DNAnucl
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DNA sequence effects on the structure and dynamics of nucleosome

Project coordinator: A. Arneodo
Laboratoire Joliot-Curie (LJC) et Laboratoire de Physique, ENS de Lyon

Partners : P. Bouvet
LJC et Laboratoire de Biologie Moléculaire de la Cellule (LBMC), ENS de Lyon

F. Argoul
LJC et Laboratoire de Physique, ENS de Lyon

The project DNAnucl is a project of fundamental research and innovation. This project has been coordinated by Alain Arneodo. It associates three teams of the Joliot Curie laboratory at ENS-Lyon : the theoretical physicists (multi-scale analysis of genomic and epigenetic data, modeling of chromatin, polymer statistical physics, signal and image processing), the experimental physicists (single molecule Atomic Force Microscopy imaging (AFM), Surface Scanning Plasmon Microcpy (SSPM), surface physics) and the molecular biologists and biochemists (synthetic DNA construction and natural DNA extraction, chromatin reconstitution, remodeling factors, histone variants). The project started in november 2006 and last for 42 months. It benefited of a financial support from the ANR of 450 000 euros for a global cost of 2 595 117 euros.

Scientific background and objectives

DNA is the physical support of genetic inheritance. Compacting DNA inside a very small volume is a general constraint for all living cells and its achievement remains an enigma both from structural and dynamical perspectives. Since the intrinsic structural and mechanical properties (bending and torsion) of the DNA polymer strongly depend on the sequence, the DNA sequence is likely to influence DNA interaction with structural proteins. This project originates from a previous comparative statistical analysis of genomic sequences that has revealed that long-range correlations (LRC) in the 10-200bp range are the signature of the nucleosomal structure and that over larger distances (> 200bp), LRC are likely to play a role in the condensation of the nucleosomal array into the 30nm chromatin fiber. We proposed that the role of LRC structural disorder is to favor the spontaneous formation of macroscopic curvature and of small DNA loops and in turn the propensity of DNA to interact with histones to form nucleosomes. In addition, these LRC might induce local rapid diffusion of those loops which would be a very attractive interpretation of the nucleosome repositioning dynamics. The main goal of this project is to take advantage of these numerical and theoretical results in order to develop original experiments coupled to the modeling of DNA compaction into chromatin, to determine to which extent LRC structural disorder favors regulation of the hierarchical structure and dynamics of chromatin in relation with replication and transcription processes.
The main objectives are : (i) modeling of elastic properties of naked DNA chains with LRC structural disorder and experimental investigation of thermodynamical properties of a few kbp long DNA chains ; ii) theoretical, numerical and experimental studies of the effect of the DNA sequence on the nucleosome formation, on its repositioning dynamics and on the collective nucleosomal organization of the 10 nm eukaryotic fiber ; (iii) visualization and modeling of nucleosome sliding induced by remodeling factors coupled to experiments on the stability of nucleosomes in the presence of external agents.

Main results

The results obtained during the past 42 months confirm the fundamental role of the DNA sequence in the nucleosomal organization of the eukaryotic chromatin fiber and in turn in the chromatin mediated regulation of transcription and replication. The main results are :

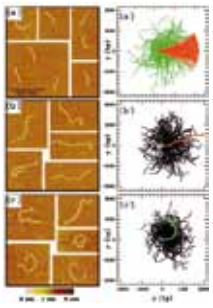

FIGURE 1 – AFM imaging of genomic DNA versus DNA simulations . AFM images of L =600 bp intrinsically straight (a) and L = 2200 bp HCV (b) and human (c) DNA molecules. DNA simulations of N = 100 chains (L = 2200 bp) : 2D frozen chains with uncorrelated (H = 1/2, red) and LRC (H = 0.8, green) bend angle fluctuations of amplitude σ_0 = 0:008 (a') ; 2D equilibrium conformations (black) generated with 2A = 540 bp from a frozen chain with uncorrelated disorder(H = 1/2, σ_0 = 0.02, red) (b') and with LRC structural disorder (H = 0.8, σ_0 = 0:008, green) (c').

- ✓ The generalization of the worm-like chain model to long-range correlated heteropolymers like DNA ;
- ✓ The experimental demonstration by AFM imaging that genomic LRC reduce the DNA persistence length and that this lowering more likely results from large-scale intrinsic curvature due to a persistent distribution of DNA bending sites than from some increased flexibility (Figure 1) ;
- ✓ The experimental confirmation that the spatial organization of nucleosomes observed in vivo is long-range correlated with characteristics similar to the LRC imprinted in the DNA sequence ;
- ✓ The grand canonical modeling of the effect of the DNA sequence on nucleosome formation and the demonstration that the collective nucleosome organization actually results from some parking phenomenon (thermodynamic ordering) nearby energy barriers encoded in the sequence (Figure 2) ;
- ✓ The discovery of a novel strategy of transcription regulation by the intra-gene nucleosomal organization ;

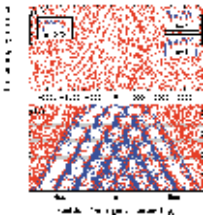

FIGURE 2 – 2D map of yeast gene nucleosome organization. (a) 2D map of local minima (red) of the experimental in vivo nucleosome occupancy profile at yeast genes; genes are ordered vertically by the distance L between the 5' and 3' nucleosomes. Insets : mean experimental (red) and one individual theoretical (blue) nucleosome occupancy profiles (Lee et al., Nat. Genet. (2007)) for "crystal" genes harboring 5 nucleosomes (right, top), 6 nucleosomes (right, bottom) and the bistable genes with 5/6 nucleosomes (left). (b) Zoom on the first 2000 genes in (a) ; on the top of the experimental data (red) are superimposed the predictions of our grand canonical physical modeling (blue) : horizontal grey-shaded bands correspond to some L- domains of bistable genes displaying high transcriptional plasticity.

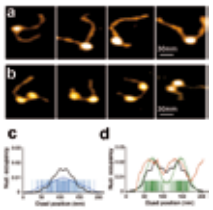

FIGURE 3 – Excluding energy barriers condition the positioning of nucleosomes consistent with equilibrium statistical ordering principles. AFM imaging in liquid of mono-nucleosomes (a) and di-nucleosomes (b) along a yeast (chr. 7) DNA fragment (L = 595 bp) containing the gene YRG105W. (c) Statistical analysis of mononucleosome positioning (N = 113 molecules) ; blue bars correspond to experimentally detected dyad locations and to their symmetrical positions with respect to the center of the fragment. The experimental nucleosome occupancy profile (blue curve) is compared to the theoretical predictions of our physical modeling after symmetrization (black curve). (d) Statistical analysis of di-nucleosome positioning (N = 62); for each image, green bars were drawn at both the 2 dyad positions and their symmetric locations with respect to the center of the fragment. The experimental nucleosome occupancy probability profile (green curve) is compared to the symmetrized theoretical profile (black curve) and to the in vivo occupancy profile obtained by Lee et al., Nat. Genet. (2007) (red curve).

- ✓ The experimental confirmation by AFM in liquid of the role of the DNA sequence (i) in the statistical positioning of nucleosomes nearby excluding energy barriers corresponding to the nucleosome free regions (NFR) observed in vivo (Figure 3) and (ii) in the nucleosome occupancy at transcription and replication regulatory sites ;
- ✓ The experimental study by AFM of nucleosome remodeling by the molecular motor SWI/SNF ;
- ✓ The conception and construction of a SSPM with radial polarization allowing the detection of nano-particles of size ranging from 10 nm to more than 200 nm, with the recent visualization of nucleosomes (Figure 4).

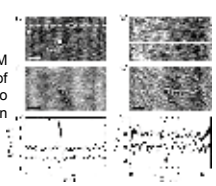

FIGURE 4 – SSPM imaging of nucleosomal particles. Nucleosomes were reconstituted in vitro from 898 bp IL2RA DNA fragments and histone octamers, and deposited on bare gold at a concentration of ~ 1 nM before visualization. (a) and (b) : Grey coded amplitude images obtained for two focusing positions. In (c) are shown some sections of (a) and (b). (a') and (b') : Grey coded phase images corresponding to the focusing positions of (a) and (b) respectively. In (c') are shown some sections of (a') and (b').

Conclusions and perspectives

The tasks achieved during the DNAnucl project provide a new vision of the role of the DNA sequence in the collective nucleosome organization that has been gaining consensus in the international scientific community. Indeed, on the contrary to the original sequence positioning dogma the dominant sequence signals are high energy barriers that locally inhibit nucleosome formation consistent with the NFRs observed in vivo. These excluding energy barriers turn out to be a major actor in conditioning the collective positioning of neighboring nucleosomes according to equilibrium statistical ordering principles. Thanks to the financial support provided by the ANR, the Joliot Curie laboratory has succeeded in its objective of giving impetus to interdisciplinary projects that associate different partners from different scientific backgrounds.
Among the perspectives that would deserve to be further encouraged and supported, we plan (i) to generalize our theoretical and experimental approaches of "intrinsic" (sequence driven) nucleosome positioning from yeast to higher eukaryotes including mammals and (ii) to extend our study to various "external factors" that affect nucleosome positioning in vivo including transcription factors, chromatin remodelers and epigenetic marks.

Impact

Publications, communications,valorisations

		Multipartners	Monopartners
Publications	Peer reviewed Book chapter	7 1	14
Communications	Conferences Vulgarization	25 (int.) 21 (nat.) 1	23 (int.) 11 (nat.)
Patents			3
International conference organization		1	

Thesis

– Sanjun ZHANG (Octobre 2003 - Janvier 2008) : Surface plasmon resonance and its applications to the probing of macromolecules on gold surfaces. (Directeur : F. Argoul).

– Hervé MENONI (Octobre 2004 – Octobre 2008) : Etude de la réparation de l'ADN dans un contexte chromatinien (Directeur : P. Bouvet).

– Fabien MONTEL (Octobre 2005 – Octobre 2008) : Etude des mécanismes de remodelage de la chromatine par microscopie à force atomique (Co-directeurs : C. Faivre-Moskalenko et S. Dimitrov).

– Julien MOUKHTAR (Octobre 2005 – Octobre 2008) : Modélisation des effets de séquence sur les propriétés thermodynamiques de chaînes d'ADN (Directeur A. Arneodo).

– Thibault ROLAND (Septembre 2006 – Octobre 2009) :Localized surface plasmon imaging : a non intrusive tool to cover nanometer to micrometer scales in biological systems (Directrice F. Argoul).

– Lamia ZAGHLOUL (Octobre 2006 – Novembre 2009) :Transcriptional activity, chromatin state and replication timing in domains of compositional skew in the human genome (Directeur B. Audit).

– Guillaume CHEVEREAU (Septembre 2007- Octobre 2010) : Thermodynamique du positionnement des nucléosomes (Directeur C. Vaillant).

Publications list

[1] J. Moukhtar, E. Fontaine, C. Faivre-Moskalenko & A. Arneodo. Probing persistence in DNA curvature properties with atomic force microscopy. Phys. Rev. Lett. 98, 178101 (2007).
[2] C. Vaillant, B. Audit & A. Arneodo. Experiments confirm the influence of genome long-range correlations on nucleosome positioning. Phys. Rev. Lett. 99, 218103 (2007).
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CONTACT : Alain Arneodo
alain.arneodo@ens-lyon.fr