Chromatin Remodelers Check in on DNA Replication

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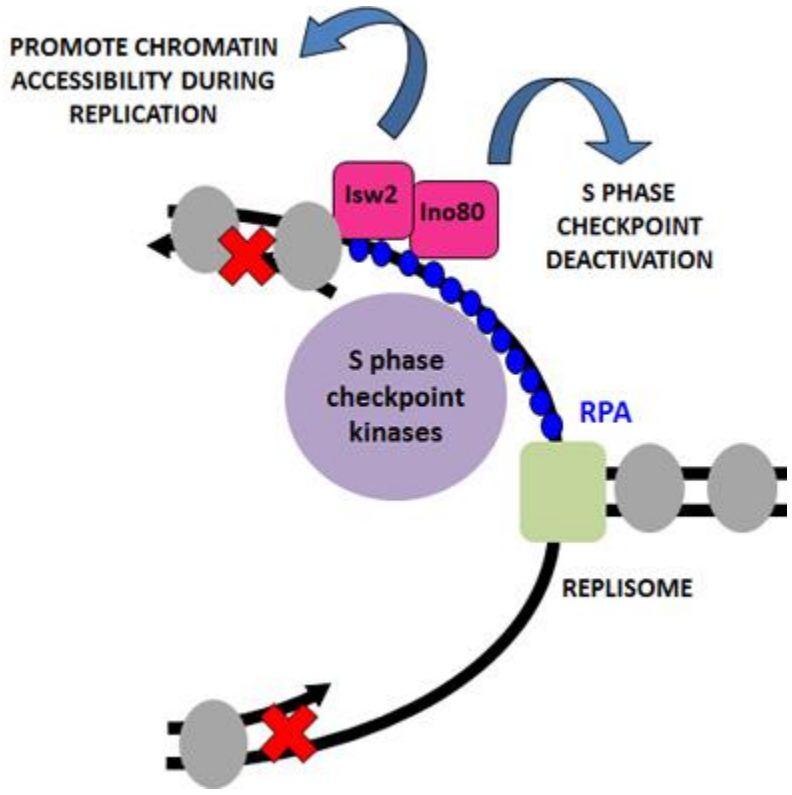

The Isw2 and Ino80 chromatin remodeling complexes associate with origins to promote chromatin accessibility, allowing deactivation of the S-phase checkpoint.

Image provided by Laura Lee

To ensure faithful transmission of genetic information, all dividing cells must replicate their DNA during S-phase of the cell cycle. However, replicating cells are particularly susceptible to DNA damage and nucleotide depletion, leading to replication stress and, ultimately, stalling of replication forks. Replication stress triggers a mechanism known as the S-phase checkpoint, which allows cells to properly complete DNA replication before proceeding with the remainder of the cell cycle. The mechanisms by which stalled replication forks activate the S-phase checkpoint in budding yeast have been studied extensively, but how the checkpoint is deactivated to allow cells to resume cell cycle progression is much less well understood. Previous work from the laboratory of Dr. Toshio Tsukiyama (Basic Sciences Division) suggested that Isw2 and Ino80 chromatin remodeling complexes had roles in attenuating the S-phase checkpoint (Au et al, 2011). To follow up this

observation, Tsukiyama lab graduate student Laura Lee and postdoctoral fellow Dr. Jairo Rodriguez performed a systematic analysis of the effect of the Isw2 and Ino80 complexes on S-phase checkpoint activity.

The authors began their analysis by assessing the levels of Rad53, a protein kinase involved in activation of the S-phase checkpoint, in wild-type (WT) cells and cells lacking Isw2, the catalytic subunit of the Isw2 complex, and Nhp10, a component of the Ino80 complex, in the presence of the DNA damaging agents hydroxyurea (HU), which results in nucleotide depletion, and methylmethanesulfonate (MMS), which causes DNA damage by base alkylation. They found that Rad53 was much more stable in the *isw2 nhp10* mutant, and that autophosphorylation of Rad53 was moderately increased by MMS treatment. The *isw2 nhp10* cells also displayed a delay in S-phase progression that was not due to incomplete DNA replication. These results suggest that Isw2 and Ino80 function to prevent overactivation of the S-phase checkpoint in the presence of MMS.

Using genetic crosses, the authors next tested potential pathways through which Isw2 and Ino80 might attenuate the S-phase checkpoint. They crossed the *isw2 nhp10* strain to strains with mutations in factors involved in replication fork protection, DNA replication, and DNA damage response pathways, reasoning that if Isw2 and Ino80 were functioning in any of these pathways, a single mutant and triple mutant would show similar MMS sensitivity, as the pathway would already be compromised in the single mutant. However, they found that the *isw2 nhp10* deletion enhanced MMS sensitivity of all strains tested, indicating that Isw2 and Ino80 function outside of these pathways.

To identify factors required for Isw1 and Ino80 checkpoint function, the authors used Rad53 autophosphorylation as a read out, reasoning as before that if a particular protein was required for Isw2 and Ino80 checkpoint attenuation, there would be no difference in Rad53 autophosphorylation between a single mutant and triple mutant. Strikingly, increased Rad53 autophosphorylation was observed in all experiments, indicating that Isw2 and Ino80 function either through unknown checkpoint proteins or through replication protein A (RPA), which could not be tested due to the severe growth phenotype of the *rpa isw2 nhp10* strain.

Based on the data thus far, the authors proposed two mechanisms by which Isw2 and Ino80 could downregulate checkpoint activity: 1) by facilitating removal of a checkpoint protein from chromatin and 2) by downregulating checkpoint protein activity. To address the first possibility, the authors analyzed the levels of chromatin-bound checkpoint factors in WT and *isw2 nhp10* cells, finding that bulk levels of these factors on chromatin was essentially unchanged.

As it was previously found that Isw2 and Ino80 are enriched at stalled replication forks, the authors wondered if Isw2 and Ino80 might affect chromatin structure at replication forks to influence checkpoint activity. Using an assay called normalized chromatin accessibility to MNase (NCAM), the authors tested chromatin accessibility across the genome in WT and *isw2 nhp10* cells. This revealed decreased chromatin accessibility at replicating regions in the double mutant.

"In our studies we found that chromatin remodeling factors are important for regulating both the checkpoint and chromatin accessibility during DNA replication," said Ms. Lee. "These results highlight the significance of chromatin remodeling factors in establishing proper DNA replication, which is essential for genomic integrity." Together, these data suggest a model in which Isw2 and Ino80 promote chromatin accessibility at replication forks to facilitate progression of replication. Further studies will undoubtedly reveal the mechanistic underpinnings of this intriguing new function for these chromatin remodelers.

Lee L, Rodriguez J, Tsukiyama T. 2015. Chromatin remodeling factors Isw2 and Ino80 regulate checkpoint activity and chromatin structure in S phase. *Genetics* 199(4):1077-1091.

See also: Au TJ, Rodriguez J, Vincent JA, Tsukiyama T. 2011. ATP-dependent chromatin remodeling factors tune S phase checkpoint activity. *Mol Cell Biol* 31(22):4454-4463.