1 Cell cycle checkpoints: safe passage through mitosis

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1. Introduction

Mitosis results in the production of two daughter cells containing identical genetic complements. Achieving this requires a carefully orchestrated series of nuclear and cytoplasmic events leading to the accurate replication and segregation of the chromosomes. A powerful combination of molecular genetics, cell biology, and biochemistry have led to the isolation of key structural, enzymatic, and regulatory components governing these events. One of the most gratifying aspects of this research has been the realization that many of these components are highly conserved throughout the phyla. Therefore many of the lessons learned from one system will probably apply to others.

The major transitions in the cell cycle are driven by the successive activation of a family of cyclin-dependent kinases (CDKs) (1–4). The CDKs are structurally related and their activity requires a physical association with cyclin. Regulation of CDK activity occurs through post-translational modifications and through associations with a conserved family of activating cyclins and a family of CDK inhibitors. Entry into mitosis occurs through the activation of a universal mitotic CDK (also known as Cdc2 and p34). Activation occurs in a stepwise fashion: first by its association with cyclin and then through the progressive alteration of the phosphorylation states of key residues. The activated mitotic CDK initiates a diverse array of cytoplasmic and nuclear events driving the cell into mitosis. The mitotic CDK also activates the anaphase-promoting complex (APC), a ubiquitin ligase responsible for the degradation of cyclin and other inhibitors of anaphase (5). This allows the cell to progress through anaphase, forming two daughter cells.

For the most part, these events satisfactorily explain the order and timely progression of events necessary for entry into and exit from mitosis. However, if errors occur or individual steps in the cell cycle are delayed, it is likely that additional regulatory mechanisms are required for the proper progression of the cell cycle. It was through addressing this issue that the concept of cell cycle checkpoints was developed (6). Checkpoints increase the fidelity of the cell cycle by monitoring the accurate completion of specific cellular events. If an event in the cell cycle is not

completed or is improperly completed, checkpoints delay progression of the cell cycle to provide time for repair or completion of the event. This inhibitory phenomenon was initially observed in a classic series of cell fusion experiments. If a cell in S phase was fused with a cell in G_2, the G_2 nucleus delayed entry into mitosis until the second nucleus had completed S phase (7). This suggested that cells which have not completed S phase produce a diffusable inhibitor of mitosis. Mutational analysis in the budding yeast, *Saccharomyces cerevisiae*, led to the first explicit description of the concept of cell cycle checkpoints and the first identification of a checkpoint gene (8, 9). These genetic studies provided the conceptual framework which led to the identification of a number of checkpoints monitoring many events throughout the cell cycle. Checkpoints have been identified in many phyla in both germline and somatic cells and are probably universal components of the cell cycle. In this chapter, we review recent work on how cell cycle checkpoints guide cells into and out of mitosis. In addition, we discuss the role of cell cycle checkpoints in maintaining the fidelity of the synchronous rapid divisions observed during early embryogenisis in many higher eukaryotes. This review is not intended to be comprehensive. Instead we highlight studies that illustrate concepts and issues central to the field.

2. Activation of the mitotic CDK controls entry into mitosis

Entry into mitosis involves a dramatic reorganization of the nucleus and cytoplasm. Centrosomes migrate to opposite poles of the nucleus and establish the microtubule organizing centers and spindle orientation. Microtubule arrays undergo a dramatic reorganization to produce the bipolar mitotic spindle. Also occurring at this time is the breakdown of the nuclear envelope and disassembly of the sheetlike network of lamins lining the inner nuclear membrane. One of the most dramatic mitotic events is condensation of the chromosomes and their alignment along the metaphase plate.

It is clear that phosphorylation plays a key role in the cellular reorganization that accompanies entry into mitosis. As a cell enters mitosis, the total amount of protein-bound phosphate increases. Much of this increase is probably due to the activation of mitotic CDK (1). Activated mitotic CDK initiates many of the nuclear and cytoplasmic rearrangements described above. For example, this complex phosphorylates the nuclear lamins which result in their disassembly at mitosis (10) (see Chapter 2 for a detailed account). In addition, mitotic CDK-directed phosphorylation is responsible for altering microtubule polymerization dynamics and the dramatic reorganization of microtubules into a spindle as the cell enters mitosis (11, 12), as described in Chapter 4. However, most of the *in vivo* substrates remain elusive and little is known about the mechanisms that provide substrate specificity to the activated mitotic CDK (13).

2.1 Activation of mitotic CDK requires an association with cyclin

There is a wealth of information concerning the mechanisms that modulate the activity of mitotic CDK and other CDKs (14). Phosphorylation and dephosphorylation of key residues and specific protein associations are the primary mechanisms of regulating CDK activity (4). As the name implies, activation of CDKs requires a physical association with cyclin (Fig. 1). Cyclins are a large family of proteins originally identified because their abundance oscillates with the cell cycle (15). Each cyclin maintains a cell-cycle-specific pattern of accumulation and rapid proteolysis. Cyclins are generally classified as G_1, S, or M based on the timing of their peak concentrations and association with specific CDKs (1, 16). The orderly progression of key transitions throughout the cell cycle is defined by the phase-specific accumulation of specific cyclin–CDK complexes. Cyclin accumulation is the rate-limiting step in key phase transitions in many cell cycles. For example, in *Xenopus laevis* extracts the accumulation of cyclin B is the rate-limiting step in the activation of mitotic CDK and entry into mitosis (17). In addition, *Drosophila melanogaster* embryos exhibit significant delays in the cortical syncytial cycles when levels of cyclin B are reduced. These delays are more severe when levels of both cyclin A and cyclin B are reduced (18). These studies provide *in vivo* evidence that cyclin A and B levels control the timing of the syncytial cycles in the *Drosophila* embryo. However, cyclin is not the rate-limiting component controlling entry into mitosis in all cell cycles. In *Drosophila* cycle 14 embryos, entry into mitosis is controlled by expression of *string* (19, 20), a

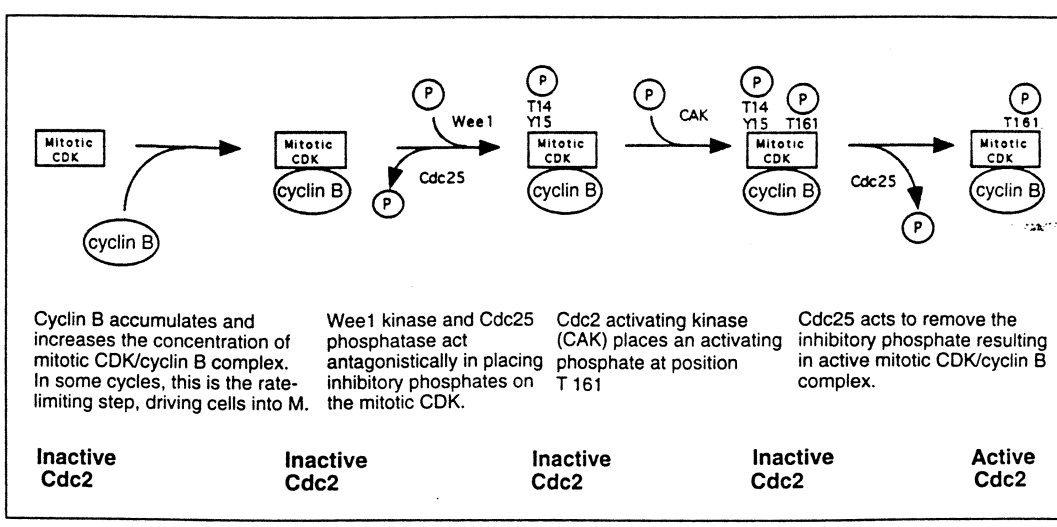

Fig. 1 The G_2–M transition in *S. pombe*. The conserved mitotic CDK ($p34^{cdc2}$) is activated through a physical association with cyclin B followed by series of steps which modify the phosphorylation state of residues Thr14 and Tyr15. The Weel kinase and Cdc25 phosphatase are key enzymes influencing the phosphorylation state of these residues. Mitotic CDK activation also requires phosphorylation on Thr161.

gene that encodes a homolog of *Schizosaccharomyces pombe* cdc25, a phosphatase that targets inhibitory mitotic CDK phosphates.

2.2 The APC mediates cyclin degradation and sister chromosome separation

In addition to regulating events required for entry into mitosis, the mitotic CDK–cyclin B complex activates a pathway leading to the ubiquitin-mediated proteolysis of cyclin B and thus its own inactivation Proteolysis is dependent on the presence of a conserved 9 amino acid–terminal domain (D-box) that targets cyclin B for ubiquitination and subsequent proteolysis (21–25). The timing of cyclin B destruction is controlled by the late metaphase activation of a multiprotein complex known as the anaphase promoting complex (APC) (Fig. 2) (5). Through an as yet undefined pathway, mitotic CDK–cyclin B activates this complex during late metaphase (26). In addition to targeting cyclin for destruction, the APC also promotes initiation of anaphase by targeting the proteolysis of proteins required for sister chromosome cohesion. Support for this latter activity comes from the observation that undegradable cyclins result in a telophase arrest in which sister chromosomes have completely separated (27, 28). In contrast, disruption of APC activity prevents separation of the sister chromosomes and entry into anaphase (5, 27, 29).

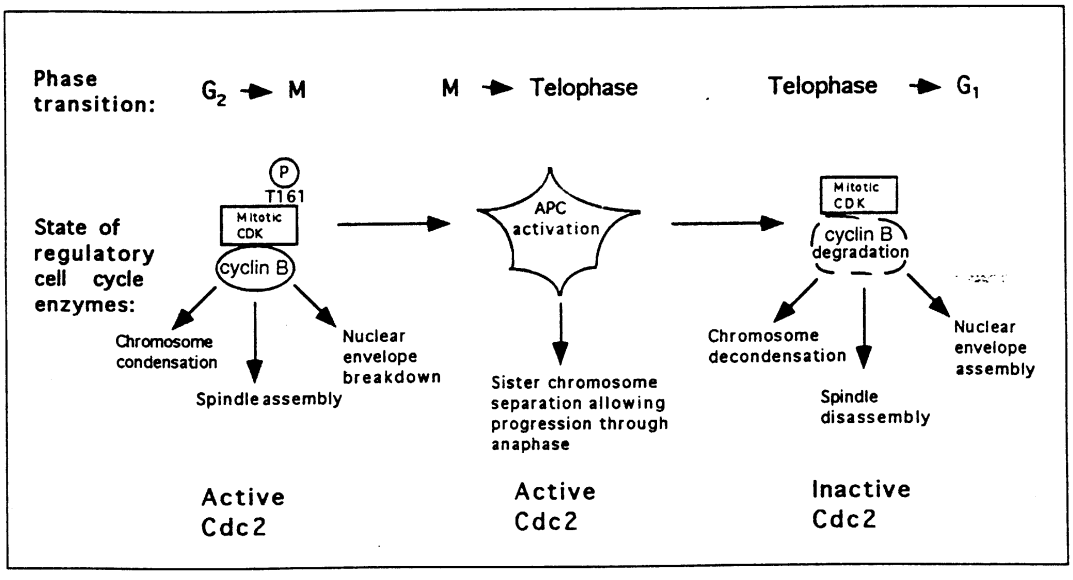

Fig. 2 A model for the role of anaphase promoting complex (APC) in driving exit from metaphase. Active mitotic CDK initiates a number of mitotic events including APC activation. APC is a large ubiquitin-ligase protein complex that inactivates the mitotic CDK by targeting cyclin B proteolysis. APC also acts earlier to promote sister chromosome separation through ubiquitin-dependent proteolysis.

2.3 Proteins that negatively regulate CDK activity

The CDK inhibitors (CDIs) are an emerging class of cell cycle regulatory proteins. In response to external environmental cues and internal signals these proteins associate with CDKs to inhibit their activity. When activated by alpha factor, *S. cerevisiae* Far 1 protein binds to and inhibits the activity of mitotic CDK–Cln2 (a G_1 cyclin). This produces a G_1 arrest (30). Another *S. cerevisiae* protein, Sic 1, binds to and inhibits an S phase cyclin–CDK complex. Sic 1 may be involved in preventing inappropriate rounds of DNA synthesis (31–33). Well known mammalian CDIs include p15, p16, p21, and p27 (34–39). p21 is a target of p53, a gene which is often mutated in human tumors (40). Interestingly p15 and p16 act by preventing the formation of the CDK–cyclin complex (34, 41). The chromosomal region to which both of these genes map in humans is often deleted in individuals with hereditary melanoma (42).

2.4 Post-translational phosphorylation regulates mitotic CDK activity

In addition to cyclin association, CDK activation requires specific phosphorylation and dephosphorylation of key residues. The ATP-binding site of *S. pombe* mitotic CDK contains threonine and tyrosine at residues 14 and 15 respectively. Phosphorylation of one or both of these residues maintains the mitotic CDK–cyclin complex in an inactive state (Fig. 1). In addition, activity of mitotic CDK requires phosphorylation at Thr161 (1, 14). This residue may influence the binding of the substrate to the CDK kinase domain. The enzyme responsible for the phosphorylation of threonine 161 has been historically referred to as cyclin-activating kinase (CAK). CAK activity is associated with the Cdk7–cyclin H complex in higher eukaryotes. However, the *S. cerevisiae* homolog of cdk7–cyclin H does not phosphorylate Thr161 *in vitro* or *in vivo* (43). Recent genetic and biochemical evidence suggests that Cak1/Civ1 is the *S. cerevisiae* kinase responsible for phosphorylating Thr161 *in vivo* (44, 45). It remains to be seen if a Cak1/Civ1 homolog is found in higher eukaryotes and if there are additional classes of physiologically important CAKs.

In *S. pombe*, the conserved phosphatase, Cdc25, and kinases, Wee1 and Mik1, act antagonistically to influence the phosphorylation state of Thr14 and Tyr15 (46, 47). Increasing the dosage of Cdc25 relative to Wee1 results in a shortened G_2 and premature entry into mitosis. Increasing the dosage of Wee1 relative to Cdc25 has the opposite effect: entry into mitosis is delayed. It may be that as a cell normally progresses through G_2, the increase in the Cdc25/Wee1 ratio regulates the timing of entry into mitosis.

3. Cell cycle checkpoints

The initiation of many events in the cell cycle depends on the proper completion of a previous event. For example, in many cells treatment with hydroxyurea, a potent

inhibitor of DNA replication, results in a G_2 delay and failure to progress into mitosis. These studies demonstrate that entry into mitosis depends on completion of DNA synthesis (8, 48). The most extensive description of dependency relationships exists for *S. cerevisiae* and *S. pombe* cell cycles. In these organisms, large numbers of mutations have been isolated that cause an initially asynchronous population of cells to arrest at a specific point in the cell cycle. Analysis of these cell division cycle (*cdc*) mutations enabled the major cytoplasmic and nuclear events of the cell cycle to be placed in one of a series of dependent pathways (49–51).

As described by Hartwell and Weinert (6), these dependency relationships may be either the result of intrinsic substrate–product relationships or may be established by adding external feedback controls enforcing the dependency relationships (Fig. 3). In the former, the product of one step in the pathway serves as a substrate for the next. Therefore if the first step does not occur or is improperly executed, the next step cannot be initiated. Alternatively, surveillance mechanisms extrinsic to the process monitor the accurate completion of each step. If a step fails or is inaccurately completed, the surveillance mechanism prevents the initiation of the next step. In this case, elimination of the surveillance mechanism eliminates the dependency relationship but does not affect the actual process. In contrast, if the dependency relationship is a consequence of a substrate–product relationship, it is unlikely that the dependency can be relieved without disrupting the process itself. Therefore finding conditions (mutations or drugs) that relieve the dependency relationship and allow cell cycle progression provides strong evidence that it is enforced by a feedback mechanism. These negative feedback controls are referred to as cell cycle checkpoints.

In many cells, exposure to low, non-lethal doses of X-irradiation produces an arrest in G_2 and cells do not enter metaphase. This demonstrates that entry into mitosis requires undamaged DNA (52, 53). In the presence of caffeine, entry into mitosis no longer requires intact DNA (54–56). The caffeine-mediated relief of this dependency relationship demonstrates that it is due to a cell cycle checkpoint rather than a substrate–product relationship.

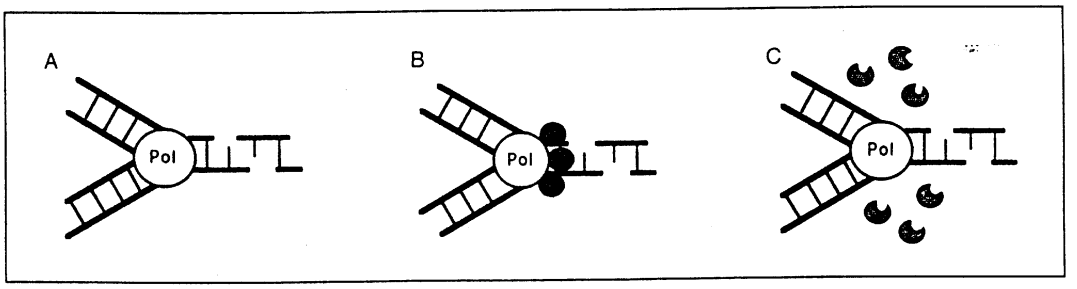

Fig. 3 Normal rates of DNA polymerization rates depend on undamaged DNA. It may be that the lesions physically prevent progression of the DNA polymerase (A, substrate product-mediated delay). Alternatively (B), the lesions may activate feedback controls that slow the polymerization rate (checkpoint-dependent delay). Identifying a condition that relieves the dependency relationship (C) demonstrates that it is the result of a negative feedback mechanism. These feedback mechanisms are called cell cycle checkpoints.

Delays, as well as arrests, in the cell cycle, indicate the presence of a dependency relationship (Fig. 3). For example, studies in mammalian cells demonstrate that DNA damage significantly reduces the rate of DNA replication (57–59). This is a direct consequence of decreasing the frequency of replicon initiation and slowing the rate of strand elongation by DNA polymerase (58). This delay demonstrates that the rate of DNA synthesis depends on undamaged DNA. It is reasonable to assume that this dependency is due to a substrate–product relationship; that is, the slowing of DNA replication is a direct result of the replication machinery navigating lesions in the DNA. However, *S. cerevisiae* mutants have been identified that fail to slow DNA replication in response to methylmethanesulfonate (MMS) induced damage (59). As these mutations relieve the dependency relationship, this indicates that the slowing of S-phase is also enforced by a cell cycle checkpoint.

In mitotic cell cycles, migration of sister centrosomes to opposite nuclear poles precedes spindle formation. In *Drosophila* embryos, mutations that disrupt separation of the centrosomes also disrupt spindle formation (60). This indicates that spindle formation is dependent on proper centrosome separation. The identification of a condition that relieves this dependency relationship is required to demonstrate that this dependency relationship is a consequence of a cell cycle checkpoint rather than a substrate–product relationship. To date such a condition has not been identified. This is not unexpected since it is reasonable to assume that centrosome separation is a necessary first step in the formation of a spindle. However, one cannot conclude that this is the result of a substrate–product relationship. In fact, there are a number of instances in other organisms in which functional spindles form in the absence of centrosomes (61–67). Only by finding a condition that eliminates the dependency relationship, is it possible to conclude that it is the result of a checkpoint rather than a substrate–product relationship.

3.1 Mutational analysis identifies *RAD9* as a DNA damage checkpoint

The first gene shown to be involved in a cell cycle checkpoint was originally recovered because of its sensitivity to X-irradiation (6). In *S. cerevisiae*, as with other cells, X-irradiation produces a delay in G_2. If this delay is the result of a cell cycle checkpoint, two classes of X-irradiation-sensitive mutations are expected: those that are defective in the enzymatic machinery required for repair and those that are defective in the checkpoint-induced delay which provides time for repair to occur (Fig. 4). Among the large collection of X-irradiation-sensitive mutants isolated in *S. cerevisiae*, both classes have been identified (6, 68). For those in the first class, the DNA damage checkpoint is intact and they exhibit a G_2–M delay in response to DNA damage, but repair does not occur. The latter class of mutants have an intact DNA repair system but fail to delay at G_2–M in response to X-irradiation (8, 67). *rad9* was the first of the latter class of mutants to be identified (6). The X-irradiation sensitivity of *rad9* mutants results from progression through mitosis with damaged DNA. This leads to inviable aneuploid daughter cells.

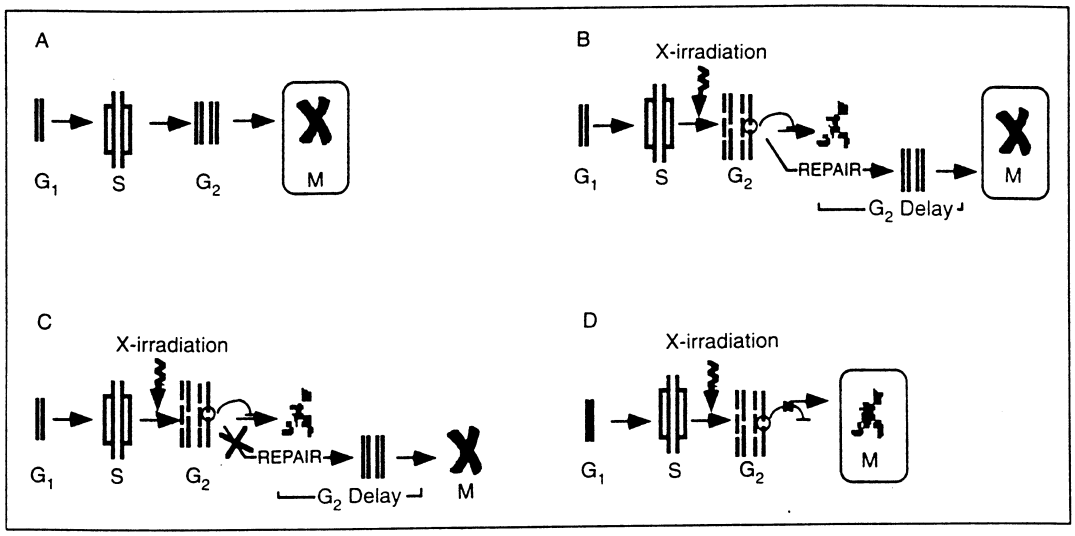

Fig. 4 Identifying DNA damage checkpoint mutations. Checkpoints, by delaying entry into metaphase, provide time for repair (compare unirradiated cells (A) with X-irradiated cells (B)). Consequently, there should be two classes of X-irradiation-sensitive mutations: those that are defective in the repair response (C) and those that are defective in the checkpoint response (D). These classes are distinguished because repair mutations, but not checkpoint mutations, cause a delay in response to X-irradiation. The boxes indicate the outcome for each situation.

This phenotype indicates that the primary function of the *RAD9* gene product is to delay the cell in G_2–M to provide time for repair of damaged DNA before progressing into mitosis. A prediction of this model is that slowing progression through G_2–M should reduce the sensitivity to X-irradiation in *rad9* cells. In *S. cerevisiae*, this experiment is readily performed because spindle formation begins directly after the completion of S phase. Consequently, exposing yeast cells to methylbenzimidazole-2-yl-carbamate (MBC), a drug that depolymerizes microtubules, dramatically slows progression through G_2–M. If *rad9* cells are treated with MBC prior to X-irradiation and are maintained in the drug for an extended period thereafter, the sensitivity to X-irradiation is greatly diminished. This indicates that if time is provided, the damaged DNA is repaired in *rad9* cells (6).

Given that *RAD9* is primarily required for the DNA damage checkpoint, it is not expected to be an essential gene. Null alleles of *rad9* are viable, but they exhibit a 20-fold increase in chromosome loss (6). The high rate of chromosome loss, however, indicates that checkpoints are required during normal growth to maintain cell cycle fidelity. This reflects the fact that cells occasionally require extra time to repair lesions that inevitably occur during S phase and normal progression through the cell cycle. *RAD9* is also involved in DNA damage checkpoints operating during G_1 and S (69, 70). Although the *RAD9* checkpoint operates throughout the cell cycle, it is specific for DNA damage and is not involved in the well-documented DNA synthesis and spindle assembly checkpoints.

3.2 Checkpoints monitor many cellular events and involve signal transduction pathways that link delays in the cell cycle to repair processes

Checkpoints have been identified at all major transitions in the cell cycle. They monitor a diverse array of events including cell size, chromosome condensation, DNA replication, DNA integrity, and spindle assembly (71). Checkpoints monitoring the latter three have been intensively investigated through mutational analysis (72–76). The mechanisms employed by checkpoints to monitor cell cycle events and induce arrest in the cell cycle are not well understood, although it is clear that signal transduction processes are involved (77). Checkpoints require sensors to respond to signals generated from an incomplete or improperly completed event in the cell cycle. Signal transduction processes amplify this signal and activate effectors that mediate cell cycle arrest. The CDKs responsible for driving the major transitions in the cell are a likely target of the checkpoints. As described previously, CDK activity is regulated by diverse mechanisms and the various array of cell cycle checkpoints may act through these mechanisms.

3.3 Ambiguities in the concept of cell cycle checkpoints

Identifying a mutation that eliminates a dependency relationship indicates that the mutated gene is involved in a cell cycle checkpoint. This operational definition has provided a valuable framework for genetically identifying components involved in monitoring cell cycle progression. However, it has also led to some confusion because mutations in the enzymatic machinery driving major cell cycle transitions have been identified that also eliminate cell cycle dependency relationships (15). For example, certain mutations in mitotic CDK eliminate the dependency of M phase on a previously completed S phase (78). In addition, mutations in DNA replication enzymes disrupt the DNA synthesis checkpoint (79). While the behavior of these mutations is in accord with the operational definition of cell cycle checkpoints, they are not in accord with the conceptual definition of a surveillance system that monitors but is extrinsic to the basic events of the cell cycle.

In general, cell cycle checkpoints are thought to monitor the cell cycle but are considered unnecessary for completion of an event or a repair process. The relief of dependence criteria has served to identify many genes involved in these checkpoints. However, for some potential cell cycle checkpoint genes a more stringent criterion has been applied: not only do mutations in these genes relieve a dependency relationship, but they can be rescued by slowing the cell cycle to provide time for repair or completion of an event (8, 78). Rescuing the mutant phenotype solely by providing additional time clearly demonstrates that the gene is not involved in the processes required for repair or completion of an event. Although it is not always practical to apply this criterion, when it is successfully applied it clearly establishes that these genes are involved in cell cycle checkpoints.

4. Lessons from budding yeast: the role of checkpoints in monitoring the completion of S phase and DNA damage

The *cdc* mutations disrupt specific events in the cell cycle and produce a phase-specific cell cycle arrest. These provide a useful set of tools to test both the phase and signal specificity of a given cell cycle checkpoint. For example, *CDC9* encodes a DNA ligase; at restrictive temperatures, *cdc9* mutants produce unligated Okazaki fragments and arrest in late S–G_2. Even in this arrested state, the mutant cells maintain viability for hours. The unligated DNA fragments produced at the restrictive temperature in *cdc9* mutants activate a cell cycle checkpoint that arrests the cell in S–G_2. This idea is confirmed by the observation that *cdc9–rad9* double mutants fail to arrest when placed at a restrictive temperature (80). Unlike the arrested cells, these double mutants rapidly lose viability. This is probably a consequence of progressing through mitosis with damaged DNA. The double mutant demonstrates that the *cdc9* arrest is dependent on the *RAD9* checkpoint. This also indicates that the *RAD9*-dependent checkpoint operates during S–G_2 in response the DNA damage.

Analysis of a series of *cdc–rad9* double mutants strengthens the notion that the *RAD9*–dependent checkpoint responds specifically to DNA damage. Of 12 *cdc* mutants tested, four were dependent on the *RAD9* checkpoint for their arrest. Three of these four encode known DNA replication enzymes (DNA ligase, DNA polymerase I, and DNA polymerase III) (81–85). In addition, the cell cycle arrest induced by hydroxyurea, a potent inhibitor of S phase, is not dependent on *RAD9*. Therefore incomplete DNA replication does not activate the *RAD9*-dependent checkpoint. Functional *RAD9* also is not required for the spindle assembly checkpoint (6). Taken together, these data indicate that *RAD9* functions during late S–G_2 in a DNA damage, but not a DNA replication checkpoint.

Screening the existing collection of radiation-sensitive mutations resulted in the identification of a second cell cycle checkpoint mutation *rad17* (80). *rad17–cdc* double mutants exhibit a pattern of phenotypes identical to that of the *rad9–cdc* double mutants. This indicates that both genes are involved in the same DNA damage checkpoint.

4.1 Synthetic lethal screens provide an efficient means of identifying additional checkpoint mutations

The synthetic lethal phenotype of specific *rad9–cdc* double mutants led to the development of a general strategy for isolating additional checkpoint mutants. The *CDC13* gene encodes a protein that binds to and protects the telomere from degradation and facilitates telomerase loading (86). *cdc13* mutations result in the accumulation of single-stranded telomeric DNA (86). This accumulation activates the arrest induced by a DNA damage checkpoint. As with other *cdc* mutant cells,

these remain viable for hours in this arrested condition. Without a functional checkpoint, these cells fail to arrest and die as a consequence of progressing through mitosis with damaged telomeres.

EMS screens for new checkpoint mutations were performed by isolating mutants that resulted in lethality in the absence of *CDC13* function (87). The screen identified four additional cell cycle checkpoint genes: *MEC1* (mitosis entry checkpoint), *MEC2/RAD53*, *MEC3*, and *RAD24*. None of these mutations delayed G_2 when exposed to X-irradiation and all exhibited an increased sensitivity to X-irradiation. Since the failed G_2 delay was an unselected phenotype, this provided strong evidence that the mutations disrupted genes involved in a cell cycle checkpoint.

Similar screens based on synthetic lethality and drug sensitivity have identified S phase and G_2 checkpoint mutations in *S. pombe* and spindle assembly checkpoints in *S. cerevisiae* (88–91). Although these screens involve different aspects of the cell cycle, they are all based on the common principle that by delaying the cell cycle, checkpoints provide time for repair and increase the tolerance of a cell to both internally and externally induced damage.

4.2 Detecting DNA damage

Although the specific signals responsible for activating DNA damage cell cycle checkpoints have not been identified, properties of these signals have been defined. The DNA damage checkpoint is activated by UV and X-irradiation, but not by drugs that inhibit DNA replication (76). That is, it is activated by signals specific to damaged rather than unreplicated DNA. The checkpoint is extremely sensitive and is activated by a single double-strand break in the *S. cerevisiae* genome (92, 93). The signals generated from DNA damage are capable of eliciting a checkpoint response throughout the cell cycle. For example, the checkpoint genes *RAD9*, *RAD17*, and *RAD24* monitor the state of the DNA during G_1–S and G_2–M (92).

Of the identified checkpoint genes, *RAD9*, *RAD17*, *RAD24*, and *MEC3* appear to be most directly involved in monitoring signals generated from DNA damage (Table 1). These genes are required for the DNA damage but not the DNA replication checkpoint (80, 87). In addition, they influence the processing of damaged DNA. As described above, *cdc13* mutations disrupt the stability of telomeric DNA and exhibit a G_2–M checkpoint-induced arrest. While arrested, these mutations accumulate telomeric single-stranded DNA. If *rad24*, *rad17*, and *mec3* are maintained in a *cdc13* background, the single-stranded telomeric DNA accumulates much more slowly (72). In contrast, *rad9–cdc13* double mutants accumulate single-stranded DNA more rapidly. In accord with these results *RAD24* encodes a protein with some homology to Rfc, a protein that binds gapped DNA, and *RAD17* encodes a putative exonuclease (72, 94). These results suggest that these genes may play a role in processing and repair of damaged DNA in addition to their checkpoint function. Damaged DNA is processed by multiple pathways: modified and crosslinked bases are often repaired by an excision-based repair process while breaks are repaired by a recombination-based process (68, 95). This processing may be required to generate signals

Table 1 Checkpoint genes

Gene	Organism	Checkpoint position	Function	Homologs	References
BUB1	*S. cerevisiae*	G_2/M		*mBUB* (mouse)	155
BUB2	*S. cerevisiae*	G_2/M			155
BUB3	*S. cerevisiae*	G_2/M			155
chk1/rad27	*S. pombe*	G_2	protein kinase	*grp* (*Drosophila*)	90, 135, 186, 193
DUN1	*S. cerevisiae*	S			194
MAD1	*S. cerevisiae*	G_2/M			3
MAD2	*S. cerevisiae*	G_2/M		*hsMAD2* (human) *XMAD2* (Xenopus)	3, 155, 168, 169
MAD3	*S. cerevisiae*	G_2/M			3, 155
MEC1	*S. cerevisiae*	G_1/S, S, S/M, G_2M	protein/lipid kinase	*rad3* (*S. pombe*) *ATM* (human) *MEI41* (*Drosophila*)	3, 155, 168, 169
MEC2	*S. cerevisiae*	G_1/S, S, S/M, G_2/M	protein kinase		87
MEC3	*S. cerevisiae*	G_2/M			87
RAD53	*S. pombe*	G_1/S, S, S/M, G_2/M	protein kinase	*cds1*$^+$ (*S. pombe*)	87, 101–103, 195
MPS1	*S. cerevisiae*	G_2/M			150
Pol2	*S. cerevisiae*	S	DNA polymerase subunit	*cds20*$^+$ (*S. pombe*)	79
Polε	*S. cerevisiae*	S/M	DNA polymerase with 3′→5′ exonuclease		79
RAD9	*S. cerevisiae*	G_1/S, G_2/M			6, 9, 69, 79, 102
RAD17	*S. cerevisiae*	G_1/S, G_2/M	3′→5′ exonuclease	*rad1*$^+$ (*S. pombe*) *rec* (*U. maydis*)	72, 80, 94, 197
RAD24	*S. cerevisiae*	G_1/S, G_2/M	weak homology to RFC	*rad17*$^+$ (*S. pombe*)	72, 87, 197

recognized by DNA damage checkpoints. In addition, activating a given checkpoint may require a specific form of processed DNA.

4.3 Monitoring completion of S phase

In *S. cerevisiae*, DNA polymerase II is a multiprotein complex required for DNA replication. The largest member of this complex is the 256 kDa polymerase encoded by *POL2* (96). Genetic analysis demonstrates that this protein possesses an N-terminal domain required for polymerase activity and a separable C-terminal domain required for the S phase checkpoint (79). Mutations have been identified that disrupt each of these functions separately.

The C-terminal domain of this polymerase is also required for complex formation with other proteins (97). Multicopy suppressor screens of C-terminal domain mutations identified the DpbII protein (98). DpbII is homologous to the product of the fission yeast *RAD4/CUT5* checkpoint gene and associates with DNA polymerase II during replication (99). Null alleles of *DpbII* demonstrate that it is essential. In addition, temperature-sensitive alleles of *DpbII* also demonstrate that the gene is required for an S phase checkpoint.

Rfc5, a component in the small subunit of the DNA replication factor C complex, was identified in a screen designed to identify genes that interact with the S phase checkpoint gene, *MEC2* (100). Replication factor C binds gapped DNA and recruits proliferating cell nuclear antigen (PCNA) (101). This DNA–protein complex recruits polymerases ε and δ to form a functional replication complex. A temperature-sensitive allele of the *RFC* gene, *rfc5-1*, was recovered because it is suppressed by overexpression of the DNA-damage gene, *MEC2*. At the restrictive temperature, *rfc5* mutations do not complete replication. In addition, they fail to arrest or delay in G_2 and progress into mitosis with incompletely replicated DNA. This results in aneuploidy and loss of viability. As with DNA polymerase II and DpbII, the *RFC* gene product is involved in DNA replication and in the checkpoint that monitors completion of DNA replication. These studies demonstrate that DNA polymerase is a key component of the S phase checkpoint (102).

4.4 Signal transduction

The properties of mutations in *S. cerevisiae MEC1* and *RAD53* genes indicate that they act as central components in the signal transduction pathway leading to checkpoint-induced cell cycle arrest (Fig. 5). *mec1* and *rad53* mutations disrupt both DNA damage and DNA replication checkpoints operating throughout the cell cycle (87). Both are required for the expression of DUN1, a protein necessary for the transcriptional response that normally accompanies checkpoint activation (102). *MEC1* is also required for a meiotic cell cycle checkpoint (87). These studies demonstrate that MEC1 and DUN1 process signals derived from multiple cell checkpoints. Consistent with their role in a centralized signal transduction process, both are essential protein kinases. In addition, they function downstream of *POL2* and *RAD9*, genes that are required early in the checkpoint response to monitor signals from improperly replicated DNA (102).

MEC1 and *RAD53* are also required for a checkpoint that operates during S phase in response to DNA damage (103). In *S. cerevisiae*, exposure to low doses of the DNA damaging agent MMS results in a six-fold decrease in the rate of S phase. This DNA damage-induced reduction in the rate of S phase has also been observed in mammalian cells. As described previously, in *mec1* and *rad53* mutants, the rate of S phase is not slowed in response to DNA damage.

MEC1 encodes a phosphatidylinositol-3 kinase and has *Drosophila* and mammalian homologs (104, 105). Mutations in the human homolog, *A-T* (ataxia telangiectasia), behave as autosomal recessives. Homozygotes experience symptoms that include progressive neurodegeneration, permanently dilated blood vessels and an elevated occurrence of cancerous tumors. There is also evidence suggesting that heterozygotes at the *A-T* locus have a slightly elevated risk of breast cancer (106). *A-T* mammalian cell lines lack G_1 and G_2 DNA damage checkpoints (107). In addition, they fail to slow progression of S phase in response to DNA damage. G_2 and S phase DNA damage checkpoints require *MEC1* and it is probably required for the G_1 DNA

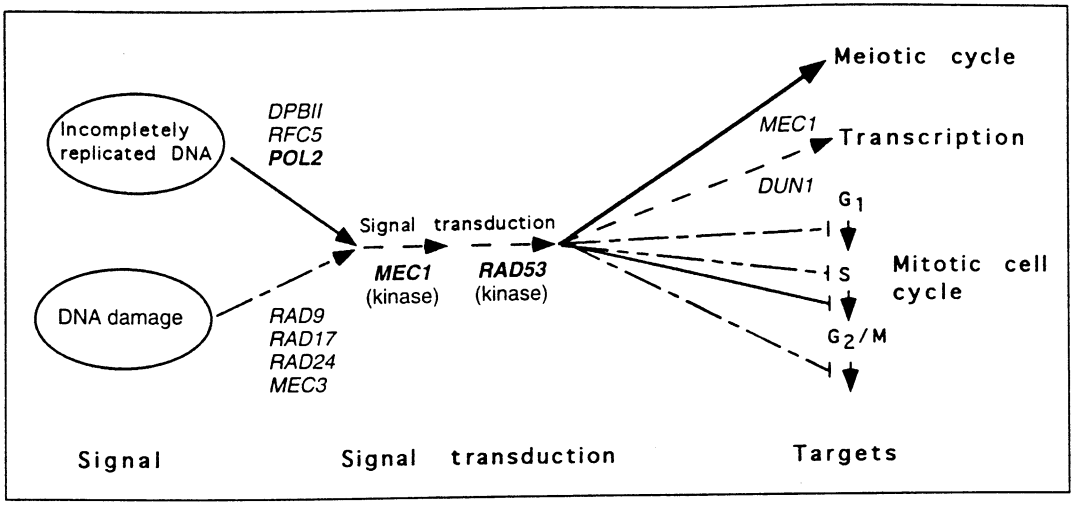

Fig. 5 Outline of the *S. cerevisiae* DNA damage and replication checkpoint signal transduction pathway. The genes indicated in bold (*POL2*, *MEC1*, *RAD53*) are essential. Common pathways are denoted by solid, striped or dashed lines. See Table 1 for references. In *S. cerevisiae*, G_2 and M phase events temporally overlap. In addition to participating in the phase-specific mitotic arrest, some of these genes are also required for meiotic checkpoint function and the transcriptional induction that accompanies checkpoint activation. Mec1 and Rad53 act as central processors in the DNA damage and replication checkpoint response.

damage checkpoint as well. Therefore the Mec1 and A-T proteins are likely to be functional as well as structural homologs.

4.5 p53 is a mammalian checkpoint gene that functions during G_1–S and G_2–M

In addition to *A-T*, the mammalian p53 and p21 genes are also required for the G_1 DNA damage checkpoint (108, 109). Cells lacking p53 are severely compromised in their ability to arrest in G_1 in response to DNA damage, while the G_1 arrest is only mildly compromised in cells lacking p21. This suggests that the mammalian G_1 arrest involves multiple pathways and that p53 may be required for a number of these pathways. This is readily explained by the fact that the p53 gene encodes a transcription factor that may regulate a battery of genes involved in the G_1 checkpoint (110). p53 exhibits rapid turnover, possibly in response to ubiquitin-dependent proteolysis (111). In response to DNA damage, both the activity and stability of the p53 protein are increased by mechanisms that are not well understood (108, 112). In cells lacking A-T, the activation of p53 in response to DNA damage is attenuated, indicating that A-T is an upstream regulator of p53 (113, 114). Like A-T, p53 is frequently mutated in human cancers, indicating that both are essential for maintaining genome stability (115–117). As well as inducing a repair response, p53 is required for DNA-damage induced apoptosis (114, 118, 119).

There is mounting evidence that p53 functions during G_2 and M as well as G_1–S. Mouse embryonic fibroblasts lacking p53 are much more sensitive to low doses of caffeine, a potent inhibitor of the G_2–M DNA damage checkpoint, than genetically matched wild-type controls (120). This indicates that p53 is involved in the G_2–M DNA damage response. Further support is provided from studies demonstrating that immortalized Li Fraumeni fibroblasts lacking p53 exhibit a defective G_2 DNA damage checkpoint response (121). Temperature-sensitive alleles of p53 have also been used to demonstrate that p53 may regulate the cell cycle at G_2–M and as well as G_1–S (122). Driving p53 expression with an inducible promoter can arrest cells at G_2–M (123). p53 undergoes a G_2–M cyclin-dependent phosphorylation that alters its DNA-binding activity (124). Concluding that p53 is involved in a G_2 DNA damage checkpoint must be tempered by the possibility that this may be a secondary effect of increased genetic instability of cells lacking p53. As will be described below, p53 is also required for the spindle assembly checkpoint.

The p21 gene is a target of the p53 transcription factor. The p21 protein is an inhibitor of the Cdk2 and Cdk4 cyclin-dependent kinases required to drive cells into S phase. p21 is activated by and requires p53 (40). Embryonic cells derived from p21 null mice are defective in their ability to arrest in G_1 in response to DNA damage (109). However, unlike p53 knockouts they do not develop tumors, nor are they defective in thymocyte apoptosis or the spindle checkpoint. It is likely that other targets of the p53 transcription factor operate during G_2 and M.

4.6 The mammalian p53 gene is involved in a spindle assembly checkpoint

Studies demonstrating that loss of p53 activity often leads to polyploidy suggests that it may be involved in checkpoints operating during metaphase as well as G_1 (110, 125, 126). To test this idea directly, wild-type and p53-deficient cells were exposed to low doses of the microtubule inhibitors nocodazole and colcemid (127). Due to activation of the spindle assembly checkpoint, wild-type cells delay in metaphase directly after exposure to nocodazole and exhibit a dramatically increased mitotic index. Cells lacking p53 exhibit only a small increase in the mitotic index in response to nocodazole, indicating that the spindle assembly checkpoint is compromised in these cells. Consequently, after 44 h exposure to nocodazole, none of the wild-type cells exhibited an 8*N* DNA content while 44% of the p53 minus cells exhibited an 8*N* content. Cells lacking p53 also undergo unregulated centrosome duplication (128). Thirty per cent of the cells derived from mouse embryonic fibroblasts contained from three to ten centrosomes. Often these additional centrosomes nucleate microtubules that associate with chromosomes and result in their mis-segregation. Whether the abnormal centrosome duplication is a direct consequence of a defective spindle assembly checkpoint in cells lacking p53 remains to be determined.

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checkpoint function by inhibiting Cdc2 activity in response to DNA damage (90, 119). Recent work suggests that Chk1 acting through Wee1 induces a G_2 arrest by maintaining Cdc2 in a tyrosine-inhibited form. Overexpression of Chk1 in undamaged cells produces a G_2 arrest with Cdc2 in a tyrosine-inhibited form. In addition, overexpression of Chk1 has no effect in cells lacking Wee1 kinase activity. Further support that Wee1 is a target of the Chk1 kinase comes from studies demonstrating that Chk1 phosphorylates Wee1 *in vitro* and that Wee1 is hyperphosphorylated in cells delayed in G_2 by exposure to UV irradiation or by overexpression of Chk1 (136). Furthermore, Chk1 activity may be responsible for Cdc25 phosphatase inhibition (137). Taken together, these studies strongly implicate Cdc2 tyrosine phosphorylation in the Chk1 mediated DNA-damage checkpoint.

4.8 Adaptation releases checkpoint-induced arrest

Irreparable DNA damage results in prolonged exposure to checkpoint-activating signals. Eventually, the cells habituate and are released from their checkpoint-induced arrest. This phenomenon, known as adaptation, is observed in many cell cycle checkpoints. Without it, cells suffering irreparable damage would remain arrested and die. Adaptation may have evolved because it allows cell cycle progression in spite of damage. This provides an opportunity, although slight, for the cells to weather the damage and survive. Although little is known about the molecular basis of adaptation, mutations that affect this process have been identified (73).

5. The role of checkpoints in monitoring spindle assembly

The mitotic spindle is an extremely complex and dynamic microtubule-based structure and its proper assembly is essential for the accurate segregation of sister chromatids. Sister centrosome separation marks the initiation of spindle assembly and determines the orientation of the bipolar spindle. The mature spindle consists of three sets of microtubules originating from each centrosome (138, 139). Polar microtubules extend from each centrosome and overlap in the middle of the spindle. These are responsible for spindle stability and separation of spindle poles during anaphase. Kinetochore microtubules extend from the centrosomes to the centromeres of each chromosome. They attach to a defined region of the centromere known as the kinetochore and play a key role in segregating sister chromatids to opposing spindle poles. Astral microtubules radiate from each centrosome into the surrounding cytoplasm and are involved in centrosome separation and spindle orientation within the cell.

Much of spindle assembly relies on the phenomenon that slowly growing and rapidly shrinking populations of microtubules co-exist simultaneously in the cell as described in detail in Chapter 4. In addition, individual microtubules frequently switch from growing to shrinking. This behavior is known as dynamic instability

(140). As a cell progresses into mitosis, the rate at which a microtubule switches fro. growing to shrinking increases (and the rate at which a microtubule switches from shrinking to growing decreases) (141). This creates an extremely dynamic microtubule population and facilitates the reorganization of the interphase microtubule array into a spindle. Spindle formation is achieved by the fact that growing microtubules are stabilized by associations with a kinetochore or other microtubules. Microtubule-based motor proteins also play a role in spindle formation and separation of the sister chromosomes during anaphase (142, 143).

Accurate segregation of sister chromosomes requires that each chromosome is properly attached to a microtubule. The kinetochore plays a key role in this process. Kinetochores are large protein complexes existing within the region of the chromosome known as the centromere (144). Centromeres and kinetochores are discussed in further detail in Chapter 5. The kinetochore provides a number of functions during metaphase, including microtubule capture, alignment and balancing of the chromosomes on the metaphase plate, and segregation and poleward movement of sister chromosomes during anaphase (145). Given these diverse tasks, it is not surprising that it is a complex organelle. Kinesin-like proteins, cytoplasmic dyneins, other microtubule-associated proteins, phosphatases, and kinases are concentrated at the kinetochore. The motor proteins appear to convert the energy of ATP hydrolysis and microtubule depolymerization into chromosome movement (146). The CENP-E motor protein is a fundamental component of the kinetochore. Injection of polyclonal antibodies against the CENP-E protein disrupts the depolymerization-driven movement of the chromosomes (147). These experiments suggest that CENP-E is the motor protein involved in coupling chromosome movement to microtubul depolymerization.

In most cells, exposure to drugs that disrupt microtubule polymerization prevents exit from metaphase (148, 149). Tubulin mutations that compromise spindle structure also produce a similar mitotic arrest. These studies demonstrate that exit from mitosis depends on a properly assembled spindle and may be the result of a cell cycle checkpoint.

5.1 Genetic identification of the spindle assembly checkpoint

Using a rationale similar to that used for the identification of DNA damage checkpoints, screens for mutants sensitive to microtubule depolymerization drugs were employed to identify genes included in the spindle assembly checkpoint. Under normal conditions a dividing cell does not require a spindle assembly checkpoint because the time required to inactivate mitotic CDK is longer than the time required to assemble a spindle. However if spindle assembly is slowed by exposing the cell to low doses of a microtubule-depolymerizing drug, a checkpoint is required to prevent exit from mitosis before the spindle is properly assembled. Through this approach, two independent screens isolated six non-essential spindle assembly checkpoint mutants: *mad1*, *mad2*, *mad3*, *bub1*, *bub2*, and *bub3* (88, 89). The drug-induced lethality is a consequence of cells progressing into anaphase in the

absence of a completely formed spindle. This produces an increased frequency of nondisjunction, chromosome loss, and inviable aneuploid daughter cells. Even in the absence of microtubule inhibitors, the cells bearing these mutations exhibit an increased rate of chromosome loss. This is expected as normally dividing cells occasionally require extra time for spindle assembly and establishment of the appropriate kinetochore–microtubule associations.

As with other cell cycle mutants, *mad* and *bub* mutants are primarily defective in their ability to delay progression through G_2–M in response to slowed spindle assembly. Therefore, providing an alternative means of slowing progression through G_2–M should eliminate the sensitivity of these mutations to microtubule inhibitors. In *S. cerevisiae*, this is achieved by the addition of hydroxyurea which inhibits DNA replication and activates an S phase checkpoint. As predicted, the sensitivity of the *mad* mutants to microtubule depolymerizing drugs is relieved by exposing the cells to low doses of hydroxyurea (92).

Another spindle assembly checkpoint mutant, *mps1*, was originally recovered as a member of a class of mutants that disrupts spindle pole formation (150). Mps1 also functions as a spindle assembly checkpoint; it fails to cause arrest in metaphase in the presence of the microtubule inhibitor nocodazole.

The identification of mutants that disrupt the spindle assembly checkpoint provides a means of rapidly determining which aspects of spindle assembly are being monitored (73). A mutation or condition that disrupts spindle assembly and results in a checkpoint-dependent metaphase arrest indicates that the process disrupted is monitored by the checkpoint. This approach shows that the checkpoint monitors a variety of aspects of spindle formation including chromosome number, centromeric DNA, centrosome duplication, microtubule polymerization, kinetochores, and microtubule motors (88, 89, 149–155). As found for the DNA damage checkpoint, different aspects of spindle assembly may be monitored. For example, the state of the centrosome and the state of the microtubules may be monitored independently by distinct spindle assembly checkpoints.

5.2 Spindle checkpoints monitor the state of the kinetochore

The conclusion that proper spindle–kinetochore interactions are required for cells to progress into anaphase is supported by live observations in mammalian tissue culture cells. Although there is considerable cell-to-cell variability in the time required for all the kinetochores to become properly attached to spindles, the interval from spindle attachment of the last free kinetochore to the initiation of anaphase is relatively constant (156). This fits with a model in which the spindle assembly checkpoint is activated by negative signals produced by free kinetochores. This idea is supported by analysis of mutations and reagents that compromise kinetochore function (157, 158). Mutations in the *S. cerevisiae* Ctf kinetochore protein activate the spindle assembly checkpoint and cause a delay in metaphase (159). Injection of anti-centromeric antibodies derived from human autoimmune sera disrupt kinetochore assembly and delay progression through mitosis (160, 161). Similar studies were

performed using antibodies directed against CENP-C, a component of the inner kinetochore (162). The injected antibodies severely disrupt and reduce kinetochore size. These cells arrest in metaphase, indicating the presence of a checkpoint monitoring kinetochore integrity.

The kinetochore can be completely eliminated through laser ablation. Surprisingly, this procedure does not elicit an anaphase delay. This clearly demonstrates that the metaphase arrest is a consequence of negative rather than positive signals produced by the kinetochore. A complete removal of the kinetochore renders it undetectable by the spindle assembly checkpoint (163).

5.3 Tension is monitored by the spindle assembly checkpoint

Micromanipulation studies in spermatocytes of praying mantids indicate that the spindle assembly checkpoint monitors tension on the kinetochores (Fig. 6) (164). The spermatocytes of praying mantids manage an XXY sex chromosome constitution by forming a trivalent in which the two X chromosomes pair and segregate from the single Y chromosome. Occasionally the trivalent breaks down because an X chromosome prematurely detaches from the Y, generating an unpaired X. These meiocytes never progress into anaphase. If the free X is placed under tension through micromanipulation, the meiocyte progresses into anaphase. These observations support a model in which a spindle assembly checkpoint monitors the tension at each kinetochore. A single kinotechore not under tension is sufficient to activate the checkpoint and prevent exit from metaphase.

5.4 Molecular changes at the kinetochore in response to tension

These studies indicate that tension alters kinetochore composition and/or conformation and that the spindle assembly checkpoint monitors these alterations. Immunofluorescence analysis using the 3F3 antibody, which recognizes a subset of kinetochore proteins only when they are phosphorylated, has provided insight into the molecular nature of this phenomenon (165). These kinetochore proteins are dephosphorylated on chromosomes that have aligned and properly attached to the spindle. The kinetochore proteins in chromosomes that have not attached to the spindle remain phosphorylated. Microinjection of the 3F3 antibody inhibits kinetochore dephosphorylation and delays entry into anaphase (166). Therefore phosphorylation and dephosphorylation of these kinetochore proteins is correlated with the presence and absence of the signal that activates the spindle assembly checkpoint.

Phosphorylation of kinetochore proteins is directly correlated with tension (167). Experiments in grasshopper spermatocytes demonstrate that detachment of a chromosome from the spindle through micromanipulation results in phosphorylation of kinetochore proteins. Conversely, applying tension to an unattached chromosome through micromanipulation results in dephosphorylation of the kinetochore

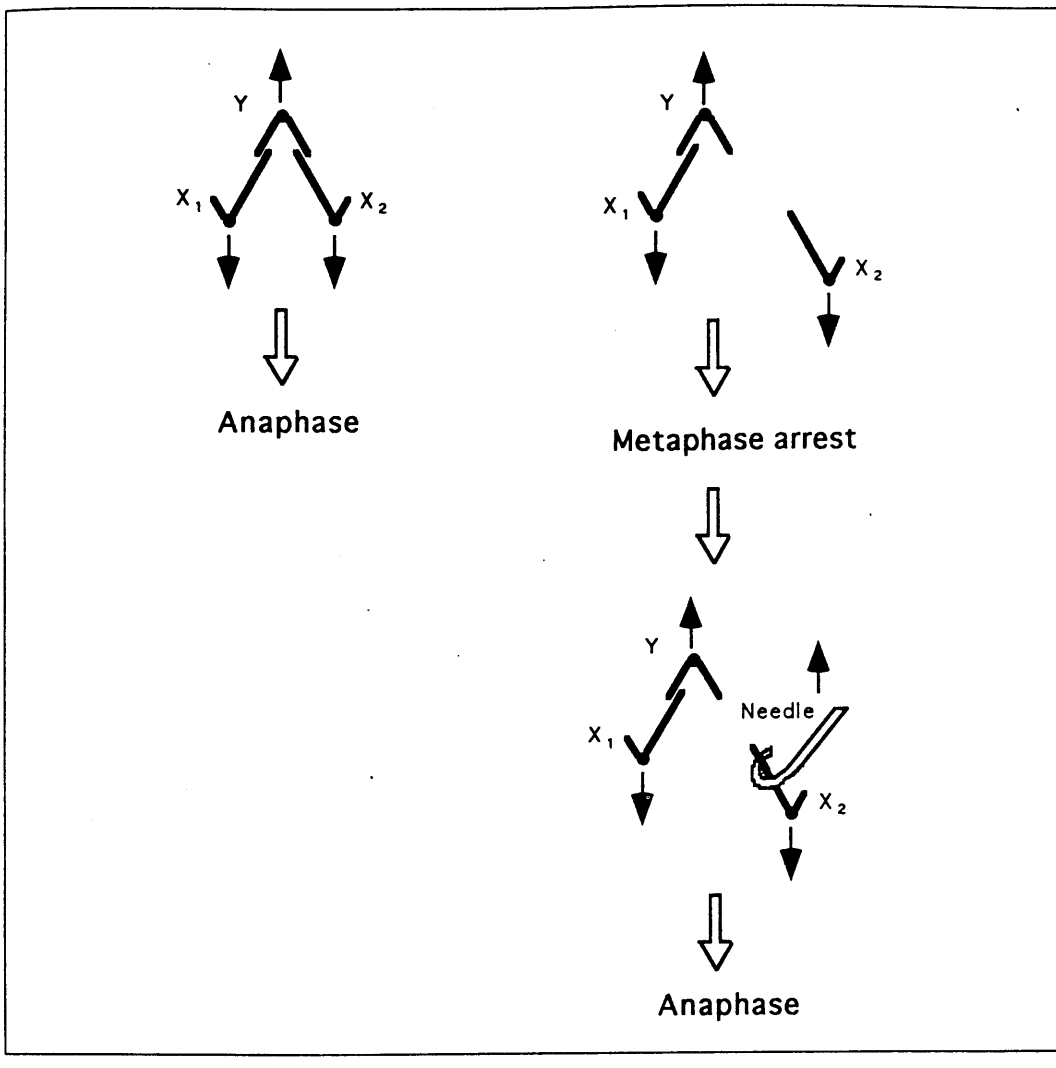


Fig. 6 The spindle assembly checkpoint monitors tension in the spermatocytes of praying mantids. Normally each arm of the Y chromosome pairs with an X chromosome, forming a trivalent in which each kinetochore is under tension. When this occurs, the cells proceed directly into anaphase. Occasionally X–Y pairing is prematurely disrupted, resulting in a free X chromosome no longer under tension. These cells remain arrested in metaphase. If the kinetochore of the free X chromosome is placed under tension through micromanipulation, the cell is release from the metaphase arrest and proceeds into anaphase.

proteins. Tension-sensitive proteins may exist within the kinetochore that regulate the phosphorylation state of these kinetochore proteins (167). The tension-mediated dephosphorylation of these proteins is necessary for release from the spindle assembly checkpoint.

Male grasshoppers contain only a single X chromosome and thus during meiosis the chromosome is unpaired and not under tension. As anaphase is not delayed in

these meiocytes, the unpaired chromosome must not be eliciting the spindle assembly checkpoint. Significantly, the X chromosome kinetochore does not react with the 3F3 antibody, indicating that its kinetochore proteins remain dephosphorylated whether or not they are under tension (167). Consequently, the unpaired X chromosome fails to activate the spindle assembly checkpoint. The observation that these exceptional unpaired X chromosomes also exhibit an exceptional 3F3 kinetochore staining further supports the notion that the checkpoint is monitoring the state of phosphorylation of kinetochore proteins.

Human and *Xenopus* homologs of the MAD2 protein exhibit a localization pattern similar to that of the 3F3 epitope. They localize to the kinetochores as the chromosomes are condensing, but localization is no longer observed once the chromosomes are attached to the spindle (168, 169). Depletion of MAD2 from *Xenopus* embryo extracts inactivates the spindle assembly checkpoint (170). Since *Xenopus* MAD2 is not phosphorylated, the relationship between *Xenopus* MAD2 and the 3F3 phosphoepitope is unclear.

In the *Drosophila* oocyte, tension is employed in a reciprocal manner to that of the grasshopper spermatocyte and is required to prevent premature entry into meiosis (Fig. 7). Analyzing the behavior of specialized compound chromosomes in the *Drosophila* oocyte demonstrates that the tension generated from physical exchange of homologs is required for the programmed metaphase arrest of meiosis I (171). It has been suggested that this distinct response to tension may be a consequence of the fact that progression through female meiosis, but not male meiosis, includes a programmed metaphase arrest (171).

5.5 In some cells, free kinetochores rather than tension activate the spindle assembly checkpoint

Tension is not the primary inducer of the spindle assembly checkpoint in all cells. Ptk cells in which all but one of the kineochores are attached to the metaphase spindle maintain an active spindle assembly checkpoint. Laser ablation of this single unattached kinetochore inactivates the checkpoint and the cells proceed into anaphase even though the sister of the unattached kinetochore is no longer under tension (163). These studies indicate that it is the presence of an unattached kinetochore, rather than the lack of tension, that activates the spindle assembly checkpoint.

5.6 The *MAD* and *BUB* genes are involved in different steps of the spindle assembly checkpoint

To prevent entry into anaphase, a spindle checkpoint requires sensors to detect an improperly formed spindle, a signal transduction pathway, and finally a target that impinges upon the enzymes driving the cell cycle. The *mad* and *bub* spindle checkpoint mutants identify components in these processes and epistasis analysis indicates that they function in a common pathway (155). The finding that Mad1

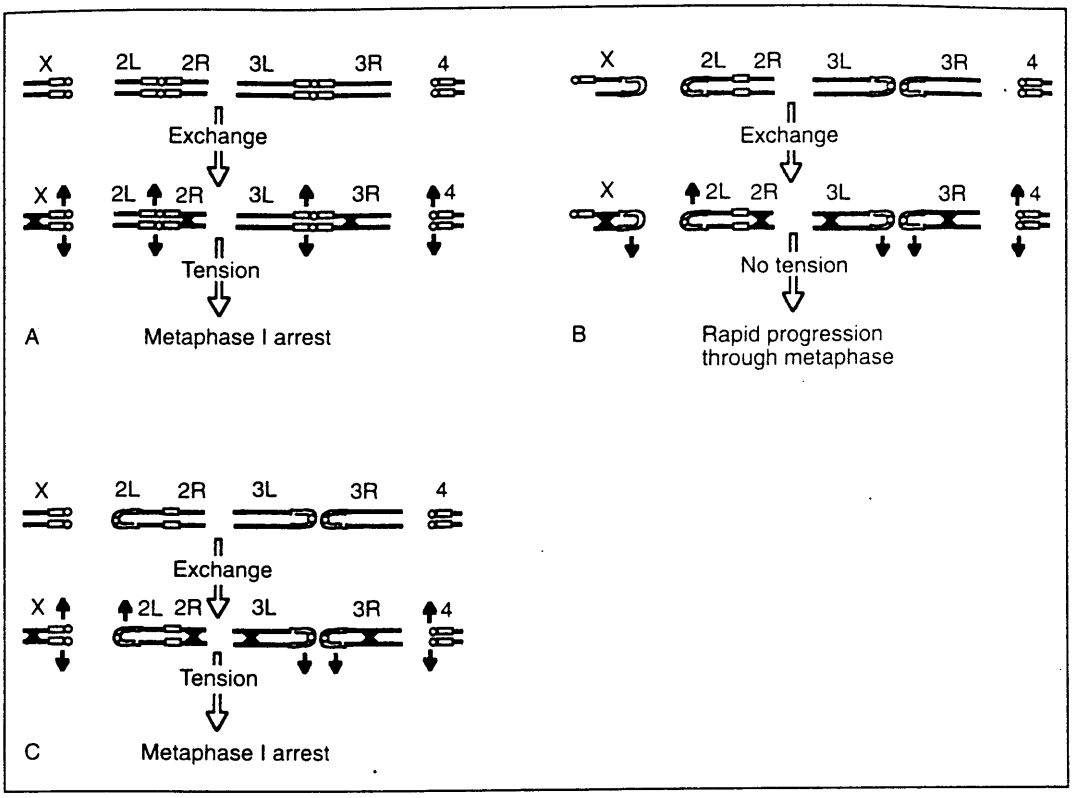


Fig. 7 In the *Drosophila* oocyte, the tension produced by recombination between homologues is required for the programmed meiosis I metaphase arrest (A). Oocytes bearing a set of compound chromosomes in which homologous recombination does not produce kinetochore tension fail to arrest in metaphase (B). Replacing the compound X chromosome with a pair of normal X chromosomes restores the metaphase arrest because recombination now produces tension at the kinetochores (C). The fourth chromosome does not undergo homologous recombination.

is hyperphosphorylated when the spindle assembly checkpoint is induced provides a means of partially ordering the action of spindle checkpoint genes (3). *MSP1*, *MAD2*, *BUB1*, and *BUB3*, but not *MAD3* and *BUB2*, are required for hyperphosphorylation of Mad1 (155). These studies indicate that *MAD2*, *BUB1*, and *BUB3* act upstream of *MAD1* while *BUB2* and *MAD3* act downstream or in parallel to *MAD1*.

MPS1 encodes a protein kinase that may be responsible for the *in vivo* phosphorylation of Mad1. *mps1* mutations disrupt spindle pole body duplication during G_1 and also disrupt the spindle assembly checkpoint (150, 172). Overexpression of *Mps1* results in hyperphosphorylation of Mad1 and a mitotic arrest that is dependent on an intact spindle assembly checkpoint. This indicates that *MPS1* functions in the same MAD and *BUB* spindle-dependent pathway and acts upstream of *MAD1* (73).

5.7 MAP kinase is required for the spindle assembly checkpoint in cell cycle extracts

Mitogen-activated protein (MAP) kinases are a family of proteins that are activated by a wide range of cellular signals and relay them via phosphorylation to other proteins. Recently, a member of this family of signaling proteins has proven to be essential for the spindle assembly checkpoint in *Xenopus* egg extracts (170, 173, 174). Minshull *et al.* found that a *Xenopus* MAP kinase, p44ERK2, is transiently activated in cycling egg extracts. When treated with MAP kinase phosphatase, these extracts in which the spindle assembly checkpoint has been activated, failed to arrest in the presence of the microtubule inhibitor nocadazole (170). Furthermore, extracts that are immunodepleted of MAP kinase fail to arrest in response to microtubule depolymerization, but introduction of recombinant MAP kinase to these extracts restores the spindle assembly checkpoint (174). Immunofluorescence studies localize the MAP kinase to the mitotic spindle during prometaphase and metaphase suggesting that it is involved in monitoring the state of the mitotic spindle and/or associated proteins (173). Other proposed roles for MAP kinase in the spindle assembly checkpoint include regulation of cyclin destruction machinery, or conversion of cyclins to non-destructable forms (173).

5.8 The APC may be the target of the spindle assembly checkpoint

As described above, a great deal is known about the protein complexes that normally drive cells into anaphase and it is likely that the spindle assembly checkpoint influences their activity to prevent entry into anaphase. Passage through anaphase and entry into G_1 is driven by the activation of a previously described large protein complex known as the APC. APC mediates sister chromatid separation and inactivation of CDC2. Through ubiquitin-mediated proteolysis, activated APC drives sister chromatid separation and cyclin B degradation. Activation of the spindle assembly checkpoint prevents cyclin degradation and sister chromatic separation. Therefore, the spindle assembly checkpoint may arrest the cell in metaphase by inhibiting APC activity. This model is in accord with the observation that mutations disrupting APC activity are epistatic to spindle assembly checkpoint mutations (74).

6. The role of checkpoints in the initial embryonic cell cycles

The eggs of many higher eukaryotes are endowed with large volumes of maternally supplied cytoplasm. Consequently early embryogenesis is characterized by cleavage divisions which divide the cytoplasm into successively smaller units. This allows for rapid, synchronous divisions alternating between M and S phases without

measurable G_1 and G_2 phases. For example, *Xenopus* embryos undergo 12 synchronous cleavage divisions each approximately 30 min in length. The cell cycles lengthen, exhibit gap phases, and become asynchronous after the twelfth division. This dramatic alteration in the regulation of the cell cycle, known as the mid-blastula transition, is activated by an increase in the ratio of nuclei to volume of cytoplasm (Table 2) (175, 176).

In *Drosophila* and other insects not only do these initial divisions occur without cell growth, they are syncytial, that is they occur without cytokinesis (177). The early *Drosophila* embryo undergoes 13 rapid synchronous nuclear divisions with extremely reduced gap phases and cellularizes during interphase of nuclear cycle 14. These divisions occur in waves that initiate near the poles of the embryo. Nuclear cycles 1–10 occur in as little as 8 min, then during nuclear cycles 11–14, the cycles gradually lengthen to 25 min.

The extent to which cell cycle checkpoints maintain the fidelity of these early divisions in *Xenopus* and *Drosophila* is unclear (8, 178). Many checkpoints operate during gap phases which have been effectively eliminated in early embyrogenesis. In addition, checkpoint-induced delays would disrupt the synchrony of these division cycles. This suggests either that the operation of checkpoints is modified or that other

Table 2 Estimates of the ratio of DNA (pg) to cytoplasm (μl) in *Xenopus* embryos. In *Xenopus* embryos, checkpoints are activated around the 700–800 cell stage (cycles 10 and 11) when the DNA:cytoplasm ratio is above 2000.

Cycle	# Nuclei	DNA content (pg)	DNA/total cytoplasm (pg/μl)[1]
1	1	6.4	3.6
2	2	12.8	7.1
3	4	25.6	14.2
4	8	51.2	28.4
5	16	102.4	56.9
6	32	204.8	113.8
7	64	409.6	227.6
8	128	819.2	455.1
9	256	1638.4	910.2
10	512	3276.8	1820.4
11	1024	6553.6	3640.9
12	2048	13107.2	7281.8

[1]Assuming the egg is a sphere of 1.5 mm in diameter, the volume is 1.8 μl and the DNA content is 6.4 pg/cell.

mechanisms are employed to maintain fidelity during these early embryonic divisions.

6.1 Relative timing of mitotic events may be the primary mechanism maintaining fidelity of division in early *Xenopus* embryos

In *Xenopus*, the mitotic cycles do not slow down until the mid-blastula transition during nuclear cycle 12. In addition, during these initial cycles entry into mitosis does not depend on completely replicated DNA and entry into anaphase does depend on a properly assembled spindle (179–181). X-irradiation does not affect the rate of progression through these initial cell cycles. This indicates that cell cycle checkpoints are not active at this time and fidelity must be maintained through alternative mechanisms. It is likely that the relative timing of basic cell cycle events is the primary mechanism by which fidelity is maintained during the initial *Xenopus* divisions (93). During these early cycles, the time required to drive the cells into mitosis is longer than the time required to complete S phase. Therefore, under normal conditions, embryos enter mitosis with fully replicated DNA. Relying on relative timing, however, leaves embryos vulnerable to X-irradiation and other treatments that extend the time required to properly complete events of the cell cycle.

As the replication machinery is titered out with increasing nuclear concentration during the later cycles in *Xenopus* embryos, S phase slows down. Relative timing is no longer an effective mechanism of ensuring that S phase is completed before the cell enters mitosis. Consequently, checkpoints are required during these later cycles.

6.2 Checkpoint control mechanisms are present but not activated in early *Xenopus* embryos

The lack of dependency relationships in the early *Xenopus* embryo could result from checkpoints not being present, or being present but not being activated. Studies using *Xenopus* embryonic cytoplasmic extracts support the latter model (182). Extracts derived from *Xenopus* eggs cycle between S and M phases. In these extracts, the length of S phase, but not M phase, increases with increasing nuclear density. In addition, the dependency of mitosis on properly replicated DNA occurs only when sperm nuclei are added to the extract. These results suggest the presence of a DNA replication checkpoint that requires a critical concentration of unreplicated DNA for activation. Entry into mitosis is prevented by inactivation of the Cdc2–cyclin complex through post-translational modification, presumably phosphorylation (182). As found in somatic cells, caffeine relieves this dependency relationship.

These results are in accordance with *in vivo* studies in which *Xenopus* embryos are injected with the DNA synthesis inhibitor aphidicolin, which delays progression of the cell cycle only after the embryo contains more than 700–800 cells (after nuclear

cycle 10) suggesting that threshold concentrations of nuclei are required for checkpoint activation (Fig. 8) (182). Analogous studies demonstrate that a spindle assembly checkpoint is also present in early *Xenopus* embryos but requires a threshold concentration of nuclei for activation (170).

6.3 The syncytial *Drosophila* nuclear cycles exhibit a number of dependency relationships

Early *Drosophila* development begins with a series of synchronous syncytial nuclear divisions (177). These divisions are rapid and alternate between M and S phases with extremely abbreviated G_1 and G_2 phases. Nuclear cycles 1–8 occur in the interior of the embryo. During nuclear cycles 9 and 10, the majority of the nuclei migrate to the cortex. Once at the cortex, they undergo four more rounds of synchronous divisions before cellularizing during interphase of nuclear cycle 14 (Table 3).

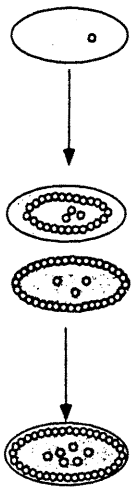
During division cycles 2–7, cell cycle oscillations do not occur in cyclin levels, Cdc2 phosphorylation, or Cdc2 activity (18). It has been proposed that during these early cycles only localized pools of cyclin closely associated with each spindle or nucleus are degraded. Cycles 8–13 exhibit more conventional oscillations in cyclin abundance and Cdc2 activity. During nuclear cycle 14, entry into mitosis is controlled by zygotic transcription of *string*, a homolog of the *S. pombe* gene *cdc25* (20).

These cortical syncytial divisions maintain a surprising number of dependency relationships. Treating syncytial embryos with drugs that disrupt spindle formation produces a metaphase arrest (183). Altered chromosome structure extends metaphase and delays entry into anaphase (178). Exposing embryos to aphidicolin, a DNA synthesis inhibitor, slows the nuclear cycles (184). Centrosome duplication also depends on DNA synthesis (185). X-irradiation delays progression through metaphase (186).

It is possible that the regulation of the *Drosophila* syncytial divisions is similar to that of the early *Xenopus* embryo: checkpoints are present but require a critical concentration of nuclei to become activated. The dependency relationships described above occur during the cortical syncytial divisions when nuclei exist in a monolayer just beneath the plasma membrane. This uniform distribution effectively increases the nucleus:cytoplasm ratio at the cortex and may be sufficient to activate checkpoints enforcing dependency relationships.

However, unpublished observations indicate that even before nuclear migration in the *Drosophila* embryo, disruption of the spindle arrests the nuclei in mitosis (J. Corbin and W. Sullivan, unpublished data). This demonstrates that the *Drosophila* spindle assembly checkpoint operates even at low nucleus:cytoplasm ratios. Similar nucleus:cytoplasm ratios would not activate the spindle assembly checkpoint in *Xenopus* embryos. The ratio of each nucleus and its sphere of tightly associated cytoplasm may be the critical ratio controlling progression through the division cycle in the *Drosophila* embryo. The observation that individual nuclei delay initiation of anaphase during the syncytial *Drosophila* divisions supports the view that regulation of the division cycle occurs at the level of a single nucleus (178). In addition,

Table 3 Estimates of the ratio of DNA (pg) to cytoplasm (μl) in *Drosophila* embryos. After nuclear migration (nuclear cycle 10) in the *Drosophila* embryo, the nuclei reside in a monolayer directly beneath the plasma membrane. This disruption effectively increases the DNA:cytoplasm ratio. Consequently, we calculated the nucleus:cortical cytoplasm ratio (the ratio of nuclei to the ~ 5 μm layer of yolk-free cytoplasm that exists at the periphery of the embryo). Approximately 30 nuclei fail to reach the cortex at nuclear cycle 10. In *Drosophila*, a value of 2000 for the DNA:cytoplasm ratio is not reached unless one considers the DNA:cortical cytoplasm ratio.



Cycle	# Nuclei	DNA content (pg)	DNA/total cytoplasm (pg/μl)[1]	DNA/cortical cytoplasm (pg/μl)[2]
1	1	0.32	0.35×10^2	
2	2	0.64	0.70	
3	4	1.28	1.39	
4	8	2.56	2.78	
5	16	5.12	5.57	
6	32	10.24	11.13	
7	64	20.48	22.26	
8	128	40.96	44.52	
9	256	81.92	89.04	
10	480	153.60	167.00	731.43×10^3
11	960	307.20	333.91	1462.86
12	1920	614.40	667.83	2925.71
13	3840	1228.80	1335.65	5851.43

[1]Assuming the egg is an ellipsoid 450 × 200 μm, the egg volume is 0.92×10^{-2} μl and the diploid DNA content is 0.32 pg.
[2]Assuming the cortical cytoplasm extends 5 μm in from the surface, the cortical volume is 0.021×10^{-2} μl.

chromosome loss during the early divisions results in individual nuclei losing division synchrony with neighboring nuclei (187).

6.4 A DNA replication/DNA damage checkpoint may operate during the late syncytial divisions of *Drosophila*

Interphase increases in length during the late syncytial divisions of *Drosophila* (188). This is thought to be a direct consequence of the increase in time required to complete DNA replication, as maternally supplied replication factors are progressively depleted by an exponentially increasing population of nuclei. As S phase lengthens with each division cycle, feedback controls may be required to prevent entry into mitosis until DNA replication is completed (Fig. 8).

A number of experiments suggest that the *Drosophila* maternally supplied gene product Grp is required for this feedback control. In normal *Drosophila* embryos,

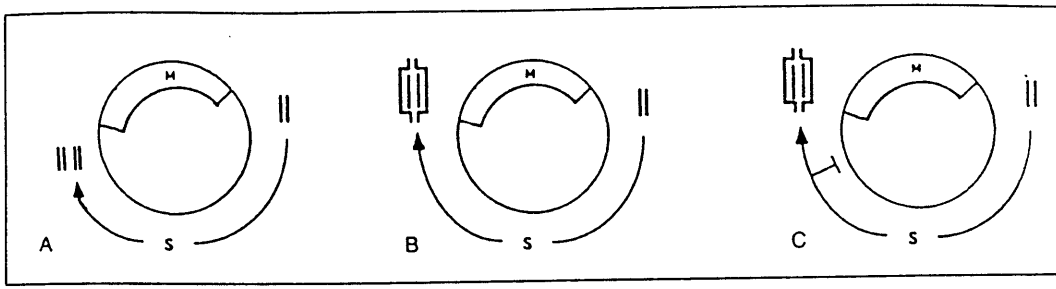


Fig. 8 (A) In the initial embryonic cycles, relative timing ensures that DNA replication is completed before the cell enters mitosis. (B) Later, DNA replication slows down and relative timing no longer solves the completion problem. (C) Eventually, DNA replication checkpoints may be activated to delay entry into mitosis until DNA replication is complete. The *Drosophila grp* gene, a homologue of *S. pombe chk*1, may be required for this checkpoint function during the syncytial cortical divisions.

interphase becomes progressively longer during the late syncytial cycles. This lengthening of interphase does not occur in *grp*-derived embryos (189). Embryos derived from *grp* mutant mothers are specifically disrupted during the late syncytial cycles when there is a dramatic increase in the length of interphase and presumably S phase (190). X-irradiation of normal syncytial embryos results in sister telophase nuclei snapping back and fusing with one another. This is probably a consequence of dicentric bridges formed from damaged chromosomes inappropriately progressing through mitosis. Unirradiated *grp*-derived embryos exhibit a similar snap-back and fusion of sister telophase nuclei during nuclear cycle 12. These fusions are probably a consequence of nuclei in *grp*-derived embryos progressing into mitosis with incompletely replicated DNA. In accordance with this model, *grp*-derived embryos exhibit a five-fold increase in DNA damage relative to normal embryos (186).

Molecular and biochemical analyses also support a model in which *Grp* functions in a developmentally regulated interphase checkpoint during the late syncytial divisions. *Grp* encodes a serine/threonine kinase with extensive homology to the *chk1*/*rad27* gene of *S. pombe* (186). *chk1*/*rad27* is a G_2 DNA-damage checkpoint gene, as previously mentioned. A number of studies indicate that *chk1* inhibits Cdc2 activity in response to DNA damage (135–137). Overexpression of Chk1 in undamaged cells produces a G_2 arrest with Cdc2 in the tyrosine-inhibited form. There is evidence that Grp may induce an interphase delay during the late syncytial divisions by modulating the levels of tyrosine phosphorylation on Cdc2. Wild-type embryos accumulate high levels of an inhibited phosphorylated isoform of Cdc2 during interphase of nuclear cycle 14. This does not occur in *grp*-derived embryos (186). Whether this effect is direct or the consequence of a failure to progress through normal developmental transitions that occur at this time remains to be determined. As in *Xenopus* embryos, the Grp checkpoint is probably present and functional throughout the early divisions but it may require a threshold concentration of unreplicated DNA for activation.

6.5 In the syncytial *Drosophila* embryo, checkpoints link delays in the cell cycle to nuclear elimination

The above studies indicate that at least some cell cycle checkpoints function during the rapid synchronous divisions of early embryogenesis in both *Xenopus* and *Drosophila*. This result is paradoxical because the delays induced by cell cycle checkpoints would disrupt the synchrony of these early divisions and thus disrupt early development. This issue was addressed by examining the effects of an abnormally long and rearranged *Drosophila* chromosome on the embryonic syncytial divisions (178). This chromosome dramatically increases the error rate of the syncytial embryonic divisions but has little effect on the error rate of the later larval neuroblast divisions. Live analysis demonstrates that in embryos bearing this long chromosome individual nuclei in the syncytial population of dividing nuclei often delay initiation of anaphase. The telophase products of nuclei delayed in entering anaphase recede into the yolk and are effectively removed from the dividing population of syncytial nuclei. Many studies demonstrate that the products of a broad range of division errors are eliminated in a similar fashion (60, 191, 192). In many cases, removal is preceded by a delay in anaphase. These delays suggest the presence of a checkpoint operating during the cortical syncytial divisions. However, unlike many somatic cell cycle checkpoints which link delays to repair processes, these checkpoints link delays to elimination processes. This results in the increase of the fidelity of the population of dividing nuclei by culling abnormal nuclei. This process is analogous to the checkpoint-induced apoptic response observed in mammalian systems (172).

7. Future directions

Checkpoints are central to maintaining the fidelity of the eukaryotic cell cycle. The identification and characterization of a number of checkpoint components have established the outlines of the signal transduction pathways by which they operate. However, much remains unknown about many aspects of these pathways. In no case has the signal responsible for activating a checkpoint been defined at the molecular level. For example, although it is clear that the spindle assembly checkpoint monitors kinetochore tension, the kinetochore proteins involved in the checkpoint response are only just beginning to be identified. Identifying and understanding at the molecular level how these proteins respond to tension is certain to be a focus of future investigation. In addition, many steps in the checkpoint signal transduction pathways remain to be defined and the targets producing cell cycle arrest remain elusive.

In response to irreparable damage, a process known as adaptation occurs; the checkpoint-induced arrest is overridden and the cell inappropriately progresses through the cycle. This process is largely unexplored. Little is known about the genetic basis of adaptation and the factors that control the timing of the adaptive

response. In addition, the molecular basis of release from the checkpoint-induced cell cycle arrest remains to be determined.

Mutational analysis of checkpoints demonstrates that cell cycle checkpoints are essential for maintaining division fidelity in higher eukaryotes. Disruption of mammalian cell cycle checkpoints often leads to tumorigenesis. Checkpoint genes, such as p53, maintain fidelity of large populations of cells by either inducing an arrest and repair response or an apoptotic elimination response. Little is known about the environmental and genetic factors influencing which alternative is chosen. In addition, the relationship between these two responses has not been determined. For example, it is not known whether the cell cycle arrest and apoptotic pathways are independent processes or whether they represent branchpoints of a common pathway.

Given the role of cell cycle checkpoints in maintaining division fidelity in mammals, it will be important to identify those checkpoint genes that are functionally as well as structurally conserved as these are likely to be involved in core aspects of the checkpoint process. Analysis of these conserved checkpoint components in model genetic organisms will provide the most direct insight into their role in mammals.

Higher eukaryotic development is quite resistant to both externally and internally generated damage. It may be that developmental mechanisms formally equivalent to cell cycle checkpoints are responsible for this resilience. In response to an incomplete or improperly executed developmental event, the organism may arrest development and properly complete the event before proceeding to the next developmental stage. These developmental checkpoints may also exhibit a number of other properties similar to cell cycle checkpoints. For example, they may only be required under conditions of unusual stress. Progress in this area will require the identification and characterization of key components of this process. It is likely that the general strategies developed for the genetic analysis of yeast cell cycle checkpoints will serve as an excellent guide in this new area of research.

References

1. Nigg, E. A. (1995) Cyclin-dependent protein kinases: key regulators of the eukaryotic cell cycle. *BioEssays*, **17**, 471.
2. King, R. W., Deshaies, R. J., Peters, J., and Kirschner, M. W. (1996) How proteolysis drives the cell cycle. *Science*, **274**, 1652.
3. Murray, A. W. and Hardwick, K. G. (1995) Mad1p, a phosphoprotein component of the spindle assembly checkpoint in budding yeast. *J. Cell Biol.*, **131**, 709.
4. Lew, D. J. and Kornbluth, S. (1996) Regulatory roles of cyclin dependent kinase phosphorylation in cell cycle control. *Curr. Opin. Cell Biol.*, **8**, 795.
5. King, R. W., Peters, J., Tugendreich, S., Rolfe, M., Heiter, P., and Kirschner, M. W. (1995) A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell*, **81**, 279.
6. Weinert, T. A. and Hartwell, L. H. (1988) The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science*, **241**, 317.

7. Rao, P. N. and Johnson, R. T. (1970) Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis. *Nature*, **225**, 159.
8. Hartwell, L. H. and Weinert, T. A. (1989) Checkpoints—controls that ensure the order of cell cycle events. *Science*, **246**, 629.
9. Weinert, T. A. and Hartwell, L. H. (1990) Characterization of *RAD9* of *Saccharomyces cerevisiae* and evidence that its function acts post-translationally in cell cycle arrest afer DNA damage. *Mol. Cell. Biol.*, **10**, 6554.
10. Peter, M., Nakagawa, J., Dorée, M., Labbé, J. C., and Nigg, E. A. (1990) In vitro disassembly of the nuclear lamina and M-phase specific phosphorylation of lamins by cdc2 kinase. *Cell*, **61**, 591.
11. Verde, F., Labbé, J., Dorée, M., and Karsenti, E. (1990) Regulation of microtubule dynamics by cdc2 protein kinase in cell-free extracts of *Xenopus* eggs. *Nature*, **343**, 233.
12. Verde, F., Dogterom, M., Stelzer, E., Karsenti, E., and Leibler, S. (1992) Control of microtubule dynamics and length by cyclin A- and cyclin B-dependent kinases in *Xenopus* egg extracts. *J. Cell Biol.*, **118**, 1097.
13. Kellogg, D. R. and Murray, A. W. (1995) NAP1 acts with Clb2 to perform mitotic functions and to suppress polar bud growth in budding yeast. *J. Cell Biol.*, **130**, 675.
14. Morgan, D. O. (1995) Principles of CDK regulation. *Nature*, **374**, 131.
15. Evans, T., Rosenthal, E. T., Yongblom, J., Distel, D., and Hunt, T. (1983) Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell*, **33**, 389.
16. Nasmyth, K. (1996) Putting the cell cycle in order. *Science*, **274**, 1643.
17. Murray, A. W. and Kirschner, M. W. (1989) Cyclin synthesis drives the early embryonic cell cycle. *Nature*, **339**, 275.
18. Edgar, B. A., Sprenger, F., Duronio, R. J., Leopold, P., and O'Farrell, P. H. (1994) Distinct molecular mechanism regulates cell cycle timing at successive stages of *Drosophila* embryogenisis. *Genes Dev.*, **8**, 440.
19. Edgar, B. A. and O'Farrell, P. H. (1989) Genetic control of cell division patterns in the *Drosophila* embryo. *Cell*, **57**, 177.
20. Edgar, B. A. and O'Farrell, P. H. (1990) The three postblastoderm cell cycles in *Drosophila* are regulated in G_2 by String. *Cell*, **62**, 469.
21. Glotzer, M., Murray, A. W., and Kirschner, M. W. (1991) Cyclin is degraded by the ubiquitin pathway. *Nature*, **349**, 132.
22. Kobayashi, H., Stewart, E., Poon, R., Adamczewski, J. P., Gannon, J., and Hunt, T. (1992) Identification of the domains in cyclin A required for binding to, and activation of, $p34^{cdc2}$ and $p32^{cdk2}$ protein kinase subunits. *Mol. Biol. Cell*, **3**, 1279.
23. Amon, A., Irniger, S., and Nasmyth, K. (1994) Closing the cell cycle circle in yeast-G_2 cylin proteolysis initiated at mitosis persists until the activation of G_1 cyclins in the next cycle. *Cell*, **7**, 993.
24. King, R. W. Glotzer, M., and Kirschner, M. W. (1996) Mutagenic analysis of the destruction signal of mitotic cyclins and structural characterization of ubiquitinated intermediaries. *Mol. Biol. Cell*, **7**, 1343.
25. Klotzbucher, A., Stewart, E., Harrison, D., and Hunt, T. (1996) The destruction box of cyclin A allows B-type cyclins to be ubiquitinated but not efficiently destroyed. *EMBO J.*, **15**, 3053.
26. Sudakin, V. (1995) The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis. *Mol. Biol. Cell*, **6**, 185.

27. Holloway, S. L., Glotzer, M., King, R. W., and Murray, A. W. (1993) Anaphase is initiated by proteolysis rather than by the inactivation of maturation promoting factor. *Cell*, **73**, 1393.
28. Surana, U., Amon, A., Dowzer, C., McGrew, J., Byers, B., and Nasmyth, K. (1993) Destruction of the CDC28 CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J.*, **12**, 1969.
29. Irniger, S., Piatti, S., Michaelis, C., and Nasmyth, K. (1995) Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell*, **81**, 269.
30. Peter, M. and Herskowitz, I. (1994) Direct inhibition of the yeast cyclin-dependent kinase Cdc28-Cln by Far1. *Science*, **265**, 1228.
31. Mendenhall, M. D. (1993) An inhibitor of $p34^{cdc28}$ protein kinase activity from *Saccharomyces cerevisiae*. *Science*, **259**, 216.
32. Schwob, E., Bohm, T., Mendenhall, M. D., and Nasmyth, K. (1994) The B-type cyclin kinase inhibitor $p40^{SIC1}$ controls the G_1 to S transition in *S. cerevisiae*. *Cell*, **79**, 233.
33. Donovan, J. D., Toyn, J. H., Johnson, A. L., and Johnston, L. H. (1994) $p40^{SDB25}$, a putative CDK inhibitor, has a role in the M/G_1 transition in *Saccharomyces cerevisiae*. *Genes Dev.*, **8**, 1640.
34. Hannon, G. J. and Beach, D. (1994) $p15^{INK4B}$ is a potential effector of TGF-β-induced cell cycle arrest. *Nature*, **371**, 257.
35. Xiong, Y., Hannon, G. J., Shang, H., Casso, D., Kobayashi, R., and Beach, D. (1993) p21 is a universal inhibitor of cyclin kinases. *Nature*, **366**, 701.
36. Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K., and Elledge, S. J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G_1 cyclin-dependent kinases. *Cell*, **75**, 805.
37. Zhang, H., Hannon, G. J., and Beach, D. (1994) p21-containing cyclin kinases exist in both active and inactive states. *Genes Dev.*, **8**, 1750.
38. Toyoshima, H. and Hunter, T. (1994) p27, a novel inhibitor of G_1 cyclin–Cdk protein kinase activity, is related to p21. *Cell*, **78**, 67.
39. Polyak, K. (1994) p27(KIP1), a cyclin–CDK inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell*, **78**, 59.
40. el-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, A., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) WAF1, a potential mediator of p53 tumor supression. *Cell*, **75**, 817.
41. Serrano, M., Hannon, G. J., and Beach, D. (1993) A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*, **366**, 704.
42. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., and Tavtigaian, S. V. (1994) A cell cycle regulator potentially involved in the genesis of many tumor types. *Science*, **264**, 436.
43. Cismowski, M. J., Laff, G. M., Solomon, M. J., and Reed, S. I. (1995) KIN28 encodes a C-terminal kinase that controls mRNA transcription in *Saccharomyces cerevisiae* but lacks cyclin-dependent kinase-activating kinase (CAK) activity. *Mol. Cell. Biol.*, **15**, 2983.
44. Espinoza, F. H., Farrell, A., Erdjument-Bromage, H., Tempst, P., and Morgan, D. O. (1996) A cyclin-dependent kinase-activating kinase (CAK) in budding yeast unrelated to vertebrate CAK. *Science*, **273**, 1714.
45. Kaldis, P., Sutton, A., and Solomon, M. J. (1996) The Cdk-activating kinase (CAK) from budding yeast. *Cell*, **86**, 553.
46. Russell, P. and Nurse, P. (1986) $cdc25^+$ functions as an inducer in the mitotic control of fission yeast. *Cell*, **45**, 143.
47. Russell, P. and Nurse, P. (1987) Negative regulation of mitosis by $wee1^+$, a gene encoding a protein kinase homolog. *Cell*, **49**, 559.

48. Murray, A. W. (1992) Creative blocks—cell-cycle checkpoints and feedback controls. *Nature*, **359**, 599.
49. Pringle, J. R. and Hartwell, L. H. (1981) In *Molecular biology of the yeast Saccharomyces: life cycle and inheritance*. Strathern, J. N., Jones, E., and Broach, J. (ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 1, p. 97.
50. Hirano, T. and Yanagida, M. (1989) In *Molecular and cell biology of yeasts*. Walton, E. F. (ed.). (Blackie, Glasgow), p. 223.
51. Hartwell, L. H., Culotti, J., Pringle, J. R., and Reid, B. J. (1974) Genetic control of the cell division cycle in yeast. *Science*, **183**, 46.
52. Brunborg, G. and Williamson, D. H. (1978) The relevance of the nuclear division cycle to radiosensitivity in yeast. *Mol. Gen. Genet.*, **162**, 277.
53. Burns, V. W. (1956) X-ray induced division delay of individual yeast cells. *Radiat. Res.*, **4**, 394.
54. Busse, P. M., Bose, S. K., Jones, R. W., and Tolmack, L. J. (1978) The action of caffeine on X-irradiated HeLa cells. *Radiat. Res.*, **76**, 292.
55. Schlegal, R. and Pardee, A. B. (1986) Caffeine-induced uncoupling of mitosis from the completion of DNA replication in mammalian cells. *Science*, **232**, 1264.
56. Tolmach, L. J., Jones, R. W., and Busse, P. M. (1977) Delayed inhibition of DNA synthesis. *Radiat. Res.*, **71**, 653.
57. Larner, J. M., Lee, H., and Hamlin, J. L. (1994) Radiation effects on DNA synthesis in a defined chromosomal replicon. *Mol. Biol. Cell*, **14**, 1901.
58. Painter, R. B. and Young, B. R. (1980) Radiosensitivity in ataxia telangiectasia: a new explanation. *Proc. Natl. Acad. Sci. USA.*, **77**, 7315.
59. Young, B. R. and Painter, R. B. (1989) Radioresistant DNA synthesis and human genetic diseases. *Human Genet*, **82**, 113.
60. Sullivan, W., Minden, J. M., and Alberts, B. M. (1990) *daughterless-abo-like*, a *Drosophila* maternal-effect mutation that exhibits abnormal centrosome separation during the late blastoderm divisions. *Development*, **110**, 311.
61. Heald, R., Tournebize, R., Blank, T., Sandalzopoulos, R., Becker, P., Hyman, A., and Karsenti, E. (1996) Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* egg extracts. *Nature*, **382**, 420.
62. Church, K., Nicklas, R. B., and Lin, H. P. (1986) Micromanipulated bivalents can trigger mini-spindle formation in *Drosophila melanogaster* spermatocyte cytoplasm. *J. Cell Biol.*, **103**, 2765.
63. Dietz, R. (1966) The dispensability of the centrioles in the spermatocyce divisions of *Pales ferruginea* (Nematocera). *Heredity*, **19**, 161.
64. Karsenti, E., Newport, J., Hubble, R., and Kirschner, M. (1984) Interconversion of metaphase and interphase microtubule arrays as studied by the injection of centrosomes and nuclei into *Xenopus* eggs. *J. Cell Biol.*, **98**, 1730.
65. Steffen, W., Fuge, H., Dietz, R., Bastmeyer, M., and Muller, G. (1986) Aster-free spindle poles in insect spermatocytes: evidence for chromosome-induced spindle formation. *J. Cell Biol.*, **102**, 1679.
66. Theurkauf, W. E. and Hawkey, R. S. (1992) Meiotic spindle assembly in *Drosophila* females: behavior of nonexchange chromosomes and the effects of mutations in the nod kinesin-like protein. *J. Cell Biol.*, **116**, 1167.
67. Debec, A., Szollosi, A., and Szollosi, D. (1982) A *Drosophila melanogaster* cell line lacking centriole. *Biol. Cell*, **44**, 7.
68. Haynes, R. H. and Kunz, B. A. (1981) In *Molecular biology of the yeast Saccharomyces*. Strathern, J., Jones, E., and Broach, J. (ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, p. 371.

69. Siede, W., Friedberg, A. S., and Friedberg, E. C. (1993) *RAD9*-dependent G_1 arrest defines a second checkpoint for damaged DNA in the cell cycle of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA*, **90**, 7985.
70. Siede, W., Friedberg, A. S., Dianova, I., and Friedberg, E. C. (1994) Characterization of G_1 checkpoint control in the yeast *Saccharomyces cerevisiae* following exposure to DNA-damaging agents. *Genetics*, **138**, 271.
71. Elledge, S. J. (1996) Cell cycle checkpoints: preventing an identity crisis. *Science*, **274**, 1664.
72. Lydall, D. and Weinert, T. (1995) Yeast checkpoint genes in DNA damage processing: implications for repair and arrest. *Science*, **270**, 1488.
73. Rudner, A. D. and Murray, A. W. (1996) The spindle assembly checkpoint in yeast. *Curr. Opin. Cell Biol.*, **8**, 773.
74. Wells, W. A. (1996) The spindle-assembly checkpoint: aiming for a perfect mitosis, every time. *Trends Cell Biol.*, **6**, 288.
75. D'Urso, G. and Nurse, P. (1995) Checkpoints in the cell cycle of fission yeast. *Curr. Opin. Genet. Dev.*, **5**, 12.
76. Stewart, E. and Enoch, T. (1996) S-phase and DNA-damage checkpoints—a tale of two yeasts. *Curr. Opin. Cell Biol.*, **8**, 781.
77. Hartwell, L. H. and Kastan, M. B. (1994) Cell cycle control and cancer. *Science*, **266**, 1821.
78. Gould, K. L. and Nurse, P. (1989) Tyrosine phosphorylation of the fission yeast $cdc2^+$ protein kinase regulates entry into mitosis. *Nature*, **342**, 39.
79. Navas, T. A., Zhou, Z., and Elledge, S. J. (1995) DNA polymerase ϵ links the DNA replication machinery to the S phase checkpoint. *Cell*, **80**, 29.
80. Weinert, T. A. and Hartwell, L. H. (1993) Cell cycle arrest of *cdc* mutants and specificity of the *RAD9* checkpoint. *Genetics*, **134**, 63.
81. Carson, M. J. (1987) CDC17, the structural gene for DNA polymerase I of yeast: mitotic hyper-recombination and effects on telomere metabolism. University of Washington, Seattle, WA.
82. Johnston, L. J. and Nasmyth, K. A. (1978) *Saccharomyces cerevisiae* cell cycle mutant *cdc9* is defective in DNA ligase. *Nature*, **274**, 891.
83. Sclafani, R. A. and Fangman, W. L. (1984) Yeast gene CDC8 encodes thymidylate kinase and is complemented by herpes thymidine kinase gene TK. *Proc. Natl. Acad. Sci. USA*, **81**, 5821.
84. Johnston, L. M., Snyder, M., Chang, L. M., Davis, R. W., and Campbell, J. L. (1985) Isolation of the gene encoding yeast DNA polymerase I. *Cell*, **43**, 369.
85. Boulet, A. M., Simon, M., Faye, G., Baur, G. A., and Burgers, P. M. J. (1989) Structure and function of the *Saccharomyces cerevisiae* CDC2 gene encoding the large subunit of DNA polymerase III. *EMBO J.*, **8**, 1849.
86. Garvik, B., Carson, M., and Hartwell, L. (1995) Single-stranded DNA arising at telomeres in *cdc13* mutants may constitute a specific signal for the RAD9 checkpoint. *Mol. Cell. Biol.*, **15**, 6128.
87. Weinert, T. A., Kiser, G. L., and Hartwell, L. H. (1994) Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. *Genes Dev.*, **8**, 652.
88. Li, R. and Murray, A. W. (1991) Feedback control of mitosis in budding yeast. *Cell*, **68**, 519.
89. Hoyt, A. M., Totis, L. and Roberts, T. B. (1991) *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell*, **66**, 507.

90. Al-Khodairy, F., Fotou, E., Sheldrick, K. S., Griffiths, D. J. F., Lehmann, A. R., and Carr, A. M. (1994) Identification and characterization of new elements involved in checkpoint and feedback controls in fission yeast. *Mol. Biol. Cell*, **5**, 147.
91. Al-Khodairy, F. and Carr, A. M. (1992) DNA repair mutants defining G_2 checkpoint pathways in *Schizosaccharomyces pombe*. *EMBO J.*, **11**, 1343.
92. Lydall, D. and Weinert, T. (1996) From DNA damage to cell cycle arrest and suicide: a budding yeast perspective. *Curr. Biol.*, **6**, 4.
93. Murray, A. and Hunt, T. (1993) *The cell cycle: an introduction*. W. H. Freeman, New York.
94. Siede, W., Nusspaumer, G., Portillo, V., Rodriquez, R., and Friedbert, E. C. (1996) Cloning and characterization of *RAD17*, a gene controlling cell cycle responses to DNA damage in *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **24**, 1669.
95. Freidberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA repair and mutagenesis*. ASM Press, Washington, DC.
96. Hamatake, R. K., Hasegawa, H., Clark, A. B., Bebenek, K., Kunkel, T. A., and Sugino, A. (1996) Purification and characterization of DNA polymerase II from the yeast *Saccharomyces cerevisiae*. Identification of the catalytic core and a possible holoenzyme form of the enzyme. *J. Biol. Chem.*, **265**, 4072.
97. Morrison, A., Araki, H., Clark, A. B., Hamatake, R. K., and Sugino, A. (1990) A third essential DNA polymerase in *S. cerevisiae*. *Cell*, **62**, 1143.
98. Araki, H., Ropp, P. A., Johnson, A. L., Morrison, A., and Sugino, A. (1992) DNA polymerase II, the probable homolog of mammalian DNA polymerase epsilon, replicates chromosomal DNA in the yeast *Saccharomyces cerevisiae*. *EMBO J.*, **11**, 733.
99. Saka, Y. and Yanagida, M. (1993) Fission yeast *cut5*$^+$, required for S-phase onset and M-phase restraint, is identical to the radiation-damage repair gene *rad4*$^+$. *Cell*, **74**, 383.
100. Sugimoto, K., Shimomura, T., Hashimoto, K., Araki, H., Sugino, A., and Matsumoto, K. (1996) Rfc5, a small subunit of replication factor C complex, couples DNA replicatior and mitosis in budding yeast. *Proc. Natl. Acad. Sci. USA*, **93**, 7048.
101. Mossi, R., Jonsson, Z. O., Allen, B. L., Hardin, S. H., and Hubscher, U. (1997) Replication factor C interacts with the C-terminal side of proliferating cell nuclear antigen. *J. Biol. Chem.*, **272**, 1769.
102. Navas, T. A., Sanchez, Y., and Elledge, S. J. (1996) RAD9 and DNA polymerase ϵ form parallel sensory branches for transducing the DNA damage checkpoint signal in *Saccharomyces cerevisiae*. *Genes Dev.*, **10**, 2632.
103. Paulovich, A. G. and Hartwell, L. H. (1995) A checkpoint regulates the rate of progression through S-phase in *S. cerevisiae* in response to DNA damage. *Cell*, **82**, 841.
104. Hari, K. L., Santerre, A., Sekesky, J. J., McKim, K. S., Boyd, J. B., and Hawley, R. S. (1995) The mei-41 gene of *D. melanogaster* is a structural and functional homolog of the human ataxia telangiectasia gene. *Cell*, **82**, 815.
105. Pecker, I., Auraham, K. B., Gilbert, D. J., Savitsky, K., Rotman, G., Harnik, A., Fukao, T., Schrock, E., Hirotsune, S., Tagle, D. A., *et al.* (1996) Identification and chromosomal localization of Atm, the mouse homolog of the ataxia-telangiectasia gene. *Genomics*, **35**, 39.
106. Lehmann, A. R. and Carr, A. M. (1995) The ataxia-telangiectasia gene: a link between checkpoint controls, neurodegeneration and cancer. *Trends Genet.*, **11**, 375.
107. Meyn, S. M. (1995) Ataxia-telangiectasia and cellular responses to DNA damage. *Cancer Res.*, **55**, 5991.
108. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. (1991) Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.*, **51**, 6304.

109. Deng, C., Zhang, P., Harper, J. W., Elledge, S. J., and Leder, P. (1995) Mice lacking p21 CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control. *Cell*, **82**, 675.
110. Livingstone, L. R., White, A., Sprouse, J., Livanos, E., Jacks, T., and Tlsty, T. D. (1992) Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. *Cell*, **70**, 923.
111. Scheffner, M., Huibregtse, J. M., Vierstra, R. D., and Howley, P. M. (1993) The HPV-16 E6 and E6-AP complex functions as a ubiquitin–protein ligase in the ubiquitination of p53. *Cell*, **75**, 495.
112. Ko, L. J. and Prives, C. (1996) p53: puzzle and paradigm. *Genes Dev.*, **10**, 1054.
113. Kastan, M. B., Zhan, Q., el-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J. J. (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and CADD45 is defective in ataxia telangiectasia. *Cell*, **71**, 587.
114. Lu, X. and Lane, D. P. (1993) Differential induction of transcripionally active p53 following UV or ionizing radiation: defects in chromosome instability syndrome? *Cell*, **75**, 765.
115. Nigro, J. M., Baker, S. J., Preisinger, A. C., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., *et al.* (1989) Mutations in the p53 gene occur in diverse human tumour types. *Nature*, **342**, 705.
116. Levine, A. J., Momand, J., and Finlay, C. A. (1991) The p53 tumor suppressor gene. *Nature*, **351**, 453.
117. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. (1994) Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res.*, **54**, 4855.
118. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. (1993) p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature*, **362**, 847.
119. Clarke, A. R., Purdie, C. A., Harrision, D. J., Morris, R. G., Bird, C. C., Hooper, M. L., and Wyllie, A. H. (1993) Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature*, **362**, 849.
120. Powell, S. N., DeFrank, J. S., Connell, P., Eogan, M., Preffer, F., Dombkowski, D., Tang, W., and Friend, S. (1995) Differential sensitivity of p53 (−) and p53 (+) cells to caffeine-induced radiosensitization and override of G_2 delay. *Cancer Res.*, **55**, 1643.
121. Rong, S., Donehower, L. A., Hansen, M. F., Strong, L., Tainsky, M., Jeffers, M., Resau, J. H., Hudson, E., Tsarfaty, I., and Vande Woude, G. F. (1995) Met proto-oncogene product is overexpressed in tumors of P53-deficient mice and tumors of Li–Fraumeni patients. *Cancer Res.*, **55**, 1963.
122. Stewart, N., Hicks, G. G., Paraskevas, F., and Mowat, M. (1995) Evidence for a second cell cycle block at G_2/M by p53. *Oncogene*, **10**, 109.
123. Agarwal, M. L., Agarwal, A., Taylor, W. R., and Stark, G. R. (1995) p53 controls both the G_2/M and the G_1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts. *Proc. Natl. Acad. Sci. USA*, **92**, 8493.
124. Wang, Y. and Prives, C. (1995) Increased and altered DNA binding of human p53 by S and G_2/M but not G_1 cyclin-dependent kinases. *Nature*, **376**, 88.
125. Harvey, M., Sands, A. T., Weiss, R. S., Hegi, M. E., Wiseman, R. W., Pantazis, P., Giovanella, B. C., Tainsky, M. A., Bradley, A., and Donehower, L. A. (1993) In vitro growth characteristics of embryo fibroblasts isolated from p53-deficient mice. *Oncogene*, **8**, 2457.
126. Metz, T., Harris, A. W., and Adams, J. M. (1995) Absence of p53 allows direct immortalization of hematopoietic cells by the myc and raf oncogenes. *Cell*, **82**, 29.

127. Cross, S. M., Sanchez, C. A., Morgan, C. A., Schimke, M. K., Ramel, S., Idzerda, R. L., Raskind, W. H., and Reid, B. J. (1995) A p53-dependent mouse spindle checkpoint. *Science*, **267**, 1353.
128. Fukasawa, K., Choi, T., Kuriyama, R., Rulong, S., and Vande Woude, G. F. (1996) Abnormal centrosome amplification in the absence of p53. *Science*, **271**, 1744.
129. Amon, A., Surana, U., Muroff, I., and Nasmyth, K. (1992) Regulation of $p34^{CDC28}$ tyrosine phoshorylation is not required for entry into mitosis in *S. cerevisiae*. *Nature*, **355**, 368.
130. Sorger, P. K. and Murray, A. W. (1992) S-phase feedback control in budding yeast independent of tyrosine phosphorylation of $p34^{cdc28}$. *Nature*, **355**, 365.
131. Cohen-Fix, O., Peters, J. M., Kirschner, M. W., and Koshland, D. (1996) Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev.*, **10**, 3081.
132. Yamamoto, A., Guacci, V., and Koshland, D. (1996) Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.*, **133**, 85.
133. Yamamoto, A., Guacci, V., and Koshland, D. (1996) Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s). *J. Cell Biol.*, **133**, 99.
134. Enoch, T. and Nurse, P. (1990) Mutation of fission yeast cell cycle control genes abolishes dependence of mitosis on DNA replication. *Cell*, **60**, 665.
135. Walworth, N., Davey, S., and Beach, D. (1993) Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. *Nature*, **363**, 368.
136. O'Connell, M. K., Raleigh, J. M., Verkade, H. M., and Nurse, P. (1997) Chk1 is a wee1 kinase in the G_2 DNA damage checkpoint inhibiting cdc2 by Y15 phosphorylation. *EMBO J.*, **16**, 545.
137. Rhind, N., Furnari, B., and Russell, P. (1997) Cdc2 tyrosine phosphorylation is required for the DNA damage checkpoint in fission yeast. *Genes Dev.*, **11**, 504.
138. Masuda, H. (1995) The formation and the functioning of yeast mitotic spindles. *BioEssays*, **17**, 45.
139. Sobel, S. G. (1997) Mitosis and the spindle pole body in *Saccharomyces cerevisiae*. *J. Exp. Zool.*, **277**, 120.
140. Mitchison, T. and Kirschner, M. (1984) Microtubule assembly nucleated by isolated centrosomes. *Nature*, **312**, 232.
141. Belmont, L. D., Hyman, A. A., Sawin, K. E., and Mitchison, T. J. (1990) Real-time visualization of cell cycle-dependent changes in microtubule dynamics in cytoplasmic extracts. *Cell*, **62**, 579.
142. Sawin, K. E. and Endow, S. A. (1993) Meiosis, mitosis and microtubule motors. *BioEssays*, **6**, 399.
143. Bloom, G. S. and Endow, S. A. (1995) Motor proteins 1: kinesins. *Protein Profile*, **2**, 1105.
144. Yen, T. J. and Schaar, B. T. (1996) Kinetochore function: molecular motors, switches and gates. *Curr. Opin. Cell Biol.*, **8**, 381.
145. Hyman, A. A. and Sorger, P. K. (1995) Structure and function of kinetochores in budding yeast. *Annu. Rev. Cell Dev. Biol.*, **11**, 471.
146. Desai, A. and Mitchison, T. J. (1995) A new role for motor proteins as couplers to depolymerizing microtubules. *J. Cell Biol.*, **128**, 1.
147. Lombillo, V. A., Nislow, C., Yen, T. J., Gelfand, V. I., and McIntosh, J. A. (1995) Antibodies to the kinesin motor domain and CENP-E inhibit microtubule depolymerization-dependent motion of chromosomes *in vitro*. *J. Cell Biol.*, **128**, 107.

148. Reider, C. L. and Palazzo, R. E. (1992) Colcemid and the mitotic cell cycle. *J. Cell Sci.*, **102**, 387.
149. Guenette, S., Magendantz, M., and Solomon, F. (1995) Suppression of a conditional mutation in alpha tubulin by overexpression of two checkpoint genes. *J. Cell Sci.*, **108**, 1195.
150. Weiss, E. and Winey, M. (1996) The *Saccharomyces cerevisiae* spindle pole body duplication gene *MPS1* is part of a mitotic checkpoint. *J. Cell Biol.*, **132**, 111.
151. Wells, W. A. E. and Murray, A. W. (1996) Aberrantly segregating centromeres activate the spindle assembly checkpoint in budding yeast. *J. Cell Biol.*, **133**, 75.
152. Neff, M. W. and Burke, D. J. (1992) A delay in the *Saccharomyces cerevisiae* cell cycle that is dependent upon mitotic checkpoints. *Mol. Cell. Biol.*, **12**, 3857.
153. Wang, Y. and Burke, D. J. (1995) Checkpoint genes required to delay cell division in response to nocodazole respond to impaired kinetochore function in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **15**, 6838.
154. Pangilinan, F. and Spencer, F. (1996) Abnormal kinetochore structure activates the spindle assembly checkpoint in budding yeast. *Mol. Biol. Cell*, **7**, 1195.
155. Hardwick, K. G., Weiss, E., Luca, F. C., Winey, M., and Murray, A. W. (1996) Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption. *Science*, **273**, 953.
156. Rieder, C. L., Shultz, A., Cole, R., and Sluder, G. (1994) Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J. Cell Biol.*, **127**, 1301.
157. Hegemann, J. H. and Fleig, U. N. (1993) The centromere of budding yeast. *BioEssays*, **15**, 451.
158. Lechner, J. and Oritz, J. (1996) The *Saccharomyces cerevisiae* kinetochore. *FEBS Lett.*, **389**, 70.
159. Spencer, F. and Hieter, P. (1992) Centromere DNA mutations induce a mitotic delay in *S. cerevisiae*. *Proc. Natl. Acad. Sci. USA*, **89**, 8908.
160. Bernat, R. L., Borisy, G. G., Rothfield, N. F., and Earnshaw, W. C. (1990) Injection of anticentromere antibodies in interphase disrupts events required for chromosome movement at mitosis. *J. Cell Biol.*, **111**, 1519.
161. Yen, T. J., Compton, D. A., Wise, D., Zinkowski, R. P., Brinkley, B. R., Earnshaw, W. C., and Cleveland, D. W. (1991) CENP-E, a novel human centromere-associated protein required for progression of metaphase to anaphase. *EMBO J.*, **10**, 1245.
162. Tomkiel, J., Cooke, C. A., Saitoh, H., Bernat, R. L., and Earnshaw, W. C. (1994) CENP-C is required for maintaining proper kinetochore size and for a timely transition to anaphase. *J. Cell Biol.*, **125**, 531.
163. Reider, C. L., Cole, R. W., Khodjakov, A., and Sluder, G. (1995) The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J. Cell Biol.*, **130**, 941.
164. Nicklas, B. R. and Li, X. (1995) Mitotic forces control a cell-cycle checkpoint. *Nature*, **373**, 630.
165. Gorbsky, G. J. and Ricketts, W. A. (1993) Differential expression of a phosphoepitope at the kinetochores of moving chromosomes. *J. Cell Biol.*, **122**, 1311.
166. Campbell, M. S. and Gorbsky, G. J. (1995) Microinjection of mitotic cells with the 3F3/2 anti-phosphoepitope antibody delays the onset of anaphase. *J. Cell Biol.*, **129**, 1195.
167. Nicklas, B. R., Ward, S. C., and Gorbsky, G. J. (1995) Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.*, **130**, 929.
168. Li, Y. and Benzra, R. (1996) Identification of a human mitotic checkpoint gene: hsMAD2. *Science*, **274**, 246.

169. Chen, R., Waters, J. C., Salmon, E. D., and Murray, A. W. (1996) Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. *Science*, **274**, 242.
170. Minshull, J., Sun, H., Tonks, N. K., and Murray, A. W. (1994) A MAP kinase-dependent spindle assembly checkpoint in *Xenopus* egg extracts. *Cell*, **79**, 475.
171. Jang, J. K., Messina, L., Erdman, M. B., Arbel, T., and Hawley, R. S. (1995) Induction of metaphase arrest in *Drosophila* oocytes by chiasma-based kinetochore tension. *Science*, **268**, 1917.
172. Winey, M., Goetsch, L., Baum, P., and Byers, B. (1991) MPS1 and MPS2: novel yeast genes defining distinct steps of spindle pole body duplication. *J. Cell Biol.*, **114**, 745.
173. Wang, X. M., Zhai, Y., and Ferrell, J. E. J. (1997) A role for mitogen-activated protein kinase in the spindle assembly checkpoint in XTC cells. *J. Cell Biol.*, **137**, 433.
174. Takenaka, K., Gotoh, Y., and Nishida, E. (1997) MAP kinase is required for the spindle assembly checkpoint but is dispensable for the normal M phase entry and exit in *Xenopus* egg cell cycle extracts. *J. Cell Biol.*, **136**, 1091.
175. Newport, J. W. and Kirschner, M. W. (1982) A major developmental transition in early *Xenopus* embryos. I. Characterization and timing of cellular changes at the midblastula stage. *Cell*, **30**, 675.
176. Newport, J. W. and Kirschner, M. W. (1982) A major developmental transition in early *Xenopus* embryos. II. Control of the onset of transcription. *Cell*, **30**, 687.
177. Foe, V., Odell, G. M., and Alberts, B. A. (1993) In *The Development of Drosophila melanogaster*. Bate, M. and Martinez-Arias, A. (ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, Vol. 1, p. 149.
178. Sullivan, W., Daily, D. R., Fogarty, P., Yook, K. J., and Pimpinelli, S. (1993) Delays in anaphase initiation occur in individual nuclei of the syncytial *Drosophila* embryo. *Mol. Biol. Cell*, **4**, 885.
179. Kimelman, D., Kirschner, M., and Scherson, T. (1987) The events of the midblastula transition in *Xenopus* are regulated by changes in the cell cycle. *Cell*, **48**, 399.
180. Gerhart, J., Wu, M., and Kirschner, M. (1984) Cell cycle dynamics of an M-phase-specific cytoplasmic factor in *Xenopus laevis* oocytes and eggs. *J. Cell Biol.*, **98**, 1247.
181. Hara, K., Tydeman, P., and Kirschner, M. (1980) A cytoplasmic clock with the same period as the division cycle in *Xenopus* eggs. *Proc. Natl. Acad. Sci. USA*, **77**, 462.
182. Dasso, M. and Newport, J. W. (1990) Completion of DNA replication is monitored by a feedback system that controls the initiation of mitosis *in vitro*: studies in *Xenopus*. *Cell*, **61**, 811.
183. Zalokar, M. and Erk, I. (1976) Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster*. *J. Microbiol. Cell.*, **25**, 97.
184. Raff, J. W. and Glover, D. M. (1988) Nuclear and cytoplasmic mitotic cycles continue in *Drosophila* embryos in which DNA synthesis is inhibited with aphidicolin. *J. Cell Biol.*, **107**, 2009.
185. Debec, A., Kalpin, R. F., Daily, D. R., McCallum, P. D., Rothwell, W. F., and Sullivan, W. (1996) Live analysis of free centrosomes in normal and aphidicolin-treated *Drosophila* embryos. *J. Cell Biol.*, **134**, 103.
186. Fogarty, P., Campbell, S. D., Abu-Shumays, R., De Saint Phalle, B., Yu, K. R., Uy, G. L., Goldberg, M. L., and Sullivan, W. (1997) *grp*, a *Drosophila* gene with homology to the *S. pombe chk1/rad27* checkpoint gene, is required for the fidelity of the late syncytial divisions. *Curr. Biol.*, **7**, 418.
187. Zusman, S. B. and Wieschaus, E. (1987) A cell marker system and mosaic patterns during early embryonic development in *Drosophila melanogaster*. *Genetics*, **115**, 725.

188. Edgar, B. A., Kiehle, C. P., and Schubiger, G. (1996) Cell cycle control by the nucleo-cytoplasmic ratio in early *Drosophila* development. *Cell*, **44**, 365.
189. Sibon, O. C., Stevenson, V. A., and Theurkauf, W. E. (1997) DNA-replication checkpoint control at the *Drosophila* midblastula transition. *Nature*, **388**, 93.
190. Fogarty, P., Kalpin, R. F., and Sullivan, W. (1994) The *Drosophila* maternal effect mutation *grapes* causes a metaphase arrest at nuclear cycle 13. *Development*, **120**, 2131.
191. Minden, J. S., Agard, D. A., Sedat, J. W., and Alberts, B. M. (1989) Direct cell lineage analysis in *Drosophila melanogaster* by time lapse three-dimensional optical microscopy of living embryos. *J. Cell Biol.*, **109**, 505.
192. Sullivan, W., Fogarty, P., and Theurkauf, W. (1993) Mutations affecting the cytoskeletal organization of syncytial *Drosophila* embryos. *Development*, **118**, 1245.
193. Walworth, N. C. and Bernards, R. (1996) *rad*-dependent response of the *chk1*-encoded protein kinase at the DNA damage checkpoint. *Science*, **271**, 353.
194. Zhou, Z. and Elledge, S. J. (1993) *DUN1* encodes a protein kinase that controls the DNA damage response in yeast. *Cell*, **75**, 1119.
195. Murakami, H. and Okayama, H. (1995) A kinase from fission yeast responsible for blocking mitosis in S phase. *Nature*, **374**, 817.
196. Onel, K., Koff, A., Bennett, R. L., Unrau, P., and Holloman, W. K. (1996) The *REC1* gene of *Ustilago maydis*, which encodes a 3′→5′ exonuclease, couples DNA repair and completion of DNA synthesis to a mitotic checkpoint. *Genetics*, **143**, 165.
197. Griffiths, D. J. F., Garbet, N. C., McCready, S., Lehmann, A. R., and Carr, A. M. (1995) Fission yeast rad17: a homologue of budding yeast RAD24 that shares regions of sequence similarity with DNA polymerase accessory proteins. *EMBO J.*, **14**, 5812.