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Clamping the Mec1/ATR Checkpoint Kinase into Action

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ABSTRACT

The yeast checkpoint protein kinase Mec1, the ortholog of human ATR, is the essential upstream regulator of the cell cycle checkpoint in response to DNA damage and to stalling of DNA replication forks. The activity of Mec1/ATR is not directly regulated by the DNA substrates that signal checkpoint activation. Rather the signal appears to be transduced to Mec1 by factors that interact with the signaling DNA substrates. One of these factors, the DNA damage checkpoint clamp Rad17-Mec3-Ddc1 (human 9-1-1) is loaded onto gapped DNA resulting from the partial repair of DNA damage, and the Ddc1 subunit of this complex activates Mec1. In vertebrate cells, the TopBP1 protein (Cut5 in S. pombe and Dpb11 in S. cerevisiae) that is also required for establishment of the replication fork, functions during replication fork dysfunction to activate ATR. Both mechanisms of activation generally upregulate the kinase activity towards all downstream targets.

INTRODUCTION

DNA damage resulting from internal or external insult constantly challenges cellular genome integrity. Analogous challenges are presented during DNA replication because of the presence of structural blocks or potential replisome dysfunction. Many DNA repair mechanisms exist to overcome these challenges and repair the damage. In addition, eukaryotic cells have several checkpoints that ensure an arrest of the cell cycle in order to provide an appropriate time-frame for DNA repair or for the completion of genome duplication.1 Thus, the G1/S checkpoint and G2/M checkpoint ensure the intactness of the genome prior to proceeding with DNA replication and mitosis, respectively. Stalled replication forks activate the replication checkpoint. Determining the identity and activities of checkpoint factors that function in these pathways has been an area of intense investigation in the last two decades. Many checkpoint factors function in multiple checkpoint pathways, and partial redundancy of structurally related factors for a given pathway is not an uncommon occurrence. Here, we will focus on just two of these factors that have the capacity to activate a phosphorylation cascade: the yeast checkpoint clamp Rad17/3/1, the replication and checkpoint protein TopBP1 (Cut5 in S. pombe and Dpb11 in S. cerevisiae).2,3 For a complete description of checkpoint mechanisms, the reader is referred to recent reviews (refs. 4–6).

The S. cerevisiae protein kinase Mec1 and its human ortholog ATR belong to the PIKK family of protein kinases. The founding member of this family is ATM, for ataxia telangiectasia mutated. Mutations in ATM lead to cancer predisposition and show a defect in autophagy. Mec1-ATR is also activated in response to double-stranded DNA breaks and is involved in HR (homologous recombination) repair.4,5 Mec1-ATR activity is required for DNA damage response and genome integrity.6

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appropriate. Could it be that RPA or the ss-DNA-RPA complex activates Mec1? Although phosphorylation of the Rpa2 subunit of RPA by Mec1 or by ATR is enhanced when RPA is bound to ssDNA, this does not appear to represent the sought after kinase activation step. Phosphorylation of DNA-bound RPA is still inefficient, and, more importantly, phosphorylation of other downstream targets is not enhanced by RPA-ssDNA. Possibly, binding of RPA to ssDNA induces a conformational change in Rpa2 that makes this subunit more accessible to the low state kinase activity of Mec1/ATR. Among the many downstream targets of Mec1/ATR is the yeast effector kinase Rad53, Chk1 and/or Chk2 in human, that mediates the global cellular responses ultimately resulting in cell cycle arrest, gene activation, increased DNA repair, and apoptosis. Last year, two activators of the transducer kinase Mec1/ATR were identified. These are the DNA damage checkpoint clamp Rad17/3/1 in yeast, and the essential replication initiation and checkpoint protein TopBP1 in the Xenopus system. We will review these activating systems in more detail and then draw comparisons between them.

DNA DAMAGE CHECKPOINT IN YEAST

The DNA damage checkpoint is most simply understood in the G1 phase of the cell cycle when the response is not complicated by damage at replication forks or issues relating to sister chromatid cohesion and chromosome segregation. The initiating steps of this checkpoint, as measured by phosphorylation of the effector kinase Rad53, minimally require Mec1-Ddc2, RPA, the checkpoint clamp Rad17-Mec3-Ddc1 (h9-1-1), the clamp loader Rad24-RFC (hRad17), an unknown nuclease, and the mediator Rad9. The latter is required for enhanced autophosphorylation of Rad53 and will not be considered further here. Dark repair of UV damage occurring during G1 is almost exclusively accomplished by nucleotide excision repair (NER). The process of NER leads to a bimodal incision of the damaged strand exclusively accomplished by nucleotide excision repair (NER). The results indicate that an intermediate in NER forms the signal for this checkpoint, as measured by phosphorylation of the effector kinase Rad53, Chk1 and/or Chk2 in human, that mediates the global cellular responses ultimately resulting in cell cycle arrest, gene activation, increased DNA repair, and apoptosis. Last year, two activators of the transducer kinase Mec1/ATR were identified. These are the DNA damage checkpoint clamp Rad17/3/1 in yeast, and the essential replication initiation and checkpoint protein TopBP1 in the Xenopus system. (Fig. 1). We will review these activating systems in more detail and then draw comparisons between them.

DNA REPLICAITION CHECKPOINT IN XENOPUS EXTRACTS

Previous studies in S. cerevisiae and in S. pombe had indicated a specialized role for Dpb11 and Cut5 (Rad4), respectively, in the initiation of DNA replication and in the replication checkpoint. Subsequently, similar roles were assigned to the mammalian homolog TopBP1. Whether Dpb11/Cut5/TopBP1 also functions during the elongation phase of DNA replication is still a matter of debate (discussed in ref. 20). The dual role for Dpb11/Cut5/TopBP1 in DNA replication and the replication checkpoint might suggest that this protein could actually play an early role in the checkpoint as a damage sensor. Checkpoint activation studies in S. pombe have placed
Dpb11/Cut5/TopBP1 downstream of ATR, perhaps as a mediator. However, these studies did not exclude an additional early function for this protein (reviewed in ref. 20).

The great advantages of the *Xenopus* extract system are that it allows for the study of DNA replication mechanisms and preserves the ability to respond to aberrant DNA structures to activate checkpoints. For instance, inclusion of aphidicolin, a DNA polymerase inhibitor, in the replication assay invokes the replication checkpoint that activates ATR, which in turn phosphorylates and activates the Chk1 effector kinase. *Xenopus* TopBP1 is required for establishing the replication checkpoint. Kumagai et al. tested TopBP1 as a plausible activator of ATR in the *Xenopus* system. Indeed, they showed that TopBP1 directly activates ATR kinase activity. For this purpose, they immunopurified xATR from uninduced egg extracts, and incubated it with recombinant TopBP1 and a phosphorylation target. Activated ATR showed greatly increased rates of phosphorylation of physiologically relevant substrates such as Chk1 and Mcm2, but also increased phosphorylation of the non-specific substrate PHAS-I. Surprisingly, this activation did not require the presence of any DNA substrate or RPA. How that requirement is bypassed in the purified kinase assay still needs to be determined.

TopBP1 contains multiple BRCT (BRCA1 C-terminal) domains that are known to mediate protein-protein interactions and function in the DNA damage response and DNA repair. These BRCT domains were not responsible for ATR activation. Rather, a ~300 amino acid domain situated between two BRCT motifs, and conserved in vertebrate cells, was sufficient to activate ATR. However, the function of this isolated small domain is misregulated, because gratuitous phosphorylation of Chk1 was observed in the absence of inducer when the domain was introduced into *Xenopus* extracts or over-produced in mammalian cell lines. Interestingly, a mutant form of TopBP1 with a mutation in the activating domain (W1138R) fully supported replication fork establishment when the mutant protein was added to a TopBP1-depleted extract, but failed to restore the checkpoint function of the depleted extract. These results indicate that the replicative and checkpoint functions of TopBP1 are specified in separable domains.

**LESSONS FROM DIVERSE ORGANISMS**

Do the two ATR-activating systems have common characteristics? Do they represent two parallel branches of checkpoint activation that are preserved in both organisms, or do they indicate fundamentally different pathways that have diverged from yeasts to vertebrates? In vitro, activation of yeast Mec1 by Ddc1 and of *Xenopus* ATR by TopBP1 appears to proceed similarly: the kinase activity towards all targets investigated is greatly enhanced. Both Ddc1-activated Mec1 and TopBP1-activated ATR show increased activity towards physiological targets such as Rad53/Chk1, and towards the non-specific kinase substrate PHAS-I. This suggests that the mechanism of activation is unlikely to be one in which the activator protein functions as an adaptor between the kinase and the substrate. The exceptions to this rule are Ddc2 and ATRIP, the regulatory subunits of Mec1.

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**Figure 1. Two distinct pathways to activate Mec1/ATR.** Left, activation by the checkpoint clamp in response to DNA damage; right, activation by TopBP1 in response to stalling of replication forks. The *S. cerevisiae* (sc) and human (h) proteins are shown.
and ATR, respectively. Their phosphorylation is not enhanced upon Mec1/ATR activation in vitro,2,24 nor does phosphorylation of Ddc2 or its S. pombe ortholog Rad26 require an intact clamp or Dpb11/Cut5 in vivo.25,26

This all or none activation of Mec1/ATR suggests that to a first approximation the same downstream targets are phosphorylated regardless of the method of activation, i.e., through the clamp or through Dpb11/Cut5/TopBP1. Differentiation between the two pathways would then mainly come about through temporal and spatial positioning of the target proteins. Further complexity is brought about by the action of the other damage transducing kinase Tel1/ATR.

The strong structural and functional conservation of the checkpoint clamp in eukaryotes strongly suggests that the activation mechanism uncovered for yeast also applies to vertebrate organisms. Failure to observe activation of mammalian ATR in vitro by incubation of the kinase with the h9-1-1 clamp or with hRad9, the ortholog of Ddc1, could easily reflect an absolute requirement that the clamp be loaded onto effector DNA in order to interact with and stimulate ATR. To our knowledge, these latter types of studies with purified mammalian factors have not yet been carried out.

As discussed above, Dpb11/Cut5/TopBP1 is a conserved replication and checkpoint protein in eukaryotes. However, the ATR-activating domain identified in Xenopus TopBP1 is conserved only in vertebrates, and cannot be found in yeasts, fly or worm.20 Possibly, another domain in Dpb11/Cut5 fulfills this function, or it is performed by an associated protein. Or is it possible that this type of activation does not exist in lower eukaryotes? In S. pombe, both the clamp and Cut5 are essential factors for the S-phase checkpoint suggesting the existence of a single checkpoint pathway in which both factors participate.27 However, genetic studies in S. cerevisiae point to the existence of two parallel and partially overlapping S-phase checkpoint pathways, one with Dpb11 and one with the clamp.28 In this organism, the existence of two separate activators of Mec1 does seem plausible. The roles of the clamp and of Dpb11/Cut5/TopBP1 in the S phase checkpoint may extend beyond that of the activation of ATR. Phosphorylated clamp subunit Ddc1/Rad9 interacts with Dpb11/Cut4,28,29 This complex may function during normal S phase progression to sense stalling of the DNA replication fork.

References


