Molecular Cell, Volume *27*

Supplemental Data

Nucleosome Chiral Transition under Positive Torsional Stress in Single Chromatin Fibers

Aurélien Bancaud, Gaudeline Wagner, Natalia Conde e Silva, Christophe Lavelle, Hua Wong, Julien Mozziconacci, Maria Barbi, Andrei Sivolob, Eric Le Cam, Liliane Mouawad, Jean-Louis Viovy, Jean-Marc Victor, and Ariel Prunell

Supplemental Discussion

1-Dimers removal by heparin

Heparin, a strong acidic polyelectrolyte, releases H2A-H2B dimers sequentially from mononucleosomes reconstituted on DNA minicircles, giving rise to hexasomes and tetrasomes (Figure S1A and legend). Heparin concentrations from 0.01 to 100 µg/mL were initially tested in the single-fiber experiment. The fiber length was not much affected up to 2 µg/mL, but it increased above 5 µg/mL (not shown), and nucleosomes were destroyed at 100 µg/mL (Bancaud et al., 2006). We tentatively chose a mild treatment at 1 µg/mL and obtained the red curve in Figure S1B, panel 2, not much different from the initial forward curve (blue). Following the excursion at large positive torsion, a reproducible curve quite similar to the initial green curve was generated, which could be run back and forth several times without hysteresis (purple in panel 3). The merging of purple and blue curves at negative torsions, like the merging of green and blue curves, indicates that canonical nucleosomes reform, and thus that dimers are still present. These nucleosomes, however, switch to the altered form as soon as the constraint becomes positive, presumably as a result of an heparin-facilitated transition. Nucleosome core particles were subsequently added

as histone acceptors (Voordouw and Eisenberg, 1978), leading to a ~650 nm extension of the fiber (purple curve in panel 3), i. e. ~24 nm per total nucleosome. This suggests that all nucleosomes in the fiber have been unwrapped by ~1 turn of DNA as a consequence of the loss of their dimers.

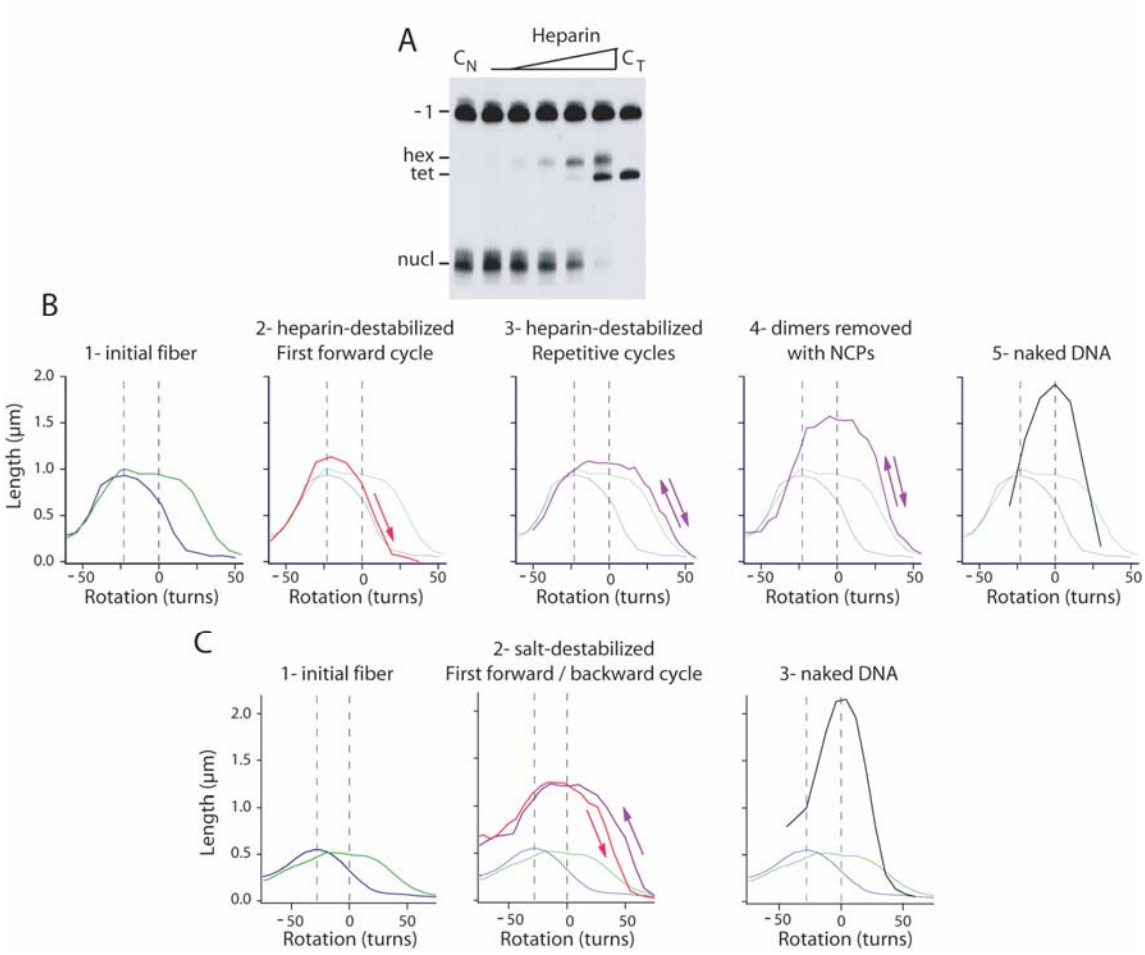

Figure S1. H2A-H2B release with heparin/core particles, or salt

(A) Mononucleosomes were reconstituted, incubated with 0, 0.4, 0.8, 1.8 and 3.9 µg/mL heparin (Sigma) in B_0 at 37°C for 10 min, and electrophoresed as described in legend to Figure 3A in main text (C_N: starting mononucleosomes; C_T: control $(H3\text{-}H4)_2$ tetrasomes). Nucleosomes (nucl) progressively vanish upon increase in the heparin concentration, to the benefit of hexasomes (hex: a single turn of DNA around the $(H3\text{-}H4)_2$ tetramer plus one H2A-H2B dimer) and tetrasomes (tet). "-1": residual unreconstituted topoisomer.

(B) Extension-*vs.*-rotation behaviour of a "190-bp" fiber at 0.35 pN through the successive steps of the assay (see Supplemental discussion). (1): Forward and backward curves of the initial fiber in B_O. (2): First forward curve in the presence of B_0+1 µg/mL heparin (red). (3): Following the application of a large positive torsion in the red curve of panel 2, the back and forth responses stabilize on the purple curve. (4): Response (purple) obtained after flushing the flow-cell with 1 µg/mL nucleosome core particles (NCPs; reconstituted on chicken ~146 bp DNA originating from native core particles with purified octamers from the same source)

in B_0 plus 50 mM NaCl, and rinsing with B_0 after a 5 min incubation. (5): Corresponding DNA response after depletion of all histones with 100 µg/mL heparin and return to B_O (black).
(C) Extension-*vs.*-rotation behaviour of a "208-bp" fiber at 0.4 pN. (1): Forward and backward curves of the initial fiber in B_0, showing a "positive" shift of +35 turns. (2): Forward response obtained after flushing the flow-cell with B_0 plus 700 mM NaCl, and rinsing with B_0 (red), and backward response after excursion at high positive torsion in the red curve (purple). No second torsion cycle was recorded. The ~700-nm increase in the fiber length correlates with a 22- (35-13) turn decrease in the "positive" shift. (3): Corresponding DNA response after depletion of all histones with 100 µg/mL heparin and return to B_0 (black).

2-Energy landscape of the transition

a) *Theory*

Forward and backward curves of the torsional response correspond to limits at which all regularly-spaced particles were assumed to be either nucleosomes or reversomes (see main text). In a steady-state equilibrium, in contrast, the two states must coexist, and the fiber length must lie in between the two curves.

If the topological contribution of the linkers, i.e. of nucleosome arrangement in the fiber, is neglected, the fiber topology (ΔLk_{fiber}) can be expressed as:

$$\Delta Lk_{fiber} = N_{nucl}\Delta Lk_{nucl} + N_{rev}\Delta Lk_{rev} \qquad \text{(S1)}$$

where ΔLk_{nucl} ~-0.4 and ΔLk_{rev} ~+0.9 are the individual DNA topological deformations of positively-crossed nucleosomes in the positive plectonemic regime of the torsional response and of reversomes, respectively, and N_{nucl} and N_{rev} their numbers. The length of the fiber and its rotation status, ΔLk_{fiber}, at steady state define one point, t_{fiber}, in between the blue and green curves of the torsion plot (scheme in Figure S2). The horizontal line going through t_{fiber} intersects the curves

at positions t_{nucl} and t_{rev}, the abscissa of which correspond to the topology of an all-nucleosome or all-reversome fiber, respectively.

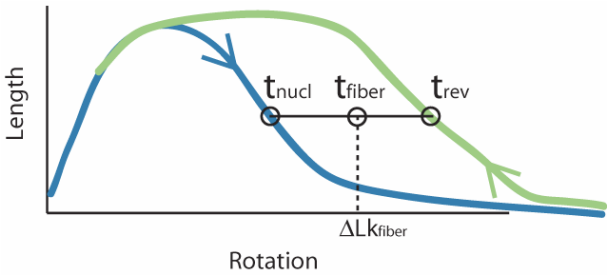

Figure S2. Schematics of the hysteretic torsion cycle

At a given rotation ΔLk_{fiber}, the steady-state extension of the fiber defines one point (t_{fiber}) located in the region delimited by the forward curve (the "all-nucleosome" fiber; blue) and the backward curve (the "all-reversome" fiber; green). The reversome or nucleosome proportions are equal to the length of the segments [$t_{nucl}t_{fiber}$] or [$t_{rev}t_{fiber}$], respectively (see Equation S2).

Equation S1 can be solved graphically, and one gets

$$N_{rev}/N_{nucl} = [t_{nucl}t_{fiber}]/[t_{rev}t_{fiber}] \qquad \text{(S2)}$$

with $[t_{nucl}t_{fiber}]$ and $[t_{rev}t_{fiber}]$ being the lengths of the corresponding segments measured on the abscissa (Figure S2).

Following the kinetic modelling (see Experimental Procedures in main text), we can compute the equilibrium constant K= k_1/k_{-1}, and derive the free energy difference, ΔG, between nucleosome and reversome:

$$\Delta G = -k_B T \ln(K) = U - F\left[l_{rev} - l_{nucl}\right] - \Gamma\left[\theta_{rev} - \theta_{nucl}\right] \qquad \text{(S3)}$$

where U is the difference in energy, F the force, Γ the torque, l_{rev} and l_{nucl} are the particles respective lengths projected on the direction of the force, and θ_{rev} and θ_{nucl} their rotational deformations perpendicular to it.

$\Delta\theta = \theta_{rev} - \theta_{nucl}$ is the "positive" shift per nucleosome undergoing the transition, i. e. 1.3 ±0.1 turns (Figure 2B in main text). The typical torque at 0.3 pN being ~3 pN.nm/rad (see below), we deduce $\Gamma\Delta\theta \sim 25$ pN.nm. If reversomes were more elongated than nucleosomes by ~10 nm, the nucleosome diameter (reversomes and open-state nucleosomes actually have the same length; see main text), we would obtain FΔ/ ~ 3 pN.nm. Thus, the force term can be neglected relative to the torque term in Equation S3, which leads to:

$$U = \Gamma\left[\theta_{rev} - \theta_{nucl}\right] - k_B T.\ln K \qquad \text{(S4)}$$

The torque cannot be measured experimentally, but it can be estimated from a fitting of the torsional curve with the worm-like rope model (Bouchiat and Mezard, 1998). In this model, the main determinant of the torque is the slope in the linear

plectonemic regime reached after ~10 turns of positive torsion are applied to the fiber (Bancaud et al., 2006). The similar slopes of forward (blue) and backward curves (green) in this regime (e. g. Figure S1B, panel 1) imply that the torques exerted on the all-positively-crossed-nucleosome or all-reversome fibers are about the same. We thus assume that the torque keeps the same value when the fiber relaxes at constant force to its steady state (see below).

In addition, the rate constants provide an estimate for the energetic barrier. We have:

$$k_1 = k_T \exp\left(-\frac{G^*}{k_B T}\right) \qquad \text{(S5)}$$

where k_T is the spontaneous fluctuation rate of dimers in a positively-crossed nucleosome (assuming the breakage of dimers docking on the tetramer is the rate-limiting step; main text and Figure 7), and G^* the activation free energy relative to the positively-crossed nucleosome. For chemical bond disruption, k_T is usually estimated to be 10^8-10^9 s^{-1} (Brower-Toland et al., 2002; Pope et al., 2005). Fluctuations occurring at the nucleosome level should be much slower, and, taking the nucleosome as a sphere of volume V~500 nm^3, one obtains $k_T \sim 3\eta V/k_B T \sim 3*10^6$ s^{-1} with η the viscosity of water. Because we consider fluctuations of dimers within a nucleosome, we expect this fluctuation rate to be on the order of 10^7 s^{-1}.

By analogy with Equation S4, and assuming the transition is driven by the external torque, one can write:

$$G^* = U^* - \Gamma(\theta^* - \theta_{nucl}) \qquad \text{(S6)}$$

with U^* being the activation energy relative to the positively-crossed nucleosome, and θ^* the rotational deformation of the reaction intermediate. θ^* should be ~0 according to our scenario (Figure 7 in main text), i.e. a flat tetrasome in the process of transiting from its left-handed to its right-handed conformation.

b) *Measurements*

i) In lower salt. Using Equation (S2), the proportion of nucleosomes during the relaxation time course can be estimated, and the kinetics can be fitted with Equation (5) in main text (Fig. 5A, insert in right panel). It comes $k_1=0.8*10^{-4}$ sec^{-1} and $k_{-1}=5.5*10^{-4}$ sec^{-1}. The torque was calculated (see above) to be ~3 (±1) pN.nm/rad, and Equation (S4) leads to U = 8 (±2) kT. In low salt conditions (B_0: 10 mM Tris-HCl [pH 7.5], 1 mM EDTA, 0.1 mg/mL BSA) and in the absence of torsional stress, the ground state in energy is the open nucleosome, and the positive conformation is then characterized by an energy difference of ~2 kT (Bancaud et al., 2006). Consequently, the energy of reversomes relative to the ground state is 10 (±2) kT. Moreover, according to Equation (S6), G^* is on the order of 26 (±3) kT. Thus, the energy difference between the reaction intermediate and the ground state is ~30 (±5) kT.

ii) In higher salt. Figure 6B in main text shows the transition observed at 0.4 pN in B_0 + 50 mM NaCl. Assuming a salt-independent topology of the reversome, and a reinitialized response in salt virtually identical to that obtained at the same force in B_0 (see Results in main text), the proportion of nucleosomes in the fiber during the relaxation time course can be deduced from the torsional response of the same fiber in B_O. The curve (inset in Figure 6B, right panel, in main text) appears to be sigmoidal rather than exponential, suggesting that some breakage of nucleosome-

nucleosome interactions occurs at early stages (Cui and Bustamante, 2000). Fitting with Equation (5) (Experimental Procedures in main text) resulted in $k_1=5.0*10^{-3}$ sec^{-1} and $k_{-1}=6.0*10^{-4}$ sec^{-1}, showing that the forward reaction is ~50 times faster than in B_0, whereas the rate of the backward reaction is similar. For a torque of 3 (±1) pN.nm/rad, we obtain U=4 (±2) kT, a value lower than the 8 kT obtained in B_0. Assuming the positively-crossed nucleosome remains unfavourable by ~2 kT relative to the open state in physiological conditions (Sivolob and Prunell, 2004), one finally obtains U~6 (±2) kT. Based on Equation (S6), the barrier free energy G^* is 21 (±3) kT, and the energy difference of the reaction intermediate relative to the ground state is ~25 (±5) kT.

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