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The role of loop extrusion in enhancer-mediated gene activation

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Gene expression patterns in complex multicellular organisms are regulated by enhancers, which communicate with their target gene promoters in three-dimensional (3D) chromatin structures. Despite advances in our understanding of the mechanisms that organize mammalian genomes into compartments and topologically associating domains (TADs), it is not well understood how specific interactions between enhancers and promoters are controlled in this 3D context. In this review, we give an overview of recent evidence that shows that a process of loop extrusion plays an important role in the regulation of enhancer–promoter communication and discuss recent insights into the molecular mechanism by which loop extrusion contributes to enhancer-mediated gene activation.

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Introduction

Enhancers are noncoding *cis*-regulatory elements that play a central role in the regulation of gene expression patterns during differentiation and development [1]. Active enhancers recruit transcription factors and coactivators, which stimulate assembly and activation of the transcription machinery at the promoters of their target genes [2]. In mammals, enhancers and their target genes can be separated by large genomic distances (10 kb– > 1 Mb) [3]. Communication between enhancers and promoters therefore depends on interactions in the three-dimensional (3D) nuclear space, which are thought to be driven by molecular affinities between the transcription factors and coactivators bound at these elements. The formation and specificity of these interactions are dependent on the 3D organization of the genome in the nucleus [4].

Mammalian genomes are organized by at least two distinct, independent mechanisms [5,6]. Compartmentalization of the genome reflects spatial separation of euchromatin and heterochromatin and distal interaction between regions with shared chromatin modifications. This is thought to be driven by microphase separation [7]. In addition, a process of loop extrusion restricted by boundary elements organizes chromosomes into local self-interacting regions, which are referred to as topologically associating domains (TADs) [8,9]. During this process, a loop-extruding factor associates with chromatin and translocates along the fiber in opposite directions. It thereby extrudes a progressively larger loop, until the factor is halted at boundary elements [10,11]. This process leads to the formation of a self-interacting domain that is demarcated by the extrusion boundaries. In the last decade, accumulating evidence has identified Cohesin as the main loop-extruding factor and CTCF-binding elements as important boundaries in mammalian cells in the interphase [12–14].

It has been shown that the ability of enhancers to activate gene promoters is largely (though not exclusively [15]) constrained to genes located in the same TAD [16]. This observation suggests that the process of loop extrusion facilitates the formation of enhancer–promoter interactions within TADs and/or prevents regulatory interactions across TAD boundaries, and thereby regulates the specificity of enhancer–promoter communication. This model predicts that global perturbation of loop extrusion causes pervasive misregulation of gene expression. Initial studies have shown that depletion of Cohesin and CTCF leads to a loss of TAD structures across the genome, thus confirming the importance of these proteins for genome organization [17–20]. However, surprisingly, the drastic changes in genome organization following Cohesin and CTCF perturbations were associated with relatively minor effects on gene expression [17–20]. The role of loop extrusion in regulating enhancer-mediated gene activation has therefore been disputed.

In the last few years, detailed analyses of the relationship between loop extrusion, enhancer–promoter interactions, and gene regulation have given new insights

into this debate. In this review, we present an overview of these studies and discuss how they contribute to a nuanced understanding of the importance of loop extrusion for the regulation of enhancer-mediated gene activation and the underlying molecular mechanism.

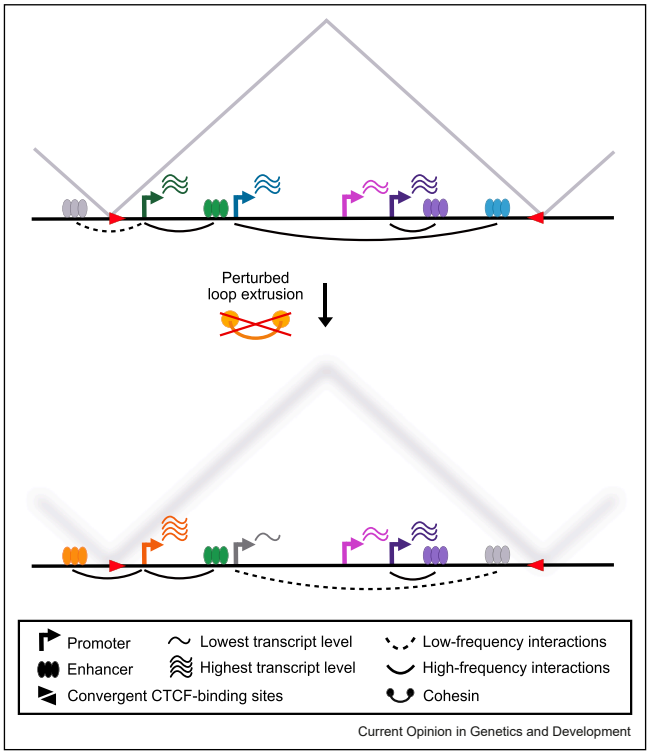
The role of loop extrusion in the regulation of gene expression

The initial Cohesin and CTCF perturbation studies were performed in steady-state conditions [13–16]. Therefore, a possible explanation for the observed mild impact on gene expression is that loop extrusion is particularly important for establishing enhancer–promoter communication during differentiation or in response to stimuli, and that molecular affinity between proteins bound at enhancers and promoters is sufficient to maintain interactions in a steady state. Furthermore, it is plausible that the impact of loop extrusion on gene expression varies across genes depending on a number of properties: (1) the extent to which a gene is dependent on enhancer-mediated activation; (2) the genomic distance between the gene and its enhancers; and (3) the location of the gene in relation to the TAD boundary (Figure 1).

In line with these hypotheses, several recent studies have shown that loop extrusion contributes to the activation of cell-type-specific, enhancer-dependent genes. For example, it has been shown that loss of Cohesin has a strong impact on the expression of inducible genes in primary macrophages in response to inflammatory stimuli, whereas expression of most constitutive genes is unchanged in the absence of Cohesin [21]. More recently, experiments involving single-molecule RNA-FISH in the same system have demonstrated that Cohesin contributes to the induction of enhancer-dependent genes by coupling the transcriptional bursting probabilities of enhancers and promoters [22]. In a similar system, it has also been shown that accurate regulation of inflammatory response genes in induced macrophages is dependent on CTCF [23]. Furthermore, a recent study in postmitotic neurons has demonstrated that Cohesin plays an important role in the regulation of genes that facilitate the maturation and activation of cortical neurons and that its depletion leads to reduced morphological complexity of neurons [24••].

Experiments in postmitotic neurons have also suggested that the degree to which genes are dependent on Cohesin for their activation is dependent on the genomic distance between their *cis*-regulatory elements [24••]. Similar observations have been made in other recent studies. For example, analyses of engineered regulatory

Figure 1



The impact of loop extrusion perturbations on gene expression depends on the relative locations of *cis*-regulatory elements within TADs. The process of loop extrusion contributes to accurate gene regulation by mediating interactions between distant *cis*-regulatory elements within TADs and separating elements in adjacent TADs (top panel). Perturbations of loop extrusion lead to a loss of TADs and can lead to changes in gene expression (lower panel). This is particularly prominent for genes located near TAD boundaries (green/orange) and genes regulated by enhancers over large genomic distances (blue/gray). Loop extrusion is less important for the regulation of genes with nearby enhancers (purple) and enhancer-independent genes (magenta).

landscapes in K562 cells have shown that the same enhancer–promoter pair is strongly dependent on Cohesin when separated by ~100 kb, mildly dependent across a distance of ~50 kb, and Cohesin-independent when separated by ~10 kb [25•]. Another recent study has shown that synthetically activated *cis*-regulatory elements in mouse embryonic stem (mES) cells can activate the expression of a target gene in absence of Cohesin over a distance of ~100 kb, but fail to robustly induce target expression over larger distances [26]. A distance-dependent function for Cohesin in enhancer-mediated gene activation suggests that loop extrusion has a facilitating role in the formation of enhancer–promoter interactions, which is more prominent for elements that are far apart in the linear genome. It

has been suggested that the degree to which long-range enhancers depend on loop extrusion varies with the strength of the enhancer [27••]. This could explain the differences between the genomic distances at which Cohesin is required for gene activation between the abovementioned studies, as each of these studies is based on different enhancer–promoter pairs in their native or a synthetic context.

It has been suggested that the importance of loop extrusion for the regulation of gene expression is not only distance-dependent, but also location-dependent [15,28]. This is supported by a recent study in HCT-116 cells, which has shown that depletion of Cohesin has a bigger impact on the expression of genes near TAD boundaries compared with genes in the center of TADs [15]. This observation suggests that Cohesin-mediated loop extrusion is important to prevent aberrant regulatory interactions across TAD boundaries, which is further supported by numerous studies that have shown that perturbations of CTCF-binding sites at TAD borders can lead to rewiring of enhancer–promoter interactions and ectopic gene activation [29,30].

Interplay between loop extrusion and enhancer–promoter interactions

In the first Cohesin and CTCF perturbation experiments, changes in genome architecture were analyzed using Chromosome Conformation Capture (3C) approaches with relatively low resolution [17–20]. Although these studies have given important insights into the role of loop extrusion in large-scale genome organization, they could not directly resolve the impact of loop extrusion perturbations on enhancer–promoter interactions. More recently, further insight into the function of loop extrusion in enhancer-mediated gene activation has been provided by analyses of enhancer–promoter interactions in the absence of components of the loop extrusion machinery using high-resolution MNase-based and targeted 3C approaches.

For example, using a combination of Hi-C and 4C-seq analysis in the context of depletion of the Cohesin release factor WAPL in mES cells, it has been shown that accurate regulation of Cohesin turnover is important for maintaining enhancer–promoter interactions [31•]. In addition, analysis of enhancer–promoter interactions at pluripotency gene loci in mES cells using a targeted MNase-based 3C approach has shown that Cohesin depletion leads to reduced interactions between enhancers and promoters that are separated by more than ~10 kb [32]. Similarly, Promoter-Capture Hi-C experiments in HCT-116 cells have indicated that Cohesin depletion leads to changes in interaction patterns and expression levels of a subset of gene promoters [28]. Furthermore, 5C analysis in Cohesin-depleted cortical neurons has shown that long-range (> 1.5 Mb) interactions between a gene promoter and its enhancers are reduced in the absence of Cohesin, whereas interactions spanning a shorter genomic distance (< 40 kb) were not severely impacted [24••].

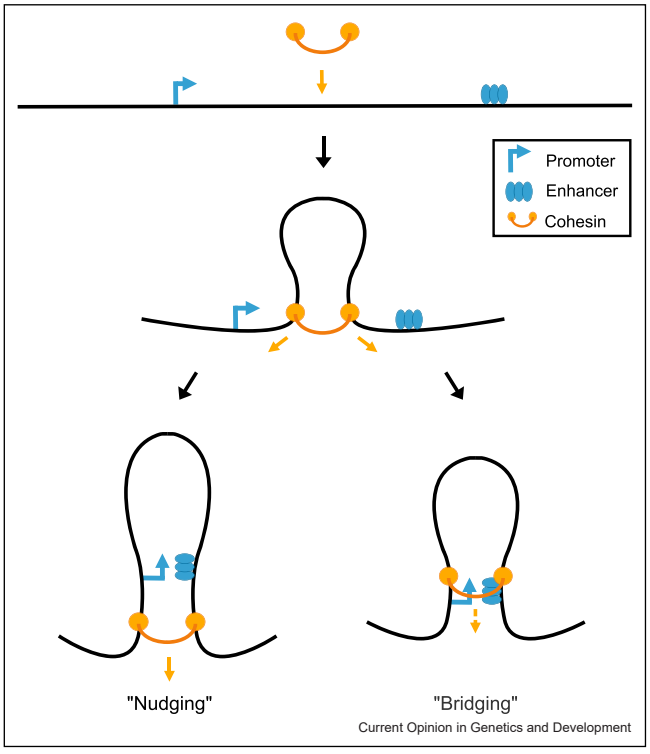
It is possible that the presence of a CTCF-binding site in close proximity to a gene promoter facilitates the formation of long-range enhancer–promoter interactions via loop extrusion. In support of this hypothesis, it has been shown that approximately 10% of genes are associated with a promoter-proximal CTCF-binding site within ± 10 kb of the transcription start site and that expression of these genes is disproportionally affected upon CTCF depletion [33]. However, analysis in cortical neurons has suggested that CTCF binding at gene promoters is not predictive of misregulation of gene expression upon Cohesin depletion [24••]. The extent to which CTCF-binding sites proximal to gene promoters contribute to enhancer–promoter interactions and the underlying molecular mechanism therefore remain unclear.

The impact of loop extrusion perturbations on the strength of enhancer–promoter interactions described in the abovementioned studies is relatively subtle. In contrast to the global loss of TADs and CTCF 'loops' in the absence of Cohesin [18–20], enhancer–promoter interactions are not completely abolished [24••,28,31•,32]. In line with these observations, it is of interest that two recent reports based on analysis with Micro-C [34] and a targeted MNase-based 3C approach [35] did not detect clear changes in enhancer–promoter interactions upon Cohesin, CTCF, and WAPL depletion in mES cells. It is possible that the discrepancy between these studies and the findings described above is related to the short depletion time (3 h) used in these studies. Together, these observations suggest that loop extrusion is particularly important for the formation and/or long-term maintenance of enhancer–promoter interactions and plays a relatively minor role in their short-term maintenance.

Mechanistic insights into loop extrusion patterns

Loop extrusion provides an efficient and systematic scanning mechanism by which *cis*-regulatory elements can find their cognate interaction partners along the chromatin fiber. In principle, active scanning in a specific region demarcated by boundary elements suffices to explain both a facilitating and insulating function for loop extrusion in the regulation of enhancer–promoter interactions. Elements within a TAD are 'nudged' to

Figure 2



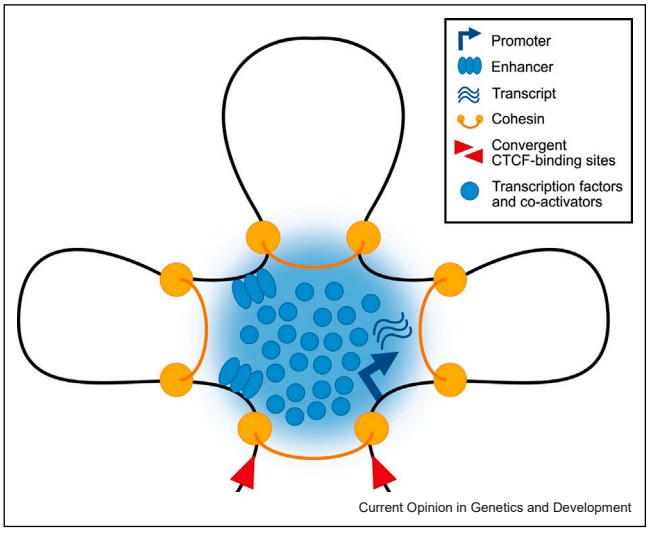
Mechanisms by which loop extrusion can contribute to the formation of enhancer–promoter interactions. By systematically scanning a demarcated region, loop extrusion brings distal *cis*-regulatory elements in close 3D proximity. In the 'nudging' model (left), Cohesin is not stalled at these elements and only functions to facilitate their initial contact, which is subsequently stabilized by molecular affinities between compatible elements. In the 'bridging' model (right), Cohesin is stalled at *cis*-regulatory elements, likely via interactions with the proteins bound at these elements, and thereby contributes more directly to their interaction.

interact via molecular affinities, whereas elements across a TAD boundary are not actively brought in proximity (Figure 2). However, it has also been suggested that loop extrusion is more directly involved in the formation and/or maintenance of regulatory interactions and 'bridges' active promoters and enhancers together (Figure 2). This implies that extruding Cohesin complexes are (transiently) stalled at active *cis*-regulatory elements. Consistent with this model, it has been observed that Cohesin colocalizes with transcription factors [36,37] and the Mediator complex [38,39], which is a ubiquitous coactivator that binds both active enhancers and promoters [40]. In addition, a recent preprint has shown that Mediator depletion leads to reduced Cohesin occupancy at enhancers and reduced enhancer–promoter interactions [41]. Similarly, depletion of RNA polymerase II has been shown to lead to a change in Cohesin extrusion patterns and weakened enhancer–promoter interactions [42–45]. Together, these studies provide support for a model in which Cohesin is stalled by large complexes bound at enhancers and promoters and thereby directly bridges these elements together.

An unresolved question regarding loop extrusion patterns concerns the sites at which Cohesin is loaded on chromatin. Since loop extrusion is thought to drive the formation of cell-type-specific sub-TADs with constitutively bound CTCF borders [38,46], it is likely that Cohesin's extrusion activity is regulated in a cell-type-specific manner. This could in principle be achieved by recruitment of Cohesin at active *cis*-regulatory elements. However, given the dynamic nature of Cohesin extrusion [47–50], it is difficult to directly study Cohesin loading. In addition, since it has been shown that the Cohesin loader NIPBL also plays a role in active extrusion [51–53], the distribution of NIPBL is unlikely to be indicative of Cohesin loading sites. A prediction following from a model in which Cohesin is loaded at enhancers is that deletion of the enhancers leads to less Cohesin occupancy at the flanking CTCF-binding sites. This has recently been demonstrated at the *Prdm14* locus in mES cells [54]. Conversely, it has also been shown that insertion of enhancers in a synthetic regulatory landscape increases Cohesin occupancy at neighboring CTCF-binding sites [25•]. Based on 3C experiments during the reestablishment of interphase genome organization following mitosis exit, it has also been suggested that Cohesin is loaded at active gene promoters [55]. However, it remains unclear whether this is specific for G1 entry or occurs throughout interphase [56].

The location at which Cohesin is loaded has implications for its extrusion patterns. If Cohesin is loaded at active enhancers (or promoters) and would extrude symmetrically, the loading site itself would not be brought into proximity with other elements along the chromatin fiber. To mediate interactions that include the loading site, Cohesin would need to remain stalled at the loading site and extrude asymmetrically. Although it has been shown that the human Cohesin complex extrudes symmetrically *in vitro* [51,52], it is conceivable that *in vivo* extrusion patterns are dependent on additional regulation. Indeed, the observation of cell-type-specific 'stripe' patterns in high-resolution contact matrices [57–60] provides support for asymmetric extrusion patterns that are driven by cell-type-specific sites at which Cohesin is loaded and stalled.

Figure 3



A model for the formation of enhancer–promoter interactions by loop extrusion and molecular affinity**.** We propose a model in which the establishment and specificity of enhancer–promoter interactions are dependent on a combination of loop extrusion and molecular affinity between the regulatory proteins bound at compatible enhancers and promoters.

Conclusion and perspectives

Accumulating evidence from the last few years highlights the importance of loop extrusion in enhancer-mediated gene activation. This provides support for a model in which enhancer–promoter interactions are dependent on a combination of loop extrusion and molecular affinity between proteins bound at compatible elements (Figure 3), which is consistent with polymer physics simulations [61]. Current evidence suggests that loop extrusion might be particularly important for the formation of enhancer–promoter interactions during differentiation or in response to stimuli and for the regulation of the specificity of these interactions. In contrast, regulatory interactions in steady-state conditions might be predominantly maintained by molecular affinity. The relative contributions of loop extrusion and molecular affinity are likely also dependent on the genomic distances between enhancers and their target genes and their relative position and proximity to TAD boundaries.

In addition to a better understanding of the role of loop extrusion in enhancer–promoter communication, progress in the past few years has also resulted in the development of innovative approaches to study 3D genome organization, including methods that can measure chromatin interactions at extremely high resolution [32,35,62] and track loop extrusion in living cells [49•]. Integration of these and other cutting-edge approaches can answer the remaining open questions regarding the dynamic nature of chromatin interactions, their relationship to transcription, and the interplay between the various mechanisms that drive genome folding.

Conflict of interest statement

The authors declare no conflict of interest.

Data availability

No data were used for the research described in the article.

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- •• of outstanding interest.

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