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RNA PolII enters the ring of Cohesin-mediated loop extrusion

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STRAP LINE

The 3D organization of mammalian chromosomes can regulate transcription. Conversely, if transcription itself influences genome structure has remained a source of debate. Using sensitive genome-wide read-outs, two recent studies describe its involvement through different mechanisms.

MAIN TEXT

Mammalian chromosomes are organized into compartments, known as TADs (Topologically Associating Domains), through a process of Cohesin-mediated loop extrusion^{1,2}. TADs and loop extrusion itself can both influence the formation of enhancer-promoter (E-P) loops, thereby regulating the activity of RNA Polymerase II (PolII) and gene transcription³⁻⁵. If PolII and transcription have reciprocal influences on 3D genome organization, and particularly loop extrusion and TAD structure, has remained debated⁶⁻⁹. This has mostly been caused by constraints imposed by the essential nature of transcription and by limits to the sensitivity of read-out. Using combinations of acute protein depletion with highly sensitive genome-wide assays, two recent studies in *Nature Genetics* have determined that PolII actively shapes 3D genome organization, which involves two different mechanisms^{10,11}.

Mammalian interphase chromosomes are compacted by a cyclical process of loading of the Cohesin complex on the chromosome, followed by bi-directional extrusion of a DNA loop and subsequent dissociation of Cohesin from the DNA^{2,5}. Without this compaction, the formation of E-P loops at distances of over 100 kilobase (kb) is impeded^{3,4}. The DNA-binding CTCF insulator protein can block the extruding Cohesin complex; thereby creating a physical boundary for the creation of E-P loops^{1,2,5}. In genome-wide Chromosome Conformation Capture experiments (*i.e.* Hi-C and Micro-C studies)^{1,7}, the region in-between two neighboring boundaries—that is permissive to the formation of E-P loops—appears as a TAD with increased intra-domain contacts. The extrusion of loops within TADs therefore promotes the formation of E-P loops, whereas CTCF-mediated blocking at boundaries impedes E-P loop formation.

If PolII itself has an impact on 3D genome organization, and particularly in the regulation of loop extrusion, has been debated (see *e.g.* references 6-9). Studies that have aimed to address this question have been complicated because of two experimental challenges. First, PolII transcription is highly essential for proper cell function, which

manifests itself after a short period of time (one day or less). Pinpointing the effect of PolII, without secondary effects caused by the perturbed expression of other genes, therefore requires a rapid and drastic removal. Previous studies mostly relied on chemical inhibition of PolII, which can reduce gene body levels by around 2 to 3-fold, yet their concentrations and exposure time need to remain short to avoid secondary effects^{6,7}. Second, Hi-C technology, the method of choice to identify 3D genome organization and DNA loops at a genome-wide level, suffers from a low signal-to-noise ratio at a short range (approximately 10-100 kb). As such, its capacity to quantify the impact of PolII perturbations on EP-loops is limited.

Two recent advances have helped to overcome these limitations. The development of degron-technologies permits the inducible, rapid and highly efficient depletion of proteins, including PolII. Micro-C, a variant of Hi-C that quantifies interactions between nucleosomes, generates data with strongly reduced local background signal, thereby improving the identification of chromatin structure at short ranges⁷. Previous studies addressed the involvement of PolII in 3D genome organization using either degrons^{8,9} or Micro-C⁷, but not their combination. These studies reported a minor impact on local chromatin interactions and EP-loops⁷⁻⁹, but lacked sufficient details to definitively confirm a direct involvement of PolII. Now, two studies have combined the advantages of degron-technology, Micro-C and tailored data analysis to identify two different mechanisms whereby PolII influences the formation of DNA loops^{10,11}.

The study by Zhang and colleagues, combining a PolII-degron with Micro-C, focused on loops that were gained and lost¹⁰. As previously reported by the same group⁹, a large number of new loops between CTCF anchors was identified when PolII was depleted¹⁰. In contrast to before, many loops with significantly reduced signal were identified as well. Many of these lost loops initially linked enhancers to promoters or other enhancers, yet loops between promoters appeared mostly unaffected (see below). Interestingly, a substantial fraction of these lost loops carried a CTCF anchor on one side and PolII on the other. After PolII depletion, these loops were rewired such that they now extended up to a more distant second CTCF anchor¹⁰. In untreated cells, these loops therefore resembled the prevalent CTCF-CTCF loops, except that PolII blocked loop extrusion on one side (Fig. 1a). As a first mechanism, PolII thus acts as a roadblock for bi-directional loop extrusion.

The study by Barshad and colleagues focused on structural aspects of E-P loops¹¹. Using a tailored reanalysis of Micro-C data⁷, they revealed that E-P contacts were most enriched at the nucleosomes covering transcription start sites (TSS) of promoters and enhancers¹¹. Live-cell imaging studies have reported that enhancers rarely contact their target promoters, raising the hypothesis that E-P communication occurs within larger macromolecular complexes¹². The pinpointed signal in the Micro-C data instead argues that E-P contacts involve direct and focal interactions between small genomic segments¹¹. Yet, Micro-C does not provide information about the transient nature of such contacts, which may explain the differences between these studies.

Reanalysis of Micro-C data upon chemical PolII inhibition⁷ revealed that the reductions in E-P contacts involved highly focal genomic segments as well, indicating a structural involvement of PolII¹¹. Interestingly, this was also observed when chemical inhibition was used to reduce the amount of paused PolII. PolII pausing creates local accumulations of polymerase on both sides of TSS, albeit with considerable variation between genes (as measured using the “pausing index”)¹³. Indeed, E-P loop strength did not only scale with transcriptional activity, but also with the pausing index¹¹. Combination of Micro-C with a

83 NELF-degron, which maintains PolII in its paused state, further confirmed the importance of
84 paused PolII for E-P loop strength.

85 How can (paused) PolII then influence the formation or stability of E-P loops? In
86 Micro-C maps, so-called interaction “stripes” emanate from PolII sites, where they follow the
87 orientation of transcription. These stripes are the result of uni-directional loop extrusion,
88 which emerge when sites of Cohesin recruitment overlap sites of one-sided loop extrusion
89 blocking. Indeed, active promoters and enhancers recruit Cohesin¹⁴ and PolII degradation
90 reduces Cohesin levels at TSS⁸⁻¹⁰. In a second mechanism, PolII thus promotes the formation
91 of E-P loops by simultaneously stimulating Cohesin loading and blocking (Fig. 1b). This
92 permits promoters and enhancers to actively scan their surroundings for other functional
93 elements, providing a potential mechanistic explanation why the formation of longer-range E-
94 P loops depends on loop extrusion^{3,4}. Moreover, this raises the possibility that PolII can
95 stabilize existing E-P loops by directly promoting the loading of Cohesin onto pairs of
96 promoters and enhancers that are already in contact (Fig. 1b, bottom).

97 Nonetheless, these studies raise new questions as well. When (paused) PolII acts as a
98 roadblock for bi-directional loop extrusion it will create sites of insulation (Fig. 1a), as was
99 previously shown with genetics experiments in *Drosophila*¹⁵. If such sites can interfere with
100 E-P loop formation and transcriptional activity of neighboring genes remains to be
101 determined. The newly identified role of PolII in the formation of loops between promoters
102 and downstream enhancers (Fig. 1b) raises the question how loops with upstream enhancer
103 are established. For these loops, it may be envisioned that the presence of PolII at enhancers
104 plays a pivotal role. Indeed, this may explain why promoter-promoter loops are less affected
105 by PolII-degradation¹⁰. Additional insights into loop extrusion kinetics at promoters and
106 enhancers, particularly using live cell imaging and genome editing, will be essential to further
107 characterize these processes.

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Competing interests

The author declares no competing interests.

FIGURE LEGEND

Involvement of PolII in loop formation through two different mechanisms. **a**, extruding Cohesin (top) is blocked by CTCF and PolII (middle). Without PolII, longer CTCF-only loops are formed (bottom). **b**, Cohesin loading at PolII binding sites (top), particularly when paused, promotes E-P loop formation (middle) and possibly stabilization (bottom).