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Final report for TOPO project

Crystal structure of human TopIIIα/RMI 1 complex

The Bloom (BLM) complex composed of TopIIIα, RMI1 and 2 in higher eukaryotes in conjunction with the RecQ BLM helicase assists in the **dissolution** of converging **Holiday junctions** in a non cross-over fashion [1-3]. This specialized reaction is carried out by a typeIII topoisomerase (T**opIIIα**), with help of tightly associated accessory protein **Rmi1** [4-7] in yeast and the RMI1-RMI2 complex in metazoan cells [2]. How Rmi1 modulates TopIII function to serve in dissolving convergent **Holiday junctions** has so far remained elusive. Dissolution of homologous recombination intermediates without any genetic material exchange is a way to prevent DNA deterioration and to improve genome stability. A deep understanding at a structural level would help to decypher the molecular mechanisms underlying BLM complex function. The main goal of my proposal was to crystallize and to determine the structure of the full or partial Bloom complex. This report will the two years under the Marie Curie fellowship at the FMI in the Structural Biology lab, directed by Nicolas Thomae and presents the **3.3 Å structure** of the human RMI1 N-terminus bound to the TopIIIα topoisomerase. The structure shows that RMI1 attaches to the **gate** of the topoisomerase with help of its **oligonucleotide binding OB-fold**. Moreover the human TopIII is the first high resolution structure obtained for a type III mamalian topoisomerase showing its conserved overall toroïdal architecture as well as its catalytic site. This structure allows us now to imagine a plausible single strand DNA (ssDNA) passage model to catalyse the dissolution reaction in concert with RMI1.

Overall architecture. Several TopIII-RMI interfaces coexist due to the crystal packing but according to interface analysis (PISA) and already published data which involved the RMI1 OB domain as well as an insertion loop [4,9,10] inside this OB domain in the binding to TopIIIα, the real biological interface (confirmed recently by our EM experiments, data not shown) between the two proteins is shown in the figure 1.

The human TopIIIα protein retain all the structural features which have been highlighted by the *E.coli* Top1A [8], IIII [11,12] and the *Thermotoga maritima* Top1A [13] crystal structures. The crystallized protein is composed of four domains named 1 to 4 (fig1) : domain 1 is the toprim (primase fold) domain carrying the acidic cluster involved in the catalytic site, domain 2 forms the so called gate made by two antiparallel topofold domains [14] and generating a ~25 Å diameter hole in the center of the protein, domains 3 and 4 share the same 'Winged helix' fold (also named CAP domains for catabolite activator protein) [15]. While domain 4 does not carry any catalytic residues, domain 3 possesses the conserved catalytic Tyrosine responsible for the cutting of ssDNA [12]. It is important to note that the C-terminal region including a putative Zinc finger domain potentially involved in DNA binding and predicted to be highly similar to the *Thermotoga maritima* Top1A C-terminus is not unambigously buildable in our structure even if some electronic densities are visible. The N-terminal RMI1 is composed of a three-α helices bundle followed by a typical OB domain fold consisting in a β-barrel as already published in [9,10]. The complex has a 'Door-knocker' overall shape, RMI1 contacting TopIII at the top of the gate (mainly α9 and the β9- α9 linker) via its OB domain. The so called RMI1 insertion loop (between α3 and β3) creates two other contacting zones to TopIII, the β10, β12 and β12-β13 linker in domain 2, α10 in domain 3, and α8 and 15 as well as α8-β9 linker generating a spread interface and obstructing partially the hole made by TopIII domain 2. This interface involve hydrogen bonds as well as hydrophobic interfacing residues, and one salt bridge for a total interface area of 1319 $Å^2$.

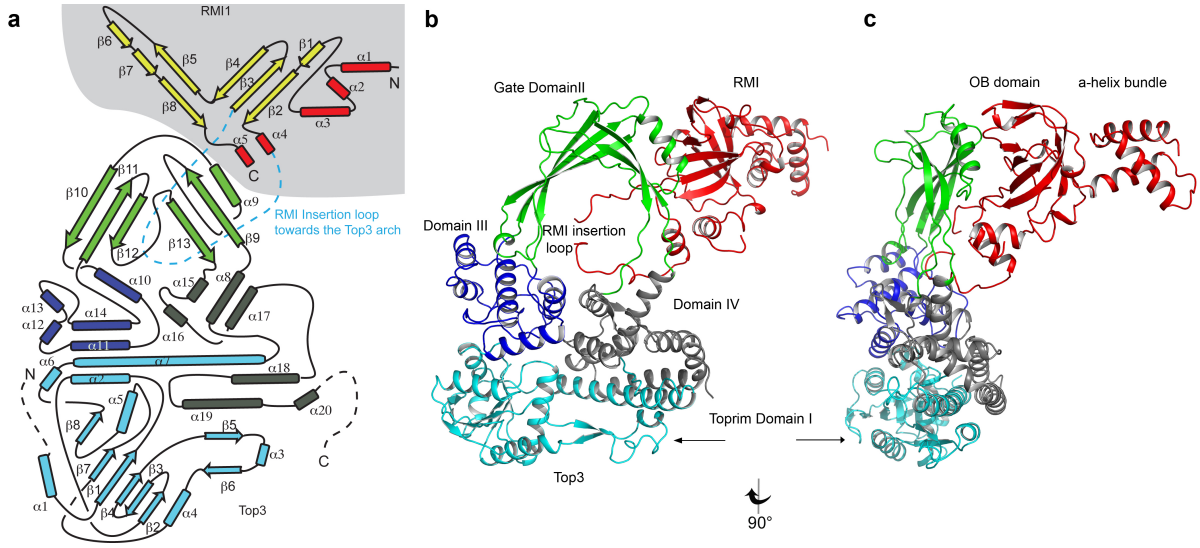

Figure 1) Overall TopIIIα-RM1 human complex architecture. a) Overall secondary structure architecture of the complex. The TopIIIα protein is coloured by domains (1 to 4, lighblue Toprim domain, in green the gate, darkblue is the 5Y-CAP catalytic domain, in grey the CAP domain 4). RMI1 is shown in the grey corner coloured in red (alpha-helices) and yellow (β-strands). The insertion loop creating the main interface area is represented in lightblue dashed line. All dashed lines correspond to partially built regions. b) Unfinished model represented in cartoon from the TopIIIα side. Same colour code than a except for RMI 1 shown in red here. c) TopIIIα-RMI1 complex side view.

New mechanistic insights. The novelty of this work is to see where RMI1 sits on TopIIIα. RMI1 contacts TopIIIα at the top of the gate via the upper part of the OB domain giving to the complex a general **'Door-knocker'** shape. The RMI1 insertion loop adopts an alternative conformation compared to the unbound RMI1 [9,10] structure and plunges in the middle of the TopIIIα gate creating two additional zones of contact. A comparison to the *E.coli* Top3 crystal structure highlights a striking difference. In *E.coli* the central cavity has two ledges formed by the 241-255 loop at the top of the gate and the decatenation loop at the intersection of domain 3 and 4 [11] which are not present in the human TopIIIα structure. TopIIIα appears then to be a structural hybrid of typeIA and typeIII topoisomerase, with an enzymatic body (domains III and toprim) having **typeIII** character, while the gate is more closely related to **typeIA** enzymes. Interestingly RMI1 sits exactly where those missing loops should be as if RMI1 would convert a type1A to a type III. This idea is consistent with the fact that RMI1 is known to stimulate the decatenation activity of TopIIIα. The RMI insertion loop restricts the diameter of the central gate suggesting a model whereby the RMI1 loop modulates the substrate specificity allowing **only ssDNA to** enter the gate. Moreover it has been shown that RMI strongly stimulates the decatenation activity [17,18] and is indispensable for Holiday junction dissolution. We propose a putative model where RMI would act in a concerted manner by increasing the efficiency of the catalysis, perhaps via the created interface at the top of TopIII domain 4. RMI would also modify the strand passage activity either by modifying the open-closed equilibrium of the topoisomerase itself or by accelerating the time spent by the passenger DNA inside the TopIII gate due to the RMI insertion loop obstruction. In order to investigate the role of the RMI insertion loop, I developped last year two types of experiments. In collaboration with Petr Cejka, I set-up a *in vitro* test of DNA relaxation and catenation reproducing the previous observation that RMI1 stimulates catenation (see figure 2) and inhibits relaxation in the context of the ternary Top3-RMI1-Sgs1 complex [17]. The aim now is to purify some RMI1 insertion loop mutants to assign a functional role to the

RMI1 insertion loop. I am also working on an *in vivo* experiment by generating genetically modified yeast strains (*Saccharomyces cerevisiae*). All the strains are now made and a genotoxic sensitivity screen is on going. On the ΔRMI 1 strain, we can already detect a sensitivity to MMS (Metyl MethaneSulfonate). In order to see if the phenotype can be explained by the insertion loop, we engineered a RMI1 modified strain where the RMI1 insertion loop is flanked by two TEV sites. By transforming this strain with a TEV overexpression plasmid whose expression is under the control of a galactose promoter, we can tune the removal of this specific loop in the yeast cell by plating the transformant strains on galactose plates. We already did a first genotoxic screen and detected a light sensitivity to MMS once the insertion loop has been removed, a phenotype close to what we have seen for the ΔRMI 1 (data not shown). All together, these preliminary results tend to show that the catenation/decatenation activity stimulation due to the presence of RMI1 can be summarized by the RMI1 insertion loop.

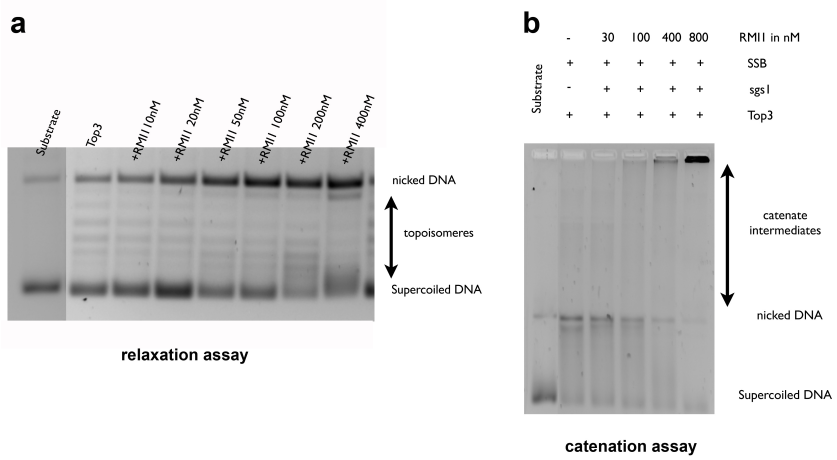

Figure 2) ***In vitro*** **DNA relaxation and relaxation assays**. a) RMI1 inhibits the DNA relaxation activity of Top3. b) RMI1 stimulates the catenation of a substrate plasmid. To allow the reaction all components are required, SSB (single strand binding protein) Sgs1 and Top3 while the concentration of RMI1 is increasing.

Towards a structure of the complete yeast STR complex.

BLM /sgs1 helicases are expected to provide the ssDNA substrate to TopIII at a replication fork. A structure of such a complex would allow to better understand the concerted role of the three proteins in dissolving Holliday junctions. In the last year, big efforts have been made to crystallize a ternary complex including a BLM helicase fragment. As the BLM binding epitope to TopIII is predicted to be in an poorly structured region of the helicase, we mapped the minimal binding epitope by a combination of limited proteolysis coupled to size exclusion chromatography and mass spectrometry analysis. This fragment, identified as the extreme N-terminal 56 first amino acids, can be coexpressed with the TopIII /RMI complex showing a tight binding (see midterm report). Some diffracting crystals have been obtained and a dataset processed up to 4.6 Å. Unfortunately the quality of the caculated maps did not allow to determine the BLM binding epitope. Due to the low stability, homogeneity of the complex and the low reproducibility of the crystals, we aim now to identify among several orthologs (*Xenopus laevis*, *Mus musculus*) a more suitable complex to lead crystallographic studies.

Finaly, electronic microscopy (EM) is foreseen in order to study the entire Bloom complex in budding yeast (Top3-RMI1-Sgs1) as well as in human (TopIIIα-RMI1-RMI2-BLM) possibly in presence of various DNA substrates as Holiday junctions or similar substrates mimicking the homologous recombination intermediates. The first bottleneck to overcome has been to

produce the full length Sgs1 in complex with Top3-RMI1. As co-expression is mandatory to produce a stable complex in insect cells, we designed polycistronic bacmids (figure 3a) containing the full-length tagged proteins (flag or MBP-Sgs1/Strep-Top3/His-RMI1 for *S.cerevisiae* complex, and flag-BLM/strep-Top3α/His or strep-RMI1/RMI2 for the human complex) and a DsRed fluorescent reporter. We optimized the purification process for the yeast complex, which has been subjected to a strep affinity step, an ion exchange column allowing to separate the Top3-RMI1 complex from the ternary complex (see fig 3 a, b and c), a size exclusion chromatography and a Grafix sedimentation separation (with weak glutaraldehyde cross-linking) for homogeneity.

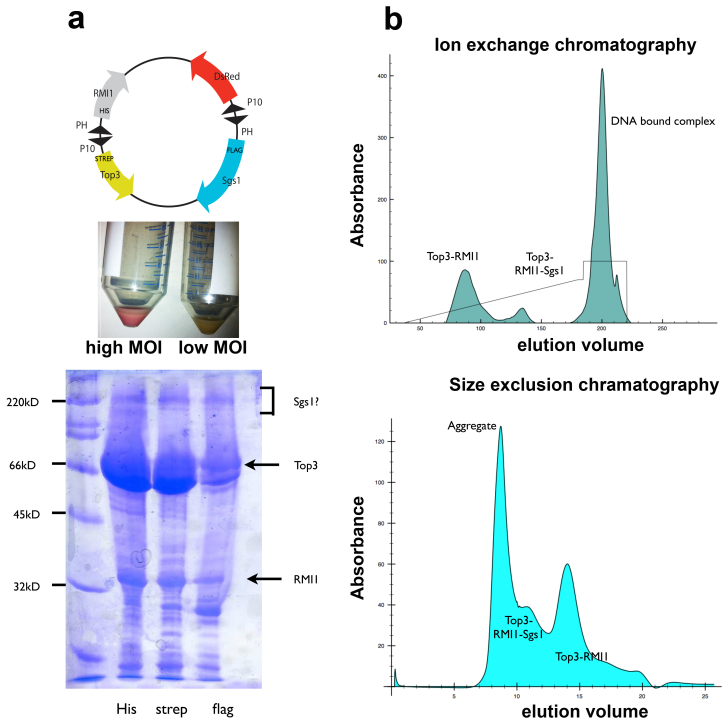

Figure 3) Expression of the yeast full Bloom complex. a) Polycistronic bacmids used to express the full Bloom complex. The pellet of the cells show the Ds Red colour especially when low MOI (Multiplicity Of Infection) are used for infection. The SDS-PAGE gel represents a pull-down experiment : flag tag is on Sgs1, Strep tag is on Top3 and His tag is on RMI1, showing the physical interaction between the three components of the Bloom complex. b) The two additional purification steps allowing to purify the ternary complex in order to perform EM analysis.

The complex has been imaged and the particle selection is ongoing in order to reconstruct in 3D a model of the full complex. A systematic comparison between human and yeast complexes as well as combination of EM and crystallography (with this already solved structure and possibly the structure of a ternary complex) should allow us to propose a model of the full Bloom complex at a reasonable resolution to deeply understand the mechanistic behind.

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