Sae2 integrates CDK and checkpoint phosphorylation to coordinate MRX cleavage with checkpoint attenuation

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Abstract:

DNA double-strand breaks (DSBs) are highly dangerous lesions that must be correctly repaired to avoid loss of genetic information or chromosome rearrangements. Eukaryotic cells can repair DSBs by homologous recombination (HR), which uses an intact homologous DNA sequence as a template for repair. HR requires the generation of 3' single-stranded DNA (ssDNA) overhangs through a process known as DNA end resection. This process is initiated by an endonucleolytic incision of the 5'-ending strand at a short distance from the DNA terminus, which is catalyzed by the evolutionarily conserved Mre11-Rad50-Xrs2/NBS1 complex (MRX in *Saccharomyces cerevisiae*, MRN in mammals). At the same time, DNA damage elicits a checkpoint response depending on the Mec1/ATR and Tel1/ATM kinases, which detect the presence of single-stranded DNA and DSBs and activate the effector kinase Rad53/CHK2.

Yeast Sae2 plays a dual role in the DNA damage response by suppressing Rad53 activation and stimulating DNA end clipping via the MRX complex. Using AlphaFold3-based modelling and mutational analysis, we show that Mec1/Tel1-dependent phosphorylation of Sae2 at T90 or T279 is sufficient to restrain Rad9-Rad53 interaction and Rad53 kinase activation. Cells expressing a non-phosphorylatable Sae2 double mutant (T90A T279A) display persistent Rad53 activation, whereas phosphomimetic Sae2 variants (T90E or T279E) restore normal checkpoint inactivation.

Structural modeling and charge-reversal genetics indicate that electrostatic interactions between phosphorylated T90/T279 of Sae2 and Rad53 residue R70 are critical for this regulation. In addition, T279 phosphorylation, but not T90, cooperates with cyclin-dependent kinase (CDK)-dependent phosphorylation of Sae2 S267 to promote MRX-dependent resolution of hairpin DNA structures and processing of meiotic double-strand breaks (DSBs). A Sae2 T279E phosphomimetic partially rescues both hairpin cleavage defects and DNA damage sensitivity of *tel1*Δ cells, indicating that Tel1 promotes MRX activity primarily through Sae2 T279 phosphorylation.