Fall 2025

Biochemistry and Molecular Biology Brown Bag Series

Kamal Dev

Postdoctoral Researcher

A missense mutation in Rad3^{ATR} bypasses Rad9-Rad1-Hus1 (9-1-1) phosphorylation to activate Cds1^{CHK2} under replication stress in Schizosaccharomyces pombe (Fission yeast)

Tuesday, September 30, 2025

11:00 AM

Location 135 Oelman Hall

Lab: Yong-jie Xu, Ph.D.

Boonshoft School of Medicine
WRIGHT STATE UNIVERSITY

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https://science-math.wright.edu/biochemistry-and-molecular-biology

Abstract

Under replication stress, DNA replication forks can slow, pause, or even collapse, which activates DNA replication checkpoint (DRC) and DNA damage checkpoint (DDC) pathways for maintenance of genome integrity and cell survival. Mutations of the checkpoint pathways cause genome instability, a hallmark of cancer. In *S. pombe*, Rad3 kinase (the ATR homolog) together with its binding partner Rad26 monitors replication stress and activate the replication checkpoint by phosphorylating several substrates, including Mrc1-Thr645/653, Rad9-Thr412, and Cds1-Thr11. The phosphorylation defective mutations of mrc1 and rad9 impair Cds1 phosphorylation, thus sensitizing the cells to hydroxyurea (HU), and methyl methane sulfonate (MMS), by inhibiting DNA replication and causing DNA damage respectively. Although the role of phosphorylated Mrc1 in promoting Cds1 activation is well established, the function of phosphorylated Rad9 in this context has remained less clear. To address this problem, we randomly mutagenized the genome in the rad9-Thr412Ala mutant and screened a suppressor mutant that confers HU resistance in rad9-T412A, as well as in rad9-ΔC (Δ411-426). Consistent with the drug resistance, phosphorylation of Cds1-Thr11 by Rad3 is restored. Whole genome sequencing identified a single missense mutation within rad3, and subsequent experiments demonstrated that overexpression or genomic integration of the mutant rad3 allele confers HU resistance and restored Cds1-Thr11 phosphorylation in rad9-ΔC cells. In vitro kinase assays further revealed that the mutant Rad3 protein is constitutively active even in the absence of replication stress, and this hyperactive form of Rad3 can also rescue the HU sensitivity seen in rad26 mutants with N-terminal mutations. These results collectively demonstrate that a rad3 constitutive mutation can bypass the canonical requirement for 9-1-1 complex phosphorylation, thereby directly activating Cds1 and stabilizing replication forks under stress conditions.