Compaction de l'ADN : relations entre conformation et activité transcriptionnelle

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Résumé :
L'ADN est un polymère pouvant subir un changement d'état d'une conformation de pelote libre et étirée vers un état compact et ordonné sous l'effet d'une modification de la composition chimique de l'environnement (concentration en polycations, polymères neutres, etc.). Ce repliement – ou compaction de l'ADN - est discret et réversible à l'échelle de chaînes individuelles et linéaires, et concerne spécifiquement les molécules suffisamment longues (plus d'un millier de paires de bases) alors que les molécules plus courtes se comportent comme des bâtons rigides et ne sont pas pliables. Nous avons comparé l'activité transcriptionnelle d'ADN longs (40 kbp) et courts (140 bp) pour différentes concentrations en agents condensants, et constaté une inhibition complète de la production d'ARN dans le seul cas des molécules longues. Le couplage de mesures biologiques et d'observations directes des chaînes par microscopie de fluorescence nous a permis de montrer une corrélation entre l'activité transcriptionnelle des ADN et leur conformation [1]. D'autre part, la transition est continue pour les ADN circulaires, et l'état compact est moins dense que dans le cas des ADN linéaires. En comparant l'activité transcriptionnelle d'ADN courts (4kbp) et longs (106kbp) dans leurs versions linéaires et circulaires, nous montrons qu'une topologie circulaire empêche l'inhibition totale de la transcription par compaction [2]. La longueur des chaînes apparaît comme un paramètre important dans le contrôle de la transcription. Nous discutons ces résultats dans le cadre de l'auto-régulation de l'activité génétique in vivo.

Abstract :
Various kinds of chemicals, such as polycations or neutral polymers, are known to cause long DNA chains collapse from a random-coil state into an ordered, folded state. This phenomenon, known as DNA compaction, generally occurs in a reversible, all-or-none manner for long linear DNA molecules (>>kbp), while short DNA fragments (-400 bp) behave like rigid rods and cannot undergo such folding transition. We found that the transcriptional activity of large DNAs can be completely inhibited by the addition of condensing reagents, whereas under the same conditions short fragments still show active transcription. Fluorescence microscopic observations showed a clear correlation between the higher-order structure of templates and their transcriptional activity [1]. While linear DNA exhibit an all-or-none transition resulting into a highly compact state, the folding of circular DNA occurs as a continuous transition and results in a rather loosely compact state. We compared the transcriptional activities of circular and linear versions of short (4kbp) and long (106kbp) DNA and found that circularity prevents the complete inhibition of transcription with compaction [2]. Length appears as a critical parameter involved in the control of transcription. These results are discussed in relation to the self-regulation of genetic activity in vivo.

Introduction :

It is generally admitted that large-scale morphological changes in the higher-order structure of chromosomal DNAs induced by the modification of DNA-histone interactions are closely related to biological functions such as transcription. Concurrently, at the sequence level, DNA biochemical functions are controlled by specific interactions involving proteins or regulatory factors, thus forming a complex network of key-lock relationships. However, the activation and inhibition of genes becomes fuzzy in individual cellular systems suggesting that processes beside specific key-lock interactions play important roles in the control and protection of genetic information. This study is aiming at understanding of the significance of changes in DNA higher-order structure induced by non-specific environmental chemicals, and identifying pertinent physical parameters involved in this process.

Part 1. All-or-none conformational transition of large DNA chains

Large DNA molecules exhibit a conformational transition between elongated coiled and folded compact states upon the addition of condensing reagents including biophysical species. Fluorescence microscopic observations of single linear DNA molecules revealed that chains individually undergo the folding transition in a biphasic, or all-or-none manner. This property appears when the DNA molecule can be considered as a semi-flexible polymer, i.e. when chain length exceeds a few kilo base pairs, corresponding to the length of genetic DNAs. On the other hand, shorter DNA fragments behave as rigid rods and undergo a folding transition (Fig.1).

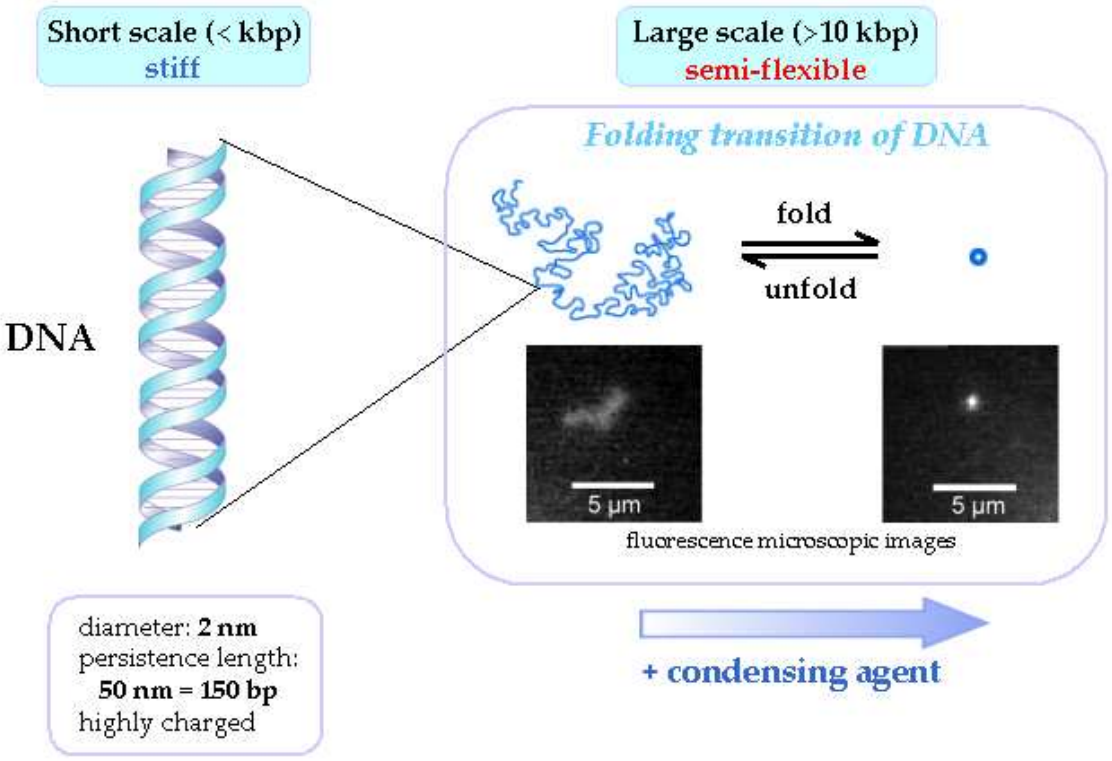

Fig.1.

Part 2. On/off switching of transcriptional activity for large linear DNA chains

We examine how changes in the higher-order structure affect the transcriptional activity of a long linear DNA, Lambda ZAP II (40kbp), as compared to a short template (140 bp) including the same promoter, using a polycation (4+), spermine, and a neutral polymer, poly(ethylene glycol) (PEG) as condensing reagents. Long templates exclusively show a dramatic change in transcriptional activity, resulting to complete inhibition, upon addition of condensing reagents, i.e. compaction of DNA (Fig.2.), [1]. Hence, DNA folding in an on/off manner also results in the on/off switching of transcriptional activity. The significant increase (10^3-10^4 times) in DNA segment density together with the transition prevents the binding and sliding of enzymes for RNA polymerization.

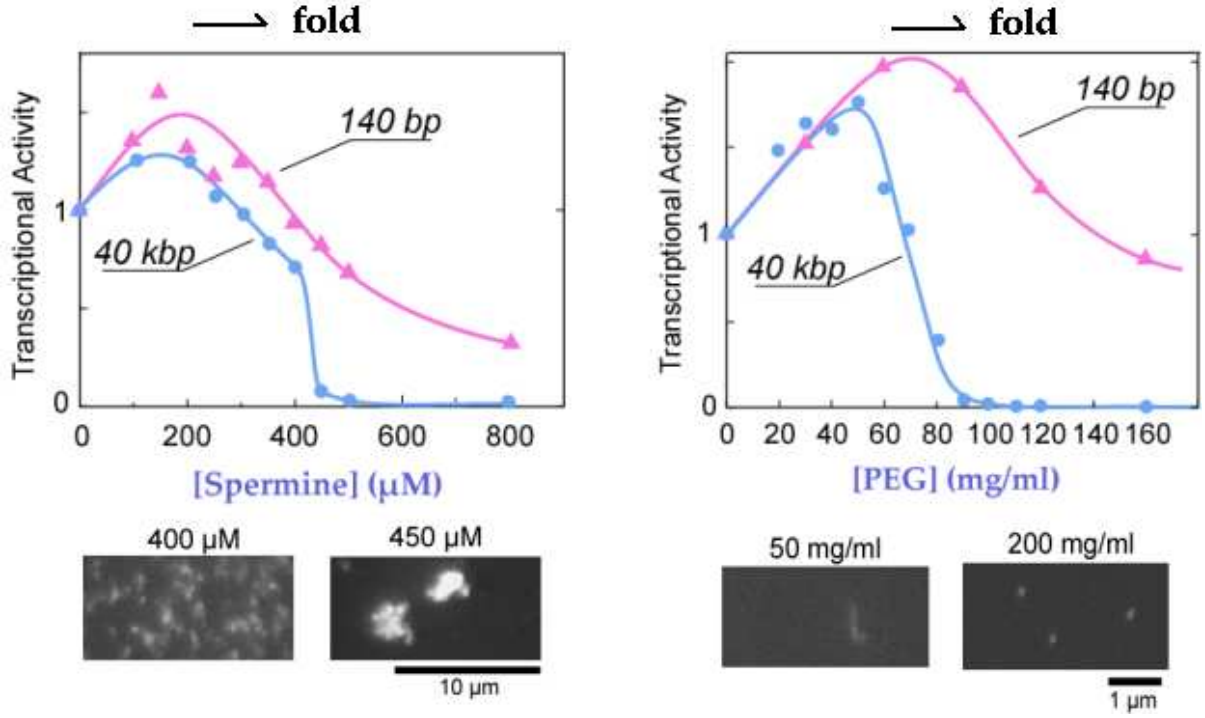

Fig.2.

In parallel to these experiments performed at high DNA concentrations, we performed single-molecules observations: on Fig.3, DNA and RNA products are stained with different fluorescent dyes, and RNA transcripts (red) are visible only on elongated templates, confirming that a transcription promoter located near the center of large linear DNA is inactive in the compact state [3].

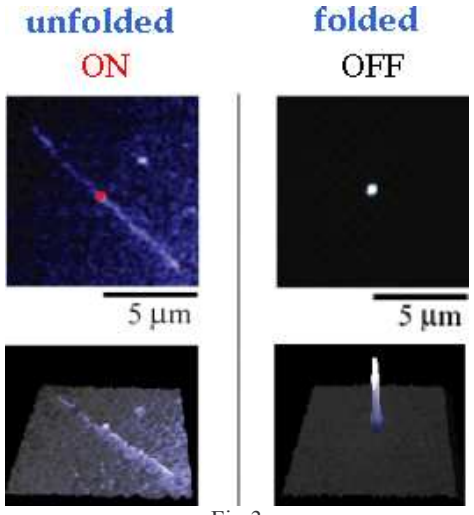

Fig.3

Part 3. Linear vs. Circular templates

In contrast to linear DNA chains, circular DNA exhibit a coninuous transition resulting in a rather loosely compact state [4]. We performed transcription on long (106 kbp) and short (4kbp) DNA for their linear and circular versions, and observed a decrease of transcriptional activity associated with the conformational transition in all cases, howerver complete inhibition is reached in the sole case of long, linear templates (Fig.4.), [2]. The differences between the activities of linear and circular DNA increases with chain length.

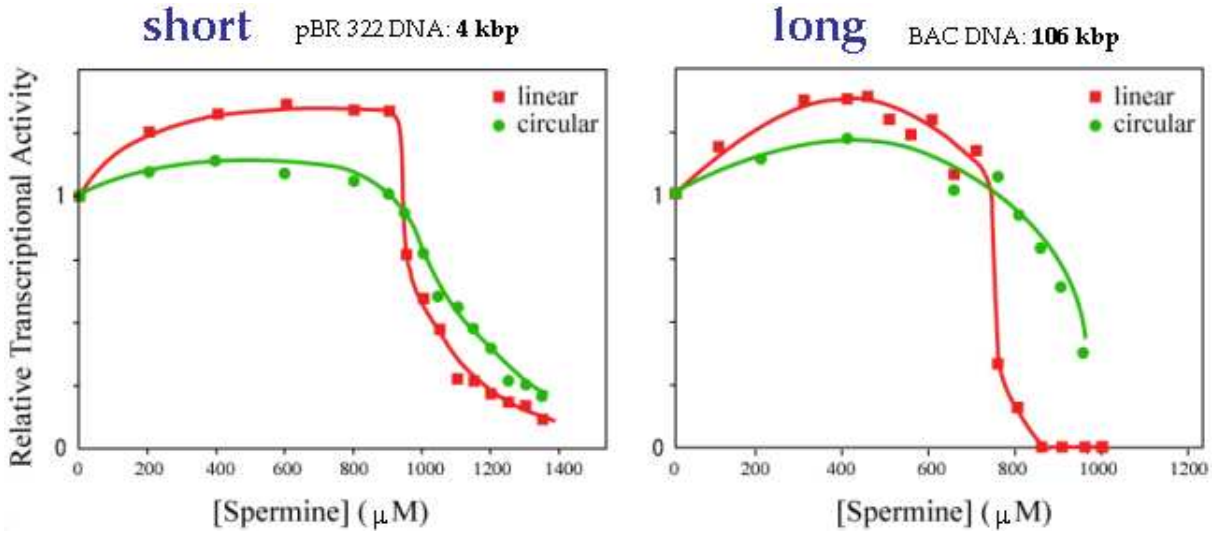

Fig.4

At lower spermine concentrations (around 500μM) i.e. before the conformational transition, we observed an enhancement of transcription. This effect is attributable to the decrease of DNA negative charge reducing the energy requirement for binding between negatively charged RNA polymerase and DNA. Since this enhancement was stronger in the case of linear chains, transcription may also be facilitated by a modification of DNA chain rigidity through spermine weak binding. It should be noted that during gene expression in eukaryotes, the partial unfolding of DNA from its tight packing around histones results in the opening of a transcription loop with two fixed ends, therefore equivalent to a closed circular structure.

Part 4. Conclusion & Perspectives

In the present study, we have shown that transcriptional activity is completely inhibited upon drastic compaction on large, linear DNA molecules. In contrast, circular templates exhibit a soft transition with a higher surviving activity. Circular DNA with superhelicity undergoes a continuous-type transition into a loosely compact state, possibly generating local torsional stress along DNA chains. Such local stress may weaken the double-stranded structure and enhance enzymatic activity.

It is well known that different compacting parameters lead to different mesoscopic structures of compacted DNA: can we tune the biochemical reactivity of DNA by simple control of its physical state? A correspondence chart between DNA higher-order conformation and activity may reveal the possibility to control transcriptional intensity (Fig.5).

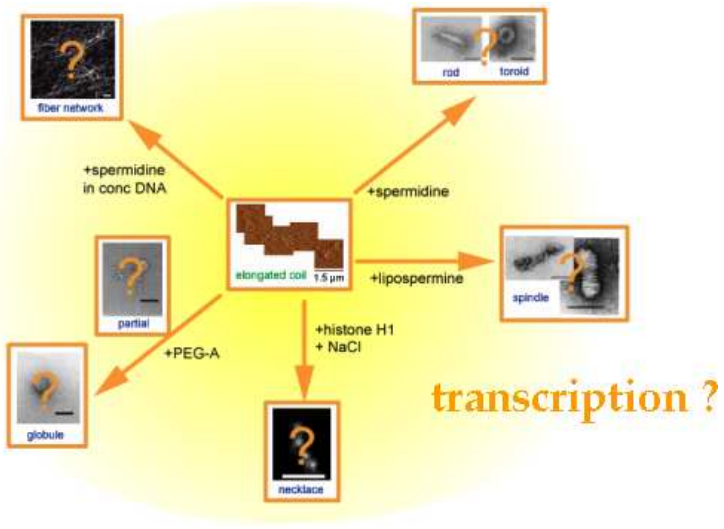

Fig.5

References

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