RSC remodeling of oligo-nucleosomes: an atomic force microscopy study

Fabien Montel, Martin Castelnovo, Hervé Menoni, Dimitar Angelov, Stefan Dimitrov and Cendrine Faivre-Moskalenko

Supplemental Material

1- Few mononucleosomes are induced by RSC action on dinucleosomal templates

We show here the sliding reaction of dinucleosomes as a function of RSC volume, including the counting of mononucleosomes during RSC mobilization of dinucleosomes (Fig. S1a). Mononucleosomes are observed only in 2 positions: either within 601 position or at the end of DNA template. For each condition, the mononucleosome proportion corresponds to the total number of mono-nucleosomes. Fig. S2 displays the detailed composition of mononucleosome population throughout the sliding reaction of Fig.S1a.

For zero or low RSC/nucleosome ratio, only 601 positioned mononucleosomes are found, that correspond to slightly undersaturated dinucleosome reconstitution. This number is less than 10% of the total number of nucleosomal template at room temperature. Note that for large reaction coordinate, most of the mono-nucleosomes are end-positioned.

We observe mainly two features: (i) the proportion of mononucleosomes does not increase as soon as RSC produces new states (#2 to #5), but only when states #4 and #5 become significant.

(ii) the rate of new mononucleosome appearance seems to follow the rate of production of state #5. This suggests that new mononucleosomes are mainly produced through the RSC action on state #5 nucleosomes by ejection of one end-positioned nucleosome of the di-nucleosomal template.

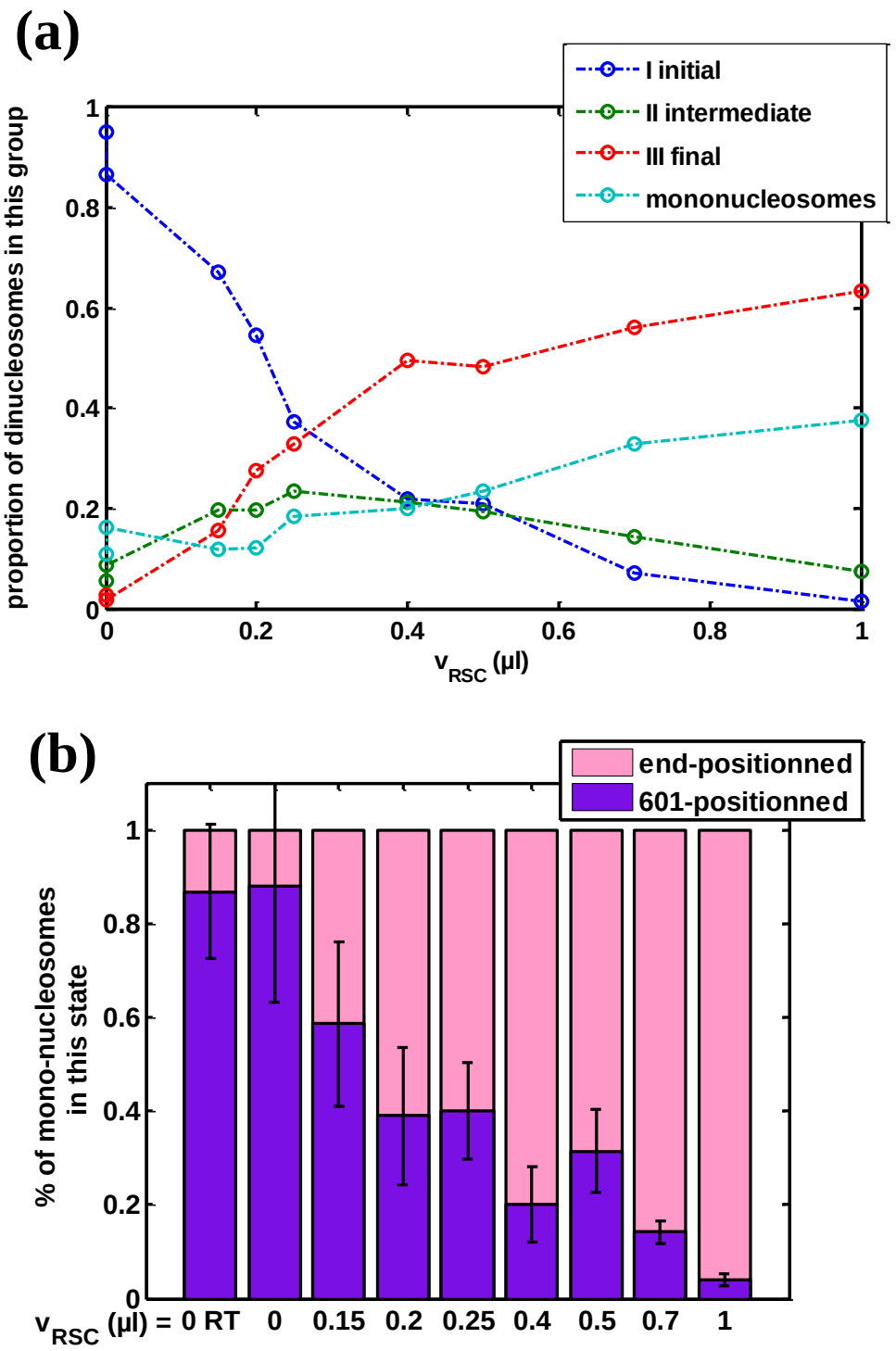

Figure S1 : Population distribution of di- and mononucleosomes during RSC action.
(a) The proportion of dinucleosomes in each group of states (I initial, II intermediate and III final) as well as the proportion of mononucleosomes is reported as a function of RSC volume. (b) For mononucleosomes, the total amount shown on fig.S1a is decomposed into the proportion that is 601-positioned and the other part that is end-slided.
Note that v_{RSC} = 0.4µl corresponds to a 1/40 RSC to nucleosome ratio.

2- RSC does not need a free DNA end to slide mononucleosomes.

To complement the results presented in the manuscript on streptavidin labeled mononucleosomes sliding by RSC, we performed experiments with circular templates by adding a new biotin tag to the other end of DNA (compared with the previous situation with only one biotin tag at an end and therefore one single binding site for the streptavidin.).

With this double-tags construction and for low streptavidin concentrations, the major conformation of reconstituted nucleosomes is circular one (Fig. S2a), one streptavidin being attached to both ends of the DNA. We performed RSC remodeling reaction on this nucleosomal substrate, and the results are presented in supplemental Figure S2. For linear streptavidin labeled templates, 93% of the nucleosomes are slided in the +RSC condition. We would like to note that this population is actually composed of 65% mononucleosomes slided away from the streptavidin (state #α) and 35 % mononucleosomes slided against the streptavidin (state #β) as it is shown in fig. 6b of the manuscript. For circular templates, there is a single slided position that is against the streptavidin (the template is not anymore oriented), and 88% of the mononucleosomes in the +RSC condition are in this state. The main conclusion about these new experiments is that RSC does not need any DNA end in order to mobilize nucleosomes, showing similar efficiency for both linear and circular templates in the same sliding conditions (same RSC/nucleosome ratio, ATP concentration, Temperature and incubation time, etc).

The high sliding efficiency observed for circular template also strengthens the message of our work about the arrest of RSC-induced nucleosome motion by physical obstacles: indeed the only physical obstacle in this construction being able to stop the RSC-induced motion is the streptavidin.

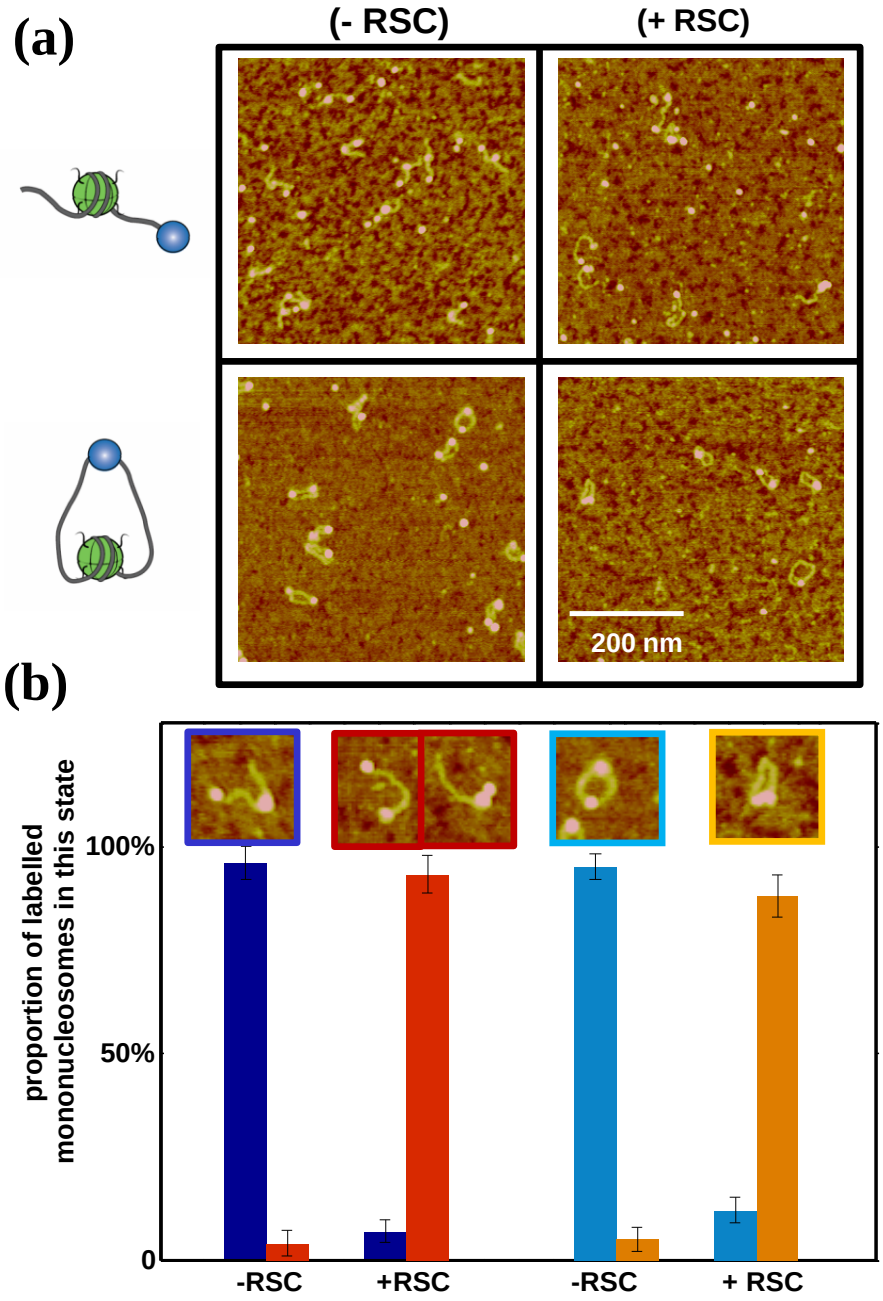

Figure S2 : Comparison of RSC sliding efficiency for linear (single biotin tag) or circular (double biotin tag) streptavidin labeled mononucleosome .
(a) typical AFM images of single tag linear (top) and double tag circular (bottom) streptavidin labeled mononucleosomes without (left) or with (right) RSC.
(b) Counting of streptavidin labeled mononucleosomes in each state: linear-601-positioned (dark blue), linear-end-positioned (red), circular-601-positioned (light blue), circular-end-positioned (orange). Typical AFM image of each stat is shown with the corresponding colored frame. The number of mono-nucleosomes analyzed in this experiment is: N(-RSC) = 468 and N(+RSC) = 766 for linear streptavidin labeled mononucleosomes, N(-RSC) = 499 and N(+RSC) = 277 for circular streptavidin labeled nucleosomes.

Supplemental methods: *Both ends of the 601 DNA template (of total length 356 or 311 bp) is biotin labeled using 5'-biotinylated primers during PCR amplification of the 601 DNA*

fragment from (plasmid pgem.). Biotinylated DNA is then added to histone mix for salt dialysis mononucleosome reconstitution. Streptavidin labeling, mononucleosome sliding and AFM imaging is performed as described in the Material and Methods section of the manuscript.