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DNA damage checkpoint in budding yeast

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Eukaryotic cells have evolved a network of control mechanisms, known as checkpoints, which coordinate cell-cycle progression in response to internal and external cues. The yeast *Saccharomyces cerevisiae* has been invaluable in dissecting genetically the DNA damage checkpoint pathway. Recent results on post-translational modifications and protein–protein interactions of some key factors provide new insights into the architecture of checkpoint protein complexes and their order of function.

Keywords: budding yeast/cell cycle/checkpoints/DNA damage/DNA replication

Note: Throughout this review, the *Schizosaccharomyces pombe* genes are indicated with a superscript 'Sp'.

Introduction

Checkpoints are genetically controlled surveillance mechanisms that ensure the interdependency of cell-cycle events (for reviews see Hartwell and Weinert, 1989; Murray, 1992; Elledge, 1996; Paulovich *et al*., 1997; Weinert, 1998). Both intrinsic and extrinsic checkpoints can be envisaged: intrinsic mechanisms act in each cell cycle under unperturbed conditions to ensure the proper temporal order of events, while extrinsic mechanisms are activated only when alterations are detected. The DNA damage checkpoint represents the subset of extrinsic surveillance mechanisms that are triggered in response to DNA insults. The activation of this pathway leads to the induction of a set of genes required for the resolution of the damage (Aboussekhra *et al*., 1996; Kiser and Weinert, 1996) and to a temporary inhibition of cell-cycle progression, in order to prevent replication and segregation of damaged DNA. Failure to respond properly to DNA alterations can lead to increased genomic instability, which is one of the most prominent hallmarks of cancer cells (Hartwell and Kastan, 1994).

The cell cycle is transiently arrested at different stages depending on the phase at which DNA alterations occur (G_1, S and G_2). Three responses have been characterized in budding yeast, which are known as the G_1/S, intra-S and G_2/M DNA damage checkpoints. It has become clear that even though the players might be different, the general mechanism underlaying the DNA damage checkpoint response is the same in the different phases of the cell cycle.

The recognition of DNA damage

The first step in the DNA damage and replication checkpoint pathways is the recognition of particular DNA structures or alterations. One of the crucial questions in this field is to define the signals that activate the checkpoint. In fact, genotoxic agents cause many types of primary lesions that can be converted to secondary lesions during replication of a damaged template. DNA replication across a single-strand nick is likely to cause replication fork collapse and production of a double-strand break, while single-strand gaps can be generated if replication is arrested in front of a covalently modified base, and DNA synthesis resumes downstream of the damage.

It is thus evident that eukaryotic cells must have evolved a complex network of systems that allow them to respond to this variety of DNA perturbations. All of these various lesions could be directly recognized by a number of checkpoint proteins, either alone or in specialized subcomplexes, or they could be processed to a common intermediate that triggers the checkpoint activation. Since the checkpoint response might also be influenced by the stage of the cell cycle at which the damage occurs, multiple sensors probably recognize the signals in specific phases of the cell cycle.

Most of the key players in the checkpoint response in *Saccharomyces cerevisiae* have been identified and have structural and functional equivalents in *Schizosaccharomyces pombe* and in human cells, thus providing an important contribution to the understanding of checkpoint controls in all eukaryotes (Table I).

The emphasis within the field is currently on defining the biochemical properties, and the functional and structural interactions among checkpoint proteins. In budding yeast, the *RAD9*, *RAD17*, *RAD24*, *MEC3* and *DDC1* gene products are specifically required for a proper DNA damage response, and are proposed to act at an early step of damage recognition at any stage of the cell cycle. Conversely, the DNA replication proteins Pol ε and Rfc5 appear to sense both replication blocks and DNA damage during DNA synthesis (Navas *et al*., 1996; Sugimoto *et al*., 1997). Pol ε is a replicative DNA polymerase, while Rfc5 is a subunit of the Replication Factor C (RF-C) complex which, after binding to template-primer junctions, loads the proliferating cell nuclear antigen (PCNA) clamp onto DNA, thereby recruiting DNA polymerases to the site of DNA replication. The RF-C subunits are structurally related to each other and to the *RAD24* gene product. Genetic and biochemical studies indicate functional and physical interactions between RF-C and Rad24 (Lydall

Table I. DNA damage checkpoint genes are evolutionarily conserved

Function	*S.cerevisiae* gene	*S.pombe* gene	Human gene
Protein kinase (?)	*MEC1*	*rad3*	*ATM, ATR*
	RAD9	*rhp9/crb2*	
	MEC3		
	DDC1	*rad9*	*HRAD9*
	RAD17	*rad1*	*HRAD1*
	RAD24	*rad17*	*HRAD17*
Protein kinase	*RAD53*	*cds1*	
DNA polymerase ε	*POL2*	*cdc20*	*POLε*
	DPB11	*cut5*	
Replication Factor C	*RFC5*		*HRFC38*
Replication Factor A	*RFA1*	*rad11*	*HRPA1*
DNA primase	*PRI1*		*PRIM1*
	PDS1	*cut2*	

and Weinert, 1997; Shimomura *et al*., 1998), suggesting that the function of RF-C in the checkpoints may not be restricted to S phase. Based on the role of RF-C in DNA replication, it is tempting to speculate that the RF-C–Rad24 complex may play a role in loading checkpoint or repair proteins onto damaged DNA.

The observation that *RAD17*, *RAD24*, *MEC3* and *DDC1* belong to the same epistasis group, while *RAD9* is in a group on its own (Lydall and Weinert, 1995; Longhese *et al*., 1997), suggests that Rad9 and the Rad24 group of proteins act in different branches of the checkpoint pathway. Indeed, *RAD9* and *RAD24* have different effects on the accumulation of single-strand DNA at telomeres in *cdc13* mutants, indicating that correct balance of their activities is required for proper processing of at least some peculiar type of lesion (Lydall and Weinert, 1996). It has been shown recently that Mec3 and Ddc1 physically interact *in vivo*, and that Rad17 is needed for this interaction (Paciotti *et al*., 1998). A similar complex also exists in *S.pombe*, where Rad1^{sp} (homologous to Rad17) interacts with Hus1^{sp}, and Rad9^{sp} (homologous to Ddc1) is required for complex formation (Kostrub *et al*., 1998). This indicates that the structural organization of at least some checkpoint protein complexes has been conserved during evolution.

Ddc1 is phosphorylated in response to DNA damage, and its modification correlates with DNA damage checkpoint activation. Damage-induced Ddc1 phosphorylation is totally dependent on a functional *MEC1* gene and also partially requires the Rad24 group of proteins (Longhese *et al*., 1997; Paciotti *et al*., 1998). Mec1 is an essential checkpoint factor which has been evolutionarily conserved in eukaryotes. It belongs to the PI-3 kinase family, which includes, among others, Tel1, Rad3^{sp}, mammalian ATM and ATR, and known protein kinases, such as the DNA-dependent protein kinase (DNA-PK) (reviewed in Elledge 1996; Weinert, 1998). Rad3^{sp} has an associated protein kinase activity (Bentley *et al*., 1996) and, although a direct biochemical demonstration is still lacking, Mec1 is also likely to act as a protein kinase.

The finding that Ddc1 phosphorylation depends on Mec1 and on the proteins encoded by genes of the *RAD24* epistasis group suggests that Mec1 may participate, together with Rad24, Rad17, Mec3 and Ddc1, at an early step of the DNA damage recognition process (Figure 1). The observation that Rad9 is not required for Ddc1 phosphorylation supports the notion that Rad9 might act in a different branch of the DNA damage response pathway. However, it has been recently found that Rad9 is phosphorylated after DNA damage and that this modification depends on Mec1, Tel1 and the Rad24 group of proteins (A.Emili, personal communication; N.Lowndes, personal communication; Sun *et al*., 1998). Rad9 phosphorylation appears to be physiologically relevant since it correlates with checkpoint activation, and phosphorylated Rad9 preferentially interacts with Rad53 (Emili, personal communication; Sun *et al*., 1998). Together with the above reported observations, the fact that Rad9 and Ddc1 are required with Mec1 to phosphorylate Rad53 in response to DNA damage (Sanchez *et al*., 1996; Sun *et al*., 1996; Paciotti *et al*., 1998), and that Mec1 is necessary for Rad9 and Ddc1 phosphorylation, suggests that the checkpoint response may be more complex than a simple linear pathway.

Similar to what has been proposed for Rad3^{sp} (Bentley *et al*., 1996; Carr, 1997), we suggest that Mec1 might be able to recognize specific DNA or protein–DNA structures. This function could be influenced by interaction with checkpoint proteins like Rad9 and the Rad24 group, which could also confer a target specificity to Mec1 (Figure 1). The last hypothesis is supported by the finding that Mec1 and Rad9, but not the Rad24 group of proteins, are required for DNA damage-dependent phosphorylation of Pds1, an inhibitor of the metaphase to anaphase transition which is also involved in G_2/M DNA damage checkpoint (Cohen-Fix and Koshland 1997; Paciotti *et al*., 1998).

Intrinsic DNA damage checkpoint

Another interesting aspect is the possible existence of an intrinsic DNA damage signal in a normal cell cycle, in the absence of external cues. Indeed, the replication process by itself can be genotoxic. Replication errors occur stochastically during nucleotide incorporation, and structural intermediates normally arising during unperturbed DNA replication, such as unwound DNA and single-stranded regions, are more fragile than double-stranded DNA organized in a chromatin structure. In addition, single-strand and double-strand breaks are generated by the nicking–closing activity of DNA topoisomerases, which are required to remove torsional stress ahead of the replication forks.

Since the DNA replication process generates DNA structures that may be similar to some of those produced by DNA damage, it is important to define whether the checkpoint can be activated by DNA synthesis *per se*, or whether it becomes activated only when DNA is damaged or replication is altered. It has been found recently that Ddc1 is periodically phosphorylated during unperturbed cell cycles concomitantly with entry and progression through S phase, and that this modification is still dependent on Mec1 and Mec3 (Longhese *et al*., 1997; Paciotti *et al*., 1998). Furthermore, Ddc1 and Mec3 are also physically associated in the absence of DNA damage, which leads us to the hypothesis that a ‘guardian complex’ may constantly monitor the integrity of the genome. From these data we propose the existence of an intrinsic checkpoint signal during unperturbed DNA replication. In S phase the checkpoint response may be in a pre-activated

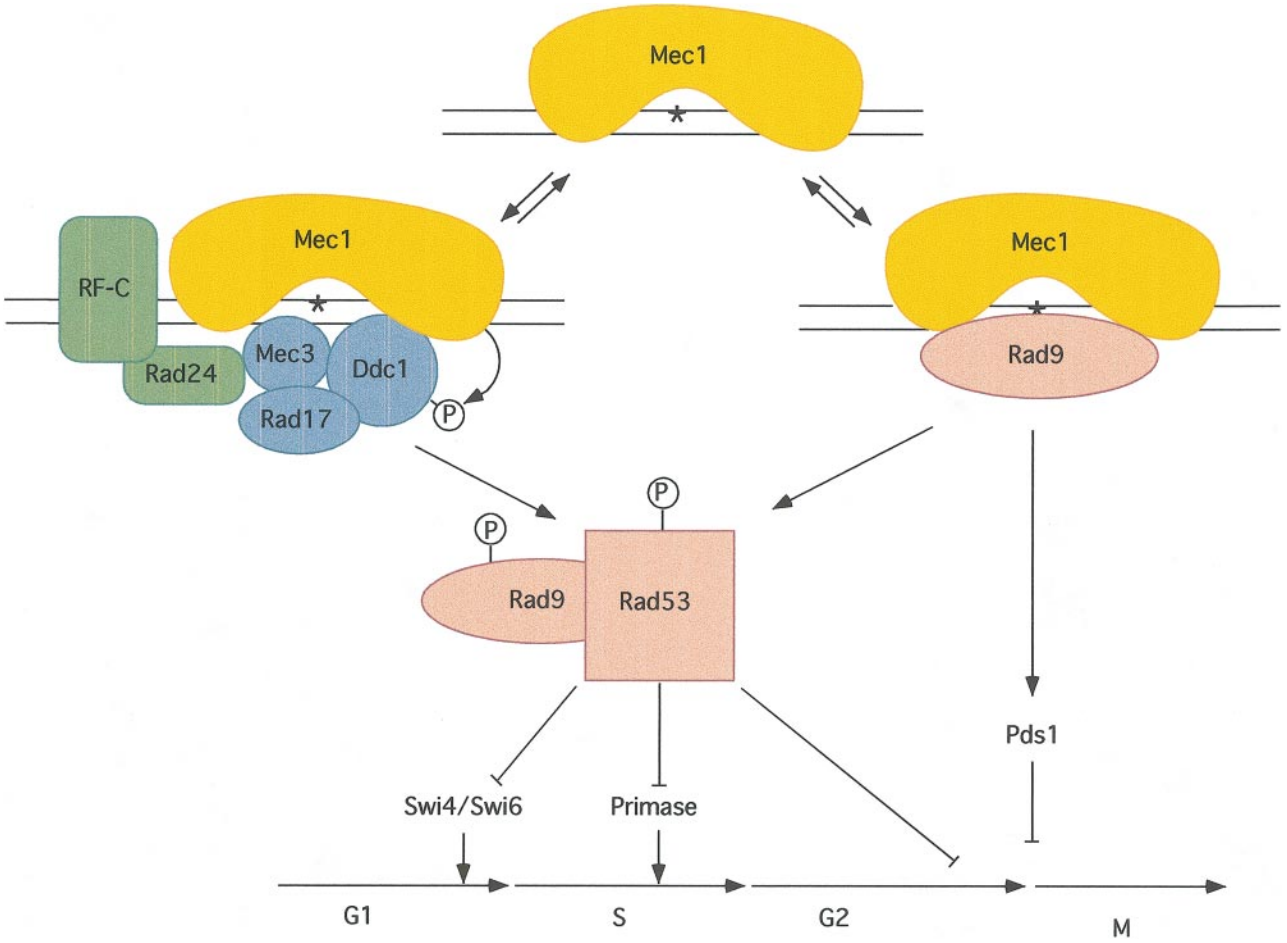

Fig. 1. Model of the DNA damage checkpoint response in budding yeast. Proteins that are known to interact physically are shown in the same colour. All other interactions are speculative. No interaction with DNA has been demonstrated, except for RF-C. * indicates damage to DNA. See text for details.

state, and complete activation, which slows down DNA synthesis, would be prevented since replication intermediates are continuously processed and correctly resolved.

Transduction of the DNA damage signals

Rad53 is an essential protein kinase playing a central role in the DNA damage checkpoint pathway that is phosphorylated and activated in response to DNA alterations sensed by Mec1, Rad9, the Rad24 class and pol ε (Sanchez *et al*., 1996; Sun *et al*., 1996; de la Torre-Ruiz *et al*., 1998). Activated Rad53 may then modulate the activity of target proteins by subsequent phosphorylation events. Rad53 seems to be involved only in a subset of the DNA damage checkpoint pathways controlled by Mec1. In fact, it has been recently found that *pds1 rad53* double mutants are more defective than the single mutants in the G_2/M DNA damage checkpoint, suggesting that parallel pathways may be required to regulate the G_2/M transition independently (R.Gardner, C.Putnam and T.Weinert, personal communication). Mec1 and Rad53 are necessary for phosphorylation of different protein targets in response to checkpoint activation. In fact, Mec1, but not Rad53, is required for Pds1 phosphorylation in G_2 (Cohen-Fix and Koshland, 1997), and for DNA damage-induced phosphorylation of the 34 kDa subunit of RP-A, a single-stranded DNA-binding complex essential for DNA replication (Brush *et al*., 1996). Conversely, Rad53 is required, together with Mec1, to activate the Dun1 kinase activity, leading to transcriptional induction of a number of repair genes (Elledge, 1996). Rad53 is also needed to phosphorylate the transcriptional regulator Swi6, inhibiting the activity of the Swi6–Swi4 complex that is required for expression of G_1 cyclins (Sidorova and Breeden, 1997). This Rad53-dependent inhibition of *CLN1-2* transcription may be one of the mechanisms required for delaying bud emergence and entry into S phase after DNA damage in G_1 (Figure 1).

Also, the rate of DNA replication is slowed down when yeast cells, progressing synchronously through a single cell cycle, are chronically damaged by methyl methan sulfonate (MMS) treatment. The finding that many checkpoint mutants replicate damaged or undamaged DNA at comparable kinetics led to the discovery of the intra-S checkpoint, and ruled out the possibility that DNA lesions alone are responsible for slowing down the replication machinery (Paulovich and Hartwell, 1995).

Components of the replication apparatus can act either as sensors or targets of the intra-S DNA damage checkpoint. Indeed, mutations affecting Pol ε, DNA primase, the large subunit of RP-A and the Rfc5 subunit of RF-C alter the cellular response to DNA perturbations during S phase (Longhese *et al*., 1996; Navas *et al*., 1996; Marini *et al*.,

1997; Sugimoto *et al*., 1997). Since Rfc5 is required for Rad53 phosphorylation, it probably acts early on in the DNA damage recognition process. Conversely, DNA primase acts downstream of Rad53, which suggests that this enzyme can be one of the final targets of the checkpoint pathway which couples DNA replication to the DNA damage response. This assumption is consistent with the biochemical properties of DNA primase, whose priming activity is required to bypass a DNA lesion. Rad53 might inhibit DNA primase in order to prevent re-initiation of DNA synthesis downstream of the damage (Figure 1).

Although most of the molecular details of the DNA damage checkpoint mechanisms are still unknown, some of the recent results discussed here provide new insights into the architecture of checkpoint protein complexes and their post-translational modifications. Moreover, experimentally testable models of the interconnections between cell cycle, DNA repair and DNA replication are beginning to emerge. Finally, most of the checkpoint mechanisms that have been identified using budding yeast as a model organism have been conserved throughout evolution and can potentially be exploited to search for therapeutic agents with increased selectivity for cancer cells.

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