and THOMPSON, J. M. (1993) *J. Cell Sci.* 104, 1155–1162
34 ROTHENBERGER, S. *et al.* (1996) *Brain Res.* 712, 117–121
35 BAKER, E. N., RUMBALL, S. V. and ANDERSON, B. F. (1987) *Trends Biochem. Sci.* 12, 350–353
36 PLOWMAN, G. D. *et al.* (1983) *Nature* 303, 70–72
37 FOOD, M. R., ROTHENBERGER, S., GABATHULER, R., HAIDL, I. D., REID, G. and JEFFERIES, W. A. (1994) *J. Biol. Chem.* 269, 3034–3040
38 BAKER, E. N. *et al.* (1992) *FEBS Lett.* 298, 215–218
39 RICHARDSON, D. R. and BAKER, E. (1991) *Biochim. Biophys. Acta* 1091, 294–302
40 RICHARDSON, D. R. and BAKER, E. (1994) *J. Cell. Physiol.* 161, 160–168
41 KELLER, G. A., SIEGEL, M. W. and CARAS, I. W. (1992) *EMBO J.* 11, 863–874
42 ROTHBERG, K. G., YING, Y., KOLHOUSE, J. F., KAMEN, B. A. and ANDERSON, R. G. W. (1990) *J. Cell Biol.* 110, 637–649
43 MAYOR, S., ROTHBERG, K. G. and MAXFIELD, R. (1994) *Science* 264, 1948–1951
44 SCHNITZER, J. E., McINTOSH, D. P., DVORAK, A. M., LIU, J. and OH, P. (1995) *Science* 269, 1435–1438
45 RIJNBOUTT, S., JANSEN, G., POSTHUMA, G., HYNES, J. B., SCHORNAGEL, J. H. and STROUSS, G. J. (1996) *J. Cell Biol.* 132, 35–47
46 DANIELSON, E. M. and VAN DEURS, B. (1995) *J. Cell Biol.* 131, 939–950
47 KENNARD, M. L., RICHARDSON, D. R., GABATHULER, R., PONKA, P. and JEFFERIES, W. A. (1995) *EMBO J.* 14, 4178–4186
48 UEDA, F., RAJA, K. B., SIMPSON, R. J., TROWBRIDGE, I. S. and BRADBURY, W. B. (1993) *J. Neurochem.* 60, 106–113
49 GARRATT, R. C. and JHOTI, H. (1992) *FEBS Lett.* 305, 55–61
50 DUNN, K. W., McGRAW, T. E. and MAXFIELD, F. R. (1989) *J. Cell Biol.* 109, 3303–3314

References added in proof

51 ROTHENBURG, B. E. and VORLAND, J. R. (1996) *Proc. Natl Acad. Sci. USA* 93, 1529–1534
52 STEARMAN, R., YUAN, D. S., YAMAGUCHI-IWAI, Y., KLAUSNER, R. D. and DANCIS, A. (1996) *Science* 271, 1552–1557

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The spindle-assembly checkpoint: aiming for a perfect mitosis, every time

William A. E. Wells

Checkpoints reduce the frequency of errors in cell division by delaying the progress of the cell cycle until certain processes are complete. The spindle-assembly checkpoint prevents the onset of anaphase until a bipolar spindle is present and all chromosomes are attached to the spindle. Evidence from yeast and mammalian cells suggests that kinetochores are at least one source of the signal that stops the cell cycle. Recent studies in budding yeast have begun to define the signal-transduction pathway involved in the spindle-assembly checkpoint, but details of the endpoint of the pathway, where these signals interact with the cell-cycle machinery, remain to be characterized.

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The eukaryotic cell cycle is driven by the regular activation and inactivation of cyclin-dependent kinases (CDKs). These oscillations can be modified by the requirement that certain events be completed before others are started; the systems responsible for this monitoring of the progress of the cell cycle are called checkpoints. They contribute to the fidelity of the cell cycle by allowing time for DNA replication and repair before the cell enters mitosis and for alignment of chromosomes on the spindle before the cell initiates anaphase. When these periodic events are incomplete, the checkpoint systems block further transitions in the cell cycle (see Ref. 1 and recent reviews on the cell biology[2] and genetics[3] of various checkpoints). If the checkpoints fail to function, genetic errors arise much more frequently. Mutation of the genes encoding proteins involved in checkpoints may be an important event in the genesis of cancer cells, allowing genetic changes to accumulate more readily, although the stages at which checkpoint controls are lost may differ for different tumour types[4]. In this review, I survey what is known about the spindle-assembly checkpoint. This is the mechanism or group of mechanisms that ensures that a bipolar spindle is present and that all the chromosomes are attached to the spindle before the cell initiates anaphase and progresses into the next cell cycle.

In common with other systems in the cell that respond to internal or external conditions, a checkpoint should consist of three functional units: a sensor, a signal-transduction cascade and an effector (Fig. 1). In the case of the spindle-assembly checkpoint, the sensor monitors events such as attachment of chromosomes to the mitotic spindle and, if there is a defect or the process is not complete, generates a signal. The initial signal is then amplified in a signal-transduction cascade and eventually modifies the cell-cycle machinery such that the cell cycle is halted until the defect is rectified. Studies in various organisms have led to a partial understanding of the sensor and signal-transduction cascade, but the nature of the endpoint of the pathway remains unknown.

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Discovery of the spindle-assembly checkpoint

Different experimental systems have proved useful in studying various aspects of this checkpoint. Budding yeast has the advantage of powerful genetics and a relatively simple kinetochore (the protein complex that assembles on the centromere and is responsible for chromosome attachment to the spindle), whereas the larger nuclei of mammalian cells make it possible to micromanipulate individual chromosomes and to observe chromosome movement directly.

The first indication of the existence of a spindle-assembly checkpoint came from the ability of microtubule-depolymerizing drugs such as nocodazole to arrest cells in mitosis. Cell-cycle arrest by nocodazole treatment could reflect the loss of the mechanical apparatus (the microtubules of a bipolar spindle) needed for progression through mitosis. Alternatively, the cell-cycle arrest may reflect the functioning of a checkpoint that monitors an aspect of spindle structure and stops the cell cycle if the spindle is perturbed. If a spindle-assembly checkpoint exists, mutants defective in the components of the checkpoint should progress through mitosis despite the presence of microtubule-depolymerizing drugs. In budding yeast, two genetic screens for mutants that fail to arrest in microtubule-depolymerizing drugs (benomyl or benzimidazole) yielded six genes potentially involved in the spindle-assembly checkpoint: *MAD1*, *MAD2* and *MAD3* (mitotic arrest deficient[5]) and *BUB1*, *BUB2* and *BUB3* (budding uninhibited by benzimidazole[6]). Cells harbouring mutations in these genes fail to arrest when the spindle is disrupted by microtubule-depolymerizing drugs and go through an aberrant, error-prone mitosis before entering the next cell cycle. Benomyl treatment of the mutants causes widespread chromosome loss, but even in the absence of benomyl the *mad* mutants have an increased rate of chromosome loss[5]. This is presumably caused by a failure to delay in response to occasional spontaneous errors in chromosome attachment, and confirms the proposed surveillance function of the checkpoint mutated in *mad* and *bub* mutants.

Is a lengthy checkpoint arrest the only possible response to destruction of the spindle? In those organisms and cell types that do arrest, the arrest can range from being essentially irreversible to being brief or non-existent[7,8]. Furthermore, evidence from studies on the DNA-damage checkpoint in yeast suggests that checkpoints can be downregulated in some cells in response to irreparable mistakes, thus saving the cell from a permanent, futile arrest, although the ensuing mitosis may involve catastrophic errors[9]. Another response to microtubule-depolymerizing drugs, which is seen in some mammalian cells, is apoptosis[10,11]. The requirement for the tumour suppressor p53 both for the functioning of checkpoint arrests (in G1 phase[12] and mitosis[13]) and for an apoptotic pathway (in response to radiation damage[12]) provides additional evidence suggesting that apoptosis may be an alternative response to detection of the same initial defect.

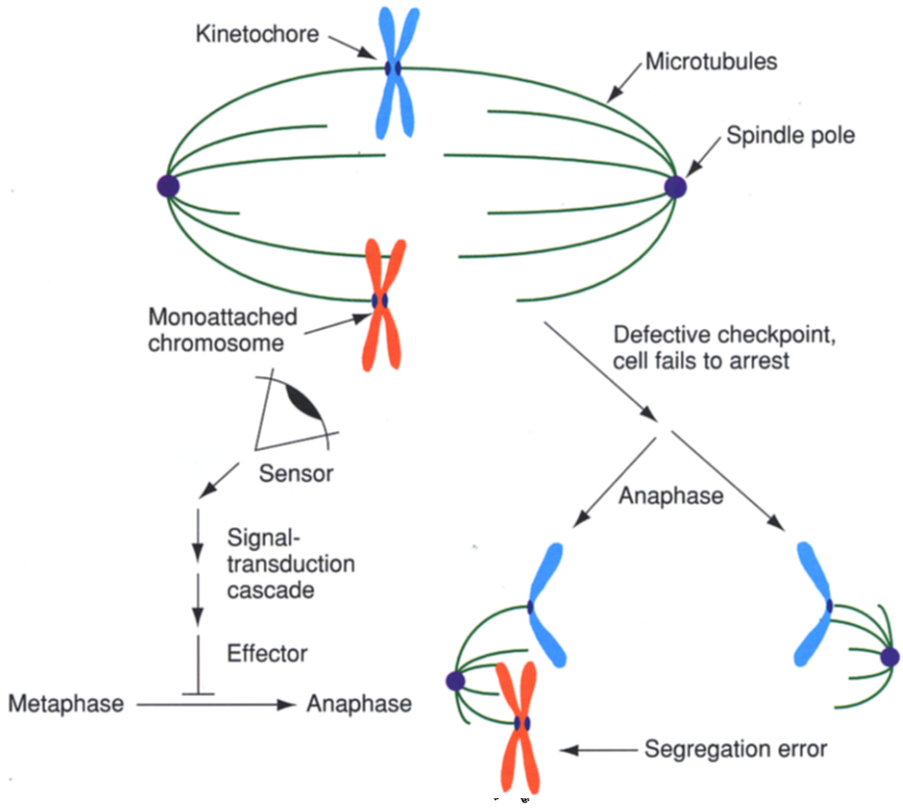

FIGURE 1

The spindle-assembly checkpoint. This system monitors one or more features of the mitotic spindle. When a defect is detected (such as a monoattached chromosome), a signal is generated that, after passing through a signal-transduction cascade, prevents progression of the cell cycle in some way that is not yet understood (left-hand pathway). This results in the cell remaining in mitosis (in a metaphase-like state) as the onset of chromosome segregation (anaphase) is inhibited. There is therefore time for a spindle to be assembled and for all chromosomes to attach correctly to the spindle. In the absence of a functional checkpoint system (e.g. in a yeast mutant), errors are not detected. Owing to the lack of a mitotic delay, there is no time to correct these errors, and this results in incorrect chromosome segregation (right-hand pathway).

What structure is being monitored by the spindle-assembly-checkpoint sensor?

Mitosis is a complicated process involving many different components, and the checkpoint sensor could monitor the status of any number of them. The sensor could detect free-tubulin levels, the function of the microtubule-organizing centre, the bipolarity of the spindle, or kinetochore attachment to microtubules. Also, the integrities of many of the mitotic components that may be sensed are interdependent; for example, the generation of monopolar spindles also creates free kinetochores. This has resulted in the present uncertainty as to whether the spindle-assembly checkpoint consists of one or more systems, as not all experimenters using microtubule-depolymerizing drugs may be studying the same phenomenon.

One experimental aim, then, is to isolate the possible perturbations to the mitotic apparatus so that each one can be studied separately. This can be achieved in yeast by manipulating chromosome structure directly, for example by mutating the short centromeric sequence, leaving the spindle intact. Mitotic delays are seen in budding yeast in the presence of mutant, partially functional centromeres[14] and dicentric chromosomes[15]. Also in budding yeast, chromosomes that have difficulty in attaching to the spindle because of their small size or high numbers appear to activate the checkpoint. Thus, the presence of short, linear minichromosomes or an excess of short, circular minichromosomes causes a *MAD*-dependent cell-cycle delay, even though the spindle is not visibly disrupted[16]. Finally, unambiguous results from mitotic mammalian cells have demonstrated that single, unattached kinetochores can delay the cell cycle[17]. Hence, disruption of the attachment of chromosomes to the spindle by several means can cause the spindle-assembly-checkpoint sensor to generate a cell-cycle-arrest signal. This suggests that kinetochores, the sites on the chromosomes that mediate attachment, may be the features of the spindle that are monitored.

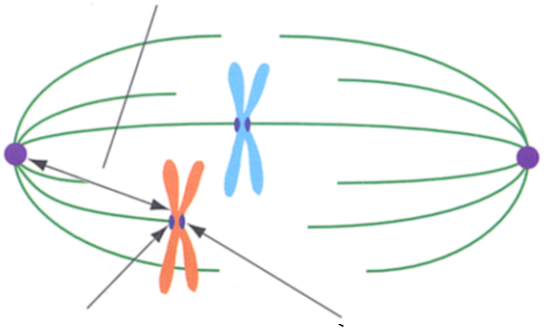

FIGURE 2

Mechanisms by which the spindle-assembly checkpoint may detect monoattached chromosomes. There is evidence supporting the models shown in both (b) and (c), but not in (a). (a) A monoattached chromosome (at the left of the spindle) lies, on average, closer to the spindle pole than does a chromosome with a correct, bioriented attachment (centre of spindle). This proximity could be detected in some unknown way. (b) Once chromosomes are attached to the spindle, the kinetochore fibres exert forces pulling the chromosome towards the spindle pole. The occupied kinetochore of a monoattached chromosome is not under tension as there is no opposing force from attachment of the other sister kinetochore to the opposite spindle pole. A tension-sensitive protein in the occupied kinetochore could generate a checkpoint signal to halt the cell cycle. The production of this signal can be prevented experimentally by exerting force on the chromosome by use of a glass needle to mimic an opposing spindle attachment[25]. The checkpoint is then bypassed. (c) The unattached kinetochore could generate a signal directly, by virtue of its free microtubule-binding sites. Support for this model comes from experiments in which the free kinetochore is ablated by a laser. The resultant monoattached chromosome is unable to signal to the spindle-assembly checkpoint[30]. Note that if the model in (b) is correct, the ablation of the free kinetochore would not prevent production of the checkpoint signal as the signal originates from the occupied kinetochore, which is still intact.

Do the protein components of the budding yeast kinetochore give any clues as to how kinetochores could be monitored? Two such components are Ctf13p and Ndc10p (for a review, see Ref. 18). A defect in a kinetochore component that results in partial functioning of the kinetochore should activate the checkpoint, resulting in an arrest in mitosis, and this is the case when the gene encoding Ctf13p is mutated. This arrest is dependent on the functioning of at least some of the *MAD* and *BUB* gene products[19]. If the structure of the entire kinetochore is destroyed, however, the kinetochore will be incompetent to signal that there is a defect. This may explain why *ndc10* mutant cells do not delay in mitosis[18], even as chromosome segregation fails. It is also possible that Ndc10p has a more specific role in the detection of chromosome-attachment defects and the subsequent signalling to the checkpoint. If this were true, it should be possible to isolate mutants deficient for the checkpoint function, but competent for the chromosome-segregation function. Such a separation of essential and checkpoint functions by specific mutation has been described for DNA polymerase ε in the DNA-replication checkpoint[20].

The more complex mammalian kinetochore has proved far less tractable than the budding yeast kinetochore. Mitotic arrest, presumably due to the engagement of a checkpoint, occurs upon the injection of antibodies to CENP-C, a structural component of the kinetochore. In such cells, the disrupted kinetochores are small or non-existent, with few associated microtubules[21]. One important consideration when comparing yeast and mammalian checkpoints is that, in mammals, multiple microtubules can be seen interacting with a single kinetochore, in contrast with the single kinetochore microtubule in budding yeast. Therefore, 'attachment' may not be the all or nothing affair that it is in budding yeast; a partially attached kinetochore may be as competent to generate a signal to stop the cell cycle as is a fully detached kinetochore.

Mechanisms of sensing defects in kinetochore attachment to the spindle

Mammalian, rather than yeast, cells can be used to approach the problem of how defects in kinetochore attachment to the spindle are sensed by the checkpoint as, in this system, better cytology can be teamed with chromosome micromanipulation. There are several possible ways in which defective kinetochore attachment, resulting in monoattached chromosomes, could be sensed (Fig. 2). Monoattached chromosomes are, on average, closer to one pole of the spindle, so proximity to the centrosome has been suggested as a signal[22], although no evidence has been obtained for or against this model. Second, monoattachment will result in an absence of

the tension normally generated by an opposing kinetochore fibre, and this could be sensed either at the centrosome or, more likely, at the kinetochore. Finally, free kinetochores have few or no microtubules attached, and this could generate a signal.

The tension-based mechanism has been studied most intensively so far. Tension was invoked originally to explain the stability of the attachments of chromosomes that have attached successfully to both spindle poles. This stability can be conferred to a monoattached chromosome by exerting tension with a micromanipulating needle to mimic the force associated with the second attachment[23]. The idea of there being tension at the kinetochore seems reasonable as close observation of monoattached chromosomes by video microscopy reveals alternate stretching and compression of the kinetochores as the chromosomes spontaneously reverse their direction of movement[24]. Tension also appears to play a role in the timing of the metaphase-to-anaphase transition, at least in meiosis. In mantid spermatocytes, a univalent X chromosome can delay anaphase onset in meiosis. Applying tension to the chromosome with a glass needle simulates attachment to the opposite spindle pole, and the cells immediately initiate anaphase[25], thus overcoming the arrest. In meiosis in *Drosophila melanogaster* females, however, the presence of tension has the opposite effect. In this case, tension from the intertwining of recombined homologues is necessary to cause a delay in the cell cycle until passage of the oocytes through the oviduct[26].

Possible biochemical evidence for a tension-sensitive checkpoint comes from an unknown phosphoprotein target recognized by the 3F3 antibody. This monoclonal was originally raised to whole, thiophosphorylated frog extracts. The 3F3 antibody stains all kinetochores to some extent, but unattached kinetochores stain more strongly[27]. Strong staining may therefore be an indicator of a checkpoint signal that arises because of a lack of tension. Support for this interpretation comes from studies using grasshopper spermatocytes, in which chromosomes can be micromanipulated such that one chromosome pair has both kinetochores attached to a single pole. The high 3F3 staining on these kinetochores, which are not under tension, can be diminished by exerting an opposing force on one chromosome by use of a glass needle[28]. The mitotic delay observed upon injection of 3F3 antibodies (a treatment that does not affect chromosome congression and, therefore, probably does not affect chromosome attachment) may occur because the antibodies prevent the dephosphorylation of the epitope and so lock the checkpoint on[29].

Although the properties of the 3F3 epitope were first characterized in mitotic cells[27,29], the more direct evidence for tension as a signal, derived from the micromanipulation experiments described above involving 3F3 staining[28] and the timing of anaphase onset[25], was all obtained in meiotic cells. So, is tension a likely checkpoint sensor in mitotic cells? Some evidence in mammalian mitotic cells supports the idea that the signal for the spindle-assembly checkpoint arises from the kinetochores of monoattached chromosomes that have bound microtubules ('occupied kinetochores') but that lack tension. Reduced tension at the kinetochore may be the cause of the delay in the completion of mitosis seen when taxol, a microtubule-stabilizing drug, is added to certain mammalian cells after the last kinetochore attaches[17]. The only visible effect of the taxol is the suppression of chromosome movements; kinetochores remain attached and the chromosomes are aligned correctly at the middle of the spindle. It is still difficult to rule out more subtle perturbations in kinetochore–microtubule interactions such as the exposure of a few free microtubule-binding sites at the kinetochore.

Although the experiments described above suggest strongly that the spindle-assembly checkpoint detects tension at occupied kinetochores, recent studies using laser ablation of kinetochores in mammalian mitotic cells have identified an alternative source for the inhibitory signal, namely, unoccupied kinetochores[30]. If the unattached kinetochore of the last monoattached chromosome in these cells is destroyed by laser ablation, the cell no longer delays as usual but rapidly enters anaphase. Whatever the explanation for the failure of the remaining kinetochore (which is now no longer under tension) to stop the cell cycle, the presence of a checkpoint signal before ablation of the free kinetochore suggests that free kinetochores constitute a major checkpoint signal. The complete answer may lie in a combination of the two models, in which a lack of tension is sufficient to generate some free microtubule-binding sites in the kinetochore, and it is these free binding sites that signal to the checkpoint.

If the sensor is detecting a combination of tension and attachment of kinetochores, is the relative importance of the two sensing systems always the same? It may be that tension is more important in meiosis, where there is no structural constraint forcing the kinetochores of homologues to lie back to back. In meiosis, it is relatively easy for both meiotic chromosomes of a pair to become attached to the same pole, and this arrangement would not activate a purely attachment-driven checkpoint. In mitosis, the paired sister chromatids lie naturally back to back, so it is unlikely that they would become attached to the same pole. In this case, full attachment to the spindle is a good indicator of fidelity. It is also possible, although perhaps unlikely, that mantid spermatocytes[25] represent a unique solution for a unique system (a meiosis involving trivalent sex chromosomes).

Experiments with sea urchin zygotes suggest that the detection of other features besides chromosome attachment may elicit a checkpoint arrest in mitosis. The creation of monopolar spindles in sea urchin zygotes leads to a significant delay in anaphase onset. If an intact, bipolar spindle is present in the same cytoplasm, however, the cell cycle is no longer delayed, even if the spindle contains a greatly reduced number of microtubules[31,32]. These cells are insensitive, therefore, to any kinetochore signal, but still have a positive requirement for a bipolar spindle. The reason that nocodazole treatment leads to

TABLE 1 – GENES REQUIRED FOR FUNCTIONING OF THE SPINDLE-ASSEMBLY CHECKPOINT

Gene[a]	Properties of protein	Refs
MAD1	Non-essential coiled-coil protein, hyperphosphorylated in cells treated with anti-microtubule drugs	34
MAD2	Non-essential, small, novel	–[b]
MAD3	Non-essential, short region of homology with Bub1p	–[c]
BUB1	Non-essential protein kinase, binds to and phosphorylates Bub3p	35
BUB2	Non-essential, homologous to fission yeast cdc16	45
BUB3	Non-essential, binds to and is phosphorylated by Bub1p	35
MPS1	Protein kinase, essential for spindle-pole-body assembly	36
p44ERK2	MAP kinase, necessary for checkpoint in *Xenopus* extracts	37
p53	Tumour-suppressor protein; well-characterized role in G1-phase radiation checkpoint; function in spindle-assembly checkpoint poorly defined	12,13

[a]All from budding yeast excepting *p44ERK2* and *p53*.
[b]R-H. Chen and A. W. Murray, pers. commun.
[c]K. Hardwick and A. W. Murray, pers. commun.

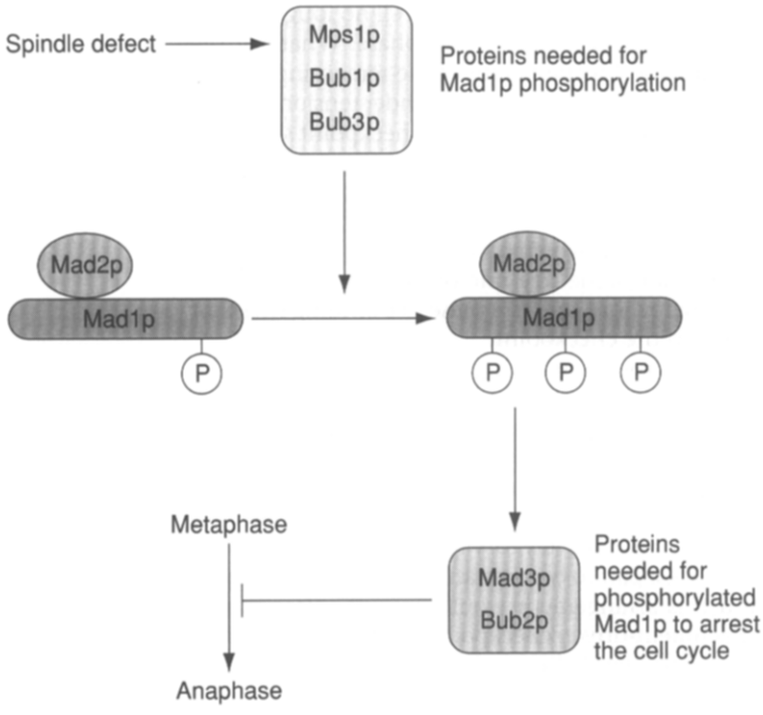

FIGURE 3

One model for signal transduction in the spindle-assembly checkpoint. Grouping of proteins in the boxes does not imply a physical interaction, but indicates merely that their relative order in the pathway cannot be determined as yet. Mps1p, Bub1p and Bub3p are necessary for the hyperphosphorylation of Mad1p in the presence of nocodazole[34], although they may not all lie in a strictly linear pathway. Mad1p is associated with Mad2p and may be directly phosphorylated by Mps1p (R-H. Chen, K. Hardwick and A. W. Murray, pers. commun.; E. Weiss and M. Winey, pers. commun.). Mad3p and Bub2p are not required for Mad1p hyperphosphorylation, but are required for cell-cycle arrest. They may lie downstream of Mad1p (as diagrammed) or in a parallel pathway.

mitotic delay in these cells[33] is likely to be that the bipolarity of the spindle is disrupted, not that kinetochores are detached. As most studies use high concentrations of microtubule-depolymerizing drugs, which affect both the spindle and chromosome attachment, it is difficult to determine whether this system exists in all cells, and whether it is separate from, or overlaps, the chromosome-attachment checkpoint described above. A recent study using lower concentrations of nocodazole in budding yeast has implicated Bub2p in a checkpoint detecting defects other than the failure of chromosome attachment, such as an aberrant spindle structure[19].

The signal-transduction cascade

Once a defect has been detected, a signal must be transmitted to delay the cell cycle. Characterization of a number of the *MAD* and *BUB* genes suggests that they function in the area of signal transduction (Table 1). A combination of genetic and biochemical studies suggests that Mad1p is centrally located in this signal-transduction cascade. It is a 90-kDa coiled-coil protein that becomes hyperphosphorylated in response to microtubule depolymerization[34]. This hyperphosphorylation still occurs in *mad3* and *bub2* mutants, but not in *mad2*, *bub1* and *bub3* mutants, suggesting that the products of the latter genes act upstream of Mad1p, while Mad3p and Bub2p function downstream or in a parallel pathway (Fig. 3). There are two candidate kinases that may phosphorylate Mad1p. Bub1p is a kinase; it phosphorylates Bub3p *in vitro*, and Bub3p also binds to and activates Bub1p (Ref. 35), but Bub1p has not been shown to have kinase activity *in vitro* against Mad1p. The other possibility is the Mps1 protein kinase, which has an essential function in construction of the spindle but is also necessary for the checkpoint[36]. Unlike other mutants in spindle-pole-body assembly (*cdc31*, *ndc1* and *mps2*), *mps1* mutants fail to arrest at the restrictive temperature (when the cell contains only a monopolar spindle), whether or not nocodazole is present. As Mad1p is not hyperphosphorylated in these conditions, and Mps1p can phosphorylate Mad1p *in vitro* (K. Hardwick, E. Weiss, A. W. Murray and M. Winey, pers. commun.), Mps1p probably lies directly upstream of Mad1p.

Are protein kinases involved in the spindle-assembly checkpoint in organisms other than yeast? Experiments using *Xenopus* extracts have identified p44ERK2, a member of the mitogen-activated protein (MAP) kinase family of protein kinases, as a necessary component of the spindle-assembly checkpoint in this organism[37]. *Xenopus* oocytes do not arrest when treated with nocodazole, but a checkpoint arrest can be achieved in oocyte extracts by also adding a high density of sperm nuclei. This mimics the high nuclear : cytoplasmic ratio present later in development (after the mid-blastula transition), when the cells become sensitive to nocodazole. The involvement of a MAP kinase in a cell-cycle arrest is not unprecedented; a thiophosphorylated, activated MAP kinase can mimic the stable arrest of frog oocytes that usually occurs in development in metaphase II of meiosis owing to the activity of cytostatic factor (CSF)[38]. The mitotic-checkpoint arrest is not identical to the well-studied CSF arrest, however, as it is not relieved by the addition of Ca^{2+}; thus not all lessons from one type of arrest may be transferable to the other type.

The endpoint: stopping the cell cycle

The ultimate effect of the spindle-assembly checkpoint is to prevent the onset of anaphase and thus the exit from mitosis. Biochemically, the end of mitosis is marked by the inactivation of $p34^{cdc2}$ via the degradation of its associated B-type cyclins. Therefore, the checkpoint may delay the cell cycle by preventing cyclin B destruction. How might this be achieved? Cyclin B contains an N-terminal destruction box that targets it for degradation by ubiquitin-mediated proteolysis (for a review, see Ref. 39). A large (20S) complex that contains at least the Cse1, Cdc16, Cdc23 and Cdc27 proteins[39] is necessary for cyclin B destruction. This complex is a candidate for regulation by the checkpoint as, unlike other components of the proteolysis machinery, it is active only during mitosis. However, as the complex has only been identified recently, there is no information yet as to what components of the complex might be regulated, or whether the complex is regulated at all. The localization of Cdc27 (Ref. 40), cyclin B and $p34^{cdc2}$ to the centrosome and, to a lesser extent, the spindle suggests that the spindle may be, at least in some organisms, the site of cyclin destruction. Once again, it is not known whether this has any regulatory consequences for the timing of cyclin destruction.

Is the only job of the spindle-assembly checkpoint to prevent cyclin B destruction? If this were true, preventing cyclin B destruction should mimic the checkpoint and arrest the cell in metaphase. Expression of a non-degradable, mutant form of cyclin B that lacks the destruction box causes cell-cycle arrest in mitosis, but chromosome separation still occurs[41,42]. By contrast, inhibition of the ubiquitination system by the use of antibodies [to Cdc27 (Ref. 40)], methyl-ubiquitin[42] or by mutation of genes involved in the system (*CDC16*, *CDC23*, *CDC27*, *PRG1*, *CIM3* and *CIM5* in budding yeast) causes a metaphase arrest. This suggests that the ubiquitination machinery degrades, in addition to cyclin B, a protein responsible for sister-chromatid cohesion. Thus, when neither cyclin B nor the putative sister-chromatid 'glue' protein are degraded, the cell cycle halts at metaphase, rather than after chromosome separation.

The checkpoint may modify separately both B-type cyclins and the 'glue' protein to protect them from destruction, or it may inactivate (or prevent the activation of) the destruction machinery (Fig. 4). Phosphorylation remains a possible mechanism for cyclin B protection as, although mutation of the phosphorylation sites on frog cyclin B1 has been found to have no effect on $p34^{cdc2}$ activation or cyclin degradation[43], possible effects on checkpoints have not been investigated. If the destruction machinery is modified, the modification must be very specific, such that this machinery can still act on some of its substrates. This scheme is necessary to account for the fact that cyclin A is destroyed earlier than cyclin B in unperturbed cell cycles[44] and is destroyed while cyclin B is protected in a checkpoint arrest[37,44].

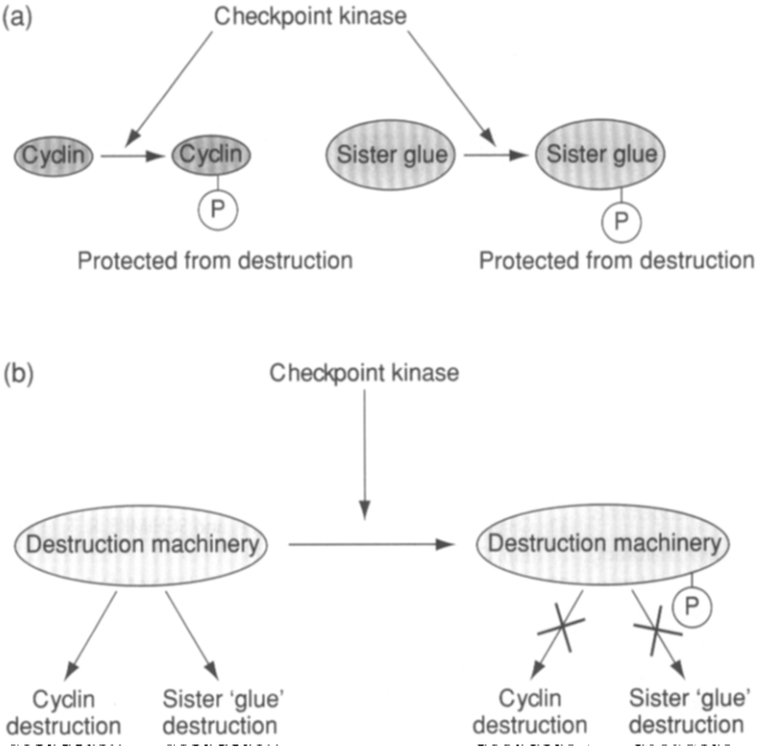

FIGURE 4

Two models for the terminal stage of the spindle-assembly checkpoint. For the sake of illustration, the checkpoint is shown as altering the cell-cycle components by phosphorylation, although other mechanisms such as the binding of an inhibitor protein are equally likely. (a) The checkpoint modifies cyclin B and so protects it from degradation, preventing the inactivation of $p34^{cdc2}$–cyclin-B-kinase that normally occurs as cells exit mitosis. The checkpoint also modifies and protects a proposed sister-chromatid 'glue' protein, preventing anaphase onset (sister-chromatid separation). (b) The checkpoint modifies the ubiquitination machinery, such that it is no longer able to destroy either cyclin B or the sister-chromatid glue. The checkpoint could prevent activation of the ubiquitination machinery or inactivate a component of the ubiquitination apparatus.

The future

Although studies in mammalian cells have identified the kinetochore as the site at which at least one spindle-assembly-checkpoint signal is generated, we do not know what proteins are responsible for this event. Identification of the 3F3 epitope or localization of one of the yeast checkpoint proteins to the kinetochore would provide a starting point for a biochemical understanding of the checkpoint sensor. Ultimately, such a characterization should include the description of a change that occurs upon binding of the kinetochore by microtubules or upon the development of tension at the kinetochore. Genetic studies in yeast will be useful in determining whether there are additional pathways, as they should allow different mitotic defects to be studied individually in a way that is not possible with microtubule-depolymerizing drugs. An understanding of how the checkpoint prevents the onset of anaphase may have to await a more detailed description of the proteins that destroy cyclin B before it can be determined whether such proteins are involved in, or modified by, the checkpoint. Nevertheless, a closer examination of modifications of cyclin B itself is already possible. And finally, expansion of our scant knowledge of the events at the end of mitosis may uncover other mechanisms of regulation, such as the direct modulation of the activity of $p34^{cdc2}$–cyclin-B complexes by means other than cyclin B proteolysis.

References

1 HARTWELL, L. H. and WEINERT, T. A. (1989) *Science* 246, 629–634
2 MURRAY, A. W. (1994) *Curr. Opin. Cell Biol.* 6, 872–876
3 MURRAY, A. W. (1995) *Curr. Opin. Genet.* 5, 5–11
4 HARTWELL, L. H. and KASTAN, M. B. (1994) *Science* 266, 1821–1828
5 LI, R. and MURRAY, A. W. (1991) *Cell* 66, 519–531
6 HOYT, M. A., TOTIS, L. and ROBERTS, B. T. (1991) *Cell* 66, 507–517
7 SLUDER, G. (1979) *J. Cell Biol.* 80, 674–691
8 KUNG, A. L., SHERWOOD, S. W. and SCHIMKE, R. T. (1990) *Proc. Natl Acad. Sci. USA* 87, 9553–9557
9 SANDELL, L. L. and ZAKIAN, V. A. (1993) *Cell* 75, 729–739
10 MARTIN, S. J. and COTTER, T. G. (1990) *Cell Tissue Kinet.* 23, 545–549
11 SHERWOOD, S. W., SHERIDAN, J. P. and SCHIMKE, R. T. (1994) *Exp. Cell Res.* 215, 373–379
12 LIEBERMANN, D. A., HOFFMAN, B. and STEINMAN, R. A. (1995) *Oncogene* 11, 199–210
13 CROSS, S. M. *et al.* (1995) *Science* 267, 1353–1356
14 SPENCER, F. and HIETER, P. (1992) *Proc. Natl Acad. Sci. USA* 89, 8908–8912
15 NEFF, M. W. and BURKE, D. J. (1992) *Mol. Cell. Biol.* 12, 3857–3864
16 WELLS, W. A. E. and MURRAY, A. W. (1996) *J. Cell Biol.* 133, 75–84
17 RIEDER, C. L., SCHULTZ, A., COLE, R. and SLUDER, G. (1994) *J. Cell Biol.* 127, 1301–1310
18 HYMAN, A. A. and SORGER, P. K. (1995) *Annu. Rev. Cell Biol.* 11, 471–495
19 WANG, Y. and BURKE, D. J. (1995) *Mol. Cell. Biol.* 15, 6838–6844
20 NAVAS, T. A., ZHOU, Z. and ELLEDGE, S. J. (1995) *Cell* 80, 29–39
21 TOMKIEL, J., COOKE, C. A., SAITOH, H., BERNAT, R. L. and EARNSHAW, W. C. (1994) *J. Cell Biol.* 125, 531–545
22 NICKLAS, R. B. and ARANA, P. (1992) *J. Cell Sci.* 102, 681–690
23 NICKLAS, R. B. and KOCH, C. A. (1969) *J. Cell Biol.* 43, 40–50
24 SKIBBENS, R. V., SKEEN, V. P. and SALMON, E. D. (1993) *J. Cell Biol.* 122, 859–875
25 LI, X. and NICKLAS, R. B. (1995) *Nature* 373, 630–632
26 JANG, J. K., MESSINA, L., ERDMAN, M. B., ARBEL, T. and HAWLEY, R. S. (1995) *Science* 268, 1917–1919
27 GORBSKY, G. J. and RICKETTS, W. A. (1993) *J. Cell Biol.* 122, 1311–1321
28 NICKLAS, R. B., WARD, S. C. and GORBSKY, G. J. (1995) *J. Cell Biol.* 130, 929–939
29 CAMPBELL, M. S. and GORBSKY, G. J. (1995) *J. Cell Biol.* 129, 1195–1204
30 RIEDER, C. L., COLE, R. W., KHODJAKOV, A. and SLUDER, G. (1995) *J. Cell Biol.* 130, 941–948
31 SLUDER, G. and BEGG, D. A. (1983) *J. Cell Biol.* 97, 877–886
32 SLUDER, G., MILLER, F. J., THOMPSON, E. A. and WOLF, D. E. (1994) *J. Cell Biol.* 126, 189–198
33 SLUDER, G., MILLER, F. J. and SPANJIAN, K. (1986) *J. Exp. Zool.* 238, 325–336
34 HARDWICK, K. and MURRAY, A. W. (1995) *J. Cell Biol.* 131, 709–720
35 ROBERTS, B. T., FARR, K. A. and HOYT, M. A. (1994) *Mol. Cell. Biol.* 14, 8282–8291
36 WEISS, E. and WINEY, M. (1996) *J. Cell Biol.* 132, 111–123
37 MINSHULL, J., SUN, H., TONKS, N. K. and MURRAY, A. W. (1994) *Cell* 79, 475–486
38 HACCARD, O. *et al.* (1993) *Science* 262, 1262–1265
39 MURRAY, A. W. (1995) *Cell* 81, 149–152
40 TUGENDREICH, S., TOMKIEL, J., EARNSHAW, W. and HIETER, P. (1995) *Cell* 81, 261–268
41 SURANA, U., AMON, A., DOWZER, C., McGREW, J., BYERS, B. and NASMYTH, K. (1993) *EMBO J.* 12, 1969–1978
42 HOLLOWAY, S. L., GLOTZER, M., KING, R. W. and MURRAY, A. W. (1993) *Cell* 73, 1393–1402
43 LI, J., MEYER, A. N. and DONOGHUE, D. J. (1995) *Mol. Biol. Cell* 6, 1111–1124
44 MINSHULL, J. *et al.* (1989) *J. Cell Sci. (Suppl.)* 12, 77–97
45 FANKHAUSER, C., MARKS, J., REYMOND, A. and SIMANIS, V. (1993) *EMBO J.* 12, 2697–2704

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