Le système de réplication du virus T4 et son redémarrage étudiés en pinces magnétiques.

The T4 replisome and its restart: a single molecule study using magnetic tweezers.

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Summary: The replisome is a central element of any replication system and is responsible for the separation and replication of the parent DNA strands. The T4 bacteriophage system offers a simple model for replication [1]. The replisome is formed by the primosome (the helicase (gp41) and primase (gp61) complex) and two holoenzymes (the polymerases (gp43) and their accessory proteins). Many questions remain concerning these complexes, including how the helicase, primase and polymerase activities and motions are coupled. Here we use magnetic tweezers to manipulate a single tethered DNA hairpin. The substrate extension is used as a real-time reporter of the replisome-activity. We have studied the coupling between the helicase and polymerase [2]. We have also investigated how the UvsW helicase can restart a stalled replication fork [6].

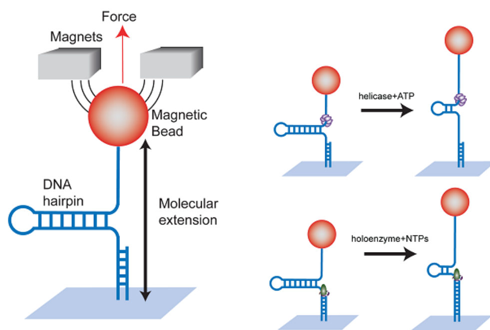

Fig.1. Schematic representation of the experimental set up.

Using a simplified version of a replication fork consisting of a DNA hairpin stretched by magnetic tweezers, we have investigated some of the components of the replisome of the T4 virus as well as their interactions. After characterizing the helicase activity alone we have followed the interplay between this helicase and the associated primase. Our results show that the primase can either dissociate from the helicase during priming or that a ssDNA loop can form between the two enzymes [3]. This is consistent with a looping mechanism, in which during priming the helicase follows translocation and a ssDNA loop is formed and released after priming is over.

The polymerase activity has been then investigated in the same DNA substrate. The hairpin substrate allows us to investigate both the strand displacement and primer extension activities of the holoenzyme. Bursts of holoenzyme activity are composed of two different phases. The first phase is given by an increasing extension generated by the polymerase displacing the 5' lagging strand in order to extend the dsDNA tail primer. The second phase corresponds to the polymerase reaching the end of the hairpin (loop region) and copying the rest of the ssDNA. We find that, as the primer extension rate depends moderately on the force, the strand displacement rate is extremely force sensitive. On the contrary, replication in presence of the helicase proceeds fast and do not depend on the applied force, demonstrating the presence of a very efficient coupling between polymerase and helicase. We have recently shown that the strand displacement synthesis of the polymerase depends upon the force applied to the fork: at high force the elongation is very efficient but at low force, the exonuclease turns the polymerase to remove the newly synthetized strand [4]. We shall show how these elements interplay in the leading strand synthesis [5]. We shall also show that we can use this behavior to reproduce Sanger sequencing at the single molecule level.

Finally we have been able to reproduce in vitro a fork reversal event produced by the UvsW helicase. This surprising enzyme can revert a stalled replication fork forming a holiday junction. With the help of a polymerase, the interrupted DNA synthesis is finished and the holiday junction is transform back to a normal fork by the same UvsW helicase [6].

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