



Chromatin remodeling : RSC motors along the DNA.

Terence Strick, Audrey Quessada-Vial

► To cite this version:

Terence Strick, Audrey Quessada-Vial. Chromatin remodeling : RSC motors along the DNA.. Current Biology - CB, Elsevier, 2006, 16(8), pp.R287-289. <10.1016/j.cub.2006.03.040>. <hal-00089431>

HAL Id: hal-00089431

<https://hal.archives-ouvertes.fr/hal-00089431>

Submitted on 18 Aug 2006

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Chromatin remodeling : RSC motors along the DNA

Terence Strick and Audrey Quessada-Vial

Biomolecular Nanomanipulation Lab

Institut Jacques Monod, Centre National de la Recherche Scientifique and Universités de
Paris VI and Paris VII
2 Place Jussieu 75251
Paris Cedex 05 France
strick@ijm.jussieu.fr

Single-molecule experiments show that the RSC chromatin remodeling complex, a member of the SNF2 ATPase family, induces formation of a negatively supercoiled DNA loop by active translocation.

Chromatin remodeling complexes such as RSC and SWI/SNF use ATP hydrolysis to modify nucleosome structure and thereby regulate DNA function (for a review see [1]). This could involve altering the position or stability of nucleosomes along DNA, for instance, via physical interaction with the histone or even modification of its octamer composition [2]. An oft-discussed possibility is that chromatin remodeling complexes could translocate DNA, directly pushing nucleosomes along or off of the DNA and even perhaps modifying the underlying higher-order DNA structure to further alter the binding stability of the nucleosome [3,4]. This hypothesis is supported by experimental work showing that DNA structures, such as supercoiling and looping, can be induced by chromatin remodeling complexes [3,5]. Understanding how chromatin remodeling can affect gene regulation would also require additional insight into the kinetics of the process: how quickly can such changes be generated, and how stable are they? These questions are not easily answered using classical biochemical techniques, but new experimental work using real-time single-molecule DNA nanomanipulation begins to address these issues.

In a recent issue of *Molecular Cell*, Lia et al. [6] describe using a magnetic-trap based single-molecule DNA nanomanipulation setup to study the interactions between a single RSC complex and a single, 3.6 kb linear DNA molecule. Single-molecule DNA nanomanipulation has gained widespread interest for its applicability to the real-time study of protein-DNA interactions. By attaching one end of a linear DNA molecule to a glass coverslip and the other end to a small bead, the DNA can be mechanically manipulated (i.e. stretched and twisted) by acting on the bead with a magnetic tweezer. The end-to-end extension of the nanomanipulated DNA is determined in real-time by measuring the position of the bead above the surface, and is a robust metric for the conformational state of the DNA molecule. Such single-molecule techniques are particularly useful for the study of DNA translocases, enzymes which use the energy of nucleotide hydrolysis to drive themselves along the DNA in a directional manner. Indeed whereas some translocases such as RNA polymerase advance base-by-base [7], rotate

with the DNA double-helix [8] and leave behind an easily identifiable, easily quantifiable biochemical product, the majority of translocases may in fact couple their motion to DNA structure in a nontrivial fashion and not produce anything more than ephemeral, physical work (i.e. movement along DNA) [9]. Using an appropriate experimental geometry, enzyme translocation can be simply detected in real-time by monitoring the resulting changes in the nanomanipulated DNA's end-to-end extension.

When Lia *et al.* placed the nanomanipulated DNA in the presence of RSC complex and ATP, they observed transient shortenings in the length of the molecule, corresponding to ATP-fuelled translocation of hundreds of base pairs through a DNA-bound RSC complex and the resulting formation of a DNA loop. This indeed implies that the RSC complex has at least two DNA binding sites : one which actually remains fixed on the DNA, and the other which translocates the DNA, driving loop formation. Loop sizes were normally distributed with a mean of about 500 bp (about 110 nm), and even kilobase-sized loops were occasionally observed. Loop extrusion was rapid, short-lived and reversible: it typically took place in a second or so, then remained fixed for several seconds before reversing either by abrupt dissociation of the complex or reverse translocation of the motor. Loop length increased moderately with increasing ATP concentrations. Low RSC concentrations and nicked DNA were chosen to ensure that the shortenings were due to a single RSC complex translocating (and not rotating) the DNA molecule. This choice was confirmed by AFM observations of a single RSC complex inducing loop formation on a single DNA in the presence of ATP. Furthermore, gel analysis of combined RSC/topoisomerase reactions suggests these loops are unconstrained and do not result from stable wrapping of DNA about the protein.

Lia *et al.* also found that these reversible shortenings were largely dependent on the applied stretching force: increasing the force decreased the size of the loops, such that no loop formation could be detected against a force of about 2 piconewtons. Note that the weak forces used here (from 0.1 to 2 piconewtons) do not deform the regular B-DNA structure of the nanomanipulated molecule, and are expected *in vivo*. Thus the RSC motor complex truly translocates DNA, even despite a (moderate) opposing mechanical force, and this mechanical work is coupled to a rate-limiting step of the enzyme cycle (otherwise the reaction would not be affected by changes in the applied force).

By working with unnicked DNA molecules to evaluate the effect of DNA supercoiling on formation of the loops, Lia *et al.* also analyzed how RSC causes DNA to rotate as it is actively transported through the complex. They found that during loop extrusion positive supercoils form in the untranslocated portion of the DNA and that, consequently, negative supercoils are formed in the translocated loop. Although this means that DNA rotates in a right-handed fashion through the translocating portion of the RSC complex, coupling between DNA rotation and translocation is not "one-to-one" for RSC. Unlike DNA-groove-tracking proteins such as RNA polymerase [8] or the EcoRI type I restriction endonuclease [Seidel], which rotate the DNA a full turn for every ten base-pairs translocated, here the rate of DNA rotation appears bounded between twenty and fifty degrees for every ten base-pairs translocated. This suggests that the stepsize of RSC along DNA is about twelve base pairs (or multiples thereof). It is noteworthy that similar behaviour was observed with the FtsK bacterial chromosome partitioning complex [9].

Surprisingly, although loop length increased with ATP concentration, supercoiling built up in the looped region appeared to decrease as ATP concentration increased. Lia *et al.* suggest this could be due to torsional “slippage” of the DNA as it rotates through the enzyme: as the motor velocity increases perhaps it cycles too quickly to fully couple translocation and rotation, allowing supercoils to “leak out” of the loop. In addition, less supercoiling was found in the looped region formed on a positively supercoiled DNA template than that formed on negatively supercoiled DNA. Because it is energetically costlier to translocate positively supercoiled DNA than negatively supercoiled DNA, this could also be a result of increased slippage in conditions where loop growth is less favourable.

Using a single-molecule DNA nanomanipulation technique, Lia *et al.* succeeded in demonstrating that the RSC chromatin remodeling complex can actively and reversibly translocate DNA, causing a negatively supercoiled loop of DNA to form. This offers experimental support for several models according to which this remodeler may act to reorganize nucleosomes: they may indeed be displaced by the passage of the RSC complex, or they may be destabilized by the transient changes in topology and mechanical strain of the DNA they are associated with. These experiments offer a tantalizing vision of the way in which chromatin remodelers work with DNA. Although the reaction may be different in the presence of nucleosomes, the observation that the BRG1 and Brm catalytic subunits of human SWI/SNF homologs generate similar DNA distortion as RSC points to a common mechanism for these ATP-fuelled molecular machines.

REFERENCES

- [1] Becker, P.B. and Hörz, W. (2002). ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* 71: 243-273.
- [2] Flaus, A. and Owen-Hughes, T. (2004). Mechanisms for ATP-dependent chromatin remodeling: farewell to the tuna-can octamer? *Curr. Opin. Genet. Dev.* (14) :165-173.
- [3] Havas, K., Flaus, A., Phelan, M., Kingston, R., Wade, P.A., Lilley, D.M., and Owen-Hughes, T. (2000). Generation of superhelical torsion by ATP-dependent chromatin remodeling activities. *Cell* 103,1133-1142.
- [4] Saha, A., Wittmeyer, J., and Cairns, B.R. (2002). Chromatin remodeling by RSC involves ATP-dependent DNA translocation. *Genes Dev.* 16, 2120-2134.
- [5] Bazett-Jones, D.P., Cote, J., Landel, C.C., Peterson, C.L., and Workman, J.L. (1999). The SWI/SNF complex creates loop domains in DNA and polynucleosome arrays and can disrupt DNA-histone contacts within these domains. *Mol. Cell Biol.* 19, 1470-1478.
- [6] Lia, G., Praly, E., Ferreira, H., Stockdale, C., Tse-Dinh, Y.C., Dunlap, D., Croquette, V., Bensimon, D., and Owen-Hughes, T. (2006). Direct observation of DNA distortion by the RSC complex. *Mol. Cell.* 21, 417-425.

- [7] Abbondanzieri, E.A., Greenleaf, W.J., Shaevitz, J.W., Landick, R., and Block, S.M. (2005). Direct observation of base-pair stepping by RNA polymerase. *Nature* *438*, 460-465.
- [8] Harada, Y., Ohara, O., Takatsuki, A., Itoh, H., Shimamoto, N., and Kinoshita, K. Jr. (2001). Direct observation of DNA rotation during transcription by *Escherichia coli* RNA polymerase. *Nature* *409*, 113-115.
- [9] Saleh, O.A., Bigot, S., Barre, F.X., and Allemand, J.-F. (2005). Analysis of DNA supercoil induction by FtsK indicates translocation without groove-tracking. *Nat. Struct. Mol. Biol.* *12* : 436-440.
- [10] Seidel, R., van Noort, J., van der Scheer, C., Bloom, J.G., Dekker, N.H., Dutta, C.F., Blundell, A., Robinson, T., Firman, K., and Dekker, C. (2004). Real-time observation of DNA translocation by the type I restriction modification enzyme EcoR124I. *Nat. Struct. Mol. Biol.* *11*, 838-843.