

lncRNA and gene looping

What's the connection?

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Abbreviations: lncRNA, long non-coding RNA; 3C, chromosome conformation capture; FISH, fluorescence *in situ* hybridization; RNAP II, RNA polymerase II; ER α , estrogen receptor alpha; HUVEC, human umbilical vein endothelial cell; LCR, locus control region; eRNA, enhancer RNA; CTCF, CCTC-binding factor; TAD, topologically associating domain; PALM, photoactivated localization microscopy; CS, consensus site

Recent functional studies have unveiled the significant role chromatin topology plays in gene regulation. Several lines of evidence suggest genes access necessary factors for transcription by forming chromatin loops. A clearer picture of the players involved in chromatin organization, including lncRNA, is emerging.

Introduction

The architectural landscape of the nucleus, while lacking in membranated compartments, has a profound influence on gene regulation. Chromosome conformation capture (3C) technologies and fluorescence *in situ* hybridization (FISH) techniques have revealed elements that are distally located either on the same or separate chromosomes, to be proximal in the three dimensional nucleus.¹⁻⁴ Contact between these elements, of which the most characterized being between an enhancer and a promoter or between two or more promoters, has been shown to be cell- and context-specific with functional consequences.^{3,5,6} Mechanisms by which these contacts are established and maintained, and their effect on the transcription of genes involved, are areas of intense scrutiny in recent years. Here we discuss some of the latest developments in these areas with a focus on an emerging class of long non-coding RNA (lncRNA), enhancer or activator RNA, which may play a vital role in loop-mediated transcriptional activation.

Multigene complexes provide a topological framework for coordinated transcription

In the last two decades, numerous studies revealed a varied distribution of nascent transcripts and RNA polymerase II (RNAP II)-enriched foci throughout the nucleus of eukaryotic cells, indicating that transcription is spatially confined in a limited number of discrete sites.⁷ These studies have led to the hypothesis that multiple active genes, by means of chromatin loops, cluster to spatially discrete nucleoplasmic sites rich in RNAP II for transcription to occur.^{3,7} In support of this model, 3C-derived methodologies have shown distally-located, transcriptionally active genes to be in contact with one another. Recent genome-wide investigation of chromatin interactions, or the interactome, mediated by RNAP II^{3,4,8} revealed widespread stimulus-responsive promoter-promoter interactions between co-regulated genes, with an average of ~9 genes³ per interaction complex. While these data may be prone to artifacts and therefore should be approached with caution, these interactions could be modeled into a hierarchical network of chromatin communities with functional compartmentalization using a systems approach.⁹ Together, these observations gave insight into the role of such “multigene complexes” in providing a topological framework for the transcription of co-regulated genes and how transcription is organized in three dimensional space.

Further glimpses into how chromatin contact may moderate transcriptional regulation came with an observation of an estrogen-inducible gene *GREB1* and its several interacting partners in MCF-7 cells. Strikingly, genes that do not directly bind the transcription factor ER α at their promoters seemed to access ER α by forming gene loops with *GREB1*, whose promoter directly binds ER α , leading to transcriptional activation of those genes.³ RNAi-mediated knockdown of ER α led to loss of contacts, as well as a reduction in transcription, between all interacting genes in this multigene complex.³ A tempting hypothesis arising from these results was that chromatin loops provide a topological framework for “synergistic” transcription,³ whereby genes cooperate to share necessary factors to reach higher levels of transcription than otherwise possible.

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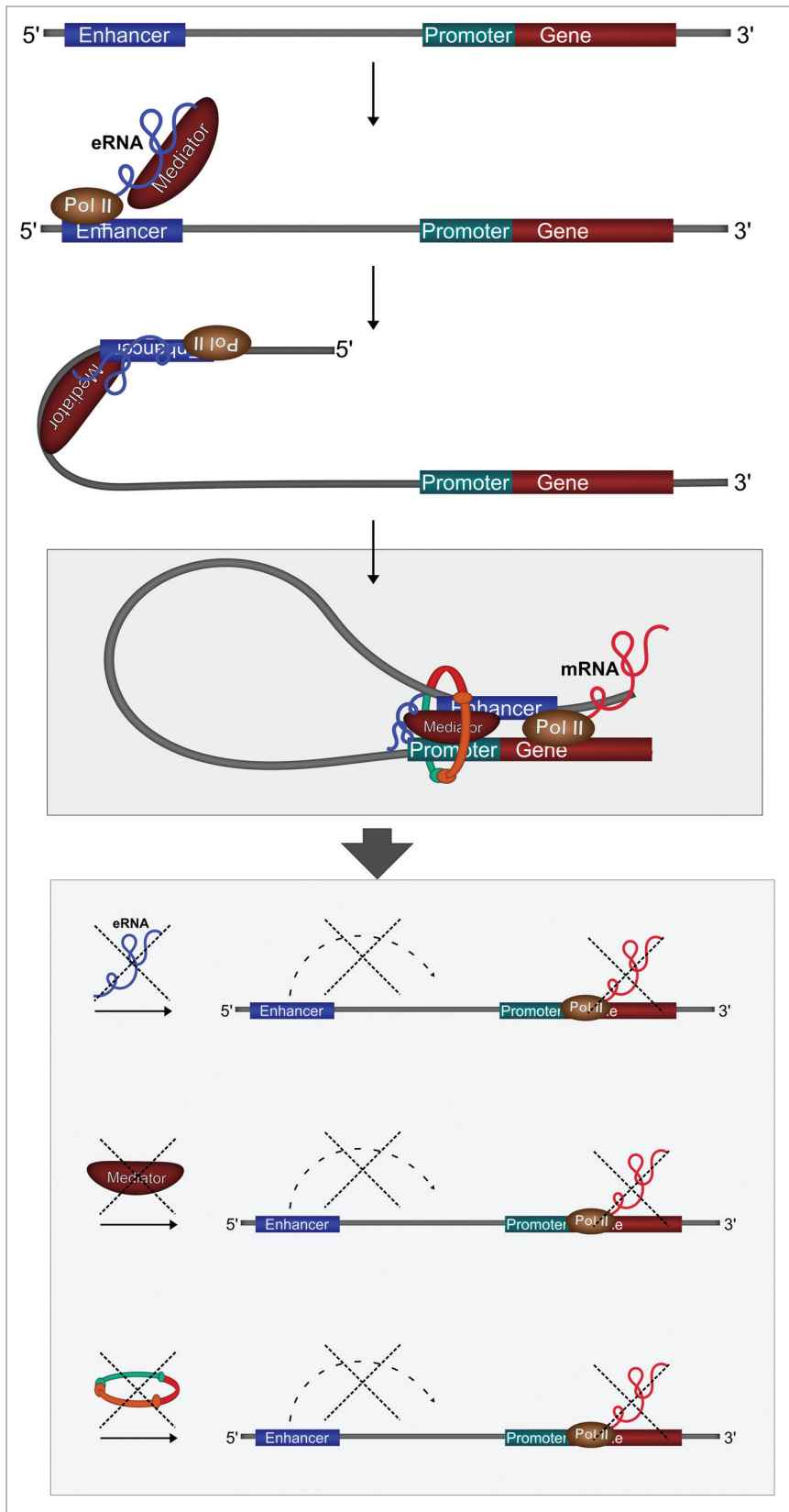


Figure 1. Enhancer-promoter interactions. eRNA associating with Mediator may induce chromatin looping for enhancer-promoter contact, leading to transcriptional activation from the target promoter. Contact may be stabilized by cohesin. Disruption of either eRNA, Mediator or cohesin may lead to loss of contact and transcriptional deactivation.

interacting genes by microscopy. Loop disruption was achieved by introducing double strand breaks using TALE nucleases in a well characterized NF- κ B-regulated multigene complex that includes *SAMD4A*, *TNFAIP2*, and *SLC6A5* genes in HUVECs.⁶ Unexpectedly, these assays revealed a hidden hierarchical transcriptional organization between the interacting genes. In particular, disruption of the *SAMD4A* gene loop led to a dramatic reduction of *TNFAIP2* and *SLC6A5* transcription.⁶ Meanwhile, disruption of the *TNFAIP2* gene loop led to the reduction of only *SLC6A5* transcription, whereas disruption of the *SLC6A5* gene loop did not influence the transcription of either *SAMD4A* or *TNFAIP2*.⁶ These findings provide direct evidence that loop-mediated chromosomal contacts precede and regulate the transcription of interacting genes. Speculating about the dynamics behind these interactions, the “collector function” hypothesis was proposed, by which *SAMD4A* collects *TNFAIP2*, which in turn collects *SLC6A5*, whereby NF- κ B and RNAP II are passed on from the dominant to the subordinate gene promoters at each round of collection.⁶ Furthermore, repair of the sheared *SAMD4A* loop with a foreign sequence resulted in the restoration of co-transcription of the three genes,⁶ providing additional evidence that chromatin loops provide the topological framework for transcription of co-regulated genes, in a sequence independent manner.

The collector function hypothesis is supported by recent evidence from live super-resolution (PALM) microscopy, which demonstrated RNAP II clustering is dynamic and transient, with an average lifespan of 5.1 s.¹⁰ De novo clustering of RNAP II observed in the study¹⁰ may correlate to the phase where polymerase is delivered from one promoter to the other. RNAP II clustering is followed by sudden disassembly,¹⁰ indicative of a mobile transcriptional elongation step.

Enhancers and their transcripts: topological elements of transcription

To directly address the role of chromatin contacts on gene regulation, Fanucchi et al.⁶ discretely disrupted chromatin loops at sites of contact and monitored the transcriptional status of

Chromatin looping also brings regulatory enhancer elements to their target promoters to activate transcription.^{4,5} A classic paradigm is the physical interaction between the distal enhancer

locus control region (LCR) and the target β -globin promoter, located over 50kb away from the LCR.^{5,11} The LCR- β -globin interaction has been repeatedly shown to result in the transcriptional activation of the β -globin target gene by bringing proteins critical to transcription into physical proximity with each other. In cells unable to form this enhancer-promoter loop, forceful tethering of Ldb1, a transcription factor thought to mediate the LCR- β -globin interaction,¹¹ to the β -globin promoter showed that formation of the LCR- β -globin loop underlies transcriptional activation.⁵ These findings unveiled enhancer-promoter interactions as indispensable topological constituents of mammalian transcription.

Previous studies have indicated that mechanisms of enhancer functioning involved the recruitment of transcription factors to promote the detangling of repressed chromatin and facilitate the assembly of transcriptional machinery on target genes.¹² A remarkable recent observation is the bi-directional transcription of a novel class of lncRNA termed enhancer RNA (eRNA), also known as activating RNA,^{13,14} arising from enhancer loci throughout the genome.^{15,16} Stimulus-dependent transcription of eRNA has been correlated with transcriptional activation of nearby coding genes, hinting at the possibility that eRNAs may be a major regulator of transcription.¹⁵⁻¹⁹ This has been supported in functional studies in which inhibition of eRNA led to reduction in mRNA levels from specific neighboring genes,^{13,14} as well as loss of specific enhancer-promoter contacts (Fig. 1).^{20,21} eRNAs have been shown to be both positively and negatively regulated to influence transcription from target promoters, with specificity between the eRNA and the target in both cases.^{20,21}

Many questions arise with respect to the molecular function of eRNA activity. Is eRNA function derived from sequence specificity, structural motifs or a combination of both? Is enhancer-promoter geographical proximity the crucial aspect of eRNA function? At what maximal or minimal distance can they act and how is this related to looping topology? Indeed fundamental questions remain unanswered. Genome editing of enhancer sequences should shed light on these issues. It has been proposed that eRNAs exert their activity through interaction with Mediator, whose depletion abrogates DNA bending and a loss of phosphorylation of H3S10 and subsequent enhancer-promoter loops resulting in diminished transcription of eRNA target genes (Fig. 1).¹⁴ This is supported by evidence from disease-causing mutations in Mediator subunit MED12, which cause the reduced ability to associate with eRNAs.¹⁴ Cohesin, known to form rings to connect two segments of DNA, has also been shown to stabilize eRNA-induced enhancer-promoter interactions and, therefore, influence target gene transcription (Fig. 1).²⁰ These observations are further supported by the demonstration of physical interactions between Mediator, cohesin and its loading factor Nipbl at looping promoter-enhancer regions, which is associated with specific expression programs in different cell-types.²² Taken together, this set of findings suggests a possible mechanism by which eRNA first binds Mediator to cause chromatin looping (Fig. 1), followed by association of Mediator to Nipbl and cohesin at the chromatin contact site to stabilize the loop, in order to drive transcription from the promoter.

Mediator, CTCF, and cohesin stitch the genome together

Studies to identify the architectural proteins shaping the three dimensional genome have revealed CTCF-binding factor (CTCF), Mediator and cohesin to have widespread and specific roles across the genome.²³⁻²⁶ Consistent with the above-mentioned role in enhancer-promoter interactions, Mediator and cohesin were found to specifically bridge short-range, cell-type-distinct interactions.²⁴ On the other hand, long-range interactions were found to be bridged by CTCF and cohesin.^{24,25}

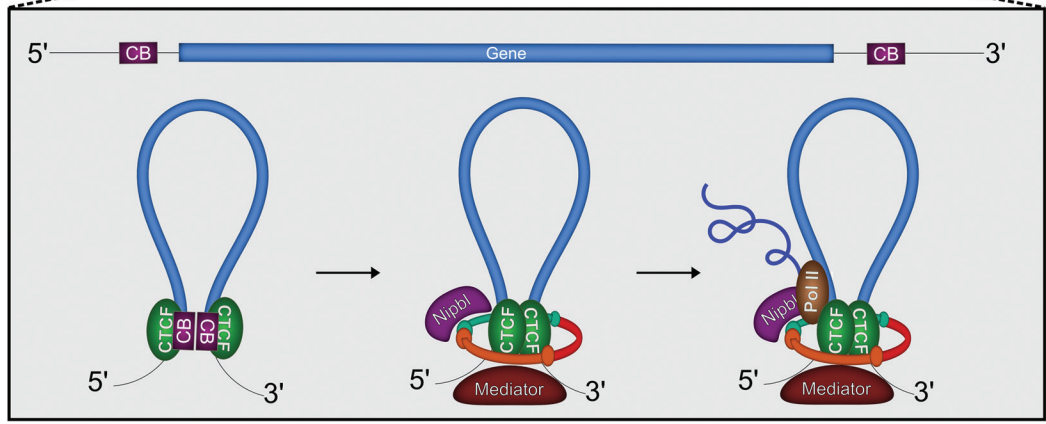
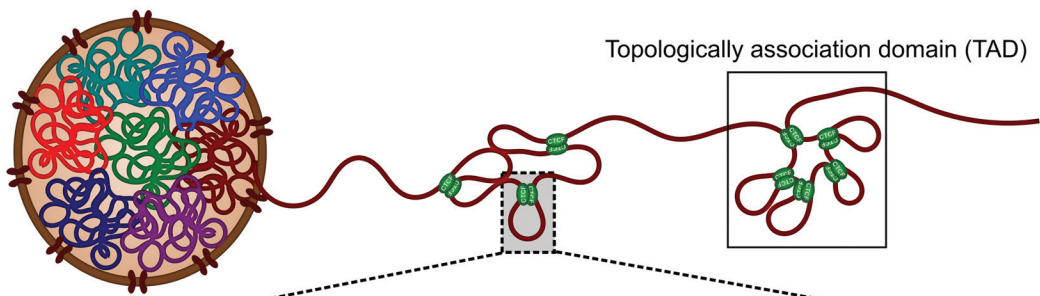
High-throughput chromosome conformation capture in *Drosophila*, mouse and human cells have suggested that genomes are segregated into discrete megabase-sized local domains termed “topologically associating domains” or “topological domains” (TADs) (Fig. 2A).²⁷⁻²⁹ The highly stable and invariant nature of these domains suggests that they are a pervasive and intrinsic mechanism of chromatin organization within genomes. A characteristic feature of TADs is the enrichment of intra-domain chromatin contacts.²⁷ FISH results confirm a spatial distinction between domains and contact arrangements within domains, as loci within a single domain are closer in nuclear space than those in different domains despite having similar genomic distances from one another.^{27,29} TADs therefore may provide a layer of structural regulation governing the principle of long-range chromatin contact.

Boundaries of TADs are enriched in binding sites for CTCF and cohesin complex, implicating their importance in maintaining domain integrity and loop-mediated transcription (Fig. 2A).²⁷ Experiments in cells containing a deletion that spans the boundary between the *Xist* and *Tsix* TADs in the X-chromosome inactivation center, directly demonstrated that the loss of boundary had led to the partial fusion of the adjacent TADs and the formation of new and ectopic contacts, causing long-range transcriptional misregulation.²⁹ Furthermore, depletion of CTCF and cohesin has revealed that these factors may contribute differentially to domain organization and transcriptional regulation.^{25,26} Particularly, disruption of cohesin reduces local chromatin interactions although TADs remain intact, whereas depletion of CTCF leads to a reduction in local intradomain interactions but also to an increase in interdomain interactions.^{25,26} In each case, different classes of genes are misregulated, indicating that each factor has a distinct capacity in chromatin organization and gene regulation.²⁶

Prospects

Given the potential differential contribution of CTCF and cohesin in chromatin organization, could the same be true for Mediator and lncRNA? More specifically, are lncRNAs only associated with organizing enhancer-promoter contacts? Transcriptome studies have identified approximately two-thirds of the mammalian genome to be pervasively transcribed into lncRNA.³⁰ Although some lncRNAs have been shown to possess regulatory functions,³¹ targets of most lncRNAs and mechanism by which they work remain elusive. Considering the extensive nature and the specificity of chromatin contacts across the genome, and in light of eRNAs thus far providing the specificity in one category of looping

A



B

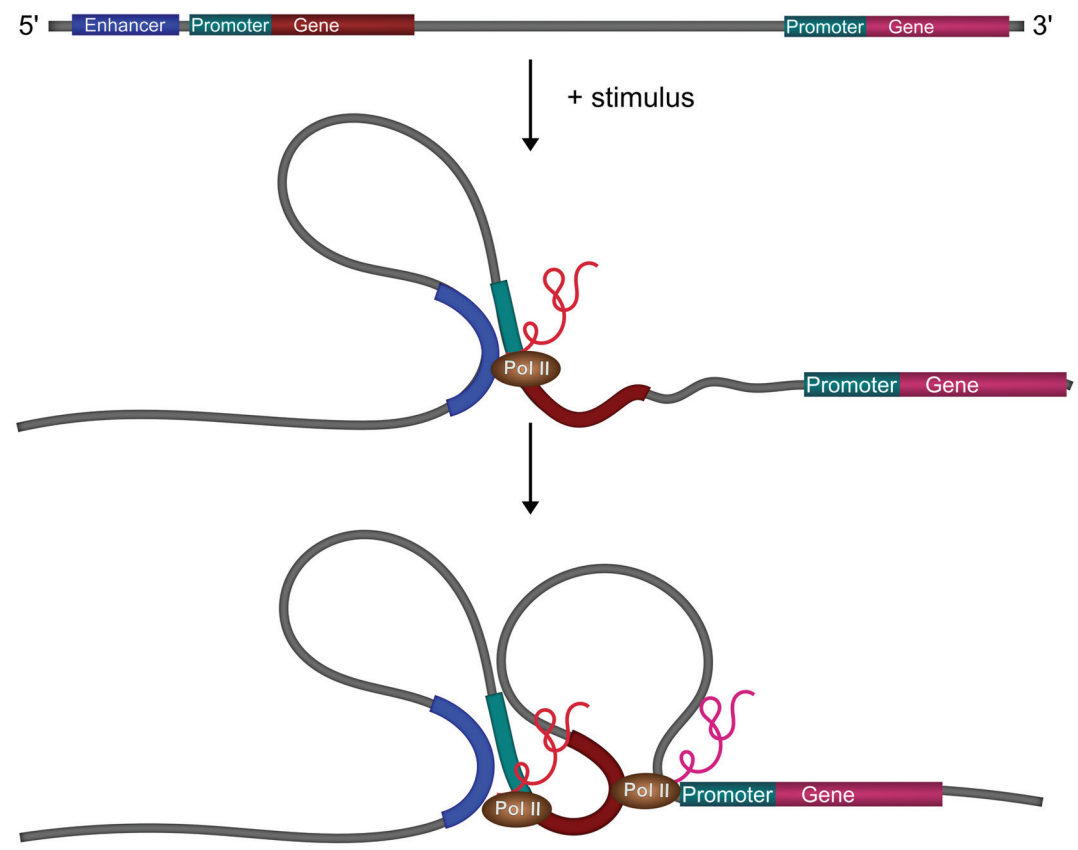


Figure 2 (opposite). Topological domains and multigene complexes. **(A)** Chromosomes are organized into subchromosomal domains, referred to as TADs. CTCF, a sequence-specific DNA-binding protein, binds to consensus sites (CS) that frequently flank genes.²⁵ At CS sites, the multiprotein cohesin 'ring-like' complex (including the Smc1-Smc3 heterodimer, Rad21 and Scc3/SA1/SA2) is loaded onto chromatin by Nipbl. The mediator complex may also be recruited to CTCF and/or cohesin occupied chromatin to stabilize loop topology, and regulate transcription initiation and elongation.²² **(B)** Enhancer-promoter and promoter-promoter interactions in a single multigene complex. eRNA may possibly hold together the entire multigene complex.

interactions, it is tantalizing to envision lncRNAs, not limited to eRNAs, holding the key to chromatin topology and gene regulation.

Interactome studies have shown RNAP II-mediated chromatin contacts can involve multiple enhancers and promoters in the same multigene complex (Fig. 2B).^{3,4} Accounting for the fact that enhancers are critical in driving transcription from target promoters, and given the possible hierarchical relationship existing between promoters,⁶ it is not farfetched to conceive of eRNAs as important regulators of transcription in multigene complexes.

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Further research using combinations of genome editing techniques, labeling of RNA in live cells, biochemical and microscopy approaches will be required to gain insight into the details of chromatin organization and its influence on transcriptional regulation. To this end, we recommend single-cell assays to account for potential cell-to-cell variability in chromatin contacts in assessing what are often rare events.⁶ For microscopy, we predict light sheet-based super-resolution techniques in live cells will permit visualization at the necessary spatiotemporal resolution allowing insight into these processes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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