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Review

Block copolymer concepts of how transcription organizes the stem cell genome

Yuzhi Bao[1], Shaoqian Ma[2], Xiaohua Shen[2] and Lennart Hilbert[1,3]

Stem cells display a highly dispersed genome organization that supports flexible gene regulation. Here, we present block copolymer concepts to explore how transcriptional activity from specific genomic regions, or 'blocks', shapes and controls several features of this architecture. Nascent transcripts tethered to chromatin can disrupt compaction and promote the formation of a micro-dispersed state of euchromatin, explaining one typical feature of the stem cell genome. A second feature is long-lived transcriptional clusters, which form via condensation at super-enhancer blocks and mediate both long-range interactions and local transcription factor accumulation. Lastly, we conceptualize promoters and gene bodies as a two-block polymer, for which sequential switching on and off of the polymer blocks controls the association and subsequent release of developmental genes with the long-lived clusters. The presented block copolymer framework provides explanations as well as hypotheses of how transcription-associated processes contribute to distinct features of stem cell genome organization.

Addresses
[1] Institute of Biological and Chemical Systems, Karlsruhe Institute of Technology, 76344 Eggenstein-Leopoldshafen, Germany
[2] State Key Laboratory of Membrane Biology, Beijing Frontier Research Center for Biological Structure, Tsinghua-Peking Joint Center for Life Sciences, School of Basic Medical Sciences, Tsinghua University, Beijing 100084, China
[3] Zoological Institute, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany

Corresponding author: Hilbert, Lennart (lennart.hilbert@kit.edu)
Lennart Hilbert (𝕏 @lennarthilbert.bsky.social), Yuzhi Bao (@yuzhibao.bsky.social)

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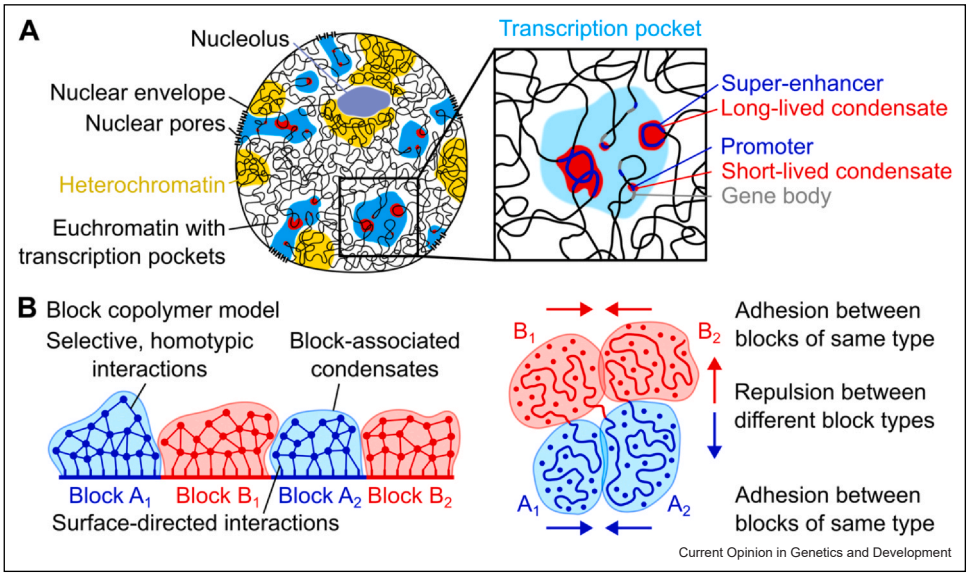
Introduction

The three-dimensional (3D) organization of the genome and transcription in stem cells and pluripotent embryos shows several unusual features. These features occur more rarely in other cells and seem to correspond to developmental functional requirements of stem cells (Figure 1a). Here, we use block copolymer models to conceptualize how transcription of different genomic elements and the interaction of these elements with biomolecular condensates contribute to these stem cell–typical features across different scales.

A block copolymer is a polymer made up of distinct segments, each with selective affinities for different molecular species. These segments represent genome regions that exhibit selective interactions for different liquid-phase condensates, allowing spatial partitioning based on condensate preferences (Figure 1b). Selective homotypic ('same-to-same') interactions drive the association of interacting molecules into condensates, which selectively form at specific polymer regions due to surface-directed interactions (the block regions act as 'condensation surfaces') [2].

Here, we use the generic concept of block copolymers and selectively interacting condensates to conceptualize several stem cell–typical features of the 3D organization of the genome and transcriptional regulation. Note that, in each case, the exact types of polymer blocks and their particular arrangement along the chromosome are specified differently for each given context. We will first address the role of chromatin-associated transcripts in A/B compartmentalization and the micropatterning of transcription pockets within euchromatin (Figure 1a). Further, we discuss the formation of prominent and long-lived RNA polymerase II (Pol II) clusters, which form inside transcription pockets by localized condensation on 'super-enhancers' (Figure 1a). Lastly, we present a promoter-gene body two-block model that conceptualizes how visits of developmental genes to such clusters are coordinated with the main steps of transcriptional control. Except for A/B compartmentalization, all described features have a prevalence of occurring in stem cells or early embryonic development. We therefore denote these features, especially when occurring in combination, as stem cell–typical. The

Figure 1



Stem cell–typical organization of the genome and transcription, and the block copolymer sorting concept. **(a)** When viewed by light and electron microscopy, the nucleus is compartmentalized at the scale of a few 100 nm into a transcriptionally permissive compartment (A compartment) and a largely transcriptionally repressed compartment (B compartment). Heterochromatic genomic domains contribute to the B compartment and associate with the nuclear lamina and nucleoli. Euchromatin domains phase-separate from heterochromatic domains, thereby forming the A compartment [1]. Within the more permissive, euchromatic A compartment, transcribed regions demix from nontranscribed regions and localize to RNA-rich microenvironments, or 'transcription pockets'. In stem cells, transcription pockets contain long-lived, prominent transcriptional clusters located at super-enhancer regions. **(b)** The different copolymer blocks of type A and type B are arranged in a linear sequence, acting as binding platforms with selective affinities for different particle species. These interacting particles exhibit homotypic interactions, thereby undergoing liquid-like phase separation. Driven by adhesion between condensates of the same type, polymer blocks of the same type associate into a compartment pattern.

proposed block copolymer models can, nevertheless, be conceptually transferred to other cell types, even though the block assignment needs to be adjusted to each particular setting, as we illustrate with selected examples.

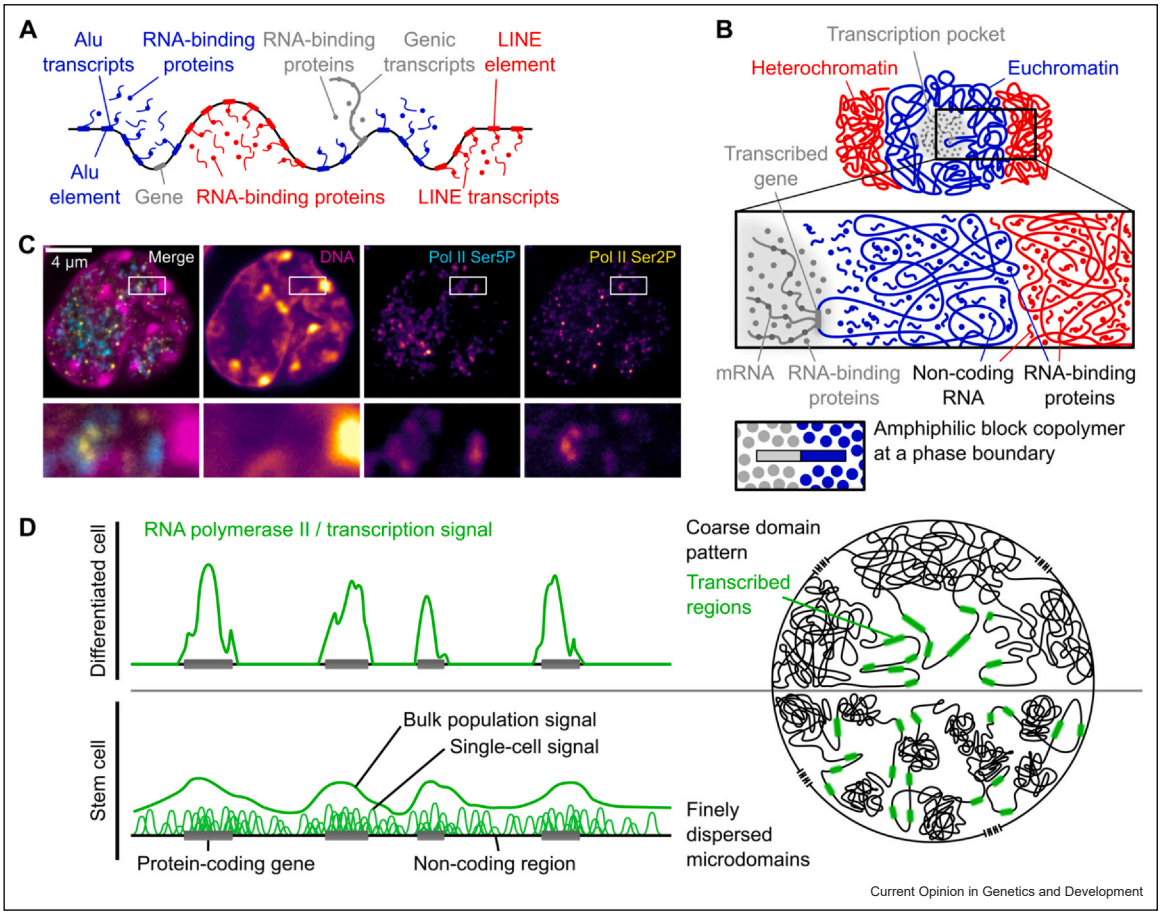
Transcription instantiates block regions that drive micropatterning of hetero- and euchromatin

To set the playing field of overall 3D genome organization, we first address polymer concepts for establishing a primarily heterochromatic B compartment and a primarily euchromatic A compartment. A/B compartmentalization is widely conserved across species and cell types and is established dynamically after cell division and during early embryonic development [3]. Note that A/B compartmentalization is not a stem cell–typical feature but serves to illustrate the block copolymer concept.

Transcripts from the heterochromatic repetitive element LINE-1 (L1, comprising ~ 20% of the mouse genome) and the euchromatic element B1 (Alu-like, comprising ~ 3.6%) play a central role in this compartmentalization, consistent with these elements' broad genomic coverage [3]. In the genomic DNA sequence, these elements occur in a clustered pattern, occupying mutually exclusive regions spanning several megabases (Figure 2a) [3]. These repetitive elements contribute to A/B compartment formation via RNA transcripts that 'decorate' the genomic regions containing the respective repetitive elements (Figure 2a). A block copolymer perspective can conceptualize how these decorating transcripts feed back into compartmentalization. Here, nascent transcripts act as binding points for molecular partners undergoing liquid-like condensation. For example, the heterochromatin protein HP1α undergoes phase separation *in vitro* in the presence of L1 transcripts [3]. Block-specific tethering via nascent RNA transcripts, such as L1, enables condensate-mediated sorting of heterochromatic and euchromatic regions into distinct compartments (Figure 2b,c) [3]. Supporting this picture, heterochromatic and euchromatic domains exhibit ripening behaviors typical of liquid-like phases [1]. Further, the interactions of euchromatin and heterochromatin with BRD4 and HP1α condensates, respectively, are consistent with predictions from elastocapillary theory regarding polymers interacting with phase-separated liquids [6].

Previous block copolymer models explained the epigenetically driven folding of megabase-scale (compartment domains) by a fine-tuned combination of

Figure 2



Transcription from block-like genomic regions contributes to A/B compartmentalization and to stem cell–typical micro-dispersal of euchromatin. **(a)** Heterochromatic and euchromatic regions can be conceptualized as polymer blocks (LINE element– and Alu element–rich blocks, respectively). The affinity of these blocks to specific interacting RNA-binding proteins (RBPs) is established via nascent transcripts decorating different genomic regions, resulting in directed adhesion of condensates [3]. **(b)** Nascent transcripts anchored at transcription sites connect transcribed genomic regions to RNA-rich domains, contributing to the dispersal of euchromatin, similar to an amphiphilic two-block copolymer. **(c)** Representative image showing heterochromatin domains, euchromatin dispersal, and localization of recruited RNA polymerase II clusters (Pol II Ser5P) as well as elongating RNA polymerase II (Pol II Ser2P) within dispersed euchromatin in the nucleus of a mouse embryonic stem cell. **(d)** Transcription occurs at elevated levels throughout intergenic genomic regions in stem cells and is even more pronounced after the deletion of the Pol II C-terminal domain [4]. Single-cell sequencing reveals that the transcription sites in intergenic regions alternate between individual cells [5]. We hypothesize that this spreading of transcription to intergenic regions could contribute to the stem cell–typical micro-dispersed state of chromatin.

nonspecific and epigenetically selective interactions between epigenetically defined blocks [7]. The resulting dynamic multistability regime of polymer folding reproduced an experimentally observed 3D folding pattern that stochastically connects multiple domains with identical epigenetic marks into larger compartments. Subsequent models that assigned polymer blocks on the basis of sequencing results and the position of 3D chromatin loops became increasingly powerful at predicting 3D contact maps [8]. These models also allowed additional insights into the topology of chromosome entanglement and suggested the involvement of liquid-like phase separation phenomena in genome folding. Notably, while not specific to stem cells, these models also suggested that regions containing highly expressed genes are excluded from dense domains containing primarily inactive regions of chromatin [8].

Also, when assessed by microscopy in stem cells, the euchromatin compartment appears internally separated into transcriptionally inactive euchromatin domains interspersed with transcription pockets. These transcription pockets accumulate RNA transcripts bound by RNA-binding proteins (RBPs) and exclude chromatin with low transcriptional activity, leading to locally lowered chromatin concentrations within transcription pockets (Figure 2b,c) [9–11]. Nascent transcripts, tethered to chromatin via elongating Pol II, form RNA–RBP

complexes and thus partition with the RNA–RBP complexes inside the transcription pocket (Figure 2b). From the polymer perspective, active transcription sites exhibit the necessary characteristics of an amphiphile. Amphiphiles typically comprise two block segments with 'opposing' affinities, resulting in their ability to micro-disperse liquid-phase systems [12] (Figure 2b).

Several studies show a 3D genome organization in stem cells that is distinctly more dispersed than in differentiated cells, both at the nanometer-scale in terms of chromatin fiber packing [13] and across higher scales of chromatin folding [14–16]. For euchromatin in mouse embryonic stem cells (mESCs) and zebrafish embryos, transcription stabilizes transcription pockets and euchromatin domains into a finely dispersed, mutually exclusive microdomain pattern at the scale of a few 100 nm [10]. Chemical perturbations that abolish transcript tethering via engaged Pol II result in marked coarsening of this domain pattern, analogous to coarsening in canonical amphiphile-containing systems at insufficient amphiphile concentrations [12]. Given the two-block, alternating pattern of eu- and heterochromatin segments, the question emerges whether this bivalency could establish amphiphilic particles, despite a deviation from the conventional picture of a simple two-block amphiphile. Indeed, polymer simulations and experiments in human cells and fruit fly embryos revealed two effects associated with amphiphile action: kinetic stabilization against coarsening and deformation of HP1α condensates [17].

This dispersing effect of transcription might relate to repetitive elements that undergo transient activation specifically during pluripotency, and whose transcription contributes to overall genome organization and developmental potency (HERV-H, MERVL, L1) [18–20]. Similarly, targeted activation of L1 transposons amplified developmental 3D contacts necessary for embryonic development [21]. Functional relevance can be seen for hominoid-specific L1 elements in hiPSCs, which escape repression and influence neural differentiation and support organoid growth [22].

In mESCs, nascent transcripts were also detected in a much larger portion of the genome — 50 times more than in differentiated cells — including heterochromatin [5]. Single-cell analysis showed nascent transcripts consistently covering euchromatin, but their locations in heterochromatin varied between individual cells (Figure 2d) [5]. This apparent confinement of transcription to genic regions upon differentiation becomes more challenging in species with greater gene spacing. Notably, greater gene spacing correlates with an increased number of 7-amino-acid repeats in the Pol II CTD across species [23]. Conversely, acute degradation of the Pol II CTD lifts transcriptional confinement, activating otherwise silent intergenic and repetitive regions and biasing cells toward totipotent states typical of early development [4]. The broad, transient activation of transcription — especially in intergenic regions — appears to expand cell fate options, leading us to hypothesize that widespread intergenic transcription sites may drive the micro-dispersed stem cell genome state (Figure 2d).

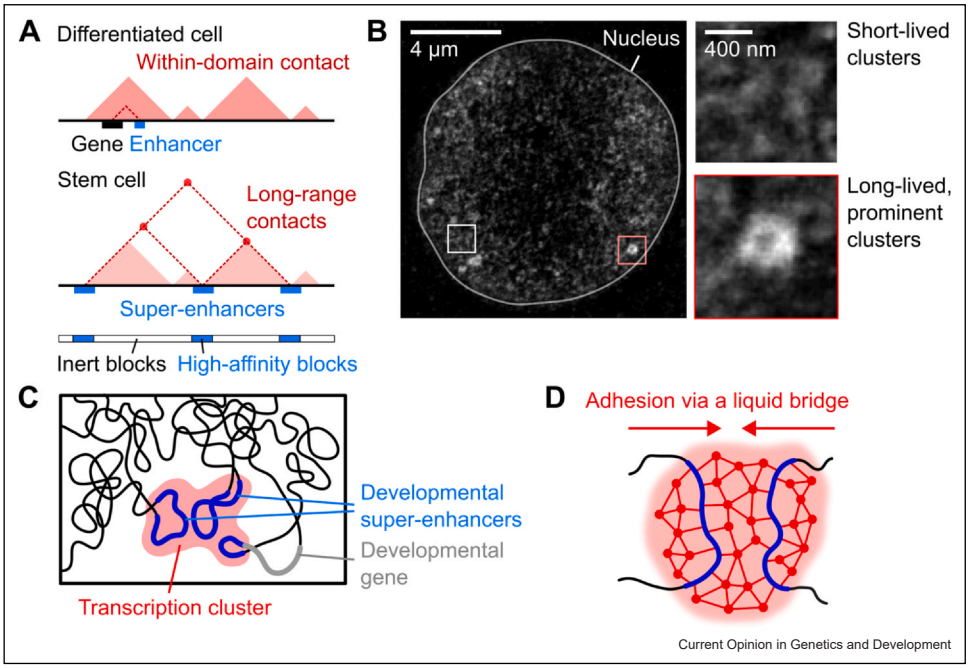
As differentiation progresses, the genome becomes more compartmentalized, and domains become more defined [15,16]. In terminally differentiated chicken erythrocytes, this leads to hypercompartmentalization, with a centrally located B compartment [24]. Transcription is reduced and shifts from genes toward short RNAs, while genes enriched in Pol II that has initiated but not transitioned into transcript elongation ('recruited Pol II') move towards the nuclear periphery. Taken together, progressing differentiation correlates with an increasing confinement of transcription to smaller stretches of the genome. In line with a model where transcription establishes polymer blocks that induce dispersal of chromatin, this progressive confinement of transcribed regions is accompanied by a coarsening of chromatin with progressing differentiation. Accordingly, the role of Pol II, RNA, and transcription to establish dispersing genomic blocks can be transferred to cells in different degrees of differentiation. The stem cell–typical micro-dispersal of chromatin, however, results from a relaxed confinement of transcription to only small parts of the genome, so that this dispersing effect spreads through larger regions of the genome in less differentiated cells.

Condensates forming at gene-regulatory elements establish three-dimensional contacts over long genomic distances

Enhancer-gene interactions are central to transcriptional control, with contacts between enhancers and promoters typically occurring within contact domains spanning several 100 000 base pairs (bp) (Figure 3a) [26]. Stem cells and early embryos feature clusters of enhancers, termed 'super-enhancers', which form long-range 3D contacts anchored at domain boundaries, sometimes spanning several domains and bridging up to several million bp [27,28] (Figure 3a). Such long-range contacts are anchored at transcriptional hubs enriched in Pol II and transcription factors [27,29,30]. Microscopy reveals these hubs as large, stationary clusters (several hundred nanometers in diameter) near super-enhancers, residing within transcriptional pockets at stationary positions for several tens of minutes (Figure 3b) [10,25,31]. In mESCs, there are about 15 such clusters per nucleus, and in pluripotent zebrafish embryos, ≈30 per nucleus, set against a background of smaller, transient transcriptional clusters common to most cell types [25,31–33].

From a block copolymer perspective, the formation of these long-lived transcriptional clusters can be understood as the

Figure 3



Long-range super-enhancer and promoter contacts form via simultaneous association with transcription factor clusters. **(a)** Within stem cells, super-enhancers associate into cliques over the distance of several topologically associating domains (TADs, indicated in red), exceeding the range of enhancer-gene contacts seen also in differentiated cells. For the stem cell case, a conceptual sketch comprising inert blocks and high-affinity blocks representing super-enhancers is shown. **(b)** Representative stimulated emission double depletion (STEDD) microscopy image showing prominent transcriptional clusters in a pluripotent zebrafish embryo nucleus with immunolabeled recruited RNA polymerase II (Pol II). The marked boxes and detail zooms show point-like Pol II clusters (short-lived) as well as the prominent, stem cell–typical clusters (long-lived), which span several 100 nm in size [25]. **(c)** Stem cell–typical long-range contacts are frequently established via looping of super-enhancers and genes to stem cell–typical clusters, which are enriched in transcriptional activators, recruited Pol II, and RNA transcripts. **(d)** Liquid-like condensates form via a combination of (i) homotypic interactions among transcription factors and (ii) interactions of transcription factors with regions carrying 'active enhancer' marks. In consequence, condensation occurs most prominently at currently active super-enhancers, which can enter into sustained contact via a 'liquid bridge' formed by fusion of super-enhancer–associated condensates.

condensation of transcriptional machinery into liquid-like assemblies. This condensation is helped by specific high-affinity block segments within an overall 'inert polymer' (Figure 3a). This model is based on observations that super-enhancer epigenetic marks like H3K27ac act as scaffolds for the co-condensation of enhancer-associated factors (such as mediator and BRD4) and Pol II into prominent clusters [31,34,35] (Figure 3c). Studies in frog egg extract and in zebrafish embryos confirmed that transcriptional condensates can form at regulatory chromatin and mediate the adhesion of several strands via the fusion of condensates, yielding a shared 'liquid bridge' (Figure 3d) [25,36]. Similar long-range tethering and localization of genomic regions was also shown in engineered intracellular systems exhibiting optically controlled phase separation [37,38].

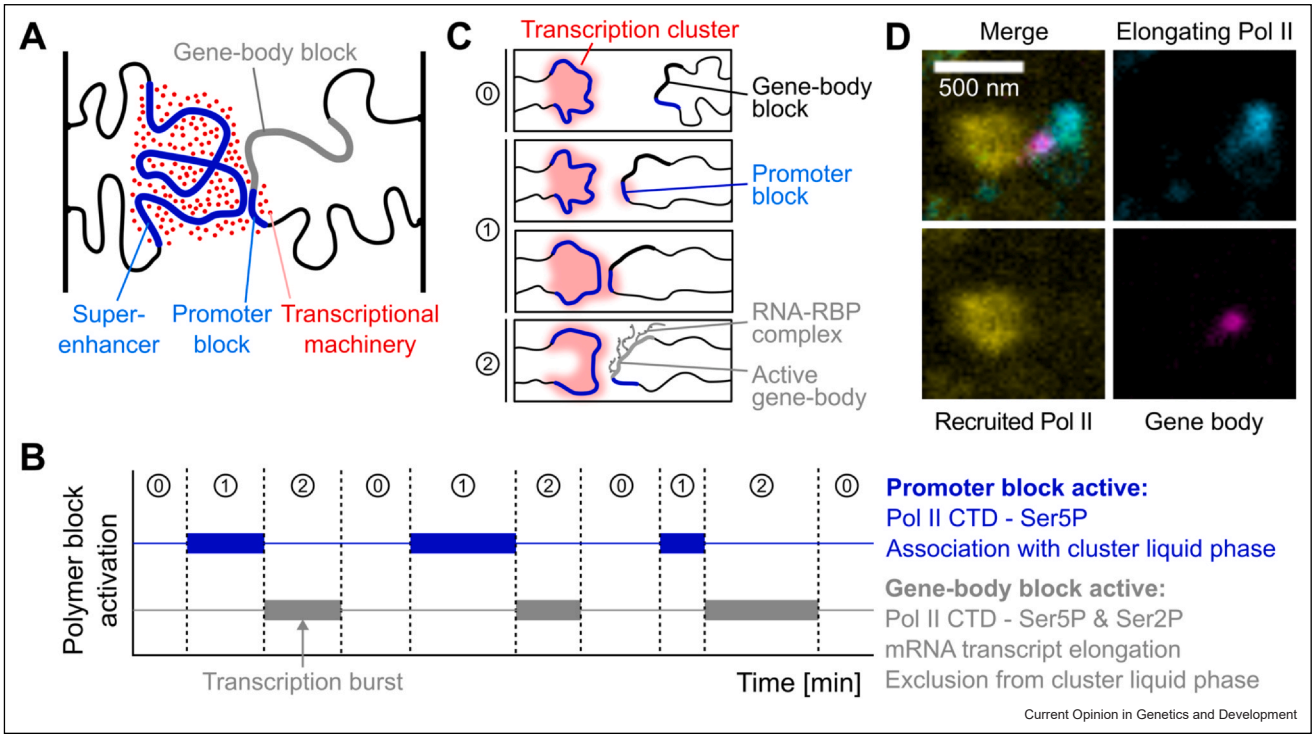
Long-range contacts, including those crossing contact domains, are not exclusive to stem cells. Initially, contacts with ranges over several 10 000 bp were identified for the mouse β-globin locus [39], followed by detection of contacts exceeding several million bp distance at Pol II clusters [40]. In the inflammatory response in human umbilical vein endothelial cells (HUVECs) that is mediated by tumor necrosis factor alpha (TNF-α), hierarchical gene-regulatory contacts over approximately 50 million bp occur [41,42]. Knocking down enhancer RNA at the TNF-9 super-enhancer in mouse macrophages or its human homolog lowers TNF-α levels and improves rheumatoid arthritis symptoms [43]. Recent studies show that certain sequence elements can equip regular enhancers with super-enhancer or long-range capabilities also in differentiated cells or multipotent progenitors [44,45]. Thus, even though super-enhancers are frequently found and studied in stem cells, similarly extensive regulatory elements can occur in other cell types. For shorter regulatory elements, previous works already applied the concept of polymer blocks with affinities for specific proteins and RNAs, successfully explaining enhancer–promoter contacts over canonical interaction distances of less than one million bp. For example, a model that assigns polymer block regions on the basis of a minimal set of epigenetic data predicted

experimentally observed 3D folding configurations and dynamics from cell populations as well as single cells [46]. Notably, this model explicitly treats proteins that transiently bind to and crosslink different polymer segments, allowing the prediction of experimentally validated enhancer-promoter folding configurations for different transcriptional states [46]. Crucial application value of such predictive polymer models exists in understanding the gene-regulatory mechanisms leading from structural variants, such as deletion, duplication, or inversion, to clinically observed disease phenotypes [47]. These examples illustrate that the fundamental concept of regulatory elements as polymer blocks has explanatory value also for differentiated cells and disease mechanisms.

Promoter and gene body act as switchable polymer blocks that coordinate gene-cluster visits

A polymer model comprising a 'promoter block' and a 'gene-body block' can explain how a gene associates with and is released from the spatially stationary, super-enhancer–associated clusters (Figure 4a). Live imaging of long-range enhancer–promoter interactions in embryos and stem cells shows approaches to 200–300 nm distance during periods of transcriptional activation [48–51]. For short-range regulation (less than 100 000 bp sequence distance) and in differentiated cells, enhancer–promoter distances of 100–200 nm were observed [52,53]. In fruit fly embryos, microscopy shows that multiple super-enhancers group into cliques separated by several hundred nanometers, which are sporadically visited by genes [51,54]. This spacing of 100 nm or more during enhancer–promoter interactions suggests that super-enhancer–associated condensates act as 'adhesive spacers': transcribed genes are placed close to the margins of, but excluded from the liquid-like material making up these clusters [25,55,56]. In block copolymer terms, placing a region with super-enhancer-like epigenetic properties near a gene can promote this type of association (Figure 4a). CpG islands upstream of promoters, especially when hypomethylated and enriched

Figure 4



Temporal switching of a promoter block and a gene-body block connects the step-wise control of transcription with visits of a gene to a super-enhancer–associated cluster. **(a)** An enhancer-like 'promoter block' placed upstream of the 'gene-body block' drives the association of the gene to a super-enhancer–associated cluster via the liquid bridge mechanism. **(b)** Illustration of the temporal sequencing of promoter block activity (1), gene-body block activity (2), and return to an uninduced state (0, both blocks inactive) on the scale of several minutes. Periods during which a given block is active are indicated by the numbers above a temporal segment and also by a thick block in blue (promoter block) or gray (gene-body block) painted into the respective segment to better visualize the temporal sequencing. Vertical positions of blue and gray lines and blocks are shifted solely to facilitate interpretation and hold no physical meaning. **(c)** Resulting coordination between gene visit and transcription hub unfolding. **(d)** Representative confocal micrograph showing gene-cluster interaction associated with transcription in a pluripotent zebrafish embryo nucleus. Fluorescence labels are for recruited RNA polymerase II (Pol II Ser5P), transcribing RNA polymerase II (Pol II Ser2P), and a developmental gene, *klf2b*.

for H3K27ac during early development, can take this role, enabling long-range contacts between genes and enhancers [57,28]. Likewise, hypomethylated L1 elements in hiPSCs but also composite transposons in differentiated cells can function as promoter- or enhancer-like adhesive blocks, supporting long-range, RNA-dependent gene regulation as a more general concept [22,58].

Short enhancer-like blocks upstream of promoters can mediate gene-cluster association through a liquid bridge mechanism. The relatively short sequence length of such enhancer-like blocks implies infrequent and transient association of genes with the relatively longer super-enhancer elements — as is observed experimentally (Figure 4a) [51,54,59]. Also, gene proximity to a super-enhancer and the start of transcription are often not synchronized, and long-range enhancer-gene contacts and transcription change asynchronously through early development [29,31,59]. This asynchronous behavior can be explained by how transcription control steps alter the liquid-phase properties of the Pol II CTD (Figure 4b). During initiation, Ser5 phosphorylation of the CTD increases Pol II's liquid-phase affinity, promoting clustering with transcription activators and association with initiation condensates [23,56,60,61]. In stem cells, the long-lived super-enhancer condensates can take the role of such initiation condensates. Following initiation, Pol II transitions into transcript elongation, which requires an additional CTD repeat motif phosphorylation at the Serine 2 position (Ser2P) by cyclin-dependent kinase 9 (CDK9). The additional Ser2P modification and the newly synthesized RNA transcripts convert the liquid-phase properties of Pol II, leading to exclusion of the transcribed gene from the initiation condensate [62–64]. This loss of compatibility can be conceptualized via a second polymer block, which represents the gene body and is placed adjacent to the enhancer-like block (Figure 4a). Finally, by activating the adhesive enhancer-like block and the repulsive gene block sequentially, initiation and elongation become linked to gene-cluster association and release, respectively (Figure 4b,c). This coordination of initiation with cluster association and elongation with release from clusters was indeed seen by expansion microscopy [33] and in a pseudo-time reconstruction of gene-cluster visits (Figure 4d) [59,2].

The necessity of a gene to visit a prominent, stationary cluster for activation can be explained via the sequestration of activating factors. A short enhancer-like block attracts elevated but low levels of activators, facilitating adhesion to the super-enhancer cluster but not transition into elongation (Figure 4c, 1). Once the gene associates with a larger cluster, the higher local concentration of activators — especially CDK9 — triggers elongation and release of the gene from the cluster (Figure 4c, 2) [65,66]. The two-block model alone does not explain experimentally observed enhancer specificity [40,41], which can, however, be attributed to selective partitioning of transcription factors, determined by charge patterns in intrinsically disordered domains [67].

Considering the two-block promoter-gene body architecture, the question arises as to how far a single gene that transiently visits a transcriptional cluster could take the role of a dispersing amphiphilic particle. Here, a pseudo-time analysis revealed that, in coordination with the transcriptional activation of a gene that engages a transcriptional cluster, the cluster transitions for a few seconds to a more dispersed morphology and relaxes to a droplet-like shape as the transcriptionally active gene moves away from the cluster [59]. Additionally, higher levels of transcription elongation correlate with dispersal of transcriptional clusters in embryonic cells, which can be recapitulated by both polymer models as well as synthetic DNA mimics of bivalent amphiphilic particles that disperse transcriptional condensates [68].

Recent polymer models also support the idea that transcription of specific regions, or blocks, can alter folding patterns and dynamics. In one case, gene activation induced localized, dynamic refolding at the minute time scale, which could only be resolved due to a simulation resolution of 1 kb and was obscured at larger length scales [69]. In another model, Micro-C data with a few kb resolution could be reproduced by simulations that track single Pol II complexes progressing through the gene body [70]. Here, transient interactions between the engaged Pol II complexes established within-gene 3D interactions, resulting in the formation of a micro-compartment around the gene body that is largely shielded from extra-genic interactions. These examples illustrate that models that represent the effects of transcription as dynamically changing polymer blocks likely have explanatory power across various stem cell and differentiated cell types, but need to be adjusted to each given regulatory scenario [80]. Super-enhancers appear as a distinguishing feature of stem cells, acting as temporally persistent polymer blocks that serve as scaffolds for long-lived accumulations of activating factors, with which regulated genes can transiently interact.

Complementary mechanisms and models

This review focused on how protein-RNA condensates can organize the genome and control transcription. Sequencing-based mapping (Hi-C) has identified cohesin-mediated loop extrusion as another main mechanism underlying genome organization [71]. Recent improvements in mapping resolution (Micro-C) underline that, in fact, an intricate interplay of cohesin-mediated processes as well as protein and Pol II clustering-related processes drives enhancer–promoter interactions

[72–75]. For example, cohesin-mediated loop extrusion antagonizes Pol II and transcription during loop formation; computational models suggest that the range of 3D contacts increases when cohesin-mediated extrusion is absent [72,73]. Cohesin is also implicated in bridging-induced phase separation, where a cross-linker and a sufficiently long polymer interact, as shown *in vitro* [76]. Consistent *in vivo* observations indicate that cohesin keeps euchromatin domains demixed from each other [77]. Supercoiling of DNA by an interaction of transcription with cohesin-mediated loop extrusion has also been implicated in genome folding [78]. Lastly, recent studies outside the stem cell context show that the disruption of multienhancer hubs does not significantly affect the regulatory function of individual enhancer–promoter pairs [79]. Accordingly, while the block copolymer model helps conceptualize stem cell genome organization, readers should remember that it is a simplification, and other mechanisms and complementary models should be considered.

Microscopy technical descriptions and data sets

Figure 2c: mESC labeled by immunofluorescence, data acquired by instant Structured Illumination Microscopy (iSIM), average z-projection (five consecutive confocal sections, z step 0.2 µm, samples kindly provided by Carmelo Ferrai). Figure 4d: zebrafish embryos labeled by oligopaint DNA fluorescence *in situ* hybridization followed by immunofluorescence, data acquired by iSIM, single confocal section (unpublished data, Hilbert laboratory). Figure 3b generated from publicly available data: https://doi.org/10.5281/zenodo.4973062.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Perplexity and Consensus to search the scientific literature, discuss parts of the review content, draft individual sentences, and edit the manuscript. After using these services, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare no competing interests.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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