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# 1 RNA PolII enters the ring of Cohesin-mediated loop extrusion

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## 6 STRAP LINE

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

## 11 MAIN TEXT

12 Mammalian chromosomes are organized into compartments, known as TADs (Topologically  
13 Associating Domains), through a process of Cohesin-mediated loop extrusion<sup>1,2</sup>. TADs and  
14 loop extrusion itself can both influence the formation of enhancer-promoter (E-P) loops,  
15 thereby regulating the activity of RNA Polymerase II (PolII) and gene transcription<sup>3-5</sup>. If PolII  
16 and transcription have reciprocal influences on 3D genome organization, and particularly  
17 loop extrusion and TAD structure, has remained debated<sup>6-9</sup>. This has mostly been caused by  
18 constraints imposed by the essential nature of transcription and by limits to the sensitivity of  
19 read-out. Using combinations of acute protein depletion with highly sensitive genome-wide  
20 assays, two recent studies in *Nature Genetics* have determined that PolII actively shapes 3D  
21 genome organization, which involves two different mechanisms<sup>10,11</sup>.

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

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

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

45 Two recent advances have helped to overcome these limitations. The development of  
46 degraon-technologies permits the inducible, rapid and highly efficient depletion of proteins,  
47 including PolII. Micro-C, a variant of Hi-C that quantifies interactions between nucleosomes,  
48 generates data with strongly reduced local background signal, thereby improving the  
49 identification of chromatin structure at short ranges<sup>7</sup>. Previous studies addressed the  
50 involvement of PolII in 3D genome organization using either degrons<sup>8,9</sup> or Micro-C<sup>7</sup>, but not  
51 their combination. These studies reported a minor impact on local chromatin interactions and  
52 EP-loops<sup>7-9</sup>, but lacked sufficient details to definitively confirm a direct involvement of PolII.  
53 Now, two studies have combined the advantages of degraon-technology, Micro-C and tailored  
54 data analysis to identify two different mechanisms whereby PolII influences the formation of  
55 DNA loops<sup>10,11</sup>.

56 The study by Zhang and colleagues, combining a PolII-degraon with Micro-C, focused  
57 on loops that were gained and lost<sup>10</sup>. As previously reported by the same group<sup>9</sup>, a large  
58 number of new loops between CTCF anchors was identified when PolII was depleted<sup>10</sup>. In  
59 contrast to before, many loops with significantly reduced signal were identified as well.  
60 Many of these lost loops initially linked enhancers to promoters or other enhancers, yet loops  
61 between promoters appeared mostly unaffected (see below). Interestingly, a substantial  
62 fraction of these lost loops carried a CTCF anchor on one side and PolII on the other. After  
63 PolII depletion, these loops were rewired such that they now extended up to a more distant  
64 second CTCF anchor<sup>10</sup>. In untreated cells, these loops therefore resembled the prevalent  
65 CTCF-CTCF loops, except that PolII blocked loop extrusion on one side (Fig. 1a). As a first  
66 mechanism, PolII thus acts as a roadblock for bi-directional loop extrusion.

67 The study by Barshad and colleagues focused on structural aspects of E-P loops<sup>11</sup>.  
68 Using a tailored reanalysis of Micro-C data<sup>7</sup>, they revealed that E-P contacts were most  
69 enriched at the nucleosomes covering transcription start sites (TSS) of promoters and  
70 enhancers<sup>11</sup>. Live-cell imaging studies have reported that enhancers rarely contact their target  
71 promoters, raising the hypothesis that E-P communication occurs within larger macro-  
72 molecular complexes<sup>12</sup>. The pinpointed signal in the Micro-C data instead argues that E-P  
73 contacts involve direct and focal interactions between small genomic segments<sup>11</sup>. Yet, Micro-  
74 C does not provide information about the transient nature of such contacts, which may  
75 explain the differences between these studies.

76 Reanalysis of Micro-C data upon chemical PolII inhibition<sup>7</sup> revealed that the reductions  
77 in E-P contacts involved highly focal genomic segments as well, indicating a structural  
78 involvement of PolII<sup>11</sup>. Interestingly, this was also observed when chemical inhibition was  
79 used to reduce the amount of paused PolII. PolII pausing creates local accumulations of  
80 polymerase on both sides of TSS, albeit with considerable variation between genes (as  
81 measured using the “pausing index”)<sup>13</sup>. Indeed, E-P loop strength did not only scale with  
82 transcriptional activity, but also with the pausing index<sup>11</sup>. Combination of Micro-C with a

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

85 How can (paused) PolIII then influence the formation or stability of E-P loops? In  
86 Micro-C maps, so-called interaction “stripes” emanate from PolIII 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 Cohesin<sup>14</sup> and PolIII degradation  
90 reduces Cohesin levels at TSS<sup>8-10</sup>. In a second mechanism, PolIII 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<sup>3,4</sup>. Moreover, this raises the possibility that PolIII 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) PolIII 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*<sup>15</sup>. 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 PolIII 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 PolIII at enhancers  
104 plays a pivotal role. Indeed, this may explain why promoter-promoter loops are less affected  
105 by PolIII-degradation<sup>10</sup>. 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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124 **Competing interests**

125 The author declares no competing interests.

126 **FIGURE LEGEND**

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