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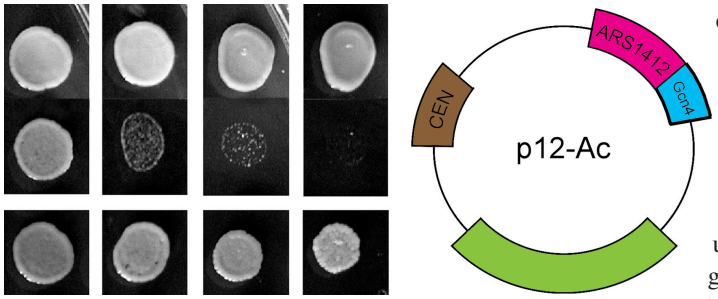
Early origins sound S phase alarm

Early risers turn on the alarm that prevents genomic instability stemming from problems with DNA replication, say Caldwell et al. The findings show that those DNA replication origins that are activated earliest are needed to trigger the S phase checkpoint.

The S phase checkpoint is turned on during every cell cycle when DNA polymerases stall upon encountering lesions in the DNA template. The checkpoint keeps polymerases on the replication fork, prevents the fork's intricate DNA structure from collapsing, and delays S phase progression into mitosis. In the meantime, the cell has time to find ways around the lesion, for instance by using different polymerases or switching template strands.

In the new work, the authors sought to determine how this checkpoint is activated. They hypothesized that early replication origins might be needed, based on evidence that early origins fire even if cells are not fully ready for replication—if dNTP levels are too low, for example.



In the absence of the DNA damage checkpoint, treatments that activate the S phase checkpoint kill off (left to right) yeast that lack early firing replication origins (middle row). Adding episomes with early firing origins (p12-Ac) rescues the mutants (bottom row).

Those early origins do not fire in a yeast mutant that lacks a kinase called Dun1 (the basis for the misfiring is so far unknown). The group now shows that, when exposed to treatments that normally activate the S phase checkpoint, *dun1* mutants rely on the DNA damage checkpoint for survival. The dependence belies the cells' inability to activate the S phase checkpoint and the resulting damage to the genome that must be repaired before mitosis.

The addition of just one or two functional early origins restored the mutants' S phase checkpoint. The working origins, which were introduced into the yeast cells on episomes, fired normally in the *dun1* mutants. The group hypothesizes that these episomal origins might fire because they lack a chromatin structure that somehow cripples endogenous *dun1* origins.

Forcing the extra origins to fire later blocked their ability to turn on the S phase checkpoint. So did deletion of their centromeres, suggesting that the checkpoint can be activated by proteins that participate in fork pausing, as occur when forks encounter large protein complexes on centromeres or actively transcribed regions. JCB

Caldwell, J.M., et al. 2008. *J. Cell Biol.* 180:1073–1086.

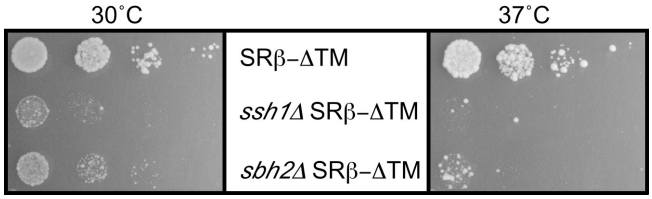
The SR finds the translocon

Results by Jiang et al. reveal an elusive interaction that leads to protein translocation into the ER.

As proteins destined to enter the secretory pathway are translated, they are brought to ER channels known as translocons. The process begins when the signal sequence that tags secreted proteins first emerges from the ribosome. This peptide is bound by the signal recognition particle (SRP), which must then find its receptor (SR). Models dating back a decade propose that empty translocons are identified via interactions with the SR.

This SR–translocon interaction has not been definitely shown, however. Because both components lie in the ER membrane, experiments designed to identify the interaction have been difficult. The transient nature of the interaction—once the ribosome docks on the translocon, the SR departs—has not helped matters. But the group's new genetic evidence backs the prevailing model.

A genetic trick was provided by a yeast mutation that results in a soluble version of SR, via deletion of its transmembrane segment. Translocation efficiency was reduced in the mutants, suggesting that SR was finding the translocon at a slower rate, because it must search in three dimensions rather than just in the plane of the ER membrane.



Yeast with a soluble SR mutant are still viable (top), but the mutation becomes deadly when combined with the loss of the entire Ssh1 translocon (middle) or just its β subunit (bottom).

When the Ssh1 secondary translocon, or just its β subnunit, was deleted from the soluble SR strain, the cells were severely crippled in translocation and growth. This genetic interaction suggests that soluble SR locates translocons through interactions with translocon β subunits. Deletion of the β subunit of either the primary or secondary translocon alone does not impair translocation in cells with wild-type SR, but loss of both causes a severe defect. JCB

Jiang, Y., et al. 2008. *J. Cell Biol.* 180:1149–1161.