DNA REPLICATION

Unlocking the secrets of fork arrest

A powerful, high-throughput single-molecule approach to probe the nanoscale mechanical properties of the Tus–*Ter* protein–DNA complex reveals that the Tus–*Ter*-induced lock in unzipping at the nonpermissive face requires only DNA strand separation and involves a progressive strengthening of the Tus–*Ter* complex.

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Large-scale DNA transactions, specifically transcription and replication, are carried out by highly licensed, highly processive molecular systems. Once the machinery is launched, it is stopping it at the end of its task that becomes the essential step[1]. Tus–*Ter* protein–DNA complexes help coordinate the arrest of converging replication forks in *Escherichia coli*. The replication fork, or replisome, is a complex machine containing DNA helicase (DnaB), DNA polymerase (polymerase III) and numerous scaffolding components; assembled at a fork in DNA, it moves at rates of 1,000 bp/s, unzipping and replicating both DNA strands as it advances. During replication in *E. coli*, two replication forks progress bidirectionally from a common origin (*oriC*) on the circular chromosome toward the terminus region opposite *oriC*, where they encounter DNA terminus (*Ter*) sites. *Ter* is a polar ~23-bp sequence specified by a consensus, with a strictly conserved GC base pair in the sixth position (GC6). The terminator protein, Tus, binds as a monomer to the *Ter* site. When encountered from the 'permissive' side, the Tus–*Ter* complex is not an obstacle to replication, but when encountered from the 'nonpermissive' side, it provides an effective stop to a replication fork (**Fig. 1**). Ten *Ter* sites are distributed near the midpoint of the chromosome, with the permissive side facing *oriC*. A faster replication fork will first pass through the initial, permissively oriented *Ter* sites it encounters, and then progress beyond the midpoint of the chromosome and encounter a nonpermissively oriented *Ter* site that can arrest its movement. Two mechanisms, which are not mutually exclusive, have been proposed to contribute to polar arrest of the replisome by Tus–*Ter*: the Tus–*Ter* locking, or mousetrap, model[2] and the protein-protein interaction model, focusing for instance on an interaction between Tus and DnaB[3].

In this issue, Berghuis *et al.* describe a single-molecule DNA strand separation assay using a hairpin construct bearing a single *TerB* site, manipulated in a magnetic trap and in the presence of Tus[4]. In the assay, the magnetic trap applies a mechanical force to the hairpin DNA to open it, in a recapitulation of double-stranded DNA unwinding during replication-fork progression. Force-induced hairpin unwinding is typically halted by a strong protein-DNA interaction along the DNA, and both the position of the blockade along the DNA and its lifetime can be measured by determining the end-to-end extension of the blocked hairpin and the dwell time for that blocked state, respectively.

Berghuis *et al.* report that in the presence of Tus, force-induced hairpin unwinding was effectively blocked when the TerB site was in the nonpermissive orientation, implying that protein-DNA interactions are at least partly responsible for blocking replication-fork progression[4]. The hairpin locked by a Tus–*Ter* complex resisted force-induced unwinding for timescales on the order of several minutes—in comparison with the millisecond timescales for unwinding by the replisome. It is also possible that dynamic interactions with an actual replisome may further affect the process.

Hairpin unwinding proceeded essentially unimpeded when the TerB site was in the permissive orientation. Mutation of the DNA-binding domain did not produce any dramatic effects, confirming this domain is not indispensable to lock formation. Mutations of the residues in or around the lock domain that interact with the conserved C of the GC6 base were shown to affect a hierarchy of locking stages, in effect allowing the authors to progressively pick the lock formed by the Tus–*Ter* complex.

A high-throughput force-spectroscopic approach identified and characterized three different stochastic dwell timescales for the lock, indicating that there are three (or more) sequential states involved in the formation of the Tus–*Ter* lock: short-lived, semi and full locks. The authors propose that at relatively elevated forces, short-lived and infrequent locked states (~1 s and ~1 min) correspond to intermediate states in the process of full lock formation. The most frequently observed structures are long-lived (~10 min), reflecting formation of a complete lock and the actual efficiency of the lock. A simple model accounting for these results includes three irreversible and progressive states along the path to formation of the final long-lived and stable lock.

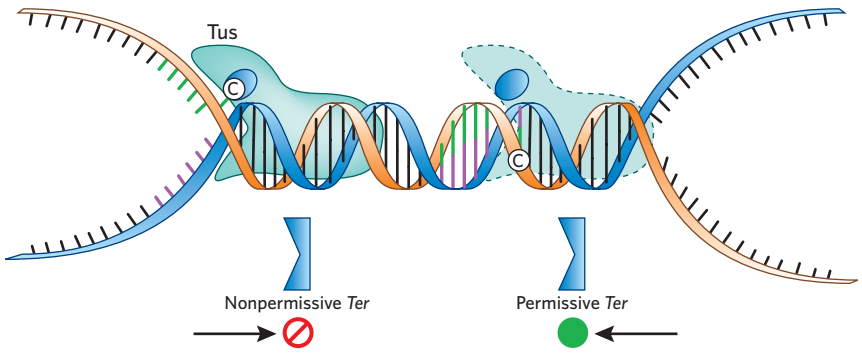

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Figure 1 | Schematic depiction of Tus protein locked onto *Ter* site in the nonpermissive orientation owing to binding to the flipped-out C6 base. Base flipping presumably takes place upon DNA unwinding by DnaB just as the replisome comes up against the lock. In the permissive orientation the full strength of the lock is not deployed as the protein is presumably displaced from *Ter* by the replisome before the C6 base is flipped out and the lock formed.

Reconstitution of strand separation on a *Ter* site has allowed Berghuis *et al.*[4] to reveal the intrinsic strength of the Tus–*Ter* lock, supporting the view that physical replisome arrest by a stable obstacle may be sufficient to initiate fork disassembly and replication termination. This could be a general solution to a complex problem; in *B. subtilis* a similar process using an unrelated but functionally identical protein, RTP, is observed. Likewise, dynamic interactions between the full replisome and the Tus–*Ter* complex may be critical to successful establishment of the Tus–*Ter* lock[5], underscoring the detail and complexity involved in replisome arrest[6]. Ultimately, beyond replisome arrest, there remains still another mysterious step: the merging of the replication forks[7].

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Competing financial interests

The authors declare no competing financial interests.