Top1 and Top2 promote replication fork arrest at a programmed pause site

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Programmed fork pausing is a complex process allowing cells to arrest replication forks at specific loci in a polar manner. Studies in budding yeast and other model organisms indicate that such replication fork barriers do not act as roadblocks passively impeding fork progression but rather elicit complex interactions between fork and barrier components. In this issue of *Genes & Development*, Shyian and colleagues (pp. 87–98) show that in budding yeast, the fork protection complex Tof1–Csm3 interacts physically with DNA topoisomerase I (Top1) at replication forks through the C-terminal domain of Tof1. Fork pausing at the ribosomal DNA (rDNA) replication fork barrier (RFB) is impaired in the absence of Top1 or in a *tof1* mutant that does not bind Top1, but the function of Top1 can be partially compensated for by Top2. Together, these data indicate that topoisomerases play an unexpected role in the regulation of programmed fork pausing in *Saccharomyces cerevisiae*.

The replication of eukaryotic genomes is a daunting task, involving the activation of thousands of origins from which replication forks progress bidirectionally until the completion of DNA synthesis. The nucleus is a crowded environment, and replication forks frequently pause when they encounter obstacles (Magdalou et al. 2014). Paused forks activate the DNA replication checkpoint to signal replication stress (RS) and are either rescued by converging forks or restarted by homologous recombination (Pasero and Vindigni 2017). Basically, replication forks can encounter two types of roadblocks. Unscheduled fork arrest can be caused by DNA lesions, protein–DNA cross-links, highly expressed genes, or structured DNA. This type of fork impediment has been implicated in genomic instability. In addition, programmed fork pausing has been reported at a variety of loci (Ivessa et al. 2003). Unlike accidental arrests, programmed pause sites induce fork arrest in a polar manner through an active process involving a cross-talk between the RFB and replisome components (Magdalou et al. 2014).

Programmed pause sites have been best characterized at the rDNA array and tRNA genes of the budding yeast *Saccharomyces cerevisiae* (Hizume and Araki 2019). Replication fork arrest at the rDNA RFB depends on Fob1, a protein that binds specific sequences downstream from the 35S gene and blocks forks progressing in a head-on orientation relative to transcription (Kobayashi 2006). Remarkably, fork arrest at the rDNA RFB also depends on Tof1 and Csm3, two replisome components that form a complex with the checkpoint mediator Mrc1 and are required for normal fork progression (Calzada et al. 2005). Fork progression through the rDNA RFB depends on Rrm3, a 5′–3′ DNA helicase of the Pif1 family that removes Fob1 from the rDNA and other nonhistone protein barriers (Ivessa et al. 2003). Since the deletion of *RRM3* restores fork pausing in *tof1Δ* mutants (Mohanty et al. 2006), it has been proposed that Tof1 counteracts the "sweepase" activity of Rrm3 (Fig. 1A) through a process regulated by Cdc7 (Bastia et al. 2016). However, this rescue is only partial, suggesting that Tof1 also controls fork arrest through a mechanism that is independent of Rrm3.

In this issue of *Genes & Development*, Shyian et al. (2020) report the use of an unbiased forward genetic screen to identify novel factors acting with Tof1 to regulate fork pausing at the rDNA RFB. This so-called "cowcatcher" screen is based on the fact that fork stalling at the rDNA RFB affects the stability of *ADE2* and *URA3* markers inserted in the rDNA array. Using this approach, they identified *TOP1* as one of the two genes increasing rDNA instability when mutated in *tof1Δ* mutants. They also showed that Top1 interacts physically with the C-terminal part of Tof1 and is recruited to replication sites in a Tof1-dependent but Mrc1-independent manner. Interestingly, both Top1 and the C-terminal domain of Tof1 were required for pausing at the rDNA RFB and tRNA genes, supporting the view that Tof1 recruits Top1 to the replisome in order to promote fork arrest.

At first sight, the fact that Top1 is required for stable fork pausing is intriguing, as Top1 normally promotes

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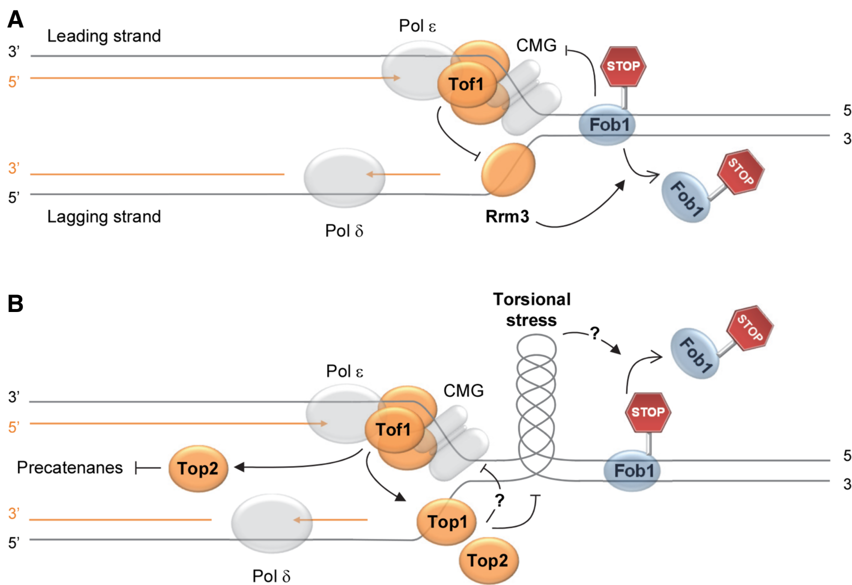

Figure 1. Mechanisms of programmed fork pausing at the rDNA RFB in budding yeast. (*A*) Replication fork pausing at the rDNA RFB depends on the Fob1 protein and on the Tof1–Csm3 complex, which interacts also with Mrc1 (orange heterotrimeric complex). Fob1 can be displaced by the 5′–3′ helicase Rrm3. In the classical "antisweepase" model, Tof1 prevents the sweepase activity of Rrm3 from displacing Fob1 from the RFB. (*B*) In the novel sTOP model proposed by Shyian et al. (2020), Tof1 interacts physically with Top1 and Top2 to promote fork pausing. In the absence of Top1, the accumulation of positive supercoiling at the RFB could displace Fob1. Alternatively, the interaction between Tof1 and Top1 could actively slow down the fork through a mechanism potentially involving Pol ε and that remains to be characterized. It is worth noting that the antisweepase and sTOP mechanisms are not mutually exclusive and likely cooperate to regulate fork arrest at the rDNA RFB.

fork progression by relaxing positive supercoiling in front of the replisome. One possible explanation for this requirement is that an excess of torsional stress may displace Fob1 when the fork reaches the RFB and therefore inactivates it (Fig. 1B). Alternatively, Tof1 may detect the presence of Top1 engaged on supercoiled DNA ahead of the fork and may slow down the fork by a mechanism that remains to be determined (Fig. 1B). It has been reported recently that the DNA polymerase ε (Pol ε) modulates the activity of the CMG helicase to promote fork arrest at the rDNA RFB in an in vitro assay (Hizume et al. 2018). Since Pol ε was identified together with Top1 in the cowcatcher screen, it is tempting to speculate that Tof1 could sense the presence of Top1 on DNA with its C-terminal domain and slow down the replisome in a Pol ε-dependent manner. This interaction between Tof1 and Top1 could also slow down forks encountering Top1 cleavage complexes (Top1cc) trapped on DNA in the presence of the Top1 inhibitor camptothecin (CPT). This view is supported by the fact that both *tof1Δ* cells and *tof1* mutants lacking the C-terminal domain that interacts with Top1 are hypersensitive to CPT.

Finally, the investigators reported that Tof1 also interacts physically and functionally with topoisomerase II (Top2), as this enzyme partially compensates for the loss of RFB activity in the absence of Top1. This observation is important as, unlike Top1, Top2 is also required for the decatenation of sister chromatids. Precatenanes accumulate when positive supercoiling is transferred behind the fork upon fork rotation. Interestingly, Tof1 also plays a key role at the replisome in preventing excessive fork rotation (Schalbetter et al. 2015). Altogether, these findings suggest the existence of a complex interplay between DNA Pol ε, the Tof1–Csm3 complex, and topoisomerases in the regulation of programmed fork pausing. They also shed new light on the mechanisms by which DNA supercoiling may affect fork progression, arrest, and rotation. Since the Tof1–Csm3 complex is conserved in eukaryotic cells, it would be important to determine whether its human counterpart, TIMELESS–TIPIN, also acts with TOP1 to regulate fork pausing.

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