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REPLICATION

Hog1 in conflict resolution

> Hog1 … directly ensures that replication is delayed by phosphorylating Mrc1

Collisions between ongoing transcription and replication can increase recombination events and therefore genome instability. Thus, when high levels of transcription are induced, for example during a stress response, it is important to suppress replication. We now learn that, in budding yeast, the stress-activated protein kinase Hog1 can coordinate transcription and replication by initiating a novel S phase checkpoint in response to osmotic stress.

Hog1 is known to be activated in response to high osmolarity, inducing an 'osmostress' response that includes broad changes in gene expression. Duch *et al.* asked whether Hog1 might also target a replication factor. They showed that, *in vitro*, it specifically phosphorylates Mrc1 (mediator of replication checkpoint 1), which is an important factor for coupling replication enzymes. Hog1 also phosphorylated Mrc1 *in vivo* during osmostress, and this required three MAPK consensus sites, Thr169, Ser215 and Ser229. Mrc1 and Hog1 were also found to interact in co-immunoprecipitation and yeast two-hybrid assays, confirming that Mrc1 is a genuine substrate of Hog1.


CORBIS

They then asked what the consequences of Hog1-mediated phosphorylation of Mrc1 are using a non-phosphorylatable Mrc1 mutant ($Mrc1^{3A}$, in which the three MAPK consensus sites were mutated to Ala). Using two-dimensional gel electrophoresis, they found that Mrc1 phosphorylation during osmostress delays replication fork progression and reduces the association of Mrc1 with the replicative enzyme DNA polymerase ε. DNA combing analysis also showed that osmostress delayed firing at early and late origins by preventing origin association of the Cdc45 helicase with early origins, and this required Mrc1 phosphorylation. Inhibition of Mrc1 phosphorylation prevented the normal S phase delay seen in yeast upon osmotic stress.

Why is it important to delay S phase during osmostress? The authors showed that expression of $Mrc1^{3A}$ resulted in increased genomic instability following osmostress, whereas a Hog1 phosphomimetic mutant, $Mrc1^{3D}$, did not. $Mrc1^{3D}$ also delayed S phase even in the absence of stress. Using a plasmid reporter system, they saw that the increased instability seen in the $Mrc1^{3A}$ mutant during osmostress correlated with increased transcription-associated recombination.

This shows that Hog1 has a dual role during osmotic stress; in addition to inducing changes in gene expression, it directly ensures that replication is delayed by phosphorylating Mrc1. In this way, it minimizes collisions between the transcription and replication machinery and avoids deleterious recombination events. It is notable, say the authors, that Hog1 targets Mrc1, which is also regulated by the S phase checkpoint that is induced by DNA damage. Indeed, this novel Hog1-dependent S phase checkpoint is independent of Rad53 and Mec1, key components of the DNA damage checkpoint, supporting the idea that the two checkpoints have complementary roles in ensuring that replication proceeds with the appropriate caution.

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ORIGINAL RESEARCH PAPER Duch, A. *et al.* Coordinated control of replication and transcription by a SAPK protects genomic integrity. *Nature* 25 Nov 2012 (doi:10.1038/nature11675)