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Perspectives on the DNA damage and replication checkpoint responses in *Saccharomyces cerevisiae*

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ABSTRACT

The DNA damage and replication checkpoints are believed to primarily slow the progression of the cell cycle to allow DNA repair to occur. Here we summarize known aspects of the *Saccharomyces cerevisiae* checkpoints including how these responses are integrated into downstream effects on the cell cycle, chromatin, DNA repair, and cytoplasmic targets. Analysis of the transcriptional response demonstrates that it is far more complex and less relevant to the repair of DNA damage than the bacterial SOS response. We also address more speculative questions regarding potential roles of the checkpoint during the normal S-phase and how current evidence hints at a checkpoint activation mechanism mediated by positive feedback that amplifies initial damage signals above a minimum threshold.

1. Introduction

The concept of DNA damage checkpoints was first developed through the identification of G_2/M arrest after X-ray irradiation in the budding yeast *Saccharomyces cerevisiae*. This arrest required the *RAD9* gene, and the sensitivity of *rad9* mutants was reduced by delaying the onset of mitosis after irradiation [1]. This led to the view that *RAD9* and similar genes define DNA damage checkpoints, which delay specific cell cycle transitions in response to DNA damage to provide time for DNA repair to occur. In *S. cerevisiae*, DNA damage checkpoints delay the G_1/S transition and block the G_2/M transition of the cell cycle [1,2]. In addition, two types of S-phase checkpoints have been defined: the DNA replication checkpoint, which arrests cell cycle progression and inhibits firing of late replication origins in response to replication stress [3], and the intra-S checkpoint, which slows DNA replication and cell cycle progression in response to DNA damage [4]. The different DNA damage checkpoints share many components and are now known to target many aspects of cellular metabolism besides cell cycle transitions. These checkpoints also likely respond to endogenous sources of DNA damage as well as exogenous sources, as checkpoint defects result in increased spontaneous genome instability [5].

A "central dogma" for the DNA damage cell cycle checkpoints has been commonly presented:

damagesignals → damagesensors → signaltransducers → effectors

In this scheme, damage is sensed by sensors, and this information is communicated through signal transducers to effectors that mediate the physiological response of the cell to the damage, including arresting or slowing the cell cycle and activating or repressing other pathways required for the eventual recovery of the cell. It has been convenient to think of checkpoints as unidirectional pathways, but this is an oversimplification. DNA repair proteins, for example, can act as both sensors and effectors. Similarly, checkpoint proteins that are components of replication complexes are both sensors and transducers and might even be effectors. This complexity suggests checkpoint responses likely involve complex regulatory networks that incorporate both feedback loops and threshold responses.

Here, we will summarize the core checkpoint machinery in *S. cerevisiae* to serve as a framework for examining some key features of checkpoint responses in greater detail. We will consider how checkpoints are activated and what is known about the effectors that are targeted by checkpoint activation. We will also consider the transcriptional response to DNA damage by reviewing which aspects of cell metabolism are transcriptionally regulated and how much of this actually represents a checkpoint response. Finally, we will also address more speculative questions regarding the potential roles of the DNA damage checkpoint during normal regulation and the use of positive feedback and threshold responses by checkpoint functions. We are particularly interested in how these checkpoint

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responses prevent genome instability. However, our goal is not to provide a comprehensive review of checkpoints but rather to highlight areas that are presently less well understood.

2. The central checkpoint pathway in *S. cerevisiae*

The key components of the common signaling pathway are several phosphoinositol-3-kinase-related kinase (PIKK) family members. *S. cerevisiae* has two PIKK proteins, Mec1, the homolog of human ataxia-telangiectasia and Rad3-related (ATR), and Tel1, the homolog of human ataxia telangiectasia mutated (ATM), that function as both damage sensors and signal transducers, but lacks a homolog of the DNA-dependent protein kinase, DNA-PKcs (Table 1). Loading of Mec1 and Tel1 onto damaged DNA does not appear to be mediated by direct recognition of DNA damage but rather by the recognition of complexes recognizing DNA damage and intermediates generated by the activities of DNA repair processes. Mec1 binds to Ddc2, the homolog of the human ATR interacting protein (ATRIP), which recognizes single-stranded DNA (ssDNA) bound by replication protein A (RPA) [6], and Tel1 binds to the DNA end-binding Mre11–Rad50–Xrs2 complex [7]. Genetically *MEC1* and *TEL1* are partially redundant; the *mec1Δ tel1Δ* double mutant cannot maintain telomeres via telomerase unlike the single mutants [8,9], has a synergistically increased sensitivity to DNA damaging agents [10], and has a synergistically increased rate of spontaneous genome rearrangement relative to the respective single mutants [5]. Both kinases preferentially phosphorylate serines and threonines preceding a glutamine residue on numerous target proteins in response to damage. For example, phosphorylation of histone H2A at Ser129 (formation of γ-H2AX) by Mec1 and Tel1 has important roles for the checkpoint response detailed below [11]. The identity of all Mec1 and Tel1 targets and phosphorylation sites is certainly not known; however, improved proteomics approaches have led to considerable progress in identifying these targets. Despite their similarities, Mec1 and Tel1 are not completely redundant. Tel1 is more important for maintaining normal telomere lengths than Mec1 [8,12]. Tel1 is more important in γ-H2AX formation at sites of DSBs in G_1, and Mec1 is more important during S and G_2 [13]. These cell cycle-dependent differences may be due to the yeast cyclin-dependent kinase (CDK) Cdk1 (also known as Cdc28) activating resection from DNA breaks via the nuclease Sae2 and thereby generating damage recognized by Mec1–Ddc2 [13,14].

Activation of PIKK family members is also influenced by the action of other damage sensors. The PCNA-like Ddc1–Mec3–Rad17 complex (the *S. cerevisiae* homologs of the Rad9–Hus1–Rad1 or 9–1–1 complex) is loaded onto partial duplex DNA via the Rad24–Rfc2-5 alternative replication factor C (RFC) complex independently of Mec1–Ddc2 [15,16]. Colocalization of Mec1–Ddc2–RPA and the 9–1–1 complex in the context of partial duplex DNA or to chromosomal arrays of Lac operator sequences in the absence of DNA damage results in Mec1 activation [17,18], indicating that DNA plays a passive scaffolding role in checkpoint activation. These results make the lack of checkpoint activation from normal telomeres that bind numerous DNA repair and DNA damage checkpoint proteins even more surprising [19]. Activation of Mec1 is also mediated by Dpb11, the *S. cerevisiae* homolog of TopBP1, and Dpb11 is synergistic with the 9–1–1 complex [20,21]. The combination of the 9–1–1 complex and Dpb11 in activation of Mec1 is highly conserved, and differs from *fission yeast*, *Xenopus*, and human systems only in that the 9–1–1 complex in those organisms cannot activate the Mec1 homolog in the absence of the Dpb11 homolog [22–24].

In addition to phosphorylating effectors of the checkpoint response (described below), the PIKK proteins also activate downstream kinases, including Chk1 and Rad53 (the *S. cerevisiae* homolog of mammalian Chk2), which presumably diffuse away from the site of their activation. In other organisms, Chk1 homologs are required to inhibit CDKs to prevent cell cycle progression in the presence of DNA damage; however, budding yeast lacks this requirement [25,26]. Budding yeast Chk1 does, however, have direct roles in suppressing the cell cycle in the context of DNA damage as described below. Phosphorylation of the Rad53 protein kinase by Mec1 and Tel1 leads to its activation and subsequent autophosphorylation; the resulting hyperphosphorylated Rad53 is frequently used as an experimental surrogate for monitoring activation of the DNA damage response. At least some inactive, hypophosphorylated Rad53, but not active hyperphosphorylated Rad53, is bound to the chromatin assembly factor Asf1 [27–29]. Rad53 also inhibits Asf1-mediated chromatin deposition *in vitro*. Despite the binding of inactive Rad53 by Asf1, deletion of *ASF1* does not give rise to a checkpoint response [27], but rather causes defects in checkpoint shut off due to its roles in chromatin assembly and modification [30].

Activation of Rad53 also depends upon Mec1 or Tel1 phosphorylating a scaffolding protein, Rad9 or Mrc1, which then binds the forkhead-associated (FHA) domains of Rad53; simultaneous deletion of both *RAD9* and *MRC1* extensively phenocopies a deletion of *RAD53* [31], and mutagenesis of Mec1 and Tel1 consensus phosphorylation sites in Rad9 and Mrc1 prevent Rad53 activation [32,33]. A Ddc2–Rad53 fusion construct alleviates many of the defects of a *rad9Δ mrc1Δ* double mutation [34], and a similar construct suppresses *mrc1Δ* defects in fission yeast [35]. Mrc1 is a component of the replication fork, has no identifiable protein domains or motifs, and seems to specifically signal replication stress [31,33,36]. Rad9, on the other hand, is more important for other types of DNA damage, although it is required for Rad53 hyperphosphorylation late in S-phase in *mrc1Δ* strains [33], which could indicate that DNA repair processes convert replication stress into damage recognized by Rad9. Rad9 contains tandem BRCT domains that bind phosphorylated residues, mediate dimerization after DNA damage-induced phosphorylation [37], and direct binding to phosphorylated histone H2A [38]. Rad9 also contains tandem Tudor domains, the latter of which recognize methylated histones [39]. In the G_1/S and intra-S checkpoints, phosphorylation of Rad9 is dependent upon methylation of histone H3 at Lys79 by Dot1 or Lys4 by Set1, which in turn is dependent upon ubiquitination of histone H2B at Lys123 by Rad6 and the Bre1/Lge1 complex [40,41]. Strains with deletion of *RAD6*, *BRE1*, both *DOT1* and *SET1*, or strains encoding the histone H2B Lys123Arg variant have defects in the G_1/S and the intra-S checkpoint after UV treatment and fail to hyperphosphorylate Rad53 [40,41]. Surprisingly, deletion of *BRE1* or *DOT1* causes only minor defects in the G_2/M checkpoint in response to a DSB,

Table 1
Homologs of the components of the central kinase cascade.

Class	*S. cerevisiae*	*S. pombe*	*H. sapiens*
Sensors	*MEC1*	*RAD3*	*ATR*
	DDC2	*RAD26*	*ATRIP*
	RAD24	*RAD17*	*RAD17*
	DDC1	*RAD9*	*RAD9*
	MEC3	*HUS1*	*HUS1*
	RAD17	*RAD1*	*RAD1*
	DPB11	*CUT5/RAD4*	*TOPBP1*
	TEL1	*TEL1*	*ATM*
	MRE11	*MRE11*	*MRE11*
	RAD50	*RAD50*	*RAD50*
	XRS2	*NBS1*	*NBS1*
	–	–	*DNA-PKcs*
Adaptors	*RAD9*	*CRB2*	*53BP1*
	MRC1	*MRC1*	*CLSPN*
Effector kinases	*CHK1*	*CHK1*	*CHK1*
	RAD53	*CDS1*	*CHK2*