REVIEW ARTICLE

# DNA replication and damage checkpoints and meiotic cell cycle controls in the fission and budding yeasts

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The cell cycle checkpoint mechanisms ensure the order of cell cycle events to preserve genomic integrity. Among these, the DNA-replication and DNA-damage checkpoints prevent chromosome segregation when DNA replication is inhibited or DNA is damaged. Recent studies have identified an outline of the regulatory networks for both of these controls, which apparently operate in all eukaryotes. In addition, it appears that these checkpoints have two arrest points, one is just before entry into mitosis and the other is prior to chromosome separation. The former point requires the central cell-cycle regulator Cdc2 kinase, whereas the latter involves several key regulators and substrates of the ubiquitin ligase called the anaphase promoting complex. Linkages between these cell-cycle regulators and several key checkpoint proteins are beginning to emerge. Recent findings on post-translational modifications and protein–protein interactions of the checkpoint proteins provide new insights into the checkpoint responses, although the functional significance of these biochemical properties often remains unclear. We have reviewed the molecular mechanisms acting at the DNA-replication and DNA-damage checkpoints in the fission yeast *Schizosaccharomyces pombe*, and the modifications of these controls during the meiotic cell cycle. We have made comparisons with the controls in fission yeast and other organisms, mainly the distantly related budding yeast.

Key words: cell cycle, checkpoint, meiosis, cdc2/CDK, yeasts.

## INTRODUCTION

In eukaryotes the cell cycle is divided into two phases, interphase and mitosis [1]. Interphase consists of G1, S and G2 phases. Mitosis can be sub-divided into prophase, metaphase, anaphase and telophase. Chromosomes condense during prophase, align during metaphase, separate during anaphase and decondense during telophase. There are several control points during the cell cycle: in late G1, called Start in yeast or the Restriction point in mammals, in late G2 and just prior to anaphase. To pass each point, cells have to fulfil several prerequisites. Before passing Start, cells can undergo two developmental programmes, i.e. entry into the mitotic cell cycle or sexual development. Adequate nutritional conditions and a critical cell size are required to traverse Start. During G2, cells have to check whether DNA replication is completed and ensure that DNA is not damaged. Before chromosome separation cells also examine whether chromosomes are aligned and spindles are formed properly. These cell-cycle checkpoints are the mechanisms that govern the order of the cell-cycle events, because if the order of the events is incorrect then a full complement of genetic information is not transmitted at cell division, which may lead to cancer in higher eukaryotes.

Much is now known about regulation of the eukaryotic cell cycle but two areas have yet to be fully understood. The first area is concerned with the molecular mechanisms acting during the DNA-replication and DNA-damage checkpoints, which block mitosis if DNA replication is incomplete or DNA is damaged. The second concerns the controls which operate during the meiotic cell cycle. In recent years, some progress has been made in both of these areas and the purpose of the present review is to summarize our current knowledge. The major focus will be on the yeasts, in which most work has been done, with emphasis on the fission yeast *Schizosaccharomyces pombe* and comparison with the budding yeast *Saccharomyces cerevisiae*. Work with other organisms such as *Xenopus* will also be discussed where particularly relevant, but for more detailed reviews of work on other organisms see [2–8].

## REGULATION OF MITOTIC CELL CYCLE BY CYCLIN-DEPENDENT KINASE (CDK) IN FISSION YEAST

There are a number of proteins which are required for cell-cycle progression. Among these, Cdc2 protein kinase (CDK1) plays a central role in the control of the mitotic cell cycle in fission yeast [9]. Loss of Cdc2 kinase activity arrests cells both before Start in G1 and in late G2 before M phase [10], and dominant forms of Cdc2 advance entry into mitosis [11]. Furthermore, Cdc2 is essential for viability and no suppressor mutations in other genes have been identified that completely rescue the disruptant of Cdc2. Based on these facts, initiation of DNA synthesis and nuclear divisions are attributed to the activity of a single CDK catalytic sub-unit encoded by *cdc2*$^+$ [12]. The equivalent gene in budding yeast is *CDC28* [13,14].

After passing Start, Cdc2 becomes complexed with B-type cyclins Cdc13 and Cig2 and the Cdc2 complex is phosphorylated on tyrosine-15 by both the Wee1 and Mik1 tyrosine kinases [15–17]. This tyrosine phosphorylation is maintained until the onset of mitosis [16,17]. Dephosphorylation of this residue is carried out by the Cdc25 tyrosine phosphatase and to a lesser

Abbreviations used: APC, anaphase-promoting complex; Cln, G1 cyclin; FHA, forkhead associated; PCNA, proliferating-cell nuclear antigen; SPB, spindle-pole body; CDK, cyclin-dependent kinase; RFC, replication factor C; MCM, minichromosome maintenance; ORC, origin–recognition complex.

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extent by the Pyp3 phosphatase [18,19]. Full activation of Cdc2 by tyrosine dephosphorylation brings about entry into mitosis. The kinase activity of tyrosine-15 phosphorylated Cdc2 is about 30% compared with that of the dephosphorylated form in starfish [20], which is consistent with its observed activities in fission yeast interphase cells [21]. This activity is sufficient to bring about DNA replication but not mitosis [22,23]. An analogous inhibitory pathway of tyrosine-19 phosphorylation of Cdc28 (equivalent to tyrosine-15 of Cdc2 in fission yeast) has been described in budding yeast. This residue is phosphorylated by Swe1 (a homologue of fission yeast Wee1) and dephosphorylated by Mih1 (a homologue of fission yeast Cdc25) [24,25]. When a bud is not formed properly or the actin cytoskeleton is defective, a morphogenesis checkpoint, which requires Swe1-mediated phosphorylation of Cdc28, operates to delay nuclear division [26–28]. However, there is no evidence that tyrosine phosphorylation determines the cell-cycle timing of mitosis as in fission yeast.

## DNA-REPLICATION AND DNA-DAMAGE CHECKPOINTS

DNA replication is accurately and temporally regulated during the cell cycle in all eukaryotes. In budding yeast, each chromosome contains multiple discrete replication origins called autonomously replicating sequences, where prereplicative complexes are assembled during M and G1 phase [29–31]. The prereplicative complexes includes the origin–recognition complex (ORC), the minichromosome-maintenance (MCM) complex and Cdc6 [29,30]. The six proteins that form the ORC bind to the autonomously replicating sequences and this binding persists throughout the cell cycle [32]. In late M phase, Cdc6 is recruited by the ORC, which in turn promotes loading of MCM proteins on to chromatin [33–38]. Activation of two protein kinase complexes, Cdc28–B cyclins and Cdc7–Dbf4, is likely to serve as the final signal activating replication fork movement [39–42]. Then the DNA-replication machinery, including DNA polymerases and proliferating-cell nuclear antigen (PCNA), initiates DNA synthesis. Similar mechanisms are likely to operate in other organisms, including fission yeast, since homologues of prereplicative-complex proteins and its regulators have been identified in many organisms [29].

In addition to the roles in DNA replication, many components of the DNA-replication machinery are required for restraining mitosis during S phase in fission yeast. These proteins are mainly involved in the initiation of DNA synthesis, including Pol$\alpha$ [43], Cdc18 (a counterpart of budding yeast Cdc6) [44], Cdt1 [45], Cut5/Rad4 [46], Orp proteins (a counterpart of ORC proteins) [47–50], MCM proteins [51], Hsk1–Dfp1/Him1 complex (a counterpart of Cdc7–Dfb4) [52–54], and replication factor C (RFC), which is required for loading of PCNA on to DNA [55,56]. The Cdt1 protein is required for recruiting the MCM proteins on to DNA [57], whereas the biochemical function of Cut5 is not known. The checkpoint function of the temperature-sensitive strains generally remains intact, whereas the complete deletion of these genes eliminates their checkpoint function, suggesting that the formation of a complex of these proteins is important for the checkpoint. However, DNA-replication proteins involved in progression of DNA synthesis are not required for this checkpoint. These include Pol$\epsilon$ [58], Pol$\delta$ complexes [59,60], fission yeast PCNA homologue [61], and PCNA interacting protein Cdc24 [62,63].

Apart from the essential DNA-replication proteins, several non-essential proteins have been identified which are specifically involved in the DNA-replication and DNA-damage checkpoints.

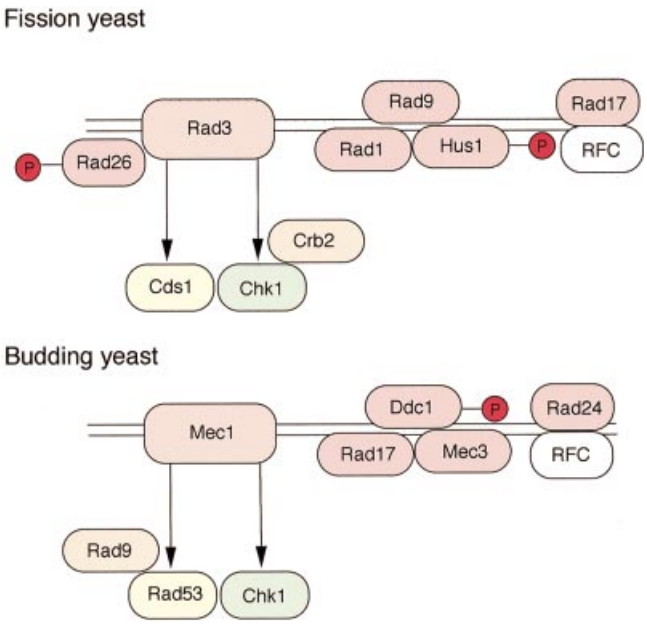


**Figure 1 Complex formation of the yeast checkpoint proteins**

Fission yeast Rad1, Hus1 and probably Rad9 form a complex regardless of the checkpoint signal. Rad3 kinase associates with and phosphorylates Rad26. These proteins are required for transmitting the checkpoint signal to the Cds1 and Chk1 kinases. Budding yeast Rad17, Mec3 and Ddc1 form a complex and Mec1 transmits the checkpoint signal to Rad53 and Chk1.

Five proteins, Rad1, Rad3, Rad9, Rad17 and Hus1, are required for both the DNA-replication and DNA-damage checkpoints in fission yeast [64–68]. From their structural or functional similarities, these proteins correspond to budding yeast Rad17, Mec1, Ddc1, Rad24 and Mec3 respectively [69]. Since the phenotypes of mutants in these genes, including sensitivity to DNA damaging agents, and the kinetics of their checkpoint defects are almost identical, these fission yeast proteins are called checkpoint Rad proteins. The fission yeast Rad26 is also a member of the family of checkpoint Rad proteins but the counterpart of this protein has not been found in budding yeast [65]. Fission yeast Rad1 is structurally similar to exonucleases, and the putative human homologue has nuclease activity [70,71] and also has a structural motif closely related to the DNA sliding-clamp protein PCNA, suggesting that it could provide processivity for DNA-repair and replication enzymes [72]. Fission yeast Rad17 has a limited homology to RFC [73]. The similarities of the checkpoint proteins to the DNA-replication proteins could indicate that they are components of complexes which interact with DNA to act as sensors for detecting DNA damage or blocks in DNA replication.

Fission yeast Hus1 and Rad1 proteins associate physically in a manner dependent on Rad9, and Hus1 is phosphorylated in a checkpoint Rad-protein-dependent manner (Figure 1) [74]. Complex formation and phosphorylation are conserved, since budding yeast Rad17, Mec3 and Ddc1 form a complex and Ddc1 phosphorylation is dependent on Mec1 (Figure 1) [75,76]. The human homologue of Rad1 also interacts physically with Rad17 [77]. However, the functional significance of these complex formations has not been found. Recently, a link between the checkpoint Rad and the DNA-replication proteins has been identified in both yeasts through the RFC protein, which physically associates with fission yeast Rad17 [56] and with

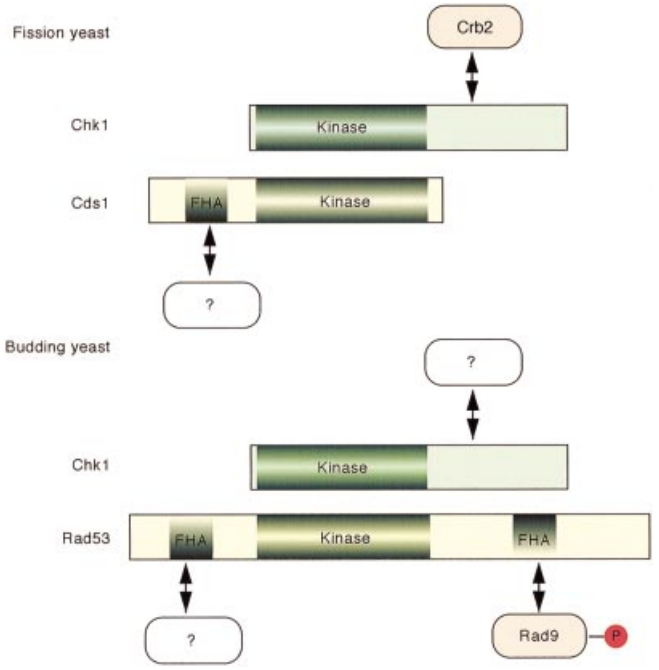


**Figure 2 Schematic representation of the yeast checkpoint kinases**

In fission yeast, Chk1 has a kinase domain in the N-terminal region, whereas in Cds1 the domain is in the C-terminal region. Cds1 has a FHA domain in the N-terminal region. Budding yeast Chk1 also has a kinase domain in the N-terminal region. Budding yeast Rad53 has two FHA domains, and Rad9 interacts with the FHA domain in the C-terminal region. Further interacting proteins have yet to be identified.

budding yeast Rad24 [78]. The fission yeast Rad3 kinase associates with Rad26, and Rad3 phosphorylates Rad26 independently of the other checkpoint Rad proteins, suggesting that Rad26 phosphorylation might be an initial response to DNA damage, although the functional significance of the Rad26 phosphorylation is not yet known (Figure 1) [79]. Rad3 is a member of the evolutionarily conserved subfamily of phosphatidylinositol 3-kinases, which includes fission yeast Tel1, budding yeast Mec1, Tel1, mammalian ATR and ATM and DNA-dependent protein kinase [80,81].

Two protein kinases, Chk1 and Cds1, provide a link between the checkpoint Rad proteins and the machinery which controls mitosis [82,83]. The Cds1 kinase is required specifically for proper recovery from a DNA-replication block, since *cds1*Δ cells stop cell-cycle progression when DNA replication is completely inhibited, but subsequently enter a premature mitosis when released from the replication block [83,84]. Cds1 also acts redundantly with Chk1 in the DNA-replication checkpoint [85–87], and Chk1 kinase itself is primarily involved in the DNA-damage checkpoint [82]. The protein kinase activity of Cds1 and the phosphorylation of Chk1 are dependent on the checkpoint Rad proteins [82,86]. Cds1 kinase is structurally similar to budding yeast Rad53 [83] and has a FHA (forkhead-associated) domain in the N-terminal region [88] (Figure 2). Budding yeast Rad53 kinase, which is required for both the DNA-replication and DNA-damage checkpoints, has two FHA domains and budding yeast Rad9 binds to the C-terminal FHA domain of Rad53, suggesting that this association is required for transduction of the DNA-damage checkpoint signal (Figure 2) [89]. The FHA domain of Rad53 in the N-terminal region is mainly involved in the DNA-replication checkpoint [90]. Budding yeast Rad9 has a region called the BRCT domain (the C-terminus of the breast cancer susceptibility gene 1 product), which is found in fission yeast Crb2/Rhp9, the tumour suppressor gene *BRCA1* and the p53 interacting protein 53BP1 [91–93]. Furthermore, Crb2 interacts with Chk1 as demonstrated by both genetic and two-hybrid analysis, although Chk1 lacks an obvious FHA domain [92]. Based on these facts, it is possible that an unknown protein binds to the N-terminal FHA domain of Rad53 or Cds1 and mediates the DNA-replication checkpoint signal. Crb2 is phosphorylated by the Cdc2 kinase not only in response to DNA damage but also during the normal cell cycle [94]. Cells having a non-phosphorylatable form of Crb2 fail to re-enter the cell cycle after DNA damage, suggesting the existence of a feedback regulation between the checkpoint protein and the cell-cycle machinery [94].

Cds1 and Chk1 play central roles in transducing the checkpoint signal from the checkpoint Rad proteins to the cell-cycle machinery (Figure 3A). The Cds1 kinase is activated by the inhibition of DNA replication [86] and then stops cell-cycle progression by acting through Wee1 [85] and Cdc25 [87]. The Chk1 kinase appears to phosphorylate Cdc25 [95] and Wee1 [96,97]. Thus both Cds1 and Chk1 phosphorylate Cdc25 and inhibit its activity [98]. Furthermore, the Chk1 phosphorylation of Cdc25 results in the physical association of Cdc25 with fission yeast Rad24 [99,100]. Fission yeast Rad24 is a member of the 14-3-3 family of proteins [101] which bind to phosphoproteins. Cdc25 complexed with fission yeast Rad24 is excluded from the nucleus in response to incomplete DNA replication and damaged DNA, suggesting that spatial organization is part of the DNA-replication and DNA-damage checkpoints [99,100].

Both the DNA-replication and DNA-damage checkpoint controls block entry into mitosis via the Cdc2 kinase [102–104], and both controls can be abolished by: (1) overexpressing *cdc25*$^{+}$ to reduce the level of phosphorylation of Cdc2 on tyrosine-15 [102,105], (2) inactivating the Wee1 and Mik1 kinases [17,103] or (3) the use of a Cdc2-Y15F mutant [103,104,106]. These studies strongly suggest that the DNA-replication and the DNA-damage checkpoints act through tyrosine-15 phosphorylation of Cdc2, although this conclusion has been controversial [104,107]. In contrast, tyrosine-19 phosphorylation of budding yeast Cdc28 is not important in the DNA-replication and DNA-damage checkpoint controls, because Cdc28-Y19F mutants arrest cell-cycle progression before nuclear division following DNA damage or inhibition of DNA replication [108–110]. In response to a DNA-replication block or DNA damage, budding yeast cells arrest with high Cdc28 protein kinase activity, short microtubule spindles and separated spindle-pole bodies (SPBs) [108–110]. If Cdc28 activity is not maintained at a high level, cells proceed through mitosis even though DNA damage is present [111]. These results suggest that the DNA-replication and DNA-damage checkpoints in budding yeast arrest cells in a metaphase-like state rather than in interphase, as is the case for most other organisms, including fission yeast.

In budding yeast, the DNA-damage checkpoint communicates to the mitotic apparatus through Pds1 and Cdc5 (Figure 3B). Pds1 is an anaphase inhibitor which is required for the DNA-damage and spindle checkpoints [112]. Pds1 is phosphorylated in response to DNA damage, a phosphorylation which is dependent on budding yeast Chk1 and Mec1 but not Rad53 [75,113]. Furthermore, budding yeast Chk1 phosphorylation depends on Mec1, and the Chk1 protein binds and phosphorylates Pds1 [114]. These studies suggest that Pds1 acts downstream of Chk1 and that Chk1 acts downstream of Mec1. Budding yeast Cdc5, a member of the polo-like family of kinases, is mainly involved in the exit from mitosis [115,116]. Overproduction of Cdc5 drives

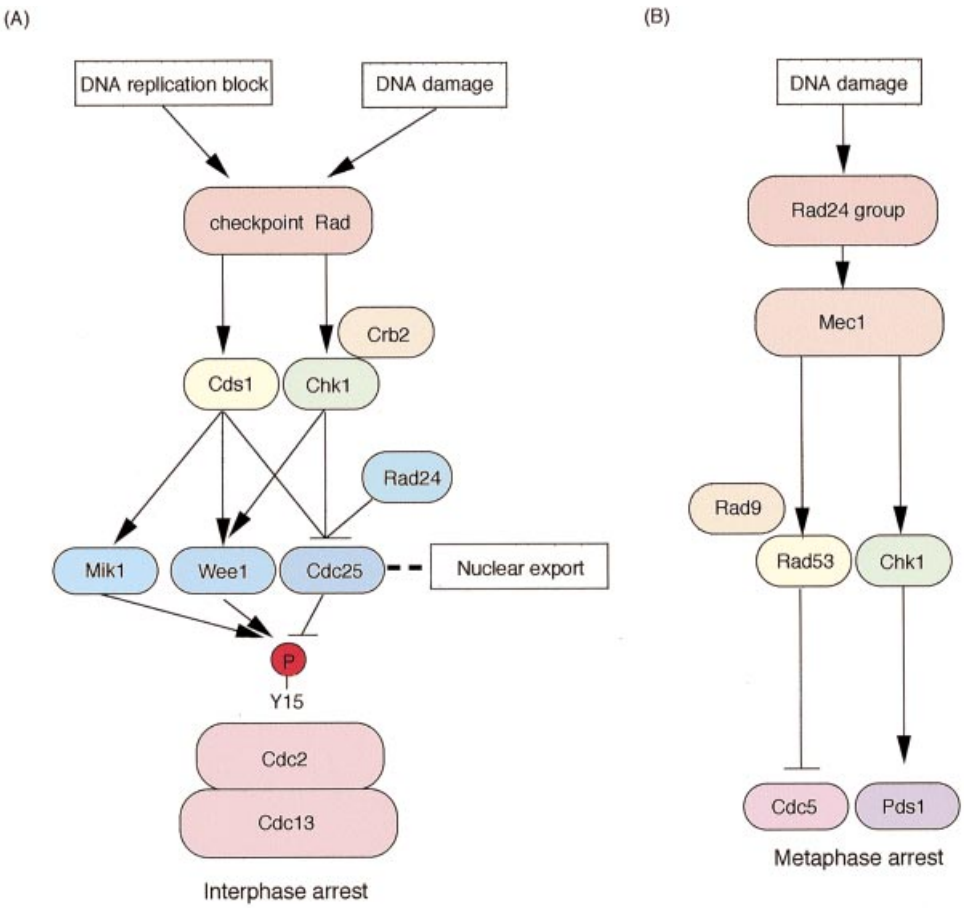


**Figure 3 Model showing how the DNA-replication and DNA-damage checkpoint signals are transmitted to the cell-cycle machinery in yeast mitotic cell cycle**

(**A**) The DNA-replication and DNA-damage checkpoints in fission yeast. (**B**) The DNA-damage checkpoints in budding yeast. See text for detailed explanations.

cells through anaphase so that they complete mitosis in the presence of damaged DNA, and inactivation of Cdc5 delays anaphase entry in *rad53* mutant cells after DNA damage [114]. It is interesting to note that the budding yeast Cdc5 kinase appears to have no obvious role for entry into anaphase during the normal cell cycle and that Cdc5 is phosphorylated in a Rad53-dependent manner after DNA damage, suggesting that Cdc5 acts downstream of the Rad53 kinase [117].

## INTRODUCTION TO MEIOSIS

In eukaryotes, meiosis not only plays a central role in the life cycle of sexual reproduction, but it is also essential for generating genetic diversity within species [118–122]. During meiosis, haploid gametes are produced from a parental diploid cell or zygote. Two successive nuclear divisions occur without S phase which halves the number of chromosomes. Fusion of two gametes restores the diploid chromosome complement. There are a number of important differences between meiosis and mitosis. The S phase in meiosis, called premeiotic DNA replication, usually takes two to five times longer than S phase in mitotic cycles [123,124]. In addition, premeiotic DNA synthesis depends on several proteins that are dispensable for mitotic DNA synthesis in both the fission yeast [6,120] and budding yeast [118]. During meiotic prophase, homologous recombination occurs about a hundred to a thousand times more frequently than during mitosis [119]. The first division of meiosis is quite different from a mitotic cell division. Sister chromatids remain associated with each other and the two copies of the same chromosome (called homologues) segregate to opposite sides of the cell. The second meiotic division immediately follows the first meiotic division without an intervening S phase. In the second meiotic division, sister chromatid disjunction takes place, as in mitosis.

## CONTROL OF ENTRY INTO MEIOSIS

In fission yeast, entry into meiosis requires a pheromone signal and nutritional starvation, primarily for nitrogen [120] (Figure 4A). Both pheromone and nitrogen starvation induce G1 arrest by inhibiting the activity of Cdc2. Pheromone inhibits the Cig2- and Cdc13-dependent Cdc2 kinase activities and induces the CDK inhibitor Rum1, which also promotes APC (anaphase-promoting complex)-dependent degradation of Cdc13 [125,126]. Nitrogen starvation enhances the degradation of both Cdc13 and Cig2, and the Cdc13 degradation is dependent on Srw1/Ste9 [127,128]. Srw1 is a regulator of the APC and is homologous to budding yeast Hct1/Cdh1 [129].

In both mitotic and meiotic cycles, periodic expression of DNA-replication genes is required for S phase. However, different components of the S phase transcriptional machinery are required to bring this about in the two cell cycles [6,130–135].

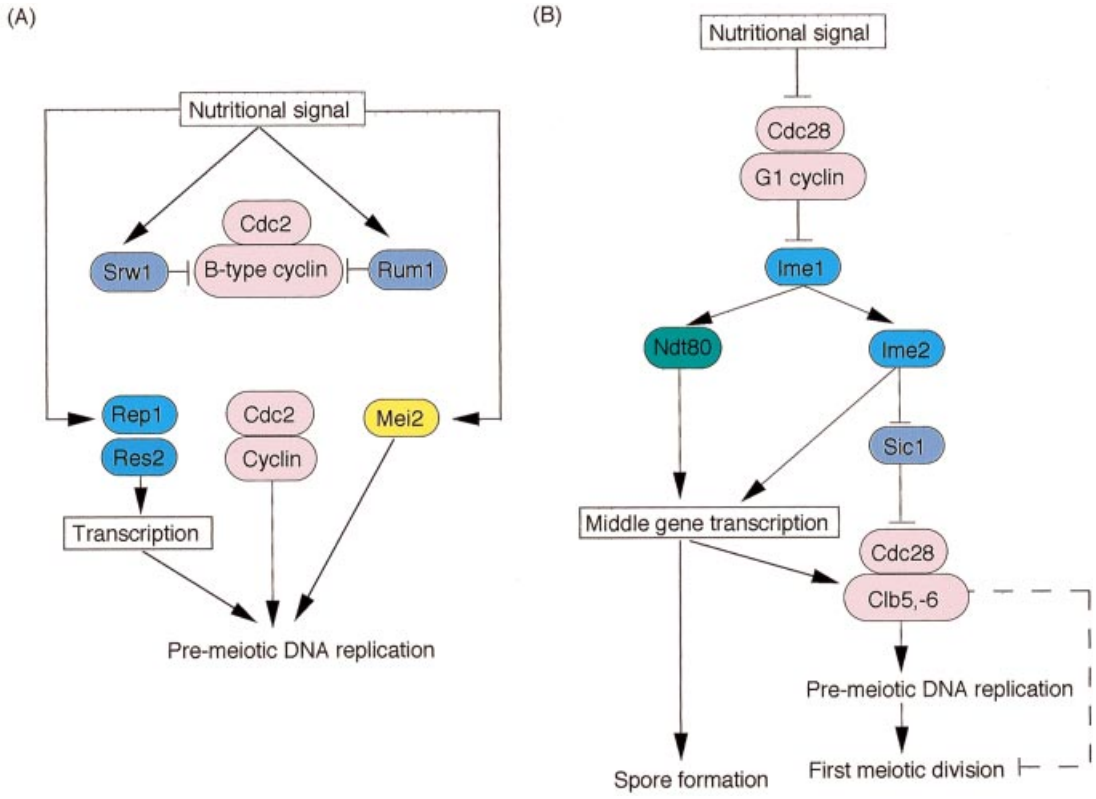


**Figure 4 Model of cell-cycle regulation in yeast meiosis**

(**A**) Entry into meiosis and premeiotic DNA replication in fission yeast. (**B**) Entry into meiosis and premeiotic DNA replication in budding yeast. See text for detailed explanations.

Activation of both the Cdc10–Res1 and Cdc10–Res2–Rep2 complexes is required for gene expression in the mitotic S phase, whereas the Cdc10–Res2 complex, possibly with Rep1, appears to play a similar role during meiosis (Figure 4A).

Rep1 is required specifically for premeiotic DNA replication, since *rep1*Δ cells arrest before premeiotic DNA replication but show no significant phenotype during mitotic cycles [136–138]. Rep1 is a zinc-finger protein with limited homology to Rep2 [134,136], and acts as an activator of the Res2–Cdc10 or Res1–Cdc10 complexes, since overexpression of *rep1*$^+$ rescues growth defects of *res1*Δ, *res2*Δ, *rep2*Δ and weakly rescues *cdc10*$^{ts}$ cells [136]. In addition, *res2*$^+$ mRNA induction is dependent on Rep1 function [136]. However, the meiotic defect of *rep1*Δ cells is not rescued by overexpression of either *res1*$^+$ or *res2*$^+$, suggesting that the Res1/Res2–Cdc10 complex is not the sole target of Rep1 [133]. Further support for this notion comes from the observation that the induction of recombination genes requires the Rep1 protein [137,138].

Interestingly, the inability of *rep1*Δ cells to carry out premeiotic DNA replication is partially suppressed if rapidly growing cells are induced to undergo meiosis [136]. After starvation, *rep1*Δ cells are completely unable to undergo meiosis. This suggests that a substitute for Rep1, probably Rep2, complements its function and allows premeiotic DNA replication to take place in these circumstances. Consistent with these results, double mutants of *rep2*Δ*rep1*Δ cells are unable to initiate meiosis [134]. In *res2*Δ cells, the premeiotic DNA-replication defect is also rescued if meiosis is induced without starvation [133]. The fact that similar proteins act in mitosis and meiosis suggests that initiation of premeiotic DNA replication might be regulated in a similar manner to initiation of DNA replication in mitotic cycles [6].

In budding yeast, Swi6, Swi4 and Mbp1 have extensive homology to fission yeast Cdc10, Res1 and Res2 respectively [6]. However, in contrast to fission yeast, double homozygous diploid mutants of either *swi4*Δ*swi6*Δ or *swi4*Δ*mbp1*Δ proceed through meiosis [139], although spore viability is low even in single *swi4*Δ or *swi6*Δ cells [140]. Very low activity of these transcriptional factors may be sufficient for progression through meiosis, but not for spore viability.

## REGULATION OF CDK IN MEIOSIS

In fission yeast, Cdc2 kinase plays a central role in the control of the mitotic cycle. Similarly to the situation in the mitotic cycle [10,141], Cdc2 is also required for premeiotic DNA synthesis [142], the second meiotic division [143–147] and possibly the first meiotic division [142] (Figure 4A). Cdc2 kinase activity increases around the beginning of premeiotic DNA replication and peaks around meiotic nuclear division [148,149]. Tyrosine-15 phosphorylation of Cdc2 appears around premeiotic DNA replication, with a concomitant increase in Wee1 protein levels [149], and decreases during meiotic nuclear divisions [148,149]. Cdc13 and Cdc25 are essential for both the first [142] and second meiotic divisions [144,145]. In fission yeast, the RNA binding protein Mei2 has a central role in the onset and progression through meiosis [120,150–152], but the relationship between Cdc2 and Mei2 is not known (Figure 4A).

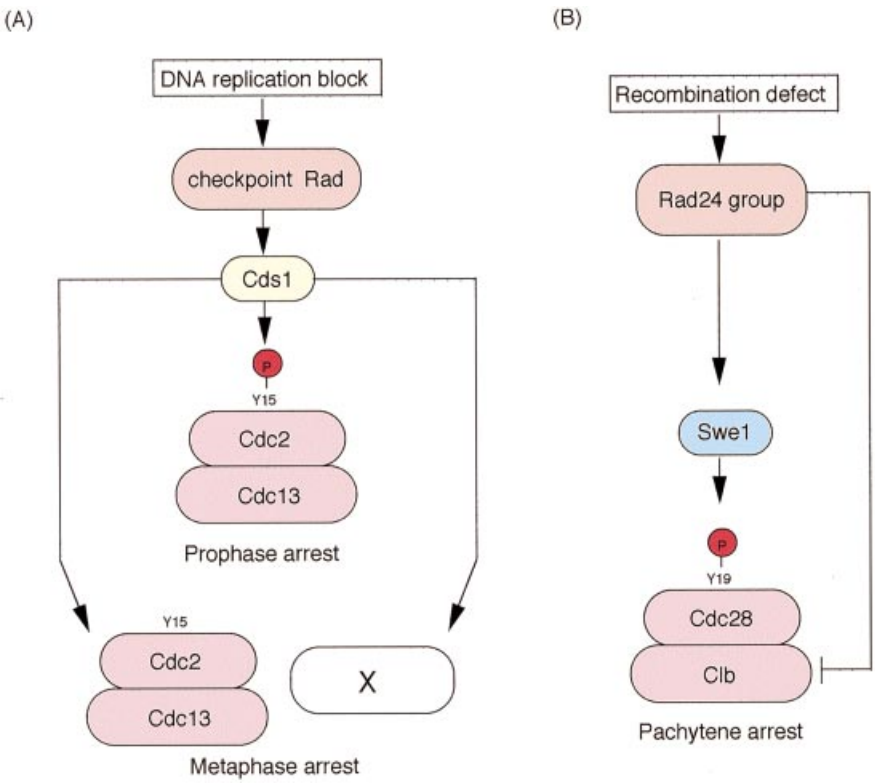


**Figure 5 Model of checkpoint regulation in yeast meiosis**

(**A**) The meiotic DNA-replication checkpoint in fission yeast. X represents an as-yet unidentified interacting protein. Reproduced from [148] with permission ©1999 Cold Spring Harbor Laboratory Press. (**B**) The recombination or pachytene checkpoint in budding yeast. See text for detailed explanations.

In contrast to the situation in fission yeast, using a temperature-sensitive budding yeast *cdc28* mutant, Cdc28 appears dispensable for premeiotic S phase but is required for meiotic nuclear divisions [153]. This seems inconsistent with the observation that overproduction of the CDK inhibitor Sic1 prevents premeiotic DNA replication [154]. It is possible that Cdc28 in this experiment is not sufficiently compromised to block premeiotic DNA replication because of the temperature-sensitive mutant, or that Sic1 may inhibit not only the Cdc28 protein kinase but also other proteins required for DNA replication. In budding yeast, G1 cyclin (Cln)-deficient cells enter meiosis regardless of nutrient conditions [155] and produce viable spores, suggesting that Clns are not required for meiosis [139]. Furthermore, Clns negatively regulate entry into meiosis by down-regulating both Ime1 transcription and protein levels (Figure 4B) [155]. Ime1 is a transcriptional activator of early genes that are essential for premeiotic DNA replication, homologous chromosome pairing and recombination [156]. As cells enter meiosis the level of the Clns decreases [155]. This negative regulation of entry into meiosis by Clns may be related to the fact that meiosis proceeds without bud formation, since Cln1 and Cln2 are involved in bud formation during mitotic cycles. The lack of Cln1 and Cln2 involvement also suggests that cell size may not regulate Start in meiosis. In meiosis, the target of Sic1 seems to be the B-type cyclins Clb5 and Clb6, because *clb5*Δ*clb6*Δ double mutants are unable to initiate premeiotic DNA replication [139,154] (Figure 4B). Surprisingly, the mutant cells proceed through meiosis with unreplicated chromosomes [154]. These facts suggest that Clb5 and Clb6 are essential for the activation of the meiotic DNA-replication checkpoint. The regulation of Sic1 degradation is different between mitosis and meiosis (Figure 4B) [139]. In mitotic cycles, Sic1 is phosphorylated by Cln-dependent CDK activity, which results in the degradation of Sic1 [157]. In meiosis, the double mutant *sic1*Δ*ime2*Δ undergoes premeiotic DNA replication, suggesting that degradation of Sic1 requires the meiosis-specific protein kinase Ime2 [139]. Ime2, which has sequence similarity to Cdc28, is essential for premeiotic DNA replication [156,158–160] and may phosphorylate Sic1 to target it for degradation. Ime2 is required to activate the early and middle meiotic genes, and is itself an early meiotic gene activated by Ime1. Middle gene products are required for the morphogenetic pathway that leads to spore formation [156]. Ndt80 is a transcription factor also required for the expression of middle and middle/late genes, as well as five of the six *CLB* genes, except *CLB2* (Figure 4B) [161,162]. All the *CLB* genes, except *CLB2*, are induced by ectopic expression of Ndt80 during the mitotic cell cycle. The fact that both *CLB* and middle gene expression are dependent on Ndt80 ensures that meiotic progression and ascospore formation are properly co-ordinated.

## MEIOTIC DNA-REPLICATION CHECKPOINT

A DNA-replication checkpoint control also operates during meiosis in fission yeast (Figure 5A) [148]. When meiotic DNA replication is inhibited with hydroxyurea, cells arrest with no spindle, unseparated SPBs and decondensed chromosomes [148].

This arrest requires the mitotic-checkpoint Rad proteins and the Cds1 protein kinase [148]. As discussed earlier, during mitotic cycles the role of Cds1 is restricted to recovery from a DNA-replication block [83]. In meiosis, Cds1 appears to play a more prominent role equivalent to that played by the checkpoint Rad proteins, since cells defective in any of these proteins proceed through meiotic nuclear divisions [148]. When DNA replication is blocked, the checkpoint maintains Cdc2 tyrosine-15 phosphorylation [148] just as in mitotic cycles [16,104,107].

Meiotic cells blocked by hydroxyurea, which harbour dephosphorylated tyrosine-15 of Cdc2, arrest with high Cdc2 protein kinase activity, separated SPBs and spindle formation [148]. This metaphase-like arrest is at a later stage of nuclear division than that seen in cells in which Cdc2 tyrosine-15 is phosphorylated, and is similar to that observed in mitotic budding yeast cells when DNA replication is inhibited [110]. The fact that certain processes occur only during meiosis, such as increased recombination rates or homologue chromosome pairing, may mean that a second checkpoint, which blocks cells in metaphase, is required during meiosis. Like the DNA-damage checkpoint in *S. cerevisiae*, counterparts of the budding yeast Cdc5 or Pds1 proteins might be downstream targets of meiotic DNA-replication checkpoint signals. Even in the fission yeast mitotic cycle there may be another DNA-replication checkpoint mechanism independent of Cdc2 regulation on tyrosine-15. Supporting this notion is the observation that the DNA-replication checkpoint remains intact in the absence of both Cdc25 and Wee1 [67]. However, it is possible that Mik1 or Pyp3 carry out this function in the absence of Wee1 or Cdc25.

In *Drosophila*, 13 rapid syncytial nuclear divisions lacking G1 and G2 phases occur during early embryogenesis. When DNA is damaged during this period, chromosome segregation but not entry into mitosis is delayed [163]. This is similar to budding yeast in which a defined G2 phase is lacking. In post-blastoderm division cycles which include a G2 phase, DNA damage delays entry into mitosis [164]. This delay requires tyrosine phosphorylation of Cdc2 as in fission yeast and other higher eukaryotes. From these observations, it is likely that, in all eukaryotes, mitosis and meiosis can be arrested either before entry into mitosis or before chromosome segregation.

In *Aspergillus nidulans*, two DNA-replication checkpoint systems have also been reported [165]. In this organism, if DNA replication is incomplete, cells harbouring non-tyrosine phosphorylated forms of Cdc2 arrest before mitosis with high Cdc2 kinase activity. At this stage, neither chromosome condensation nor spindle formation are observed. This contrasts with the situation during meiosis of fission yeast in which chromosome condensation and spindle formation occur in the presence of hydroxyurea if Cdc2 tyrosine-15 is dephosphorylated. Mutation of an APC component, BIME, in combination with non-tyrosine-phosphorylated Cdc2 can overcome the DNA-replication checkpoint. It is likely that co-ordination of both Cdc2 and APC functions is important for the DNA-replication checkpoint in this organism, and this may have some relevance to the analogous checkpoint during fission yeast meiosis.

In *Xenopus* egg extracts, non-tyrosine phosphorylatable forms of Cdc2 show a limited capacity to induce mitosis in the presence of a DNA synthesis inhibitor [166]. In addition, *Xenopus* Chk1, which is capable of phosphorylating the mitotic inducer Cdc25, is partially involved in the DNA-replication checkpoint pathway [167]. Similarly, in human cells, regulation of Cdc2 tyrosine-15 is important in the DNA-replication and DNA-damage checkpoints but is not the sole mechanism for restraining mitotic progression [168,169]. Spatial compartmentation of cell-cycle regulators is one of the mechanisms that prevent mitosis. In fact, it has been shown that exclusion of B-type cyclin from the nucleus is required for blocking mitosis after DNA damage [170,171]. At least two different mechanisms of the DNA-replication and DNA-damage checkpoints may exist in all organisms, but they may be regulated in different ways in different organisms.

## RECOMBINATION OR PACHYTENE CHECKPOINT

In many organisms, cells defective in recombination or synaptonemal complex formation arrest at the pachytene stage of meiotic prophase [121,172,173]. This arrest is called the recombination checkpoint or the pachytene checkpoint (Figure 5B) [174,175]. Pachytene is the stage of the meiotic cell cycle when premeiotic DNA synthesis is completed, the SPB is duplicated but not separated and the cell is committed to recombination. This arrest requires the DNA-damage checkpoint Rad17, Rad24, Ddc1 and Mec1 proteins in budding yeast, ensuring the order of meiotic events by preventing chromosome segregation when recombination is incomplete or the synaptonemal complex is defective [121,173,174,176] (Figure 5B). Pch2 and the silencing factor Sir2 are also required for the pachytene checkpoint but are not required for the DNA-damage checkpoint in mitotic cycles [176]. The meiosis-specific Red1 and Mek1 proteins are also required for pachytene arrest [173]. Mek1 is a protein kinase and associates with Red1, which is an essential component of the meiotic chromosomes [177,178]. However, in fission yeast there are no mutants that arrest with recombination intermediates and the recombination checkpoint has not been identified [119].

Interestingly, budding yeast Rad9, which is essential for the DNA-damage checkpoint is not required for the recombination checkpoint [174]. However, this protein is required for the arrest in meiosis imposed by inactivation of a telomeric DNA binding protein [179]. This arrest point in meiosis is at a stage that follows premeiotic DNA replication but is prior to commitment to recombination. These facts indicate that Rad9 has stage-specific roles during meiosis, although the exact mechanisms of its action or regulation are not known.

Tyrosine-19 phosphorylation of Cdc28 is also required for pachytene arrest in budding yeast, since both *swe1*Δ and Cdc28-F19 cells proceed through meiosis in the presence of double-strand breaks, although the segregation of chromosomes is delayed [175] (Figure 5B). Clb1 overproduction in *swe1*Δ or Cdc28-F19 cells eliminates this delay, suggesting that the regulation of B-type cyclin synthesis is also important in this checkpoint [175] (Figure 5B). In contrast, the budding yeast Mih1 phosphatase is required neither for mitosis, meiotic progression nor the pachytene checkpoint [175]. Upon activation of the pachytene checkpoint, the Swe1 kinase accumulates and is hyperphosphorylated; this phosphorylation is dependent on budding yeast Rad24 checkpoint protein. These facts suggest that Swe1 is a target of the pachytene checkpoint control. It is of interest that these meiotic controls are similar to those operating during the DNA-replication and DNA-damage checkpoints in fission yeast.

## COHESIN IN MEIOSIS

Physical association between sister chromosomes is required for their proper segregation during mitosis and meiosis. This attachment is provided by a molecular 'glue' called cohesin. The cohesin complex is composed of at least four proteins, Scc1/Mcd1, Scc3, Smc1 and Smc3, in budding yeast [180–183]. Similar proteins and their regulators exist in fission yeast and other

eukaryotes, indicating that this mechanism is conserved [184,185]. Loss of any of these proteins brings about premature chromosome separation even if the APC is inactivated. At the metaphase-to-anaphase transition, Scc1 is cleaved by Esp1 (Cut1 in fission yeast), resulting in sister chromatid separation [186]. Esp1 is kept inactive by binding to Pds1 (Cut2 in fission yeast) until the onset of anaphase [187], when Pds1 (Cut2) is destroyed by the APC [184,188,189].

In meiosis, after premeiotic DNA replication, homologous chromosomes are held together by recombination between homologues [180,190]. The physical attachment between homologues is provided by chiasmata, which are sites of reciprocal recombination between homologous chromosomes. Meiotic cohesin, located in the arm regions of sister chromatids, is also important for this attachment to keep the recombined chromosomes tightly connected. In addition, meiotic cohesin, located in the vicinity of the centromeric regions, is required to hold sister chromatids together. Two sister kinetochores, held by the cohesin, move to the same pole in the first meiotic division. After disappearance of the cohesin at the centromeric region, sister chromatid disjunction occurs during the second meiotic division. This step-wise loss of cohesion is essential for an orderly progression through the two meiotic divisions.

In fission yeast, one of the candidates of the meiotic cohesin is the Rec8 protein since *rec8* mutants show precocious separation of sister chromatids [191]. Fission yeast Rec8 shares homology with the budding yeast mitotic cohesin Scc1 protein [182,192,193]. The Rec8 protein is absent in mitotic cycles, but its level increases during premeiotic DNA replication and decreases around the second meiotic division [192]. During prophase I Rec8 is distributed along the length of the chromosome. Around the first meiotic division Rec8 is lost in the arm regions but is retained at the centromeres until metaphase of the second meiotic division [192,193]. This localization may explain why recombination is reduced only at the centromere regions in *rec8* mutants [191,193]. Loss of Rec8 function causes premature separation of chromosomes [191], and a detailed analysis of the *rec8* mutant revealed that the reductional segregation pattern of the first meiotic division is shifted to an equational segregation pattern [192]. This switch to an equational segregation pattern rather than random segregation is partially explained if Rec8 has an additional role in directing sister-chromatid kinetochores to the same pole [192]. In contrast, budding yeast *rec8* mutants undergo random segregation in the first meiotic division [194].

## TELOMERES

Telomeres are essential for blocking the degradation of linear chromosome ends in all eukaryotes. Telomeres have additional roles in the pairing of homologous chromosomes during the early stages of meiosis. The fission yeast Taz1 and Lot2 proteins have a critical role for the maintenance of telomere length and telomeric silencing, although disruptants of these genes can grow normally [195,196]. During meiosis in *taz1* and *lot2* mutants, telomeres fail to cluster at the SPB, resulting in a low recombination rate [196,197]. In fission yeast, the nucleus is highly mobile moving back and forth during the early stages of meiosis [198–200], a movement which is likely to be required for homologous chromosome pairing and recombination. At this stage, telomeres cluster at the SPB instead of the centromeres and reside at the leading edge of the chromosome with the rest of the chromosomes trailing behind [198,199]. Therefore the Taz1 and Lot2 proteins help chromosome pairing through telomere clustering at the SPB.

## SUPPRESSION OF DNA REPLICATION BETWEEN THE FIRST AND SECOND MEIOTIC DIVISION

One of the hallmarks of meiosis is suppression of DNA replication between the first and second meiotic division. So far, there are no mutants that can initiate DNA replication during this period in either fission yeast or budding yeast. In *Xenopus* and starfish oocytes, inhibition of the Cdc2 kinase or of the Mos function can induce DNA replication during this period [201,202]. A partial fall in Cdc2 kinase activity occurs between the two meiotic divisions, concomitant with the appearance of threonine-161-dephosphorylated forms of Cdc2 [201]; threonine-161 phosphorylation of Cdc2 is essential for its activity [12]. During this period, no tyrosine-15 phosphorylation of Cdc2 was observed [201,203]. Inactivation of Mos leads to the partial phosphorylation of Cdc2 tyrosine-15 and the dephosphorylation of Cdc2 threonine-161 [201]. These observations imply that the activity of Cdc2 between the two meiotic divisions is regulated by the phosphorylation status of both tyrosine-15 and threonine-161. Supporting this notion, ectopic induction of Wee1 induces DNA replication after the first meiotic division [204]. The regulation of Cdc2 complexes appears to be required for the suppression of DNA replication between the two meiotic divisions, and it is important to know how Cdc2 activity is regulated during this period.

## CONCLUDING REMARKS

Recent studies have revealed that mitosis and meiosis share common pathways to undergo DNAreplication and chromosome segregation. Distinctive mechanisms also exist in meiosis, probably because of high rates of recombination and suppression of DNA replication before the second meiotic division. In the near future, the whole genome sequence of several organisms, including fission yeast and humans will be known. A comparison with the budding yeast sequence will be revealing with regard to what differs and what is conserved between organisms. In particular, the comparison of the two yeasts during the mitotic and meiotic cell cycles will continue to help our understanding of the cell-cycle regulation in all eukaryotes.

We thank Jacky Hayles, Emma Greenwood, Chris Lehane, Stephanie Yanow, Annie Borgne and Takashi Toda for a critical reading of this review. H.M. was supported, in part, by the Imperial Cancer Research Fund.

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