

UvrD helicase

The little engine that could

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In classical bacterial nucleotide excision repair (NER),¹ a bulky DNA lesion such as a UV photoproduct is recognized by the UvrAB DNA damage recognition complex, and the strand containing the adduct is incised by UvrC nuclease upstream and downstream of the lesion. UvrD helicase subsequently loads at a nicked DNA site and unwinds the 12-base pair duplex containing the lesion, thereby creating a gap subsequently filled in by DNA polymerase and ligated to restore genomic integrity. Unexpectedly, another prominent role for UvrD in NER was discovered by Epshtein et al. that has major implications for how bacteria sense bulky adducts in actively transcribed DNA sequences.²

As reported in the January 16, 2014 issue of *Nature*, Epshtein et al. found that UvrD facilitates an alternative mode of transcription-coupled repair (TCR) by pulling the transcribing RNA polymerase backward to enable NER proteins gain access to the damage² (see train engine analogy, **Fig. 1**). The ability of UvrD to physically bind RNA polymerase and tow the macromolecule in the opposite direction, to template-directed translocation during RNA synthesis, provides a new paradigm for molecular motors and their action on polymer lattices. UvrD tracks uni-directionally in its own right;³ therefore, the helicase enzyme performs its Herculean task by translocating 3' to 5' on the rail opposite to that which RNA polymerase uses as a template for RNA synthesis during elongation.

RNA polymerase backtracking is known to pose a significant source of genomic instability in *E. coli*.⁴ How?

Collision of the replisome with backtracked RNA polymerase elongation complexes can lead to discontinuous replication, resulting in double-strand breaks (DSBs) that lead to genome rearrangements. This finding poses a challenge to understand the potentially conflicting outcome of UvrD-sponsored RNA polymerase backtracking. It appears that “mother nature” has placed a definite cost on implementing the UvrD-based alternative mode of TCR, taking the risk that replication machinery will encounter a backtracked RNA polymerase, resulting in a toxic DSB. Perhaps this is why multiple anti-backtracking factors (transcript cleavage factors, ribosomes)² conceivably help to keep the system in balance during specialized circumstances, such as periods of elevated DNA damage (e.g., UV irradiation). In addition, bacterial cells solicit the DNA translocase Mfd, which serves to push RNA polymerase forward and displace it from the lesion site as it recruits the DNA damage recognition protein UvrA.¹ UvrD also has a helper in TCR, the NusA elongation factor, which supports UvrD-mediated backtracking by RNA polymerase and possibly aids in recruitment of UvrAB.²

UvrD's risk-taking behavior to yank RNA polymerase backward is conceivably minimized if limited to periods when replication is subdued. Nonetheless, transcription can occur during times of DNA synthesis. DNA translocases like Mfd may help to push the backtracked RNA polymerase forward again, once the lesion is repaired to alleviate potential collisions with co-directional replication fork proteins. In eukaryotic cells, it is easy to imagine that RNA polymerase backtracking would be less hazardous in

periods outside S phase, when the cell is not actively replicating its genome; however, this remains to be seen. These issues are likely to be significant, as cells have a very low threshold for DSBs.

Genetic studies have already implicated UvrD in facilitating fork progression across transcribed sequences. Mutations in RNA polymerase subunits that affect stability of DNA: transcription complexes suppress the growth defects of bacteria lacking the replication accessory helicases Rep, UvrD, and recombination protein RecF.⁵ Genetic and biochemical studies support a model in which certain DNA helicases (Rep, UvrD, DinG) have specialized duties to displace proteins bound to DNA, or unwind R-loops to facilitate progression of the replication fork across transcribed sequences.⁶ However, the discovery that UvrD can pull RNA polymerase backward suggests that the role of UvrD in both NER and collision avoidance is more complex than previously thought.

For helicase aficionados, the subtle aspects of UvrD mechanism are intriguing. UvrD is known to load at single-stranded/double-stranded junctions and, depending on its oligomeric state, translocate on single-stranded DNA as a monomer or unwind duplex DNA as a dimer.³ Therefore, the assembly state of UvrD as it pulls RNA polymerase backward is of interest. Second, UvrD can strand switch and translocate in the opposite direction on the strand complementary to the one it was originally tracking.⁷ This behavior of UvrD suggests that at times UvrD may translocate on the damaged strand and, upon encountering the lesion, switch to the undamaged strand. Either way, as UvrD mightily tows RNA polymerase

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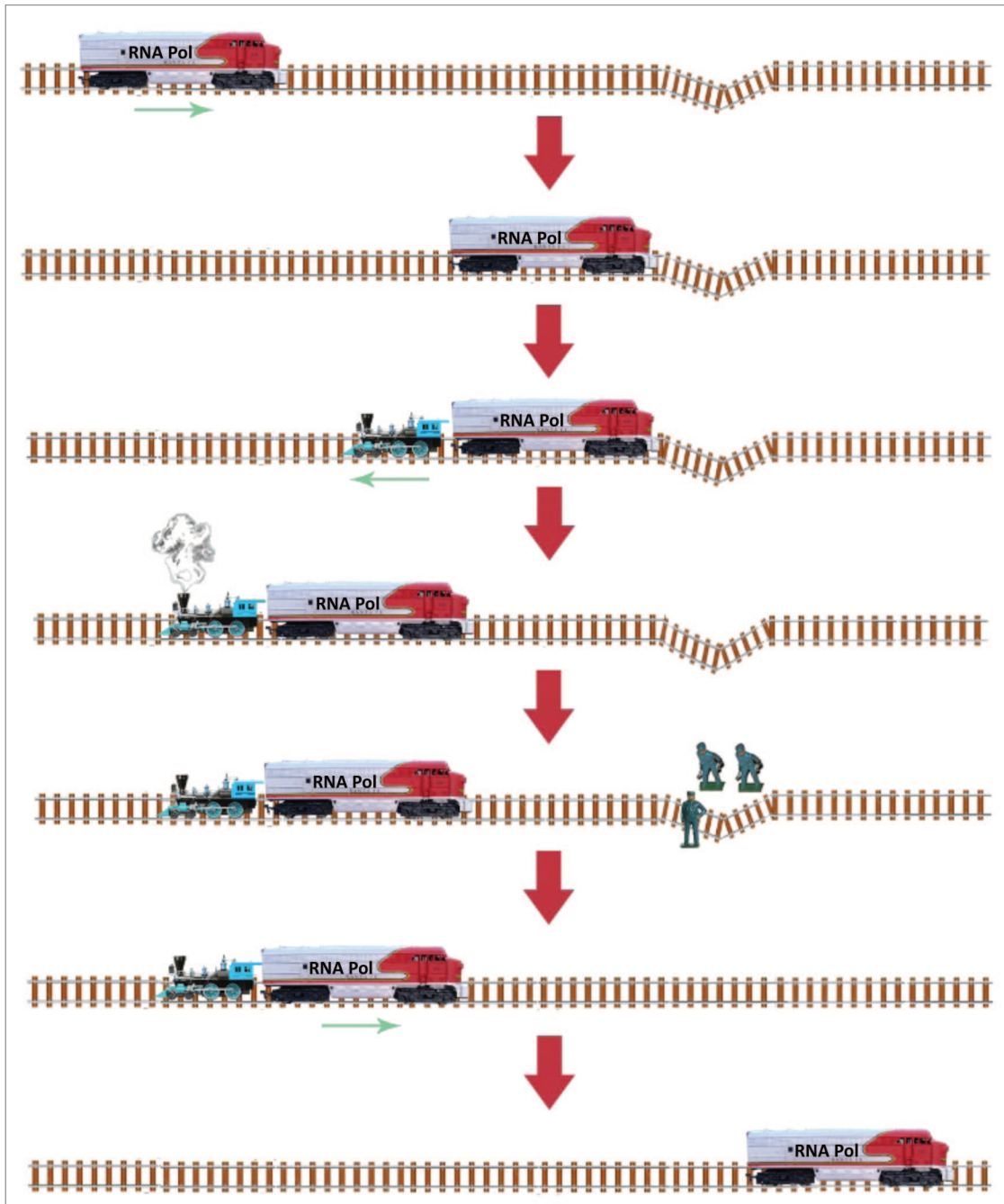


Figure 1. A simple way to envision the action of UvrD to promote RNA polymerase backtracking is the train engine analogy, in which a locomotive (RNA polymerase) encounters a railroad track buckle (DNA damage) and stalls. A second locomotive (UvrD in blue) is positioned at the rear end of the first locomotive, and the UvrD throttle is engaged to quickly and efficiently drag the stalled RNA polymerase locomotive away from the track buckle, so the repair crew can fix it. In this scenario, UvrD utilizes its force-producing ATP hydrolysis to haul the large 5-subunit RNA polymerase complex of ~400 kilodalton backward, overcoming the driving force of the elongating RNA polymerase to translocate again toward the damage and obstruct the buckle. Once the lesion is repaired, RNA synthesis can resume. Artwork by Jimmy Burril of the NIA Visual Media Section.

backward, the unwound strands rezip,² a phenomenon that is likely to contribute to the branch migration of the transcription bubble away from the lesion so the repair machinery can gain access.

*The Little Engine That Could*⁸ illustrates how a molecular motor can perform a seemingly difficult task. As the small switch engine bravely repeats “*I think I can, I think I can...*”, UvrD hauls the massive RNA

polymerase backward so the repair crew can go to work. Future studies that divulge more mechanistic insights and address how conserved the DNA repair pathway is in eukaryotes will be provocative.

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