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Science Signaling

AAAS

<< Counting Phosphates

The kinases Rad53 and Dun1 are important components of a checkpoint kinase cascade activated in response to DNA damage in yeast. Both enzymes contain forkhead-associated (FHA) domains, which bind to phosphothreonine (pThr) residues. Rad53 has four Thr residues clustered in its N-terminal SCD1 domain. Upon phosphorylation by upstream kinases, Rad53 interacts with Dun1 through the Dun1-FHA domain to activate Dun1. Although mutant Rad53 proteins that contain only one of the four SCD1 Thr residues are readily activated by upstream kinases, they cannot activate Dun1. Lee *et al*. found that a recombinant Dun1-FHA domain bound with greater affinity to Rad53-SCD1–derived phosphopeptides containing both $pThr^5$ and $pThr^8$ than to phosphopeptides that had only one of these residues, consistent with the Dun1-FHA domain, unlike that of Rad53, having not one, but two high-affinity pThr-binding sites. Treatment of yeast strains expressing a mutant *rad53* allele with a DNA-damage–inducing agent showed that the presence of both Thr^5 and Thr^8 in the Rad53-SCD1 was required for optimal Dun1 activity. Mass spectrometry studies showed the presence of monophosphorylated and diphosphorylated Rad53 proteins in response to DNA damage in vivo. Together these data suggest that whereas monophosphorylation activates Rad53, diphosphorylation of Rad53 is required to activate the Dun1-dependent arm of the DNA-damage response. — JFF

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