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SPOTLIGHT

Active RNA polymerase II curbs chromatin movement

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Chromosomes are not very mobile during interphase. In this issue, Nagashima et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201811090) propose that the overall stabilization of genome structure is achieved by loose connections between DNA regions brought about by transcriptionally active RNA polymerases.

The eukaryotic genome consists of chromosomes that fill the volume of the nucleus. Recording the movement of DNA regions in real time in living cells has shown that the mobility of DNA in the nucleus is slow, restricted, and mostly diffusive (1). The DNA strands are not found floating freely throughout nuclear space, but rather the movement of a DNA region is mostly confined to its immediate vicinity. More so, painting of individual chromosomes with chromosome-specific DNA probes using fluorescence in situ hybridization has demonstrated that the DNA strands of the chromosomes are not randomly intermingled in the whole volume of the nucleus. Instead, each chromosome is situated in its own "chromosome territory" such that chromosomes retain their individuality in the nuclear space (2; Fig. 1).

How does the DNA remain restricted in its movement in the aqueous surroundings of the nucleus? Which molecules limit the mobility of DNA over the many hours of interphase and prohibit DNA from slowly diffusing throughout the whole nucleus? How are chromosome territories up-kept? Can the mobility of chromatin regions be modified depending on the physiological state of the cell? Such questions can be answered by direct imaging of DNA motion in the nucleus of single living cells and the tracking of chromosomal regions over time. It has been postulated that various molecules can act as "glue" molecules that prevent DNA regions from freely moving around. Indeed, it was found that the soluble nucleoplasmic fraction of the lamin A protein (which is also found in a filamentous form in the nuclear lamina at the periphery of the nucleus) contributes to the maintenance of restricted DNA mobility, and that the depletion of lamin A from the nucleus increases chromatin mobility (3). That study focused on the tracking of many telomeric ends of chromosomes and examined the characteristics of genome diffusion in living cells. In this issue, detailed monitoring of chromatin dynamics was accomplished by Nagashima et al. in a global genome-wide manner under different transcriptional conditions.

Nagashima et al. (4) track the movement of numerous chromatin regions simultaneously using fluorescently labeled single nucleosomes, using a labeling method similar to one the authors previously devised (5) in which histone H2B molecules were tagged with a HaloTag in RPE-1 cells, thereby allowing the detection of individual nucleosomes in living cell conditions. As expected, the movement of the chromatin regions was found to be confined and subdiffusive in interphase, where individual nucleosomes could explore a limited area with a radius of constraint of 141 ± 19 nm. Interestingly, this movement increased when the transcriptional activity of RNA polymerase II (RNAPII; and not RNA polymerase I in nucleoli or pre-mRNA splicing) was specifically inhibited in the cells using two transcription inhibitors that reduced the levels of active RNAPIIs associated with chromatin: α-amanitin and 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB). The radii of constraints increased to 164 ± 22 nm and 149 ± 20 nm, respectively. However, when actinomycin D was used to inhibit transcription by stalling polymerases on the DNA, chromatin mobility further decreased. These measurements suggest that the restriction of DNA movement in the nucleus under regular conditions is connected to the levels of transcriptional activity of the RNAPIIs associated with chromatin.

A series of additional experiments supported the above conclusion connecting chromatin mobility and transcriptional activity. The nuclear periphery is enriched in heterochromatic DNA regions that have a low transcriptional status, in contrast to the nuclear interior that contains more euchromatic regions and much more RNAPII activity. Indeed, transcription inhibition did not increase chromatin mobility in the nuclear periphery since these areas are less associated with RNAPII. Depletion of RNAPII from cells using an inducible degron sequence that was targeted into the *POLR2A* gene increased chromatin mobility. Washing out of the inducer rescued the polymerases and suppressed the mobility. RNAPII activity was reduced under more physiological conditions by serum starving the cells for several days and driving them into the quiescent G0 phase of the cell cycle. An increase in chromatin mobility was observed. Similarly, the readdition of serum restored the constrained mobility. Finally, DNA damage caused by UV irradiation can also have a transcriptional inhibition effect. Under these conditions, DNA mobility was increased.

Taken together, these observations suggest that transcription complexes can serve as connector elements that bridge DNA regions, thereby forming a network of connections

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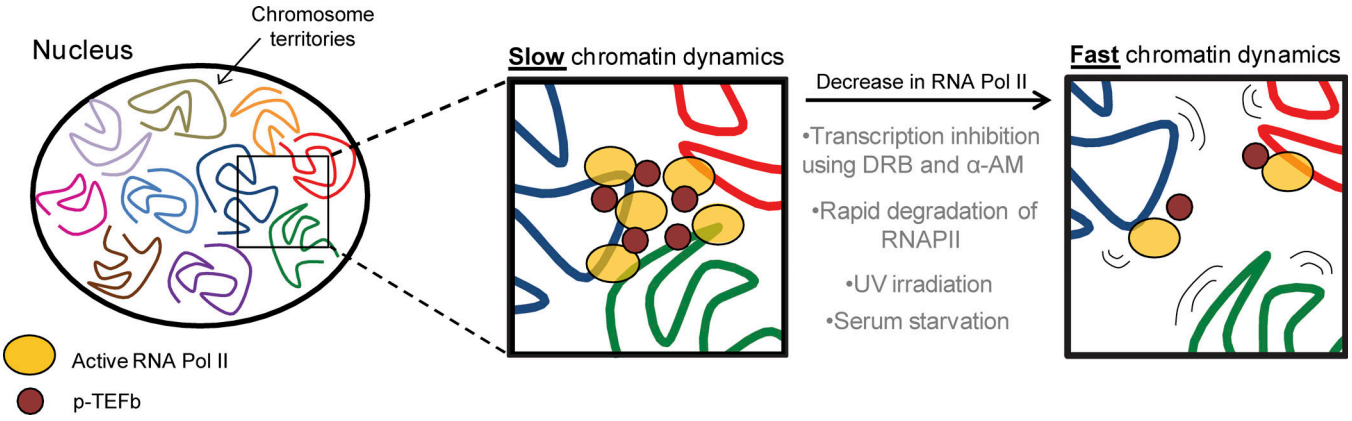

Figure 1. **The eukaryotic genome is organized in the cell nucleus as chromosome domains that form defined regions termed chromosome territories.** Nagashima et al. (4) show that chromatin can be globally stabilized by a loose network consisting of clusters of connector elements, namely, transcriptionally active RNAPIIs (yellow ovals) and clusters of p-TEFb molecules that serve as hubs (brown circles). Perturbing transcription either by inhibitors such as DRB and α-amanitin (α-AM), the degradation of RNAPII, or UV irradiation and serum starvation, increases the dynamics of chromatin regions.

constraining DNA motion (Fig. 1). Since the cell requires degrees of plasticity in response to various cues, this network would be expected to consist of loose interactions that can be altered as needed. Transcription complexes can serve this purpose since transcription activity is typically not of constitutive nature and is rather burst-like (6). Recent studies have demonstrated that elements of the transcription machinery can form clusters. For instance, the CDK9 kinase, which is part of the positive transcriptional elongation factor (P-TEFb) complex, is found in clusters (7). Therefore, CDK9 might form weak interactions between the DNA and the RNAPIIs. Indeed, Nagashima et al. (4) found that knockdown of CDK9 increased chromatin mobility, as also described for DRB that inhibits CDK9 (4). Another element in the control of RNAPII transcription is the phosphorylated form of the C-terminal domain of the polymerase. RNAPII phosphorylated on serine 5 of the C-terminal domain correlated with the changes of chromatin mobility observed in this study and is suggested by the authors to be another glue element in the formation of the constrained chromatin domains.

Formation of transcription clusters in between DNA regions is supportive of the "transcription factory" model (8) in which active RNAPIIs were found to cluster in nuclear foci of fixed cells. Subsequently, live-cell imaging studies detected these structures in living cells (9), which were shown to be enriched in the RNAPII-Ser5, marking the initiating form of the RNA polymerase (7). Changes in chromatin mobility within particular DNA regions may impact the efficiency of interactions between distant regions of DNA, as previously proposed (10). The regulation of RNAPII by phosphorylation in combination with subnuclear compartmentalization and the concentration of transcription factors to specific subregions is suggested to increase the efficiency of the transcriptional process, thereby demonstrating how transcription can be regulated both spatially and temporally.

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