Internship/PhD project

Dynaloops

Dynamics of DNA-RNA structures seen at the single molecule scale by nanopores and optical tweezers

Supervisors :
Cendrine Moskalenko et Fabien Montel (cendrine.moskalenko@ens-lyon.fr, fabien.montel@ens-lyon.fr) Laboratoire de Physique de l'ENS de Lyon.
Collaboration : Vincent Vanoosthuyse
Laboratoire de Biologie et de Modélisation de la Cellule, ENS de Lyon,).

Several activities, although essential for cell function, threaten the integrity of the somatic cell genome. Gene transcription, which produces messenger RNA from DNA, is one of these. By moving across the DNA to transcribe RNA, RNA polymerases can introduce distortions in the DNA double helix that can generate deleterious secondary structures. By interfering with primordial cellular functions such as transcription, DNA duplication or repair, these RNA-DNA secondary structures can compromise cell survival.

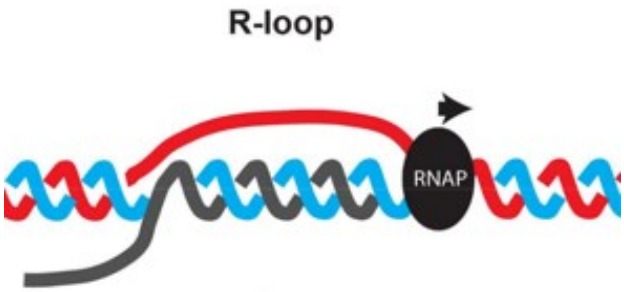

Fig.1: R-loops are formed when the nascent RNA (grey) hybridises with its DNA template (blue). The untranscribed DNA strand (in red) can no longer hybridize with the template strand (in blue).

The 'R-loop' is one of the structures that locally distorts the double helix and has the ability to compromise chromosome integrity. It is a three-stranded structure resulting from the abnormal hybridisation of nascent RNA to its DNA template (Fig. 1). Recent data have shown that only a small proportion of R-loops are cytotoxic. The intrinsic properties of these cytotoxic R-loops remain poorly understood. The objective of this project is to use a multidisciplinary biological/physical approach to better characterise and manipulate the physical properties of cytotoxic R-loops.

In a previous study the teams of V. Vanoosthuyse and C. Moskalenko showed that R-loops differ from each other in vitro by their 3D organisation [1]. Using atomic force microscopy (AFM), the team showed that the synthesis of a model cytotoxic R-loop results in the formation of complex structures of several hundred nm3 in volume containing several hundred nucleotides, whose physical properties are essentially determined by the 3D organisation of its single-stranded DNA (in red in Fig.1) and which constrain the surrounding DNA. Unexpectedly, for the same gene, the formation of R-loops can generate different structures. Similarly, sequencing of R-loops at the single-molecule level showed that the same gene could produce R-loops of different sizes. These observations suggested that each transcription cycle may generate a different type of R-loop, exposing a specific amount of single-stranded DNA.

It appears essential to determine precisely the thermodynamic and kinetic characteristics of these structures to better understand and predict their dynamics and stability. To do this, it is necessary to be able to determine the exact sequence of the single-stranded DNA involved in each structure observed.

Artificial and natural nanopores have been used in recent years as real molecular probes. The team from the Physics Laboratory of the ENS in Lyon is developing tools based on nanofluidics to study the dynamics of biomolecules. They have constructed an optical detection method that allows the transport of individual molecules through single nanopores to be followed in real time (Zero-Mode Waveguide for Nanopores, Figure 2b). These tools have enabled them to measure the transport energy landscape for DNA or RNA molecules [2,3,4,5] and viruses [6]. In addition, the team has just acquired a unique in France system coupling multi-trapping optical tweezers, a confocal microscope and a microfluidic control. For this project, it will allow us to measure the forces necessary to induce the

translocation of biomolecules and the destabilisation of RNA-DNA complexes (Figure 2a). It will also allow to quantify the variability of the structures obtained during destabilisation/reformation cycles.

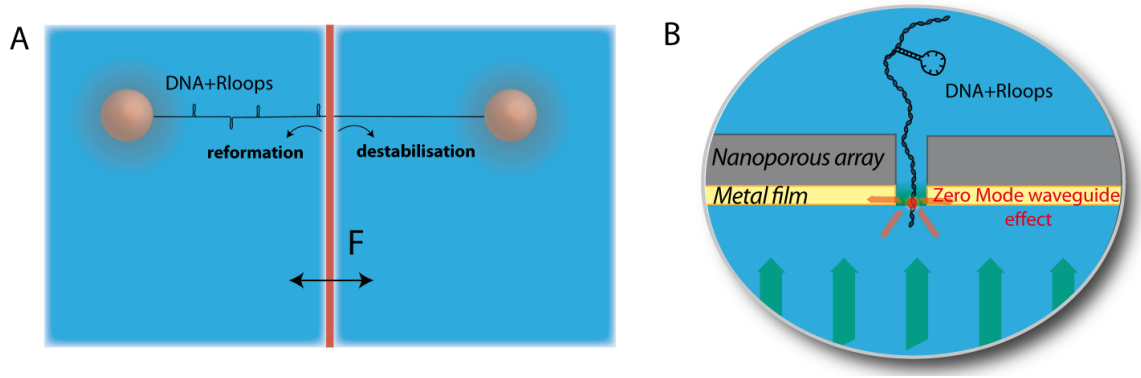

Figure 2: A) Rloops destabilisation/reformation cycle performed using the multi-trap optical tweezers (grey). A double-stranded DNA molecule (black) with one or more R-loops is trapped at both ends and manipulated to measure the destabilisation energy landscape. B) Characterisation of the Rloops structure by Zero-Mode Waveguide. Real-time, single-molecule monitoring of Rloops translocation across a nanoporous membrane will allow us to quantify their structure and stability.

The objective of the internship and/or PhD project is to use nanopore and optical tweezer techniques to determine the stability and sequence of the single-stranded DNA involved in R-loop structures at the single-molecule scale. This will allow the size and sequence of R-loops to be matched to their ability to form 3D structures, thus greatly increasing our understanding of the internal organisation and thermodynamic parameters associated with different types of R-loops.

This combined strategy will also be applied to determine the kinetics of R-loop formation during transcription by RNA polymerase and to study the role of potential cofactors. Ultimately, this work will make it possible to determine precisely the link between the physical properties of R-loops and their toxicity. It will also allow us to better predict the genomic regions likely to form toxic R-loops.

References :

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