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PERSPECTIVE

Mck1 kinase is a new player in the DNA damage checkpoint pathway

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The DNA damage checkpoint is a signal transduction cascade with three layers of kinases. Checkpoint kinases are conventionally defined as kinases that are activated by DNA damage—either directly or by upstream kinases—and phosphorylate targets that preserve genome stability. In the presence of damaged DNA, the sensor kinases (ATM/ATR in mammals and Tel1/Mec1 in budding yeast) become active and phosphorylate the effector kinases (CHK1 and CHK2 in mammals and Chk1 and Rad53 in budding yeast [1]). Rad53, in turn, activates another checkpoint kinase, Dun1 [2]. This hierarchy is likely to provide both amplification and specialization, as the substrates of each set of kinases are selectively enriched for proteins in particular areas of biology. ATM and ATR are localized to DNA breaks, and their substrates are enriched for chromatin components and repair proteins that are similarly localized (e.g., H2AX and Slx4) [3–7]. While Rad53, Chk1, and their homologues target some proteins in this category, they primarily act on a large number of substrates that are not directly adjacent to sites of DNA damage, including cell cycle regulators, such as Sld3 [8, 9] and Pds1 [10]. By contrast, Dun1's only known substrates are involved in the regulation of ribonucleotide levels [11]. Here, Liu and colleagues [12] show that the GSK3-related kinase Mck1 is directly activated by Rad53 and, like Dun1, regulates ribonucleotide biosynthesis, suggesting it too is a checkpoint kinase.

All eukaryotic organisms require an adequate concentration of deoxyribonucleoside triphosphates (dNTPs) in order to ensure accurate DNA replication and repair and to maintain genomic stability. The rate-limiting step in dNTP synthesis is catalyzed by ribonucleotide reductase (RNR), an essential heterotetrameric enzyme that mediates the reduction of ribonucleotides (rNTPs) into deoxyribonucleotides (dNTPs). In the budding yeast *Saccharomyces cerevisiae*, the large R1 subunit is composed of an Rnr1 homodimer (or Rnr1-Rnr3 heterodimer), whereas the active small R2 subunit is formed by an Rnr2-Rnr4 heterodimer [13]. The activity of RNR is tightly regulated by the cell cycle and environmental cues, which is critical since an unbalanced supply of dNTPs dramatically increases the mutation rate. Once Dun1 becomes activated, it enhances RNR activity by multiple mechanisms. First, in response to DNA damage and replication stress, Dun1 phosphorylates the Crt1 repressor. This causes it to be lost from *RNR* promoters, leading to an increase in transcription of *RNR2*, *RNR3*, and *RNR4*. However, induction of *RNR* genes upon genotoxic stress is not completely dependent upon the Dun1 kinase. In *dun1Δ* mutants, *RNR* genes continue to be significantly induced in response to DNA damage [14]. *DUN1*-independent *RNR1* induction upon DNA damage is also Crt1-independent. This is mediated by Rad53 activation of Ixr1, a DNA-binding protein that interacts with the *RNR1* promoter and activates *RNR1* transcription [15]. In addition, the yeast Rnr1 inhibitor Sml1 undergoes a *DUN1*-dependent phosphorylation that leads to Sml1

degradation [16–18]. A third mechanism regulates the subcellular distribution of the RNR subunits in yeast. Under normal conditions, the large subunit R1 is predominantly localized to the cytoplasm, whereas the small subunit R2 localizes to the nucleus, except during S phase [19]. The nuclear localization of the small subunit R2 is achieved by a dual mechanism. Under normal growth conditions, the nuclear WD40 protein Wtm1 binds to Rnr2-Rnr4 and anchors the complex to the nucleus, limiting its export, whereas Dif1 facilitates the nuclear import of the Rnr2-Rnr4 heterodimer by directly interacting with the complex. Dun1 disrupts the association between Rnr2-Rnr4 and Wtm1 in the nucleus, leading to release of Rnr2-Rnr4 into the cytoplasm, where it presumably assembles with the large subunit R1, resulting in an active RNR complex. Simultaneously, Dif1 is phosphorylated by Dun1 and degraded, thereby diminishing nuclear import [20, 21].

In this study, Liu and colleagues [12] show that the highly conserved GSK3-related Mck1 kinase is a downstream target of Rad53 and functions in the Dun1-independent RNR activation pathway. The authors suggest that Mck1 and Dun1 kinases cooperate in a nonredundant manner to provide cells with a multilayer response system to deal with various degrees of replicative stress. Using a synthetic genetic screen, Liu and colleagues [12] find that deletion of *MCK1* and *DUN1* (but not other *GSK3* paralogs) displays a synergistic sensitivity to replication stress, reminiscent of *mec1Δ* or *rad53Δ*. They show that, like Dun1, Mck1 is phosphorylated by Rad53, and genetic experiments suggest that this phosphorylation is activating. Mck1 appears to act on both *CRT1* itself and a *CRT1*-independent pathway. The authors demonstrated that Crt1 phosphorylation is significantly compromised in an *MCK1* deletion, accompanied with dissociation of Crt1 from the *RNR* promoter, resulting in induction of RNR genes. This phosphorylation is only partially redundant with Crt1 phosphorylation by Dun1. In addition, the authors demonstrate that Mck1 represses the transcription of *HUG1* in a Crt1-independent way. This observation is reminiscent of previous work showing that Hug1 acts to fine-tune RNR activity [22]. According to the authors' model, when higher levels of RNR activity are required after cells suffer a more severe condition, Mck1 will inhibit the induction of *HUG1* in a Crt1-independent manner (Fig 1).

Liu and colleagues' discovery that Mck1 is directly activated by Rad53 is particularly interesting given previous connections between Mck1 and the DNA damage response. In response to DNA damage, Mck1 phosphorylates the PKA regulatory subunit Bcy1, restraining anaphase [23]. Furthermore, Mck1 and Rad53 activities are required to promote Hst3 turnover by the ubiquitin ligase SCF^{Cdc4} to maintain genome stability in response to replication stress [24]. Interestingly, Mck1 also seems to be important to ensure proper DNA replication, prevent DNA damage, and maintain genome integrity by promoting Cdc6 degradation after DNA damage [25]. While each of the above mentioned studies suggested that Mck1 activity was important for the response to DNA damage, they did not show that it was directly activated by DNA damage, thus fulfilling the definition of a checkpoint kinase. Several other yeast kinases, such as casein kinase—and even cyclin-dependent kinase—clearly phosphorylate proteins important for the damage response; however, they are not directly activated by the checkpoint pathway [26–29]. Thus, unlike Mck1, they may be important for the damage response but are not strictly DNA damage checkpoint kinases.

Like Mck1, several mammalian kinases with previously characterized roles in other pathways also appear to moonlight in the DNA damage response. Vertebrate GSK-3 phosphorylates the oncogenic transcription factor c-Myc after ultraviolet light, targeting it for ubiquitination by SCF^{Fbw7} [30, 31]. In addition, several vertebrate MAP kinases (MAPKs) have links with the damage response. There are three subgroups of mammalian MAPKs: extracellular signal regulated kinases (ERKs), stress-activated protein kinase/jun N-terminal kinase (SAPK/JNK), and p38 MAPKs. Both MK2 and JNK can be activated in an ATM-dependent

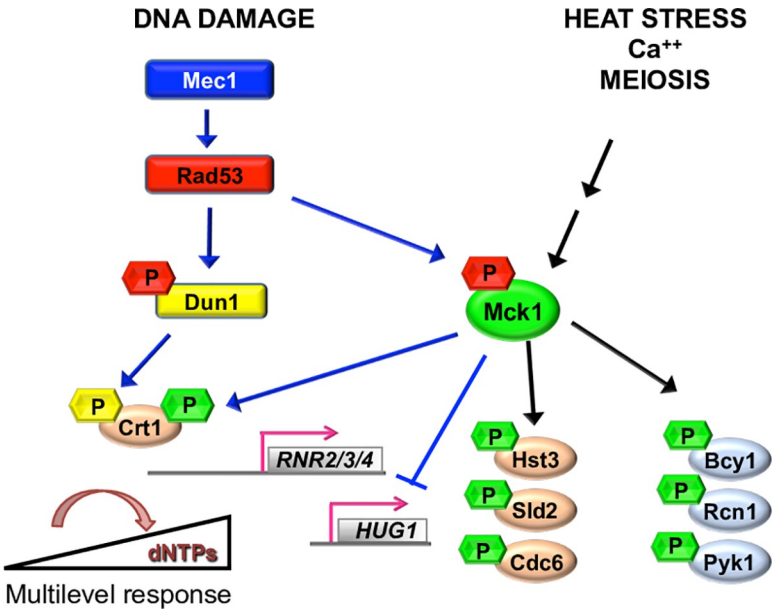

Fig 1. Multiple roles of Mck1 in response to stress. Upon DNA damage, Mck1, together with Dun1, antagonizes the repressor function of Crt1 via phosphorylation, which allows the derepression of *RNR2/3/4* transcription. Meanwhile, Mck1 inhibits the expression of the RNR inhibitor Hug1 in a Crt1-independent manner. This mechanism allows the cell to maintain appropriate dNTPs levels according to the degree of stress. Yeast Mck1 has been shown to phosphorylate the cell cycle regulators Cdc6, Hst3, and Sld2, the calcineurin regulator Rcn1 to stimulate calcineurin signaling, Pyruvate Kinase 1 (Pyk1), and the PKA regulatory subunit Bcy1 and to have roles in meiosis, sporulation, and heat stress resistance.

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manner in response to particular genotoxic stresses [32–35]. These kinase pathways are involved in the cellular response to environmental stresses but can also be modulated during apoptosis, transformation, development, immune activation, and inflammation in an ATM-independent manner. Although these kinases are not entirely dedicated to the DNA damage response, there is some evidence that other signals may input into the traditional checkpoint kinases as well. For example, ATM is thought to be activated by oxidation stress unrelated to DNA damage [36, 37]. One question that remains from these studies, however, is whether the substrates of these noncanonical checkpoint kinases vary depending upon the way in which they have been activated. In addition to the cell cycle regulators Cdc6, Hst3, and Sld2; yeast Mck1 has been shown to phosphorylate the calcineurin regulator Rcn1 [38] and Pyruvate Kinase 1 [39] and to have roles in meiosis and sporulation [40]. Mck1's targeting of Bcy1 has been shown to be regulated by heat shock [41] as well as DNA damage [23]. The fact that multiple inputs lead to activation of Mck1 leaves open the question of whether disparate signals activating it lead to phosphorylation of only a subset of its substrates, as has been characterized for other yeast MAP kinases [42], or whether one signal, such as DNA damage or heat shock, impinges upon all Mck1-regulated pathways.

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