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Mitotic DNA damage and replication checkpoints in yeast

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Abstract

Studies of the genetics of G₂/M checkpoints in budding and fission yeasts have produced many of the defining concepts of checkpoint biology. Recent progress in the biochemistry of the checkpoint gene products is adding a mechanistic understanding to our models and identifying the components of the normal cell cycle machinery that are targeted by checkpoints.

Introduction

The paradigm that checkpoints arrest the cell cycle to allow time for completion of a previous cell cycle event or repair of cellular damage has come to be seen as a fundamental part of cell cycle regulation. This is as a result of an appreciation of the widespread occurrence of checkpoints and the realization that distinctions between checkpoints and the mechanisms that normally control the timing of cell cycle transitions are often semantic. In the past few years checkpoint research has blossomed in breadth [1] and depth [2]. Checkpoints have been identified that regulate all of the major mitotic cell cycle transitions, and several of the meiotic cell cycle transitions. Checkpoint proteins also have roles in transcriptional regulation, induction of DNA repair, recovery from cell cycle arrest, adaptation and, in metazoans, apoptosis. Increasingly detailed genetic, molecular and biochemical analyses of the gene products involved in checkpoints are rapidly revealing the mechanisms by which they exert their effects.

This review covers recent developments in this field from work on *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. We focus on the cell cycle arrest functions of the G₂/M DNA damage checkpoint that prevents mitosis in the presence of damaged DNA, and the S/M replication checkpoint that prevents mitosis when DNA replication is blocked, dealing primarily with experiments that show a direct effect on cell cycle progression.

G₂/M checkpoints prevent cells from passing through mitosis. In some cases, such as the G₂ DNA-damage checkpoint in *S. pombe* and vertebrates, cells arrest in G₂ [3,4], while in others, such as the DNA-damage and spindle checkpoints in *S. cerevisiae*, cells arrest during M phase at the metaphase to anaphase transition [5,6]. But, as near as we can tell, the general purpose of G₂/M checkpoints is to prevent sister chromatid separation which, in the presence of either DNA damage or incomplete replication, is the irreversible step that turns a temporary setback into a lethal event (Figure 1a). Therefore, G₂/M checkpoints need to target an event just before sister chromatid separation. In *S. pombe* and vertebrates, the G₂/M transition, regulated by the inhibitory tyrosine phosphorylation of Cdc2^{SP}, serves this purpose [7•,8•,9]. In *S. cerevisiae*, however, the events regulated by the tyrosine phosphorylation of Cdc28^{SC}, such as

spindle pole body separation and the bud formation checkpoint, occur much earlier in the cell cycle, at around the end of S phase [10,11] (Figure b). Thus, the period between S phase and mitosis in *S. cerevisiae* is, in some ways, more akin to metaphase than to G₂. Instead of targeting the transition, the *S. cerevisiae* checkpoints target the metaphase to anaphase transition [5, 12]. To help distinguish between the overlapping nomenclature of the two yeasts, in this review *S. cerevisiae* and *S. pombe* gene and protein names are identified by super scripted sc and sp, respectively.

The *Schizosaccharomyces pombe* G₂ DNA damage checkpoint

The proteins involved in the G₂ DNA damage checkpoint can be divided into three groups: the checkpoint *rad* proteins that are also involved in the S/M replication checkpoint, the G₂ DNA-damage checkpoint-specific proteins, and the target genes that are part of the normal mitotic machinery. The checkpoint *rad* genes include *rad1^{sp}*, *rad3^{sp}*, *rad9^{sp}*, *rad17^{sp}*, *rad26^{sp}*, and *hus1^{sp}*. These are required for all the known *S. pombe* DNA checkpoints [3,13,14]. At the very least, the checkpoint *rad* gene products serve as signal transducers between the primary checkpoint signal and the cell cycle machinery. They may also be directly involved in the recognition of DNA damage, since several are similar to proteins involved in DNA metabolism (Table 1). Rad1^{sp} is similar to the *Ustilago* Rec1 exonuclease, the putative human Rad1^{sp} homolog (hRad1) displays exonuclease activity [15,16•], and Rad17^{sp} has limited similarity to replication factor C (RFC) [17]. Furthermore, *S. cerevisiae* homologs of Rad1 and Rad17 have been implicated in the processing of DNA damage [18]. The most interesting similarity is that of Rad3^{sp} to DNA-dependent protein kinase (DNA-PK) [19,20]. DNA-PK is a three-subunit enzyme that is activated by binding to double- and single-strand DNA breaks [21] suggesting that DNA-PK-like kinases may be directly involved in recognition of damaged DNA. The catalytic subunit of DNA-PK is a large and unusual protein kinase that has structural similarities to lipid kinase [19]. Other members of the DNA-PK-like family involved in checkpoint regulation include ATM (ataxia telangiectasia mutated) and ATR (ATM related) in humans, and Mec1 in *S. cerevisiae* [19,22–24]. Efficient activation of DNA-PK by DNA breaks, however, requires two regulatory subunits, Ku70 and Ku86 [21]. KU homologs have not been found in *S. pombe*, and the KU subunits in *S. cerevisiae* are not involved in DNA damage checkpoints [25•].

Are the other checkpoint Rad proteins regulatory partners of Rad3^{sp}? It is too early to say, but there is accumulating evidence that at least some of the checkpoint Rad proteins associate *in vivo*. Rad1^{sp} and Hus1^{sp} are bound together *in vivo*, and this interaction depends on Rad9^{sp}, suggesting that they may form a heterotrimer [26••].

Furthermore, Hus1^{sp} is phosphorylated in response to DNA damage and this phosphorylation depends on Rad1^{sp} and Rad9^{sp}, as well as Rad3^{sp} [26••]. Deletions of the other checkpoint *rad* genes do not effect the Rad1^{sp}–Hus1^{sp} complex. Therefore, Rad1^{sp}, Hus1^{sp} and maybe Rad9^{sp} form a stable complex independent of the other checkpoint *rad* genes.

A seventh gene, *cut5^{sp}*, may also belong to the checkpoint *rad* class but its analysis is complicated by the fact that it is essential for replication [27]. Thus, *cut5^{sp}Δ* cells are inviable and most analysis has been done with temperature sensitive alleles that may retain some residual function. Cut5^{sp} is required for both the S/M and the γ-radiation induced G₂ DNA damage checkpoint [28,29•] but, surprisingly, not for the UV-radiation induced G₂ DNA damage checkpoint [28,29•]. Whether these results indicate a real difference between the γ and UV damage checkpoints, or a complication of using non-null *ts cut5^{sp}* mutations, awaits to be determined.

One role of the checkpoint Rad proteins in the G₂ DNA damage checkpoint is to regulate the serine/threonine kinase Chk1^{sp}. Deletion of Chk1^{sp} completely inactivates the G₂ DNA

damage checkpoint [14]. Cells with the *chk1^{SPΔ}* mutation are not as sensitive to DNA damage as cells with a deletion of any of the checkpoint *rad* genes, suggesting that the checkpoint Rad proteins also have a Chk1^{SP} independent role, perhaps in activating DNA repair [14,30]. Furthermore, deletion of Chk1^{SP} does not affect the S/M checkpoint, indicating that the checkpoint *rad* genes have other targets in the S/M checkpoint, as discussed below [14,30]. Chk1^{SP} is phosphorylated in response to DNA damage and this phosphorylation depends on the checkpoint Rad proteins [31].

The nature of the interaction between Chk1^{SP} and the checkpoint Rad proteins has remained obscure, but analysis of the newly identified Crb2^{SP} protein promises to shed light on the problem. Crb2^{SP}, like Chk1^{SP}, is specific to the G₂ DNA damage checkpoint and acts downstream of the checkpoint Rad proteins [32•,33••]. Like Rad9^{SC} it contains a BRCA1 carboxy-terminal (BRCT) motif [32•,33••] also found in the p53 binding protein 1 (53BP1) and the breast cancer susceptibility gene BRCA1 [34,35]. Although it is intriguing that the BRCT motif is found in a number of otherwise unrelated proteins involved in checkpoints and cancer, its role is as yet unclear. Crb2^{SP} is phosphorylated in response to DNA damage in a checkpoint Rad protein dependent but Chk1^{SP} independent manner [33••]. This phosphorylation may release Crb2^{SP} from a large complex, since Crb2^{SP} found in lower molecular weight fractions after DNA damage. Conversely, Chk1^{SP} phosphorylation is Crb2^{SP} dependent [33••]. These data suggest that Crb2^{SP} acts downstream of the checkpoint Rad proteins and upstream of Chk1^{SP}. Thus, Crb2^{SP} may act a mediator between the checkpoint Rad proteins and Chk1^{SP}. Consistent with this idea, Chk1^{SP} and Crb2^{SP} interact in a two-hybrid assay, although this interaction has not been seen *in vivo* [33••]. Crb2^{SP} also interacts with Cut5^{SP} *in vitro*, providing a potential link between Crb2^{SP}/Chk1^{SP} and the checkpoint Rad proteins [33••].

The ultimate target of the G₂ DNA damage checkpoint is the tyrosine phosphorylation of the cyclin-dependent kinase Cdc2^{SP} [7••]. The timing of mitosis in a normal cell cycle is controlled by the phosphorylation of Cdc2^{SP} on Tyr15 which maintains Cdc2^{SP}-cyclin B complexes at an interphase level of activity, high enough to initiate replication and prevent rereplication, but too low to trigger mitosis [36]. Phosphorylation of Tyr15 is catalyzed by the Wee1^{SP} and Mik1^{SP} kinases and is removed by the Cdc25^{SP} phosphatase, and to a lesser extent the Pyp3^{SP} phosphatase [36]. At the G₂→M transition, the balance of kinase and phosphatase activity shifts in favor of dephosphorylation, Cdc2^{SP} is activated, and mitosis ensues. During DNA-damage-induced checkpoint-arrest, Cdc2^{SP} is maintained in its tyrosine phosphorylated form [7••,37•]. Mutation of Tyr15 to Phe (which cannot be phosphorylated) abrogates the checkpoint, showing that Tyr15 phosphorylation of Cdc2^{SP} is required for the checkpoint arrest [7••]. The fact that there is no residual checkpoint in *cdc2-Y15F* (in the single letter code for amino acids) cells also shows that *S. pombe* does not have a significant metaphase to anaphase DNA damage checkpoint.

In order to maintain Cdc2^{SP} in its tyrosine phosphorylated interphase form, the checkpoint must target either Cdc25^{SP}, Wee1^{SP}, Mik1^{SP} or some combination of the three. Consistent with this, Chk1^{SP} can phosphorylate both Wee1^{SP} and Cdc25^{SP} *in vitro*, although the effect of this *in vivo* has yet to be determined [37•,38••]. The role of Cdc25^{SP} as a target of the G₂ DNA damage checkpoint has been demonstrated by genetic analysis [38••]; furthermore, dephosphorylation of Cdc2^{SP} by Cdc25^{SP} is inhibited by the checkpoint [7••]. Whether such inhibition is caused by inactivation of Cdc25^{SP}, or by a mechanism that prevents access to Cdc2^{SP} of Cdc25^{SP}, was not addressed. While these data show that Cdc25^{SP} is a target of the DNA damage checkpoint, they certainly do not exclude the possibility that Wee1^{SP} or Mik1^{SP} could also be targeted.

The regulation of Cdc25^{SP} by the G₂ DNA damage checkpoint may involve the 14-3-3 protein Rad24^{SP}. The name 14-3-3 describes a large family of proteins that bind to a phosphorylated

consensus site in a variety of proteins, including Cdc25 [39,40,41•,42•]. Deletion of *rad24^{SP}* advances mitosis, which is consistent with Rad24 acting as a negative regulator of Cdc25 during normal growth, but only partially impairs the G₂ DNA damage checkpoint [43]. The remaining checkpoint function in *rad24^{SP}Δ* cells may be due to Rad25^{SP}, another 14-3-3 protein and a high copy suppressor of *rad24^{SP}Δ* [43]; however, *rad25^{SP}Δ* has no checkpoint defect and *rad24^{SP}Δ rad25^{SP}Δ* cells are inviable, suggesting these genes have important functions that are unrelated to checkpoints [43].

The *Saccharomyces cerevisiae* metaphase DNA damage checkpoint

Five genes in *S. cerevisiae*, *RAD9^{SC}*, *RAD17^{SC}*, *RAD24^{SC}*, *MEC3^{SC}*, and *DDC1^{SC}*, are required for the metaphase DNA damage checkpoint, but not the S/M replication checkpoint [12,44, 45•]. These genes can be divided into two groups, with *RAD9^{SC}* in one and the rest in another, referred to as the *RAD24^{SC}* epistasis group. This distinction is made on the basis of the additive phenotypes of *rad9^{SC}Δ* in combination with deletions of members of the *RAD24^{SC}* epistasis group [18,46•,47•]. The other two genes required for the metaphase DNA damage checkpoint, *MEC1^{SC}* and *RAD53^{SC}*, are also required for the S/M checkpoint [12,48]. They also differ in that they are essential for viability but this role is independent of their mitotic checkpoint functions (SJ Elledge, personal communication).

Mec1^{SC}, like Rad3^{SP}, is a member of the DNA-PK family of protein kinases [2]. Another member this family is Tel1^{SC}, a protein involved in telomere maintenance [49]. While, Tel1^{SC} is not required for checkpoints in *S. cerevisiae*, it can substitute for Mec1^{SC} in a number of situations [50,51•,52]. Rad53^{SC} is also a protein kinase and it contains two forkhead associated (FHA) domains, one on either end of the protein [48,53], which may be involved in binding regulatory partners. Dun1^{SC} is a Rad53^{SC} homolog that is also involved in checkpoints [54]. While the major role of Dun1^{SC} is thought to be downstream of Rad53^{SC} in transcriptional induction of repair genes, *dun1^{SC}Δ* cells do have a partial checkpoint arrest defect which may or may not be related to the role of Dun1^{SC} in transcription [54,55]. Rad53^{SC} is phosphorylated in response to DNA damage and this phosphorylation is dependent on Mec1^{SC}, Rad9^{SC}, and the proteins of the Rad24^{SC} epistasis group [52,56]. Whether Rad53^{SC} is a direct substrate of Mec1^{SC} remains to be determined.

Both Rad9^{SC} and the proteins of the Rad24^{SC} group were thought to function upstream of Mec1^{SC} [2]. Several proteins of the Rad24^{SC} group are structurally similar to proteins involved in DNA metabolism (Table 1), and are required for the processing of damaged DNA [18]. Both Rad9^{SC} and Ddc1^{SC}, however, are phosphorylated after DNA damage in a Mec1^{SC} dependent manner, indicating that they lie downstream of Mec1^{SC} [45•,51•,57•]. A simple interpretation of these results is that Rad9^{SC} and Ddc1^{SC} associate with Mec1^{SC} in such a way that they are required for Mec1^{SC} activation and serve as Mec1^{SC} substrates. This is a common situation for the regulatory subunits of kinases; however, the differences between the roles of Rad9^{SC} and Ddc1^{SC} suggest that they may be in different Mec1^{SC} complexes [47•]. There is much work to be done to substantiate such a model. Initial work has led to the characterization of Rad9^{SC} and Ddc1^{SC}. Ddc1^{SC} interacts *in vivo* with Mec3^{SC}, and this association requires Rad17^{SC} [47•]. This is reminiscent of the observation that *S. pombe* homologs of Rad17^{SC} and Ddc1^{SC}, Rad1^{SP} and Rad9^{SP}, may also interact [26•]. Phosphorylation of Ddc1^{SC} is dependent on the other members of the Rad24^{SC} group, but not Rad9^{SC}, consistent with the additive roles of Rad9^{SC} and the Rad24^{SC} group [47•]. The phosphorylation of Rad9^{SC} leads to its association with Rad53^{SC}, through the second of the two Rad53^{SC} FHA domains [51•,57•]. It is plausible that Rad9^{SC} acts as an adapter, such that, when phosphorylated by Mec1^{SC}, it recruits Rad53^{SC} to be phosphorylated in turn by Mec1^{SC}. This cannot be the only role of Rad9^{SC}, however, because phosphorylation of Pds1^{SC} is dependent on Rad9^{SC} but not on Rad53^{SC} [58•].

In order to arrest cells, the *S. cerevisiae* metaphase DNA damage checkpoint targets the stability of Pds1^{sc}. Pds1^{sc} is a rate-limiting negative regulator of anaphase that is degraded at the metaphase to anaphase transition. Expression of a nondegradable Pds1^{sc} prevents anaphase, while deletion of *PDS1^{sc}* allows premature sister chromosome separation [59,60] and also compromises the metaphase DNA damage checkpoint [5]. Pds1^{sc} is phosphorylated in response to DNA damage in a Rad9^{sc} and Mec1^{sc} dependent manner [58••]. Although the effect of Pds1^{sc} phosphorylation is unknown, it is tempting to speculate that it stabilizes the protein, leading to metaphase arrest. The phosphorylation of Pds1^{sc} is not dependent on Rad53^{sc} or Ddc1^{sc} [47••,58••]. Consistent with this observation, mutations in neither *RAD53^{sc}* or *PDS1^{sc}* alone completely eliminate the metaphase DNA damage checkpoint, although both mutations together do (R Gardner, C Putnam, T Weinert, personal communication). Hence, Rad53^{sc} is likely to have a Pds1^{sc} independent target. This Rad53^{sc} dependent arrest is also dependent on Dun1^{sc}. In this context, it is interesting to note that maintenance of Cdc28^{sc} activity seems to be required for metaphase arrest in *S. cerevisiae*. Mutations that decrease Cdc28 activity compromise metaphase arrest [61,62]. These results have led to a model in which both Cdc28^{sc} activity and Pds1^{sc} stability are required to prevent anaphase [62]. If this is true, the metaphase arrest checkpoint may act both to stabilize Pds1^{sc} via a Rad53^{sc} independent manner and to maintain the activity of Cdc28^{sc} via a Rad53^{sc} dependent mechanism.

In addition to the well studied mitotic DNA damage checkpoint, a second, mid-anaphase, checkpoint has been identified [63••]. This Rad9^{sc} dependent checkpoint is triggered by the stretching of dicentric chromosomes as anaphase begins. This suggests that there is independent regulation of anaphase A and B. The mid-anaphase checkpoint may provide a means of dissecting that regulation.

The *S. pombe* S/M replication checkpoint

The S/M replication checkpoint in *S. pombe* is defined as the checkpoint that prevents mitosis if replication is blocked by hydroxyurea (HU) [64]. HU is a competitive inhibitor of ribonucleotide reductase, which blocks replication after initiation by preventing nucleotide synthesis. Unreplicated DNA *per se* is insufficient to activate the S/M checkpoint, as mutants that cannot initiate replication nevertheless undergo mitosis [65]. Thus, to show a replication protein has a direct role in the S/M checkpoint, it is important to show that it is required for the checkpoint under conditions in which it is not required for replication. In *S. pombe*, temperature sensitive mutations in replication proteins such as polymerases and ligase often lead to a checkpoint dependent arrest with close to fully replicated DNA content. This arrest, unlike an HU induced arrest, is dependent on Chk1 and Crb2, and is genetically the same as the G₂ DNA damage checkpoint [33••,66]. While this type of checkpoint arrest is sometimes referred to as a replication checkpoint, it is distinct from the HU induced S/M replication checkpoint. A simple explanation for this type of checkpoint arrest is that the mutant cells complete bulk DNA replication and therefore do not activate the S/M checkpoint, but leave the DNA in an imperfectly replicated state that then triggers the G₂ DNA damage checkpoint.

The primary signal in the S/M checkpoint probably emanates from the replication forks themselves. In *S. pombe*, the DNA polymerase responsible for replication initiation, DNA polymerase α (encoded by *pol1^{sp}*), seems to be one of the proteins involved in sending that signal [67]. Cells with *pol1^{sp} Δ* mutations (derived from germinated spores of *pol1^{sp}/pol1^{sp} Δ* diploids) partially replicate their DNA, probably using residual maternal pol1^{sp} protein, but lack an S/M checkpoint. Thus, DNA polymerase α is required for the S/M checkpoint even after replication is initiated. The role of Cut5^{sp} in the S/M checkpoint is also independent of its requirement for replication [28,29•]. Therefore, Cut5^{sp} may also play a role in generating the S/M checkpoint signal, although this will probably remain unclear until the essential role

of Cut5^{SP} in replication is understood. Unlike *S. cerevisiae*, DNA polymerase ϵ is not required for the *S. pombe* S/M checkpoint [68•].

As in the G₂ DNA damage checkpoint, the S/M checkpoint prevents mitosis by preventing the tyrosine dephosphorylation of Cdc2^{SP} [69•]. This mechanism does not reduce the activity of Cdc2, but rather maintains it at its interphase level [70]. Genetic studies have implicated Cdc25^{SP} as a target of the S/M checkpoint [64]; moreover, as is the case for DNA damage during G₂, HU treatment decreases the rate of Cdc2^{SP} tyrosine dephosphorylation by Cdc25^{SP} [69•]. Since, as described below, Wee1 is also phosphorylated by an S/M checkpoint kinase *in vitro*, the two G₂/M checkpoints have overlapping, and possibly identical, cell cycle targets.

In addition to having the same ultimate target, the S/M checkpoint shares the upstream checkpoint Rad proteins with the G₂ DNA damage checkpoint [13,14]. It is possible that all the checkpoint *rad* genes are required for both G₂/M checkpoints because they sense the same primary signal in both cases, perhaps single stranded DNA. Two observations argue against this idea. First, DNA polymerase α ^{SP} seems to be specifically involved in generating the S/M signal. Second, the *S. cerevisiae* homologs of Rad1^{SP}, Rad9^{SP}, and Rad17^{SP} are not required for the *S. cerevisiae* S/M checkpoint. Alternatively, it has been suggested that the checkpoint Rad proteins form a complex that has separate roles in the two checkpoints, and that although different subunits function in only one or other checkpoint, they all are required for the integrity of the complex [14]. The hypothesis is supported by the fact that there are alleles of *rad1*^{SP} and *rad26*^{SP} that disrupt only the S/M checkpoint [14,71,72•], suggesting that they have separate roles in the two checkpoints.

Although the checkpoint Rad proteins are required for both S/M and G₂ DNA damage checkpoints, they have different downstream effectors for the different checkpoint signals. Specifically, Chk1^{SP} is not phosphorylated in response to HU [31]. While this does not necessarily mean that Chk1^{SP} is not activated by HU, Chk1^{SP} is not normally required for an HU induced arrest [14,30]. Instead, the protein kinase Cds1^{SP} plays the analogous role in the S/M checkpoint [73•,74•,75]. Cds1^{SP} is the putative homolog of Rad53^{SC}, although Cds1^{SP} lacks the second FHA domain that is required for the metaphase DNA checkpoint function of Rad53^{SC} [75]. This observation may explain the fact that while Rad53^{SC} acts in both checkpoints, Cds1^{SP} is specific to the S/M checkpoint. If FHA domains are involved in binding regulatory partners in a phosphorylation dependent manner, one such partner of Cds1^{SP} could be Rad26^{SP}. Rad26^{SP} interacts with Cds1 *in vivo*, although not *in vitro* [74•]. The lack of *in vitro* interaction may indicate an indirect interaction, or the need for Rad26^{SP} phosphorylation. Cds1^{SP} is phosphorylated and activated by HU treatment in a checkpoint Rad dependent manner [73•,74•]. Furthermore, HU treatment causes Cds1^{SP} to bind Wee1^{SP} and the bound Cds1^{SP} can phosphorylate Wee1^{SP} *in vitro* [73•]. The fact that the S/M checkpoint also appears to effect Cdc25^{SP} suggests that Cdc25^{SP} might also be a target of Cds1^{SP} [69•]. Deletion of *cds1*^{SP} does not cause an apparent checkpoint defect because in the absence of Cds1^{SP}, HU treatment leads to Chk1^{SP} phosphorylation and causes cells to arrest in a Chk1 dependent manner [73•,74•]. Thus, the role of Cds1^{SP} in preventing mitosis in response to replication arrest is masked by the ability of Chk1^{SP} to substitute in its absence. In the absence of Chk1^{SP}, however, Cds1^{SP} is required for cells to arrest in response to HU, revealing its true role as a checkpoint kinase.

There are plausible reasons why the checkpoint signals should be split into S phase and G₂ branches, even if they then reconverge on the same checkpoint targets. Cds1^{SP} has roles in HU resistance that are independent of its checkpoint function. In the absence of Cds1^{SP}, or any of the checkpoint Rad proteins, cells rapidly lose viability, independent of the failure to prevent mitosis [13,75]. It is thought that this loss of viability reflects a role for Cds1^{SP}, termed its

recovery function, in stabilizing stalled replication forks. In addition, Cds1^{SP} is required to slow replication when DNA is damaged during S phase [74•,76•]. Clearly, these HU recovery and S phase DNA damage functions of Cds1^{SP} would have no purpose during G₂. Conversely, Chk1^{SP} may have a role in the induction of DNA repair and such induction in every S phase may be detrimental to efficient replication.

Chk1^{SP} is required for a prolonged cell cycle arrest in HU at 37°C, close to the maximum temperature at which *S. pombe* can grow [77]. Given the dependence of the HU arrest on Chk1^{SP} in the absence of Cds1^{SP}, this observation may indicate that the Cds1^{SP} dependent S/M arrest function fails at 37°C.

The *S. cerevisiae* S/M replication checkpoint

The S/M checkpoint signal in *S. cerevisiae* seems to be sent by DNA polymerase ϵ . The role of DNA polymerase ϵ was revealed by mutations that effect the carboxyl terminus of the protein [78]. This region contains a zinc-finger motif dispensable for catalytic activity that may play a role in subunit interactions. In addition, two other proteins, RFC5^{SC} (the small subunit of RFC) and Dpb11^{SC} (the homolog of Cut5^{SP}) are also required for the checkpoint [79,80]. All three of these proteins have essential functions in replication but, in each case, it has been shown that their role in the S/M checkpoint is independent of their role in bulk replication.

As in the metaphase DNA damage checkpoint, Mec1^{SC} and Rad53^{SC} are both required to transduce the S/M signal [12]. Kinetic analysis of the cell cycle response of *MEC1^{SC}* or *RAD53^{SC}* mutant cells has yet to be done, so it is not yet known if these cells retain any checkpoint response. How the S/M checkpoint regulates mitosis is unclear. Pds1^{SC} is not required, but cells arrest with short spindles, indicating that they are blocked at the metaphase to anaphase transition [5,12]. Therefore, there must be a Pds1^{SC} independent mechanism for preventing anaphase. Since destruction of Pds1^{SC} seems to be sufficient for anaphase in the absence of the S/M checkpoint, there may be an active anaphase inhibition induced by the checkpoint that does not play a role in the normal control of mitosis.

Comparison to mammals

As model systems for study of the cell cycle, *S. cerevisiae* and *S. pombe* make a well-matched pair. The combination of *S. cerevisiae*, with its cell cycle controls at Start and metaphase, and *S. pombe* with its cell cycle control at G₂/M, cover all the major cell cycle controls seen in mammalian cells. The same is true of checkpoint control. All of the known mammalian checkpoints are present in one or both of the yeasts and the mechanisms of these checkpoints seem to be conserved. As in the yeasts, a central player in the mammalian checkpoints is a DNA-PK like protein kinase, ATM. ATM was originally cloned as the gene mutated in ataxia telangiectasia, an inherited disease that includes predisposition to cancer [22]. ATM has since been shown to be involved in many mammalian DNA damage checkpoints [24]. In addition to ATM, putative human homologs of various upstream proteins have been identified, including hRad1, hRad9, hRad17, and hHus1 [16•,26•,81,82•]. While the role of these proteins in checkpoint control has yet to be established, hRad1 and hRad17 interact in a two-hybrid assay, hinting that the human checkpoint Rad homologs may function in concert [82•].

The G₂/M cell cycle targets also seem to be conserved between the yeasts and mammals. A good example of this is in the control of the G₂→M transition. As in *S. pombe*, mammalian cells regulate the tyrosine phosphorylation of Cdc2 to prevent entry into mitosis in the presence of damaged or unreplicated DNA [8•,9]. The human homolog of Chk1^{SP} is phosphorylated in response to DNA damage and can bind to and phosphorylate human Cdc25C *in vitro* [42•, 83•]. This phosphorylation has been mapped to Ser216, the major site of Cdc25C interphase phosphorylation *in vivo*, and creates a 14-3-3 binding site [41•,42•]. These results inspired a

model in which DNA damage leads to the phosphorylation of Cdc25C by hChk1 on Ser216. The inactivation or sequestration of Cdc25C by 14-3-3 binding would then arrest cells in G₂ by preventing tyrosine dephosphorylation of Cdc2. This model is supported by the fact that, when expressed in tissue culture, Cdc25C S216A (in the single letter code for amino acids) causes a defect in both the S/M and G₂ DNA damage checkpoint [42••]. These effects are modest, however, and it seems likely that there are other levels of checkpoint regulation of Cdc25, or other mammalian G₂ checkpoint targets. In any case, much of the *S. pombe* G₂ DNA damage checkpoint seems to be conserved in mammalian cells. A metaphase DNA damage checkpoint has not been identified in mammalian cells, but they do have a metaphase spindle checkpoint that appears to be homologous to the *S. cerevisiae* spindle checkpoint [61,84,85]. Furthermore, a metaphase DNA damage checkpoint in mammalian cells could explain why overriding the G₂/M checkpoint leads to some metaphase events but not an authentic mitosis [8••,9].

Conclusion

The wealth of information on the G₂/M checkpoints in *S. cerevisiae* and *S. pombe* offers a perfect opportunity to compare and contrast. A comparison of the two yeasts and of vertebrates reveals conserved mechanisms for initiating and sending checkpoint signals. With some plausible speculation, it is possible to build model of how this mechanism may work. At the center of all checkpoints seems to lie a DNA-PK like kinase [2,24]. These kinases probably associate with different regulatory subunits to recognize the different signals generated by DNA damage, replication arrest and perhaps other, as yet unidentified, signals. In *S. pombe*, we may see a situation where the DNA damage and replication subunits have merged into an interdependent complex. The main function of the DNA-PK like kinase appears to be the activation of one or more downstream kinases that again may associate with regulatory partners. These downstream kinases are then good candidates to have the major signaling function in the checkpoint. At least in the case the Rad53^{sc} and Cds1^{sp}, they seem to have several independent targets. This much of the checkpoint machinery seems to well conserved.

By contrasting the two yeasts, and different checkpoints within the two yeasts, it is clear that conserved checkpoint signals can have diverse targets. The difference between the targets of the G₂/M DNA damage checkpoints is an extreme, but useful example. It is not important how sister chromatid separation is delayed; whatever mechanism is used during a normal cell cycle will make a good checkpoint target. A similar situation is seen for the target of the G₁ DNA damage checkpoint in *S. cerevisiae* and mammalian cells [2,4], and we may expect to find flexibility of targets at other cell cycle transitions. To what extent the differences between the checkpoint targets in the two yeast reflects a general plasticity of checkpoint targets remains to be seen. This example is just one of the many interesting questions to be answered by work in other species, inspired by the groundwork laid in yeast.

Abbreviations

ATM	ataxia telangiectasia mutated
ATR	ATM related
BRCT	BRCA1 carboxy-terminal motif
DNA-PK	DNA-dependent protein kinase
HU	hydroxyurea
RFC	replication factor C

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Figure 1. Models of checkpoint control in (a) *S. pombe* and (b) *S. cerevisiae*. These models of the checkpoint regulatory pathways represent the interpretation of a variety of genetic, molecular and biochemical data. The proximity of protein names to each other does not necessarily imply a physical interaction. (a) The position of Cut5^{SP} in two places reflects an uncertainty about its exact role. (b) The question marks represent an unknown target of the Rad53^{SC}/Dun1^{SC} pathway. It is unknown if this target is independent of the role of Dun1^{SC} in transcriptional induction.

Table 1

Yeast mitotic DNA checkpoint genes.

Checkpoint homologs*			Function/similarity	References
Other yeast		Human		
<i>S. pombe</i>				
Damage and replication				
Rad1 ^{sp}	Rad17 ^{sc}	hRad1 [‡]	Exonuclease	[3,15,16•]
Rad9 ^{sp}	Ddc1 ^{sc}	hRad9 [‡]		[3,81]
Rad17 ^{sp}	Rad24 ^{sc}	hRad17 [‡]	Limited similarity to RFC	[3,17,82•]
Rad26 ^{sp}				[14,72•,74••]
Hus1 ^{sp}		hHus1 [‡]		[13,26••]
Cut5 ^{sp} [†]	Dpb11 ^{sc}		XRCC1 like repeats	[27,28,29•]
Damage				
Chk1 ^{sp}		hChk1	Serine/threonine protein kinase	[30,83••]
Crb2 ^{sp}	Rad9 ^{sc}		BRCT motif	[33••,34]
Rad24 ^{sp}		14–3–3	14–3–3	[43]
Replication				
Pol1 ^{sp}			DNA pol α	[67]
Cds1 ^{sp}	Rad53 ^{sc}		Serine/threonine protein kinase with FHA	[53,73••,74••,75]
Targets				
Wee1 ^{sp}		hWee1 [‡]	Cdc2-Y15 kinase	[36]
Cdc25 ^{sp}		Cdc25C	Cdc2-Y15 phosphatase	[36]
<i>S. cerevisiae</i>				
Damage and replication				
Mec1 ^{sc}	Rad3 ^{sp}	ATM	DNA-PK like protein kinase	[2,52]
Rad53 ^{sc}	Cds1 ^{sp}		Serine/threonine protein kinase with 2 FHA	[48,52,53,57••]
Damage				
Rad9 ^{sc}	Crb2 ^{sp}		BRCT motif	[18,34,44,51•,57••,86]
Rad17 ^{sc}	Rad1 ^{sp}	hRad1 [‡]	Exonuclease	[12,87]
Rad24 ^{sc}	Rad17 ^{sp}	hRad17 [‡]	Limited similarity to RFC	[12,87]
Ddc1 ^{sc}	Rad9 ^{sp}	hRad9 [‡]		
Mec3 ^{sc}				[12,87]
Replication				
Pol2 ^{sc}			DNA pol ε	[78]
Dpb11 ^{sc}	Cut5 ^{sp}		XRCC1 like repeats	[79]
Rfc5 ^{sc}			RFC small subunit	[80]
Targets				
Pds1 ^{sc}			Anaphase inhibitor	[5,58••]

* Homologs that are known not to have a checkpoint function are not listed.

[†] May not be required for all damage checkpoints.

[‡] Not shown to have checkpoint function.