Primer

The mitotic spindle checkpoint

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One of the most awe-inspiring demonstrations in biology is a time-lapse video of mitosis viewed with high magnification phase contrast or Nomarski DIC microscopy. From initial chaotic movement comes the orderly alignment of chromosomes at the metaphase plate followed by a flawlessly synchronous splitting of the chromosomes and their gliding separation to the two mitotic poles. Enforcing the timing and synchrony of chromosome segregation is the job of the spindle checkpoint.

Chromosome instability

In normal cell division, each set of replicated chromosomes is usually precisely distributed to each of the progeny. However errors that lead to imbalances in chromosome segregation do occur, with catastrophic consequences. Loss or gain of chromosomes — aneuploidy — can lead to cell death or unregulated cell growth. In cancer, the outgrowth of aneuploid cells is a likely factor in the progression of tumor malignancy and in the development of resistance to therapy. In germ line cells, errors that occur in two chromosome segregation events of meiosis result in aneuploid gametes. These will become embryos with abnormal chromosome content that abort spontaneously or produce offspring with developmental defects.

The spindle checkpoint

Each mitotic chromosome, composed of two paired chromatids, moves to align at the spindle equator during prometaphase. Once all are aligned the cell is at metaphase. At anaphase, the chromatids separate and move synchronously to the spindle poles. A mechanism called the spindle checkpoint blocks anaphase onset and subsequent exit from mitosis until all chromosomes are properly attached to the spindle microtubules and aligned at the metaphase plate (Figure 1). In mammals, the spindle checkpoint is essential and ensures equal chromosome segregation at almost every cell division.

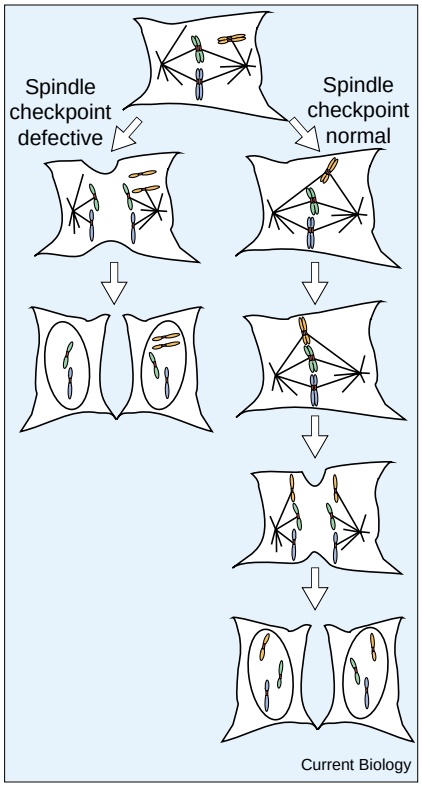
How does the spindle checkpoint system sense when all the chromosomes are attached and aligned at the metaphase plate? A cowboy moving a herd of cattle along a trail finds it easier to look behind for stragglers rather than count the many animals milling about in front of him. Similarly, the spindle checkpoint does not regard the chromosomes at the metaphase plate but scans for straggler chromosomes out in the cytoplasm or near the spindle poles. Anti-microtubule drugs disrupt the spindle and scatter the chromosomes, turning them all into stragglers and causing maximal activation of the spindle checkpoint.

In mammalian cells chromosome segregation normally takes less than an hour. While the spindle checkpoint can delay the onset of anaphase for many hours, the delay is rarely permanent. In a process termed adaptation, cells can override the spindle checkpoint and exit mitosis. The strength of the spindle checkpoint — how long cells will remain arrested in the presence of microtubule drugs — varies among cells derived from different species and among individual cell lines from the same species. Normally a mechanism induces apoptosis in cells that have passed through mitosis and have missegregated their chromosomes. However, in cancer cells the failsafe mechanisms for eliminating cells with abnormal numbers of chromosomes may be ineffective.

The kinetochore

During chromatin condensation in prophase, a pair of organelles called kinetochores form on each chromosome. The major forces for chromosome movement are imparted by the interactions of kinetochores with microtubules of the mitotic spindle. In addition to its function in chromosome motility, the kinetochore plays a vital role in signaling the spindle checkpoint. The basic idea is that ‘unsatisfied’ kinetochores, those that lack microtubule attachments or those whose attachments are unstable, signal and inhibit the onset of anaphase. Even a single unaligned

Figure 1



The spindle checkpoint inhibits premature chromosome segregation. Because chromosomes move individually to align at the metaphase plate, there are stages when some but not all chromosomes are aligned. If the spindle checkpoint is defective (left pathway) then chromatid separation can occur before all the chromosomes are aligned at metaphase. The resulting cells have imbalanced numbers of chromosomes. In the presence of the normal spindle checkpoint (right pathway), chromatid separation is delayed until all the chromosomes have aligned and chromosomes are segregated equally.

chromosome can block anaphase onset. Once the last chromosome achieves stable attachment to microtubules of the spindle, the kinetochore ceases its distress signal. After some delay to allow the final chromosome to move to the metaphase alignment, anaphase ensues. This model has been validated in yeast by genetic manipulation and in mammalian cells by laser ablation experiments. In mammalian cells, ablating the kinetochores of laggard chromosomes destroys their ability to signal the checkpoint and induces premature anaphase.

Severing chromosome cohesion

Much of our understanding of the spindle checkpoint originates from genetic studies with the budding yeast *Saccharomyces cerevisiae*. The two chromatids of a chromosome are linked by a protein complex termed cohesin. At anaphase onset, one of the subunits of that complex, Scc1, is destroyed by a protease called separase. Interestingly, in budding yeast all the Scc1 is cleaved at anaphase onset. In mammalian cells, the bulk of the cohesin falls off the chromosomes during prophase and prometaphase. A small amount remains concentrated between the kinetochores. Thus in mammalian cells, only a small amount of the Scc1 protein, presumably the kinetochore-associated portion, is proteolysed at anaphase onset.

If the proteolytic activity of separase initiates anaphase, what keeps separase activity in check until the chromosomes are aligned at metaphase? That function falls to a family of proteins called securins that exhibit weak conservation among different species. Securins are proteins that bind to and inhibit the activity of separase. When cells reach metaphase the securins are conjugated with ubiquitin and degraded by the 26S proteasome. In the timing of anaphase onset, the critical feature is regulation of the mitotic ubiquitin ligase termed the anaphase-promoting complex or cyclosome (APC/C). APC/C is a large complex of approximately a dozen proteins. At anaphase onset, APC/C targets securin for ubiquitin-mediated proteolysis, thus releasing separase. At about the same time, the APC/C also begins to induce the degradation of cyclin B. At entry to mitosis cyclin B binds and activates cyclin-dependent kinase 1 (Cdk1/Cdc2). Cdk1 is the primary kinase that maintains the mitotic state. The degradation of the cyclin B activator downregulates Cdk1 and leads to exit from mitosis.

Regulating APC/C

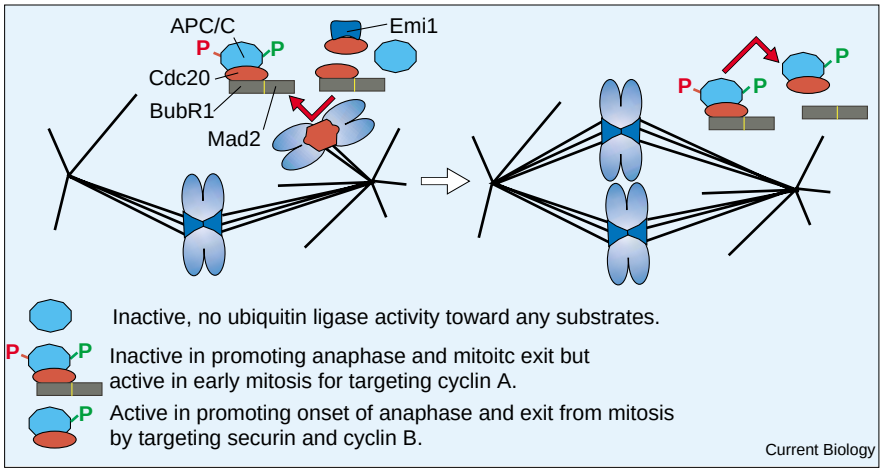
The basic model for the spindle checkpoint holds that unattached or unstably attached kinetochores broadcast a signal that inhibits APC/C-mediated degradation of securin. The pressing targets of recent investigation are the protein components of the checkpoint signal, how the checkpoint signal is broadcast, how it protects securin until metaphase, and lastly, how the checkpoint is extinguished at the appropriate time to allow chromatid separation at anaphase. Studies in budding yeast identified a number of proteins whose loss compromises the spindle checkpoint. These include Mad1, Mad2, Mad3/BubR1, Bub1, Bub3 and Mps1. All these proteins have homologues in vertebrate cells that are concentrated at kinetochores in mitosis. For most the accumulation of the protein is maximal at unattached kinetochores and diminishes as the kinetochore attaches to microtubules and moves to the metaphase plate. Two of the proteins, Mad2 and Mad3/BubR1, appear to be direct inhibitors of APC/C ubiquitin ligase activity. There is some disagreement whether these proteins are part of a single inhibitor complex or function separately. Cell fractionation suggests that an assortment of complexes of checkpoint proteins coexists within the cytoplasm. The inhibitor complexes bind a protein called Cdc20 (also called p55CDC/Fizzy). Cdc20 binds to APC/C and acts as an adaptor protein to bind substrates. Blocking Cdc20 function in yeast and in vertebrate cells inhibits progression to anaphase. It is not yet clear how the checkpoint protein inhibitor complexes act. Recent studies suggest that they do not block binding of Cdc20 to the APC/C nor the binding of substrates. Rather they appear to inhibit the ubiquitin ligase activity of the Cdc20–APC/C complex toward specific substrates. Originally it was supposed that the spindle checkpoint simply inhibited all ubiquitin ligase activity of APC/C. However in early mitosis the spindle checkpoint protects securin and cyclin B but does not hinder APC/C and Cdc20-dependent degradation of another cyclin, cyclin A.

Protein dynamics and the checkpoint

Most models of the spindle checkpoint suggest that an unattached kinetochore must broadcast a signal to the entire cell. One notion is that proteins associate with the signaling kinetochore, are modified in some way, and then move off to the rest of the cell. Tools have become available to study the movements of candidate proteins within living cells. These include tracking fluorescent proteins in living cells by high resolution fluorescence microscopy and quantifying dynamics at organelles through methods such as fluorescence photobleaching and recovery. Static images prepared by immunolabeling tended to emphasize the accumulation of checkpoint proteins at kinetochores. However, only a small proportion of the total cellular pool is found there at any one time. Most kinetochore-associated checkpoint proteins are also found diffusely distributed in the cytoplasm and associated with other organelles, particularly the spindle poles. The multiple pools of checkpoint proteins may reflect transmission of the checkpoint signal. The rapid dynamic associations of checkpoint proteins with organelles

may also be important in turning the checkpoint on and off. High resolution imaging suggests that Mad2 protein travels out of kinetochores on attached microtubules. The idea is that transport of checkpoint signaling components out of the kinetochore on microtubules may be linked to turning off the checkpoint signal. Mutant proteins with altered trafficking properties may allow us to discover how intracellular trafficking contributes to the regulation of spindle checkpoint signaling.

Figure 2



Kinetochores of unattached chromosomes block anaphase onset by facilitating the association of inhibitory proteins with the mitotic ubiquitin ligase called the anaphase-promoting complex/cyclosome (APC/C). In cells with any chromosomes lacking bipolar attachment to the mitotic spindle (left panel), unattached kinetochores catalyze changes to the APC/C, possibly phosphorylation, that promote attachment of the substrate adaptor protein Cdc20 bound with an inhibitory complex that contains BubR1 and Mad2. This form of the APC/C can ubiquitylate certain targets such as cyclin A, but cannot ubiquitinate securin and cyclin B whose destructions are required for anaphase onset and exit from mitosis. After all the chromosomes have achieved stable bipolar attachment (right panel), no further production of the inhibited form of the APC/C occurs. The inhibitor complex containing Mad2 and BubR1 is released, perhaps accompanied by removal of inhibitory phosphates on APC/C. APC/C can now target securin and cyclin B. The protein Emi1 may also play a role at early stages of mitosis by sequestering Cdc20.

Kinetochores may modify APC/C

Many early models suggested that kinetochores of unaligned chromosomes might catalyze the assembly of spindle checkpoint inhibitor complexes. However, recent studies show that inhibitor complexes are present in cells throughout the cell cycle, even in interphase. As kinetochores do not exist in interphase, it seems unlikely that they are required for inhibitor complex formation. Ubiquitin conjugation studies *in vitro* showed that only APC/C isolated from mitotic cells could be inhibited by the spindle checkpoint complexes. APC/C from interphase cells was unaffected unless it was first exposed to isolated mitotic chromosomes. These data are more consistent with a new model that suggests that during mitosis kinetochores of chromosomes that are not aligned at the metaphase plate induce a modification of APC/C causing it to become sensitive to the effects of the inhibitor complexes (Figure 2). What form might this modification of the APC/C take? One idea is phosphorylation. A variety of kinases implicated in spindle checkpoint activity have been immunolocalized at kinetochores. In addition, phosphatase activity is required for normal progression through mitosis. The 3F3/2 antiphosphoepitope antibody is a marker for kinetochores that are active in spindle checkpoint signaling. Kinetochores of chromosomes aligned at the meta-phase plate show little or no labeling with the 3F3/2 antibody while the kinetochores of unaligned chromosomes are strongly labeled. In mitosis several components of APC/C become highly phosphorylated. Both activating and inhibitory effects of kinases on the APC/C have been found. Moreover, the competitive binding of Cdc20, and a related adaptor protein that acts at the end of mitosis, Hct1/Cdh1, to the APC/C is regulated by phosphorylation. To add further complexity another protein termed early mitotic inhibitor-1, Emi1, that sequesters Cdc20 and inhibits the ubiquitin ligase activity of the mitotic APC/C has recently been found. Emi1 appears to act during early mitosis, and is itself degraded prior to metaphase.

Turning off the spindle checkpoint

One enduring controversy is the biophysical nature of the upstream event that shuts down kinetochore checkpoint signaling when micro-tubules are stably attached. In one model, mechanical tension on kinetochores generated by the bipolar attachment of spindle microtubules directly modulates enzyme activities at the kinetochore and halts checkpoint signaling. The tension hypothesis is supported by genetic studies in yeast and by micromanipulation experiments in insect spermatocytes. In insect spermatocytes, pulling on chromosomes with microneedles turns off checkpoint signaling and simultaneously downregulates kinetochore expression of kinetochore phosphoepitopes recognized by the 3F3/2 antibody. The competing model is that occupancy of microtubule-binding sites at the kinetochore is the event that turns off checkpoint signaling. This model is supported by studies in cultured mammalian cells. Treating cells with the microtubule

hyperstabilization reagent taxol reduces tension at all kinetochores but leads to accumulation of the checkpoint protein Mad2 at only a few kinetochores, presumably those in which taxol induces unoccupied microtubule binding sites. Because tension increases microtubule stability and occupancy at kinetochores, the competing hypotheses have been difficult to tease apart. There is evidence that tension and occupancy may each act in different situations within one organism, in meiosis versus mitosis in maize for example. Others have proposed that both tension and occupancy simultaneously play partial roles in the downregulation of the spindle checkpoint signal.

Microtubule motors

Central to understanding how the checkpoint is switched off is finding the proteins that communicate the presence of microtubules to the signaling components of the kinetochore. Microtubule motor molecules and their associated proteins are key candidates. Centromere protein E, CenpE, is a member of the kinesin family of microtubule motor proteins. In mammalian cells, inhibition of CenpE blocks chromosome alignment at the metaphase plate and activates the spindle checkpoint. However, in cycling extracts of activated *Xenopus* eggs, immunodepletion of CenpE eliminates the checkpoint signal itself. Another microtubule motor, dynein, is implicated by its association with two dynein-interacting proteins, Zw10 and Rod. These proteins were identified in screens for mitotic mutants in *Drosophila* and for both the *Drosophila* proteins and mammalian homologs, inhibition of either protein blocks the normal association of dynein with kinetochores and reduces the stringency of the spindle checkpoint signal. Neither CenpE, Zw10 nor Rod has a homolog in budding yeast. Thus although the signaling components of the spindle checkpoint pathway show close homology from budding yeast to mammals, the kinetochore proteins that bind to microtubules may differ.

The spindle checkpoint in meiosis

Dividing cells in both mitosis and meiosis have been used to explore the spindle checkpoint pathway. Robust spindle checkpoint systems exist in yeast meiotic divisions and in the meiotic divisions of male germ cells in insects and in mammals. However in female meiosis and in early embryonic divisions in several species, the spindle checkpoint appears less stringently applied. Evolution may have had more opportunity to experiment with meiosis particularly in oogenesis where lengthy arrests of the cell cycle are physiologically normal. In particular, mechanisms controlling the first division of meiosis may be quite different from those in mitosis or in the second meiotic division which is more 'mitotic-like'. In *Xenopus* oocytes, the first meiotic division can occur independently of APC/C ubiquitin ligase activity. In an interesting turnabout, in *Drosophila* oogenesis, tension across chromosomes causes rather than releases the normal arrest of oocytes in meiosis.

Only now are researchers beginning to investigate in higher eukaryotes how meiotic spindle checkpoint systems operate, what components are shared between mitosis and meiosis, and how the checkpoint systems differ in males and females. Understanding meiotic checkpoint systems is of obvious practical significance: in humans chromosomal imbalances occur frequently. It has been estimated that 25% of human conceptions produce aneuploid offspring. The gain or loss of chromosomes usually results in embryonic death, but certain aneuploidies such as trisomy chromosome 21 and imbalances in the sex chromosomes, result in live births of individuals with developmental defects. An age-dependent, quantitative decrease in levels of mRNA coding for checkpoint proteins was found in comparing human oocytes obtained from younger and older women. This loss of coding potential for spindle checkpoint proteins may contribute to the age-dependent increase in frequency of aneuploidy found in oocytes of older women.

Conclusion

Fantastic progress has been made in understanding the spindle checkpoint, the guardian of balanced chromosome segregation. However, several basic questions and many details remain to be worked out. In the meantime, the use of chimeric fluorescent proteins and ever better imaging technology has enhanced the stunning videos of mitosis made today, revealing ever more fascinating details of cell division and evoking greater gasps of wonder from seminar audiences. Keep watching.

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