c). These interdigitated nuclear

microtubules appear to have crossbridges that contain the kinesin-like motor protein complexes needed to drive the SPBs apart from each other. Motor proteins, particularly dynein, drive centrosomes apart in vertebrate cells<sup>31</sup> and in invertebrates<sup>32</sup>. The basis of the requirement for *CLB* kinase is not known, but it could involve direct regulation of an activity such as bridge severing, the function of kinesin-like motors, and/or others.

Intermediate states of spindle assembly between duplicated side-by-side SPBs and the formation of a short (~600 nm) spindle with SPBs facing each other (Fig. 1d) are very difficult to observe, which indicates that the transition may be rapid. No detailed structural analysis of these early events in spindle assembly has been undertaken. However, the microtubule arrays in short spindles (~600 nm), possibly in S-phase cells, have been examined by reconstruction from serial thin sections<sup>15</sup> (Fig. 1d). This study has shown that the microtubule number is roughly 20 per SPB, and that

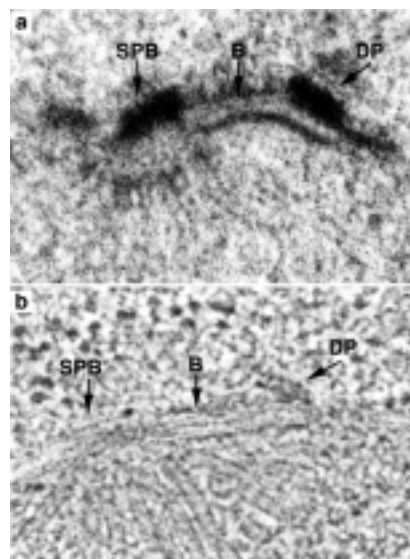
the microtubules are not organized in a way that allows clear determination of distinct functional classes, as is possible later in mitosis (see below). Nonetheless, there are sufficient numbers of microtubules to capture all the kinetochores and to have enough left over to organize the central or core spindle that connects the SPBs to each other. Despite the number of microtubules in these short spindles, the cells are not yet at metaphase. Similar results have been reported in the oomycete fungus *Saprolegnia ferax*<sup>33</sup>.

The proposition that these short, pre-metaphase spindles could potentially capture all the kinetochores raises what is possibly the most intriguing and difficult question concerning the spindle cycle in budding yeast. When do kinetochores attach to the spindle microtubules? As yeast cells contain nuclear microtubules throughout the cell cycle, it is possible that the kinetochores are always attached; this seems to be the case in *S. ferax*<sup>34</sup>. In fact, centromeric DNA in *S. cerevisiae*, as visualized both by *in situ* hybridization<sup>10</sup> and by GFP tagging of kinetochores<sup>6,7</sup>, has been found to cluster in the vicinity of the SPB. However, if kinetochores are always attached, questions arise as to how replication occurs through the early-replicating centromeric DNA<sup>35</sup>. Furthermore, attachment of kinetochores to microtubules after replication of centromeric DNA might be expected to show an interesting bias, with parent kinetochores attached to the parent SPB and new kinetochores attached to the new SPB, but no such bias exists<sup>36</sup>. Nonetheless, bipolar attachments are achieved, with no evidence (although detection could prove difficult) that transient monopolar attachments occur, as seen in other cells<sup>37</sup>. Finally, if kinetochores are attached to microtubules throughout the yeast cell cycle, the specific molecular nature of these attachments may not be identical throughout the cycle. This is one aspect of the *S. cerevisiae* spindle cycle that may be unique to fungal species that undergo closed mitosis.

### Metaphase and kinetochore splitting

Although difficult to detect, the budding yeast spindle cycle has a metaphase, which is defined as the point of bipolar attachment of chromosomes just before the onset of chromosome segregation. Metaphase is easily recognized in many cell types by visualizing condensed chromosomes at the midzone of the spindle that form the metaphase plate. Not only do chromosomes not condense sufficiently in budding yeast to see a structural change in the light microscope without *in situ* hybridization, but bipolar attachment of chromosomes does not seem to result in congression to the midzone of the spindle. Nonetheless, there is technically a metaphase in yeast, on the basis of the regulation of cell-cycle progression<sup>38</sup> and of cytological data reviewed here. Metaphase spindles are ~1–2  $\mu\text{m}$  long, and three-dimensional reconstruction from serial thin sections has documented two distinct microtubule classes — core and kinetochore<sup>15</sup> (Fig. 1e). The core microtubules form the core or central spindle that presumably pushes the SPBs apart during anaphase B and serves to keep the two SPBs in contact with each other. Kinetochores apparently attach to spindles through a single microtubule<sup>4,15</sup>. However, the plus ends of the kinetochore microtubules from the two SPBs do not meet at the spindle midzone, as might be expected for bipolar attachment of chromosomes (Fig. 1e). A trivial explanation for this observation is that metaphase is relatively rapid and was simply not represented in the few spindles that had been modelled. However, the reconstruction of spindles from cells arrested at the metaphase–anaphase transition by mutation of the *CDC20* gene revealed a minimum gap of ~0.1–0.25  $\mu\text{m}$  (or more) between the ends of the kinetochore microtubules<sup>39</sup>. These observations led to the proposal that centromeric regions of chromosomes are not joined between the sister chromatids at metaphase<sup>15</sup>.

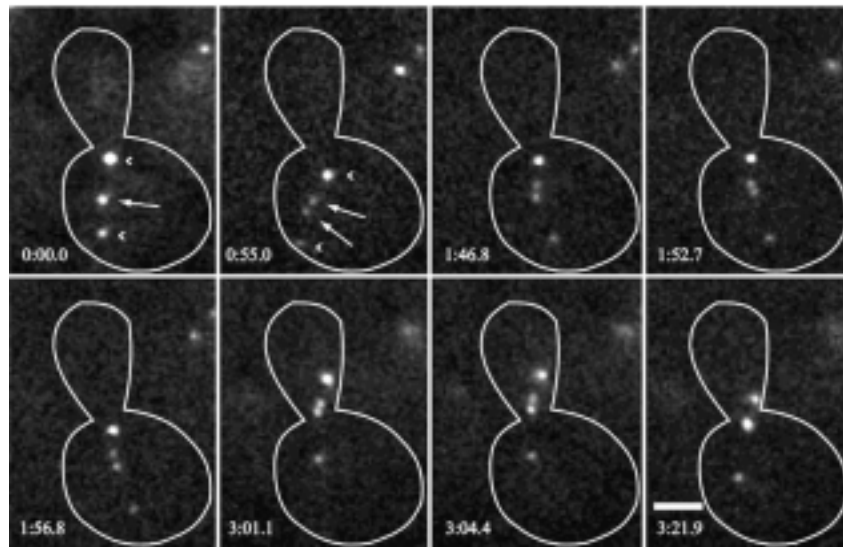
Recent analysis of living cells has shown that the centromeric regions of replicated chromosomes are separated before metaphase, whereas the sister chromatids are still attached to each other at centromere-distal sites (9–13 kilobases from the centromere<sup>7</sup>). Although such splitting of the centromeres does not occur in all cell



**Figure 2 Two views of the duplication plaque.** The duplication plaque (DP) is the new SPB assembling on the elongated bridge (B). Existing SPBs are indicated, and their associated nuclear microtubules can be seen. **a** Conventional electron-microscopic image<sup>24</sup>. **b**, Tomographic slice from an electron tomogram of the organelle<sup>17</sup>. The complete tomographic series can be viewed as a movie in the Supplementary Information. In both images the cytoplasm is at the top and the nucleoplasm is at the bottom. Micrographs are reprinted from *J. Cell Biol.* (**145**, 809–823; 1999) with permission from Rockefeller Univ. Press, and from *Mol. Biol. Cell* (**10**, 2017–2031; 1999) with permission from the American Society of Cell Biology.

types<sup>40</sup>, it has been reported in various vertebrate cells<sup>41–43</sup>. The observation in yeast has been made using tightly centromere-linked binding sites for hybrid GFP–DNA-binding proteins<sup>6,7,11</sup> (Fig. 3 and Supplementary Information). Similar observations have been made using GFP-tagged kinetochore proteins<sup>6,7</sup> and by *in situ* hybridization to normal chromosomes<sup>11</sup>. This separation of the kinetochores and centromeres at metaphase, termed ‘breathing’ or ‘splitting’, has been shown to last for 10 or more minutes, with separations of up to 0.8–1.0  $\mu\text{m}$  (half or more of the spindle length). The live-cell data fits well with previous electron-microscopic studies of spindle structure<sup>15</sup>. Furthermore, centromere separation has been reported in cells arrested at metaphase by mutation of *CDC20* (ref. 11), as predicted by electron-microscopic analysis of these spindles<sup>39</sup>. Later in mitosis, the centromeric regions reassociate, revealing interesting elastic properties of the chromosomes<sup>7,11</sup> (Fig. 3) and significant force generation. This degree of movement indicates very dynamic microtubule behaviour, as seen in other cell types, and has been documented in yeast using FRAP<sup>9</sup>. Furthermore, the change must be at the plus ends of microtubules, because FSM showed no minus-end turnover at the SPB<sup>9</sup>.

Some analyses of centromere splitting have revealed that centromeric DNA can be rather distant from the SPB (0.9  $\mu\text{m}$ ) and away from the spindle axis<sup>7</sup>, although this is not generally observed (refs 6, 11, and C. Pearson and K. Bloom, personal communication). Various electron-microscopic studies have failed to identify long, off-axis microtubules that could connect to these kinetochores<sup>4,15,17</sup>. Although there may be difficulties in fixing or visualizing such microtubules, which may also be very transient, it is possible that some observations made with extensively engineered chromosomes are not representative of the behaviour of endogenous chromosomes. For instance, it is difficult to demonstrate that the off-axis centromeric DNA is attached to a kinetochore microtubule, or that it has a normal level of condensation. Nonetheless, the demonstration of centromere splitting has resolved several unexplained



**Figure 3 Transient separation of sister chromatids.** Time-lapse images obtained using *LacI* repressor–GFP bound to a *Lac*-operator cluster 1 kb from the centromere on chromosome XI (arrows). The position of the SPBs is marked by *Spc72*–GFP (chevrons). The outline of the budded cell is shown in white. Images were obtained over a time period of roughly 3.5 min, at the indicated times. The

first and final panels show the centromeres together and the interceding six panels show the separation event. Scale bar represents 2  $\mu\text{m}$ . A time-lapse series showing a similar cell can be viewed as a movie in the Supplementary Information. This figure and the accompanying movie were supplied by C. Pearson and K. Bloom (Univ. North Carolina, Chapel Hill).

observations made using electron microscopy and allows for a model of metaphase in budding yeast that is largely consistent with data obtained using several imaging techniques. The description of metaphase in budding yeast contributes to the view that these cells have a relatively conventional mitosis.

### Anaphase movements

Budding yeast is known to exhibit both anaphase A (poleward movement of kinetochores), and anaphase B (increase in pole-to-pole distance). Anaphase B clearly contributes most to the separation of sister chromatids, as a metaphase spindle of  $\sim 1.5 \mu\text{m}$  in length can increase in length to 10  $\mu\text{m}$  (ref. 15). Furthermore, anaphase B has been found to be biphasic. In live-cell recordings, spindle elongation is initially fast, at  $1.0 \mu\text{m min}^{-1}$  or more, until a length of 4–6  $\mu\text{m}$  is reached, after which elongation pauses and then continues at a lower rate (half or less of the initial rate) to the final length<sup>44,45</sup>. A structural basis for this behaviour has not been determined. However, it has generally been observed that microtubule signal through use of immunofluorescence staining or autofluorescent GFP–tubulins is low, indicating that there are few microtubules in the long spindles of late anaphase. Indeed, three-dimensional reconstruction from serial thin sections has revealed that late spindles can have as few as four microtubules, two from each SPB. These microtubules are observed to twist around each other and interdigitate with the microtubules of the opposite polarity from the other SPB<sup>15</sup> (Fig. 1f). The packing of the microtubules is fairly regular (40-nm separation) with a distinct preference for antiparallel interactions. Crossbridge structures have been seen, by electron microscopy, between the interdigitated microtubules, and *Ase1p*, among other proteins, is localized to this region of the spindle<sup>46</sup>. The crossbridge structures may function to crosslink and stabilize the microtubules from the two SPBs. Indeed, these microtubules are known to grow at their plus ends away from the SPBs, and have been shown to exhibit little dynamic behaviour when tested by FRAP<sup>9</sup>. Whereas these few interpolar microtubules increase in length during anaphase B, many other microtubules shorten, and the control of these distinct events is not known. Not only is there shortening of kinetochore microtubules (see below), but there is also a reduction in the number of nonkinetochore microtubules from roughly six from

each SPB to the final two<sup>15</sup>. The mechanism used to cull the number of microtubules in the central spindle and then to stabilize and lengthen the selected microtubules is not known.

Concurrent with anaphase-B elongation of the spindle is the movement in anaphase A of the kinetochores towards the SPBs after the metaphase–anaphase transition (ref. 15 and C. Pearson and K. Bloom, personal communication). Shortening in anaphase A of presumptive kinetochore microtubules was reported in the HVEM study by Peterson and Ris<sup>4</sup>, and anaphase-A movement was implied for *in situ* hybridization of centromeric (CEN) DNA<sup>10</sup>. Also, GFP-tagged kinetochore proteins and CEN-linked ‘GFP dot’ markers are found directly adjacent to the SPBs at the end of anaphase B<sup>6,7</sup>. Three-dimensional electron-microscopic reconstruction of the spindles at various points in anaphase B has indicated that anaphase A was also occurring, as the presumptive kinetochore microtubules were shortening from an average length of 0.4  $\mu\text{m}$  in metaphase spindles to 0.2  $\mu\text{m}$  in late-anaphase spindles<sup>15</sup>. However, only a few kinetochore microtubules were detected on the SPBs of late-anaphase spindles (Fig. 1f). This was due to a problem with detection in the thin sections used, because more recent analysis using electron tomography allowed the recognition of residual kinetochore microtubules as short as 30 nm long, with a median length of 50 nm (ref. 17; Fig. 1g). As with anaphase A in other organisms, the relative contributions of loss of tubulin dimers at each end of the kinetochore microtubule is not known. However, FSM has revealed that there is little, if any, minus-end turnover of spindle microtubules, indicating that most of the polymer loss is from the plus ends of microtubules at the kinetochore<sup>9</sup>. How this is achieved during anaphase A is not known.

### Exit from mitosis and beginning anew

As described above, the late-anaphase spindle has very short kinetochore microtubules and a few long microtubules that interdigitate at the spindle midzone. This spindle must be disassembled before karyokinesis and subsequent cytokinesis. The fine structural details of this event are not known, although spindle breakage can be visualized by light microscopy and the long spindle microtubules are known to be disassembled from their plus ends<sup>9</sup>. The anaphase-promoting complex (APC) degrades *Ase1p* at this point in the cell cycle, a process

that is necessary for spindle disassembly<sup>47</sup>. Degradation of the mitotic cyclins, under the control of the mitotic-exit network (MEN), is necessary for cells to exit mitosis<sup>48</sup>. Haase *et al.*<sup>26</sup> have shown that degradation of the mitotic cyclins is also required for SPBs to enter the next round of duplication, a finding that hints at the existence of a licensing event at this late point in the cell cycle. Interestingly, many of the MEN proteins have been localized to the outer (cytoplasmic) plaque of the SPB, and carry out an important signalling function from this position in the cell<sup>49</sup>. An early event in the exit pathway is the activation of the *TEM1* gene product, which is thought to sense the position of the SPB (usually the new one) in the daughter cell; this is the basis of a spindle-position checkpoint that uses *BUB2* and *BFA1* (ref. 49). It seems fitting that the organelle that initiates the spindle cycle by duplicating to form the spindle poles should be so intimately involved in the very final events of the cycle, just before the formation of progeny cells.

Newly born G1-phase cells each contain a single SPB with a half bridge, as well as a single set of chromosomes. Unlike many other cell types, these interphase nuclei contain nuclear microtubules that are longer (median length 150 nm; ref. 17, Fig. 1a) than the putative kinetochore microtubules of late anaphase. On the basis of their numbers, it would seem that these could be longer forms of the remaining microtubules from late anaphase, and the kinetochores could still be attached as described above. How this microtubule lengthening occurs, how it is controlled and when it takes place are all unknown. Indeed, we have little idea of what controls microtubule dynamics in G1 phase and what function might be served by having the chromosomes attached to microtubules at this point in the cell cycle. It is plausible that this attachment is important for chromosome movement during nuclear fusion (karyogamy) in mating cells<sup>50</sup>. Nonetheless, this brings us back to where we started in the spindle cycle; a G1-phase nucleus with a single SPB that is prepared to duplicate.

## Summary

The extensive analysis of mitosis in yeast is leading to a complete molecular description of the mitotic spindle in these cells. Integrating molecular data with both fine structural data and dynamic live-cell analysis will provide a very sophisticated description of the mitotic mechanism. Several challenges remain, most notably concerning attachment of kinetochores to the spindle and management of microtubules in the nucleus such that they can accomplish different functions. Nonetheless, these efforts have provided evidence that many features of mitosis are conserved, meaning that their molecular description in yeast will be generally relevant to the study of mitosis. □

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