

No Origin, No Problem for Yeast DNA Replication

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GE Zentner

To ensure proper transmission of genetic information, cells must accurately replicate their genome during each cell cycle. In budding yeast, DNA replication initiates from well-defined origins called autonomously replicating sequences (ARSs), while in multicellular organisms replication it is thought to initiate from broader, less well-defined zones. Interestingly, some recent work has suggested unexpected plasticity in the initiation of DNA replication, particularly in organisms with discrete origins of replication. For instance, the archaeon *Haloferax volcanii* can replicate its chromosome even when its single origin is deleted, and origin-deficient *Haloferax* cells grow faster than wild-type cells (Hawkins *et al.*, 2013). Additionally, budding yeast lacking several ARSs on chromosome III show no appreciable increase in the loss of this chromosome during cell division (Dershowitz *et al.*, 2007). Despite these observations, how DNA replication can initiate in the absence of ARSs is unclear. To address this question, graduate student Naomi Bogenschutz and postdoctoral fellow Dr. Jairo Rodriguez in the lab of Dr. Toshio Tsukiyama (Basic Sciences Division) generated a yeast strain carrying a chromosome lacking seven well-characterized ARSs. They found that this strain displayed no increase in sensitivity to DNA damaging agents and that DNA replication initiated from non-canonical sites on chromosome VI, suggesting that DNA replication in budding yeast is more flexible than previously thought.

To study how yeast can replicate their DNA in the absence of ARSs, the authors generated a yeast strain in which seven ARSs on chromosome VI were deleted (hereafter referred to as *ToriΔ*). They first tested *ToriΔ* for sensitivity to replication stress by growing it in medium containing stress-inducing chemicals. Strikingly, *ToriΔ* cells showed no increase in sensitivity to replication stress, suggesting the presence of additional mechanisms for maintaining replication initiation in the absence of ARSs.

Replication is initiated by the binding of the origin recognition complex (ORC) to origins, so the authors next investigated the binding of the ORC subunit Orc2 to chromosome VI in the *ToriΔ* strain. Consistent with the recognition of ARSs by ORC, Orc2 binding was strongly reduced at each origin deleted in *ToriΔ*. However, low-level ORC binding adjacent to deleted origins was detected, indicated that ORC binding is not confined to ARSs.

To investigate how replication could initiate in the absence of ARSs, the authors profiled replication using incorporation of bromodeoxyuridine (BrdU). BrdU is an analog of the nucleotide thymidine that can be captured with an anti-BrdU antibody, allowing isolation of newly replicated DNA. This revealed robust BrdU signal around each of the deleted origins, suggesting that residual levels of ORC bound adjacent to deleted origins might be sufficient for initiation. Indeed, higher resolution analysis of initiation in *ToriΔ* revealed that replication initiated from multiple sites adjacent to the deleted origins.

These results reveal a new safeguard for replication integrity, wherein sequences adjacent to canonical origins are sufficient to initiate replication when the ARS is lost. This may indicate that DNA replication origins in budding yeast are not as discrete as previously thought, and may be more similar to metazoan origins. "Our results suggest that even in budding yeast, at least when a large number of origins fail, replication can initiate from non-canonical sites, which suggests that the difference between budding yeast and metazoan systems is not as black and white as people generally describe," said Dr. Tsukiyama. "An interesting possibility is that we may be able to use the yeast mutant carrying this chromosome as a model for metazoan-like replication initiation."

[Bogenschutz NL, Rodriguez J, Tsukiyama T](#). 2014. Initiation of DNA Replication from Non-Canonical Sites on an Origin-Depleted Chromosome. *PLOS ONE* 9(12):e114545.

See also: [Hawkins M, Malla S, Blythe MJ, Nieduszynski CA, Allers T](#). 2013. Accelerated growth in the absence of DNA replication origins. *Nature* 503(7477):544-547.

[Dershowitz A, Snyder M, Sbia M, Skurnick JH, Ong LY, Newlon CS](#). 2007. Linear Derivatives of *Saccharomyces cerevisiae* Can Be Maintained in the Absence of Autonomously Replicating Sequence Elements. *Mol Cell Biol* 27(13):4652-4663.

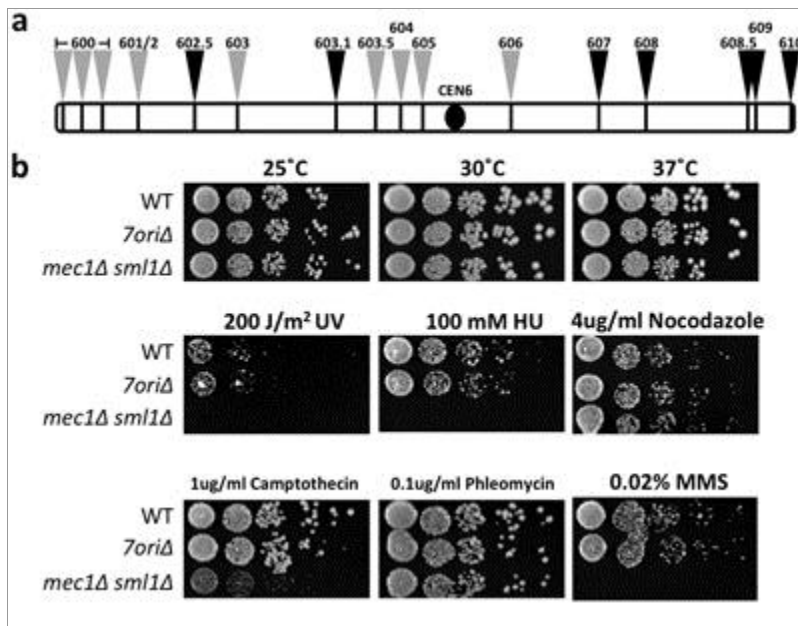


Image from the publication

(a) Schematic of chromosome VI, denoting the location of intact ARSs with grey arrows and deleted ARSs with black arrows. (b) Growth analysis of wild-type and *7oriΔ* yeast on standard medium (top row) or medium containing various stressors (middle and bottom rows). The *mec1Δ sml1Δ* strain serves as a positive control for sensitivity to replication stress.