4ct Collision between a DNA Replication Fork and an RNA Polymerase Ternary Complex

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In prokaryotic and eukaryotic organisms, DNA replication and RNA transcription proceed simultaneously on the same DNA molecule. In bacteria, the rate of DNA replication is approximately ten times faster than the rate of transcription; therefore, collisions between cellular DNA replication machinery and RNA polymerase ternary complexes are probable [1,2]. In eukaryotic genomes, close spacing between multiple replication origins and transcription promoter sites suggests that the non-destructive interplay between these bulky enzyme complexes is crucial for cell viability.

The general mechanism of DNA polymerase/RNA polymerase interaction is unknown, although previous *in vitro* studies have shown that replication forks can pass RNA polymerase molecules on DNA templates [1,2]. In addition to physical contact between the replisome and RNA polymerase ternary complex, the topology of the DNA template also plays a role [3,4]. RNA polymerase induces torque on the template DNA molecule, leading to positive supercoiling in the DNA ahead of the ternary complex. Knotted replication bubbles are observed in plasmids undergoing oppositely oriented DNA replication and transcription [3], suggesting another reason for nature to resolve head-on collisions if knotted DNA cannot be unraveled at a sufficient rate by cellular topoisomerases.

We study the collision between a DNA replication fork and an actively transcribing RNA polymerase molecule. We utilize a flow-stretched DNA assay coupled with fluorescence detection, allowing for simultaneous detection of the location of a replication fork and an RNA polymerase ternary complex on the same DNA template. This single molecule assay provides an excellent platform to study concurrent DNA replication and transcription on the same DNA molecule. In this manner, we can determine the duration of the pause (if any) in replication and the fate of the nascent RNA transcript. Furthermore, we can determine whether DNA topology or physical contact of enzymes contributes to pausing during collisions.

[1] Liu B., Wong M. L., Tinker R. L., Geiduschek E. P., Alberts B. M., *Nature*, **366**, 33 (1993). [2] Liu B. and Alberts B. M., *Science*, **267**, 1131 (1995). [3] Olavarrieta L. et al., *J. Mol. Biol.*, **322**, 1 (2002). [4] Schvartzman J. B. and Stasiak, A., *EMBO*, **5**, 256 (2003).