Chromatin architecture underpinning transcription elongation

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ABSTRACT
RNA polymerase 2 (pol2) associates with enhancers and promoters, followed by transcription initiation and subsequent pausing. Upon release, pol2 proceeds into productive elongation. A wide spread view of transcription holds that during elongation, pol2 and associated factors clear the promoter proximal region to track along the chromatin fiber until a termination site is encountered. However, several studies are compatible with alternative models. One common feature among these models is that transcription elongation results from movement of the gene along a complex consisting of pol2 and associated factors. Such a scenario predicts that active enhancers and promoters that are bound by transcription complexes, including pol2 are in dynamic physical proximity with the gene body in a manner paralleling pol2 processivity. This has indeed been observed by chromosome conformation capture under conditions of synchronous transcription. Here we discuss these observations and their implication for architectural models of transcription elongation.

Introduction
The eukaryotic genome is folded in a manner that reflects its function. Most distal enhancers form contacts with their target promoters when they are poised or fully active.1-5 In addition to gene-specific long range chromatin contacts between enhancers and promoters, the genome is organized into domains, including TADs (Topologically Associating Domains) and sub-TADs, the former being similar across lineages and even species. The techniques used to measure such contacts, such as chromosome conformation capture (3C) and its various derivations usually provide snapshots of contact averages over large cell populations.6,7 Thus, they typically lack the granularity required to resolve highly fluctuating architectural patterns that introduce cell-to-cell variability. This is particularly relevant to dynamic processes such as transcription elongation. Indeed, little is known about how chromatin folding impacts or is impacted upon during transcription. In the traditional view of transcription, pol2 is recruited to a promoter and, following a pause, proceeds to track along the chromatin template. In this model, pol2 distances itself from the promoter until it reaches the end of the transcribed unit upon which it might fall off and get recycled for additional rounds of transcription. At some genes in yeast and mammalian cells transcription-dependent 5’-3’ gene loops have been described, and it has been suggested that in yeast these loops might contribute to some form of transcriptional memory8 or govern the directionality of elongating pol2.9 Moreover, some genes move toward nuclear pores upon activation,10,11 potentially optimizing their expression.12 Recent work, which is the topic of this Extra View, examined dynamic gene folding patterns of mammalian loci during active transcription. These studies are based on 3C under conditions of synchronous transcription and suggest an alternative model for transcription elongation. Specifically, it is proposed that the DNA templates of some active genes move relative to pol2 complexes that are formed and stabilized by enhancer-promoter contacts.

Questioning the conventional model of RNA polymerase tracking

Genome wide studies of chromatin accessibility, transcription factors, and histone modifications enabled the annotation of enhancer and promoter elements.13,14 With the recognition that many enhancer elements are transcribed and that histone modifications are similar
between the two, the separation of enhancers and promoters has become somewhat blurred.\textsuperscript{15} Since enhancers typically contact the promoters they regulate via chromatin looping,\textsuperscript{3,4,16,17} both classes of regulatory elements are in similar chromatin environments. Enhancer-promoter contacts as measured by 3C are stable under conditions that inhibit transcription elongation or that reduce promoter pol2 occupancy, suggesting that, at least at the examined loci, pol2 per se is dispensable for long range contacts.\textsuperscript{18-20} In addition, Lee et al (2015) examined whether enhancer-promoter contacts change during synchronous transcription elongation at the murine Kit locus. Transcription elongation was inhibited by treatment with the p-TEFb kinase inhibitor DRB, followed by release from the DRB block. During the entire interval of transcription elongation throughout ~82kb of coding region, measured at 7 min intervals, contact frequencies between a distal enhancer located 114 kb upstream and the promoter were essentially constant.

Notably, during the same dynamic conditions, the levels of pol2 and transcription elongation factors at both the Kit enhancer and promoter were stably maintained for the duration of transcription elongation as determined by ChIP (chromatin immunoprecipitation).\textsuperscript{20} During synchronous activation of transcription by TNF-\textalpha treatment, pol2 levels at promoters were also largely maintained.\textsuperscript{21} If pol2 and elongation factors were to depart from the promoter region to track along the gene, why then is their association with the enhancer and promoter ostensibly unchanged? One possibility is that if only a small fraction of alleles is elongating at a given time, the expected reduction in pol2 enhancer/promoter occupancy during later stages of elongation would be too small to be reliably detected by ChIP. However, in the case of the Kit gene, single molecule RNA-FISH revealed that almost all cells were actively transcribing following the DRB block-release.\textsuperscript{20} A second possibility is the gene transcription continuously and rapidly undergoes re-initiation leading to the appearance of stable pol2 promoter/enhancer occupancy. In the case of the Kit locus this scenario seems unlikely as there appear to be more discrete “waves” of transcription elongation. Finally, it is possible that the gene body passes along an elongation competent pol2 complex assembled on an active enhancer-promoter scaffold (Fig. 1). This model deviates from the traditional one of a pol2 complex traveling

![Figure 1](image-url)

**Figure 1.** Architectural model of transcription elongation. In the model depicted, the DNA template moves relative to the pol2 complex that is established via enhancer- and promoter-bound transcription factors. This leads to protrusion of the newly transcribed DNA placing the enhancer and promoter in dynamic proximity with the template. On an absolute scale, whether the pol2 complex is fixed or whether it contributes to the relative movement between it and the template will ultimately be resolved by live cell imaging.
along the gene and is discussed here in light of recent discoveries.

Evidence in favor of genes moving along pol2 complexes

Dynamic gene body contacts with enhancers and promoters

The locus control region (LCR) of the β-globin locus is a strong enhancer with multiple constituent regulatory elements. The LCR is in physical proximity with the active β-globin genes.22,23 3C contacts of the LCR with the 3’ end of the active β-globin gene are sensitive to DRB treatment, while 5’ contacts are not, suggesting that the LCR contacts the gene body in a manner dependent on transcription elongation.20 Since the β-globin gene is too short for reliable spatial analysis of chromatin contacts during elongation, enhancer-anchored 3C studies were carried out at the Kit locus, which comprises a ~82kb coding region and is driven by a distal (~114kb) enhancer that loops to the Kit promoter.24 Following synchronous elongation block-release, these experiments revealed that the enhancer not only contacts the promoter but also the gene body in a manner following the same kinetics as pol2 and transcript production.20 This supports a scenario of the gene body and an enhancer/promoter-anchored pol2 complex moving toward each other. Consistent with this model, 3C detected dynamic contacts of the Kit promoter with the gene body. Similar results were observed at the CD47 locus, suggesting that this model is not a peculiarity of the Kit locus.

It has been proposed that pol2 and associated protein complexes are organized into so-called “transcription factories” that can accommodate more than one gene at a time and which represent relatively immobile structures in the nucleus.25-27 The idea of factories in their original definition as static structures to which genes diffuse is still being debated.28,29 However, the factory model shares certain features with the model discussed here, most prominently including the idea that transcribing genes move relative to a pol2 complex. Using 3C, dynamic contacts between the promoters and gene bodies matching pol2 progression of TNF-α-induced genes have been detected.21,30 Supporting the idea of mobile genes and immobile pol2 complexes is the observation that 2 TNF-α-induced genes change the relative position to each other during synchronous activation, arguing against the idea of pol2 tracking along static genes.31 However, the architectural model based on the TNF-α regulated genes is also different in some critical ways from that based on the Kit and CD47 genes. Super resolution RNA-FISH with intron probes against the 5’ end of the TNF-α-induced gene Samd4 transcript and various additional positions along the transcript at successive time points suggested that the promoter separates from the pol2 complex, extruding into the nucleoplasm and later re-engages the same complex or a different one nearby. In contrast, the second model that is based on the studies of the Kit and CD47 genes proposes that the pol2 complex remains in stable proximity with the enhancer and promoter as the gene body travels alongside its surface (see below for a more detailed mechanistic description). The observation that pol2 ChIP signals appear to be steady throughout the elongation interval at the TNF-α-induced genes as well as at the Kit and CD47 genes support the second model but don’t prove it. The gain in measured distances between 5’ and 3’ RNA-FISH probes of the TNF-α-induced Samd4 gene is harder to reconcile with the second model, but it is possible that the probes detected a fraction of transcripts that are not yet fully spliced. It also remains possible that different genes use different modes by which they operate. In either situation, however, the models are consistent with the gene moving relative to a complex consisting of pol2, elongation factors, and RNA processing complexes.

Insights from Hi-C studies

If enhancers and promoters are in proximity with the coding region during transcriptional elongation as suggested by the above studies, one might expect that previous large scale studies on chromatin contacts would have uncovered them. There are indeed hints that such contacts do occur, but the results allow for additional interpretations. For example, a Hi-C study in human fibroblast IMR90 cells reported asymmetric distributions of cis contacts from highly active promoters leaning toward gene bodies.16 However, the downstream bias in contact frequencies was maintained when transcription elongation was inhibited globally using the pharmacologic p-TEFb inhibitor flavopiridol. This downstream bias of promoter contacts might reflect the presence of intronic enhancers that form looped contacts with their target promoters. A more recent high-resolution capture Hi-C study of
~22,000 promoters also revealed an asymmetric distribution of chromatin contacts, tipping the balance toward gene bodies.\textsuperscript{3} Notably, this interaction bias occurred predominantly at active genes. However, it remains open whether this is due to gene bodies being moved along the promoter, or whether intronic regulatory elements contribute to this imbalance, as above. Another study employing both 3C and anti-pol2 ChIA-PET observed contacts between enhancers/promoters and gene bodies.\textsuperscript{32} However, in this case interactions favored exons residing in DNaseI hypersensitive sites, leading the authors speculated that this folding pattern might influence alternative splicing. The observation that the elongating (serine 2 phosphorylated) form of pol2 was enriched at the exons involved in promoter/enhancer contacts is in agreement with the model. However, whether the promoter-exon interactions are maintained or lost upon inhibition of transcription was not examined. More generally, it is likely that the lack of transcriptional synchrony or low level gene expression has diminished the ability to detect of enhancer/promoter-gene body interactions in these high throughput studies.

**Indirect evidence for contacts between gene bodies and enhancer/promoters**

Some clues regarding chromatin folding models of elongating genes can be gleaned from ChIP studies. If an enhancer contacts the coding region during transcription elongation it is possible a) that enhancer-associated factors might be crosslinked to the coding region during elongation, b) conversely, that elongation factors might be detectable at the enhancer, and c) that proteins mediating enhancer-promoter contacts might also be detectable in the coding region by ChIP. There are examples for all 3 of these scenarios. The chromatin “reader” protein Brd4, which occupies most enhancers is detected in the coding regions of genes\textsuperscript{33-35} in a manner depending on active transcription.\textsuperscript{34} Several studies observed the elongating form of pol2,\textsuperscript{36-40} certain elongation factors,\textsuperscript{38,41,42} and general transcription factors\textsuperscript{43,44} at enhancers. The elongation factor Spt5 has been detected not only in the gene body but also at the Kit promoter and enhancer. Of note, Spt5 levels at the promoter and enhancer remained essentially constant during synchronous transcription elongation.\textsuperscript{20}

Naturally, since many enhancers are transcribed to produce eRNAs, it is expected that basal transcription factors, pol2 and components of the transcription elongation machinery exist at active enhancers.\textsuperscript{45} However, there are cases in which this explanation might be insufficient. Why, for example, do the amounts (measured by ChIP) of the elongating form of pol2 (phosphorylated on serine 2) and the p-TEFb components Cdk9 and cyclin T at the active \( \beta \)-globin LCR match or exceed those measured in the coding regions?\textsuperscript{38} It is possible that this reflects a very high rate of eRNA production, and one would need to assume a high turnover rate to account for the low steady state eRNA levels. However, an alternative explanation is that the ChIP signals at the LCR result in part from the close proximity of the LCR with the transcribing gene body. Of course, it cannot be presumed that all enhancer bound factors can be similarly crosslinked to the gene body during elongation because of variable distances to DNA and the dynamic nature of the contacts during elongation. Hence, the presence or absence of ChIP “indirect peaks” are thus harder to interpret. But it is interesting to note that p-TEFb, a kinase complex that facilitates the transition from paused into the elongating form of pol2 is detected not only near promoters where it catalyzes serine 2 phosphorylation but also throughout the coding region,\textsuperscript{38,40,46} again consistent with the idea that DNA might move along a large machinery consisting of pol2 and associated complexes.

Another intriguing observation is that the architectural cohesin complex, which is enriched at many promoters and enhancers, is also found in actively transcribed regions overlapping with elongating pol2.\textsuperscript{40} This observation could be interpreted as above, supporting contacts with yet another complex, i.e. looping factors, as being stationary with the gene traveling alongside. Cohesin and mediator not only promote long range enhancer-promoter contacts\textsuperscript{40,47-49} but might also facilitate productive elongation\textsuperscript{40,50} (for Mediator, see review\textsuperscript{51}). In addition, it is tantalizing to think that cohesin might be actively involved in the elongation process by promoting the protrusion of a gene loop in which the gene body travels past the enhancer-promoter complex (Fig. 1). This is a variant of the loop protrusion model\textsuperscript{52-55} and the driving force might be the elongating pol2.

The model clearly becomes more complicated, and looped structures more intricate if one considers...
transcription re-initiation that might occur prior to completion of the first round of transcription. Also, from many promoters transcription initiates bidirectionally. Hence, one would have to propose that template reeling occurs either simultaneously from both directions or in an alternating fashion. Finally, it is not immediately obvious how regulatory elements in the coding regions of genes, such as intronic enhancers would impact this folding pattern.

**Challenges for the future**

3C based technologies have provided the lion share of information about chromosomal folding patterns. Yet, like all methods, they are fraught with limitations that affect the interpretation of any measurements they provide. One potentially confounding factor is that crosslinking efficiencies vary depending on elongation rate and protein composition along the gene. In addition, there might be biases in restriction digestion and ligation. These considerations are especially relevant in the context of dynamic interactions, such as transcription elongation. The combined use of 3C and synchronous transcription initiation or elongation block-release has improved detection of contacts of promoters and enhancers with the coding region, but none of these experiments are immune to the above technical artifacts. This necessitates the use of orthogonal technologies that are principally distinct from proximity ligation methods. High resolution imaging in fixed or live cells will ultimately be useful to test these models once the necessary resolution has been achieved to reliably detect interactions on the scale of tens of kilobases.

It is tempting to adopt the simplest architectural model of transcription elongation which proposes that the megadalton protein complexes involved in transcription elongation, chromatin remodeling, and RNA processing might not all spiral around the double helix but that instead the DNA is pulled toward such machineries. This idea is not new, of course, and has been invoked in the context of transcription factories.26,27 As pointed out above, the recent results summarized here don’t need to invoke stable pre-existing factories. To determine whether these complexes are immobile or fixed requires more live cell imaging studies and needs to take into consideration that individual molecules might be dynamically associating or dissociating with these complexes.

If a promoter makes contacts with its own gene body,20,21,30 how does this process get started? If the gene folds back on itself to allow for promoter-gene body contacts, this would initially require a sharp bend in the DNA. In this regard it is noteworthy that many DNA binding proteins, including basal transcription factors such as TBP do indeed strongly bend DNA,56 and the initial unwinding of DNA in the transcription bubble might impart further flexibility needed to initiate the dynamic folding pattern that ultimately progresses via loop protrusion.

Another important question to be addressed in the future is how widespread elongation dependent enhancer-gene body contacts are. Not all genes necessarily fall into the same patterns and their mode of elongation might be influenced by genomic organization and gene size (for example, Yao et al57). Hi-C at high temporal and spatial resolution under conditions of synchronous transcription elongation should provide global insights especially when bolstered by imaging of more representative genes.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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