Dun1 Counts on Rad53 to Be Turned On

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DOI 10.1016/j.molcel.2008.06.009

In a recent issue of *Molecular Cell*, Lee et al. (2008) demonstrate that the forkhead-associated (FHA) domain of Dun1, a Chk2-related kinase involved in DNA damage signaling, selectively binds a diphosphorylated motif found in its paralog and upstream regulator Rad53.

DNA damage elicits an evolutionarily conserved signal transduction cascade that is critical for cellular viability and preservation of genome integrity. In the budding yeast *Saccharomyces cerevisiae*, DNA damage signaling is largely under the control of the phosphoinositide 3-kinase-like kinase Mec1 (the ATR ortholog) and, to a lesser extent, its paralog Tel1 (ATM). Following its recruitment and activation at DNA lesions, Mec1 phosphorylates mediator proteins Rad9 (53BP1) or Mrc1 (Claspin) to promote the activation of effector proteins such as Chk1 (Chk1) and Rad53 (Chk2), two protein kinases that regulate DNA repair, DNA replication fork stabilization, cell-cycle progression, and transcriptional regulation (Harrison and Haber, 2006; Zhou and Elledge, 2000).

In their new work, Lee et al. (2008) investigate the important question of how Chk2-type kinases are regulated in response to DNA damage. Curiously, *S. cerevisiae*, as well as a number of ascomycetes, contains two Chk2 paralogs (Rad53 and Dun1) instead of a single Chk2 kinase (Zhou and Elledge, 2000). Rad53 and Dun1 evidently arose from an ancestral gene duplication, as they share a high degree of protein similarity. However, Dun1 lies downstream of Rad53 during DNA damage signaling, where it acts primarily to regulate the transcriptional response to DNA damage. Lee et al. (2008) set out to determine how Dun1 is activated by Rad53.

Chk2-type kinases are characterized by the presence of a serine/threonine kinase domain usually preceded by an FHA domain, the latter acting as a phosphothreonine-binding module (Durocher and Jackson, 2002). Rad53 is slightly different in this regard, as it contains two FHA domains (FHA1 and FHA2) that flank a central kinase domain (Durocher et al., 1999). In addition, Rad53, Chk2, and most of their relatives contain clusters of putative Mec1/Tel1 (ATM/ATR) target sites, termed SQ/TQ cluster domains, or SCDs (Traven and Heierhorst, 2005). These motifs are functionally important, as the SCDs of most Chk2/Rad53 relatives participate in their activation following DNA damage. The DNA damage-induced SCD phosphorylation promotes homomultimerization through an interaction with its cognate FHA domain. This binding event, in turn, results in autophosphorylation and, ultimately, in catalytic activation (Traven and Heierhorst, 2005). Intriguingly, Dun1 does not have an SCD motif, and its activation is dependent on the Rad53 SCD1 motif (Rad53 contains two SCDs, SCD1 being the most conserved). Indeed, mutation of all four threonine residues in the Rad53 SCD1 motif impairs both Rad53 and Dun1 activation in response to DNA damage (Lee et al., 2003). However, reintroduction of any one threonine residue in the mutated SCD1 motif restores the activation of Rad53 but surprisingly not that of Dun1 (Lee et al., 2003). These results therefore suggest that multisite phosphorylation of the Rad53 SCD1 motif is necessary for Dun1 activation.

This observation was the starting point for Lee et al. (2008), who first examined the binding affinity between the Dun1 FHA domain and various phosphothreonine-containing peptides derived from the Rad53 SCD1 motif. They found that the Dun1 FHA domain binds a diphosphorylated SCD1 peptide, with two orders of magnitude better affinity than the corresponding set of monophosphorylated SCD1 peptides. By contrast, the Rad53 FHA1 domain binds SCD1-derived phosphopeptides with a similar affinity, regardless of whether they are mono- or diphosphorylated. These biophysical studies hinted at a previously unsuspected capacity for FHA domains to recognize diphosphorylated peptides.

To clarify the structural basis of diphosphorylated epitope recognition by the Dun1 FHA domain, the authors determined the structure of the Dun1 FHA domain bound to the diphosphorylated SCD1 peptide using nuclear magnetic resonance spectroscopy (Lee et al., 2008). The results were striking: the Dun1 FHA domain appears to have duplicated its phosphothreonine-binding site to accommodate a second SCD1 phosphothreonine. In support of this idea, the second phosphopeptide-binding site employs conserved residues that are usually involved in phosphothreonine binding at the canonical site (Durocher and Jackson, 2002). Moreover, both phosphoresidue-binding pockets of the Dun1 FHA domain are selective for phosphothreonine and are unable to bind phosphoserine-bearing peptides, just like canonical FHA domains (Durocher and Jackson, 2002).

Remarkably, the binding behavior of the Rad53 and Dun1 FHA domains is highly reminiscent of logic gates (the authors refer to the binding behavior as phosphocounting). Logic gates are signal-processing devices that integrate two or more inputs to produce a single output. Widely used in digital circuits, they are becoming increasingly recognized as components of biological signaling networks (Hasty et al., 2002). In the context of the work of Lee et al. (2008), the Rad53 FHA1 domain can be considered to be an "OR" gate (Figure 1), as its output (peptide binding) depends on the

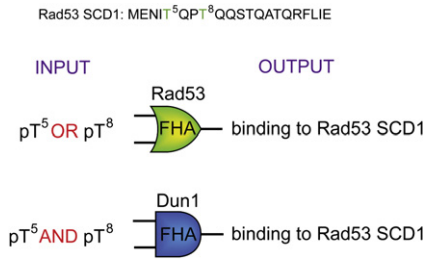

Figure 1. The FHA Domains of Rad53 and Dun1 Act as Logic Gates

Rad53 and Dun1 paralogs require binding to the phosphorylated SCD1 motif of Rad53 (its amino acid sequence is depicted) for their respective activation. The binding behavior of the Rad53 FHA domain resembles an "OR" gate (depicted using the standard distinctive symbol), as it requires phosphorylation of either threonine 5 (pT^5) or threonine 8 (pT^8) residues on the SCD1 motif. By contrast, the binding behavior of Dun1 resembles an "AND" gate, as it requires diphosphorylation of the SCD1 motif.

phosphorylation (input) of any one threonine residue. By contrast, the Dun1 FHA domain behaves as an "AND" gate, where the output depends on a diphosphorylated input (Figure 1). The obvious implication of the Dun1 AND gate behavior is that Dun1 activation must be more "selective" than that of Rad53.

Although the recognition of logic gates in signaling networks is not uncommon, it is important to remember that Dun1 and Rad53 have evolved from a common ancestor. Therefore, this work provides a glimpse into how signal transduction systems might evolve. Indeed, the evolution of an OR gate into an AND gate provides a simple means by which a paralog, such as Dun1, could become more specialized or selectively activated in response to certain stimuli.

Lastly, despite being an attractive model, the question of whether the logic gate/phosphocounting model is physiologically relevant remains open. Indeed, although the authors demonstrate that diphosphorylation of the Rad53 SCD1 motif is important for Dun1 activation, it remains unclear why Dun1 might need to discriminate between mono- and diphosphorylated forms of this peptide sequence. One possibility could be that Dun1-dependent transcriptional regulation is slow and rather costly in terms of energy; it therefore might be beneficial to not activate Dun1 every time Rad53 is activated. Such a possibility will have to be tested carefully in order to extend the significance of these elegant biophysical and genetic studies. Notwithstanding this issue, Lee et al. (2008) describe a stunning example of the plasticity and evolvability of FHA domain-ligand interactions that will likely be echoed in other systems in which multiple posttranslational modifications are "read" by specialized binding domains.

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Just the FACTs: Histone H2B Ubiquitylation and Nucleosome Dynamics

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DOI 10.1016/j.molcel.2008.06.012

In this issue of *Molecular Cell*, Fleming et al. (2008) show that histone H2B ubiquitylation and FACT function interdependently to facilitate nucleosome reassembly during transcription elongation, thereby demonstrating that histone posttranslational modifications can provide important but transient transcriptional signaling cues.

Nucleosomes, the fundamental repeating subunit of chromatin, repress transcription; they block access to underlying DNA sequences, obscuring promoters and enhancers, and also form a repeating barrier to elongating RNA polymerases. Eukaryotic genes therefore tend to be transcriptionally repressed, and accordingly, eukaryotes use an elaborate set of transcriptional regulatory mechanisms that disrupt, alter, or move nucleosomes (Li et al., 2007).

An important advance in our understanding of how chromatin regulates gene expression came with the recognition that histones are subject to a myriad of posttranslational modifications (PTMs), and that specific PTMs are frequently