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REVIEW

Nonspecific Interactions in Transcription Regulation and Organization of Transcriptional Condensates

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Abstract—Eukaryotic cells are characterized by a high degree of compartmentalization of their internal contents, which ensures precise and controlled regulation of intracellular processes. During many processes, including different stages of transcription, dynamic membraneless compartments termed biomolecular condensates are formed. Transcription condensates contain various transcription factors and RNA polymerase and are formed by high- and low-specificity interactions between the proteins, DNA, and nearby RNA. This review discusses recent data demonstrating important role of nonspecific multivalent protein–protein and RNA–protein interactions in organization and regulation of transcription.

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INTRODUCTION

Gene expression in the nucleus is orchestrated by the coordinated activity of three RNA polymerases: RNA polymerase I (RNAPI), which is responsible for transcribing the precursor of 18S, 5.8S, and 25S ribosomal RNA (rRNA); RNA polymerase II (RNAPII), which is predominantly involved in transcribing messenger RNAs (mRNAs); and RNA polymerase III (RNAPIII), which is responsible for transcribing ribosomal 5S rRNA, transfer RNA (tRNA), 7SL RNA, and various other small RNAs. Regulation of transcription is a complex process that relies on the intricate interplay of numerous proteins and RNAs that accumulate at specific loci in the nucleus. An illustrative example of such accumulation is nucleolus, the largest organelle within the nucleus, where the processes of transcription, pre-mRNA processing, and pre-ribosomal assembly take place [1, 2].

The key feature of the cell nucleus, which enables transcription and its flexible regulation, is high mobility of nuclear proteins. In 2000, data began to accumulate indicating that many proteins in the nucleolus, interchromatin granules, and chromatin are not stably bound to the structures, but rather are in continuous and relatively rapid exchange with the surrounding nucleoplasm [3, 4]. Focus of this review is on the nature of binding and exchange of the transcription factors responsible for carrying out the relatively long process of transcription. While constant binding of transcription factors to promoters could theoretically activate transcription, early experiments showed that the glucocorticoid receptor, a hormone-dependent modulator of gene expression, undergoes rapid exchange between the chromatin target site and the surrounding nucleoplasm [3]. It was later shown that both the glucocorticoid receptor and its interacting partner, glucocorticoid receptor interacting protein 1 (GRIP-1), exist in a dynamic equilibrium with the promoter and must repeatedly return to the DNA template during

Abbreviations: ARM, arginine-rich motif; CTD, C-terminal domain; DFC, dense fibrillar component; GC, granular component; IDR, intrinsically disordered region; mRNA, messenger RNA; PRC2, Polycomb repressive complex 2; RNAPI, RNA polymerase I; RNAPII, RNA polymerase II; RNAPIII, RNA polymerase III; rRNA, ribosomal RNA; tRNA, transfer RNA.

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transcription activation [5]. Other transcription factors also exhibit dynamic interactions with promoters, occupying the promoter region for relatively short periods of time ranging from fractions of a second to tens of seconds [6]. RNAPII subunits have also been shown to dynamically assemble at the promoter site [7].

High exchange rates have also been demonstrated for some rRNA transcription factors – UBF [8-10], TAFI48, PAF53, and TIF-IA/Rrn3 [9]. RNAPI subunits also exhibited dynamic behavior at the promoter, suggesting stochasticity and low efficiency in the assembly of complexes required for transcription [9].

Behavior of transcription factors is described by the 3D genome-scanning model [6], also referred to as the diffusion and affinity model. This model assumes that proteins diffuse freely and rapidly throughout the nucleus, intermittently interacting with its components. Most of these interactions are nonspecific and non-functional. However, when proteins engage with the target sites through high-affinity interactions, they could become temporarily immobilized. This, with a seemingly low probability, may result in formation of the functional macromolecular complexes.

However, this model fails to describe all quantitative regularities associated with regulation of the genome activity. For example, it has been calculated that the rate at which proteins locate their sites on DNA in bacterial cells is approximately 100 times higher than predicted based on diffusion alone [11, 12]. Several mechanisms have now been proposed that explain enhanced efficiency and accuracy of protein accumulation. At least some of these mechanisms rely predominantly on low-specificity interactions between molecules, leading, in particular, to formation of the membraneless structures now commonly referred to as biomolecular condensates. These condensates serve as repositories for the molecules essential for efficient transcription. Given that formation of condensates in many cases depends on the interaction between proteins and RNAs, this review will systematically explore the roles of proteins and RNAs involved in organization and regulation of transcription through formation of transcriptional condensates.

COMPARTMENTALIZATION OF TRANSCRIPTION

Regulation of gene transcription is primarily based on numerous high-affinity (specific) interactions of various proteins with enhancers and promoters. For example, transcription activation depends on the interaction of the Mediator complex with the enhancer and promoter, which facilitates landing of RNAPII during transcription initiation [13, 14]. However, it has been suggested in a number of studies that transcription initiation can lead to formation of rather large complexes – transcription factories – in which many transcribed genes are concentrated. This hypothesis is compelling as it holds the promise of coordinated regulation for numerous genes within a single transcription factory. Existence of such complexes is primarily supported by the microscopic data showcasing clustering of RNAPII molecules in animal nuclei [15-20]. These observations were initially made using conventional fluorescence microscopy rather than super-resolution localization microscopy, which allows precise determination of the number of molecules in a given area. The results obtained using the super-resolution microscopy indicate that transcription factories are relatively small and are predominantly composed of only a few RNAPII molecules [21].

Subunits of the Mediator complex are also found among the constituents of the transcription factories [22]. It has been suggested that a fraction as small as <10% of all clusters consisting of large assemblies of RNAPII and Mediator may correspond to the previously described clusters of super-enhancers. Regulation of certain crucial genes involves multiple enhancers, known as super-enhancers [23-27]. Existing data indicate that the large clusters of RNAPII and the Mediator complex often are co-localized with the loci containing super-enhancers [22, 28-30].

Live cell observations suggest that such transcription factories are formed as dynamic, short-lived aggregates that include RNAPII molecules [31-33]. A similar situation has been demonstrated for the transcription factors Zelda and Bicoid in the developing *Drosophila* embryos [34]. Both of these factors generate dynamic, short-lived condensates that transiently interact with active sites of Bicoid-dependent transcription thus activating it.

Finally, microscopic visualization of the transcription of long genes should be considered. Long genes form rather extended loops along which RNAPII molecules appear to move [35, 36]. These loops share similarities with the structures observed in lampbrush chromosomes [37-39]. Consequently, recent data do not align with the concept of highly ordered transcription factories; instead, they suggest the existence of highly dynamic complexes. These dynamic complexes are now commonly referred to as transcriptional condensates [40].

ROLE OF NONSPECIFIC INTERACTIONS IN THE FORMATION OF TRANSCRIPTIONAL CONDENSATES

The concept of biomolecular condensates originated in the study of membraneless organelles [41, 42]. Subsequently, this concept was extended to any com-

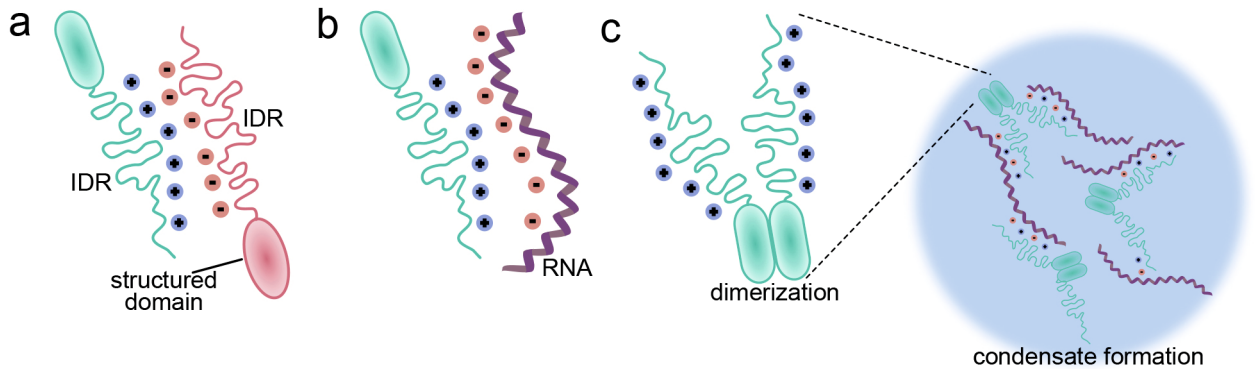

Fig. 1. Role of intrinsically disordered domains (IDRs) in formation of biomolecular condensates. a) Electrostatic interactions between the protein IDRs in the presence of domains with different charges. b) Interactions between the protein IDRs and RNA. c) Formation of a biomolecular condensate due to multivalent interactions provided by the dimers of proteins with IDRs binding RNA molecules.

plex that does not exhibit stoichiometry in its composition [43]. Formation of such complexes, irrespective of their size, relies on numerous low-affinity interactions among the molecules. Crucially, interactions involving intrinsically disordered protein domains (IDRs) play a pivotal (though not exclusive) role [44-47] (Fig. 1, a and b). Additionally, multivalent interactions involving structured domains are of significance [48, 49]. In terms of their role in the condensate formation, protein molecules are categorized into scaffold molecules, participating in multivalent interactions and contributing to condensate formation, and client proteins, which accumulate within already formed condensates [49]. Although IDRs do not exhibit high affinity, they, as well as the high-affinity RNA-binding domains, can serve as foundation for multivalent interactions – either due to extensive IDR length or through oligomerization of the protein molecules (Fig. 1c).

Ability of some proteins involved in transcription to form condensates *in vitro* suggests that transcription may be accompanied by the formation of biomolecular condensates. The most detailed data are available for some nuclear proteins, in particular for two pivotal nucleolar proteins, FBL and NPM1 [50]. Numerous other proteins engaged in the diverse aspects of genome activity regulation demonstrate capability to form condensates *in vitro*. In some instances, the analysis was limited to the IDR of these proteins, but even such data appear to be sufficient to indicate potential involvement of the proteins in the biomolecular condensate formation. This group of proteins include components of the Mediator complex and RNAPII [22, 28, 29], TAZ [51], BRD4 [28], and transcription factors OCT4 [52] and TAF15 [53].

One of the most striking pieces of evidence supporting the formation of the IDR-dependent biomolecular condensates during transcription activation comes from the study of the Wnt/β-catenin signaling pathway [54]. Upon pathway activation, β-catenin translocates to the nucleus, where it forms a complex with the LEF/TCF transcription factors. This complex recruits additional cofactors and enhances expression of the target genes. LEF1 demonstrates the ability to form biomolecular condensates with β-catenin both *in vitro* and *in vivo*, and the formation of these condensates is essential for transcription activation. Notably, LEF1 with disrupted IDR loses its activity, which can be restored by substituting with the intrinsically disordered domain of another protein (FUS). This emphasized the significance of nonspecific interactions in the transcription regulation.

Moreover, recent data suggest that IDRs can not only enhance transcription efficiency by attracting diverse proteins into the transcriptional condensates, but also improve accuracy of the promoter recognition by transcription factors. Transcription factors possess DNA-binding domains that bind to the specific short DNA sequence motifs. However, these DNA-binding domains constitute only a fraction of transcription factor sequences, with the majority being of low complexity and lacking a stable 3D structure [55-57]. As mentioned earlier, IDRs are not capable of high-affinity and high-specificity interactions. Nevertheless, these regions can “target” transcription factors to the specific genomic loci, thereby increasing specificity of the promoter recognition, when acting in concert with high-affinity DNA-binding domains [58-60].

ROLE OF RNAPII C-TERMINAL DOMAIN IN THE ORGANIZATION OF TRANSCRIPTIONAL CONDENSATES

C-terminal domain of RNAPII (CTD) plays a distinctive role in organization of transcription processes. The CTD consists of numerous heptad repeats with the consensus sequence Tyr 1-Ser 2-Pro 3-Thr 4-Ser 5-Pro 6-Ser 7 [61-63], and is characterized as an intrinsically

disordered region. Its length exhibits considerable variation across different organisms, ranging from 26 repeats in *Saccharomyces cerevisiae* to 52 repeats in humans.

When expressed in cells, the CTD accumulates at active transcription sites, indicating its potential role in the recruitment of RNAPII at target sites [64]. However, the data regarding its direct involvement in the transcription process are conflicting. Catalytic activity of RNAPII relies on the conserved enzyme subunits RPB1 and RPB2, rather than the CTD [65], so it seems logical that the RNAPII with deleted CTD can perform transcription *in vitro* but not *in vivo* [66]. Multiple investigations have explored this issue, with the most recent study utilizing Raji cells suggesting that the CTD is not required for transcription in live cells [67]. The findings presented in this study suggest that the CTD could be required not for the transcription itself, but rather for the precise localization of RNAPII at the target sites, as well as for regulation of the transcription-related processes and transcript maturation.

Both human and yeast CTD sequences are able to form condensates *in vitro*, resulting in spherical aggregates that contain intact RNAPII [68]. Importantly, the ability of RNAPII to cluster in the nucleus correlates with the number of heptad repeats: shortening the human CTD to the length of the yeast CTD reduces concentration of the accumulated RNAPII and its association with chromatin in human cells. Conversely, increasing the CTD length enhances clustering of the RNAPII molecules [69]. Modeling confirms that the CTD length promotes binding of RNAPII molecules to the promoter and delays their release from it, while the CTD–CTD interactions facilitate concentration of numerous RNAPII molecules at the single point. Several studies also suggest that CTD is involved in the recruitment of the Mediator complex [22, 28, 70]. Disruption of the Mediator complex results in disassembly of the large complexes of hypophosphorylated RNAPII [71].

The studies cited above suggest that formation of biomolecular condensates accumulating RNAPII, various transcription factors, and other proteins could represent a crucial mechanism for enhancing transcription processes. However, this mechanism might be less effective in *S. cerevisiae*. The CTD in this organism is much shorter than, for example, in humans, where the longer CTD leads to the stronger CTD–CTD interactions and formation of the less dynamic condensates [68]. This distinction could potentially explain why the RNAPII molecules in *S. cerevisiae* nuclei do not form clusters, a characteristic feature of the human cell nuclei [72]. Observations of individual RNAPII molecules in the living yeast cells demonstrated that CTD plays a role in restricting diffusion of RNAPII within the nuclear region enriched with active genes, although no visible condensate formation was evident. Shortening of the CTD resulted in the reduced diffusion restriction, increased target site search time, and impaired formation of the preinitiation complex. According to the authors, these differences could be attributed to the unique organization of transcription within a small yeast cell nucleus, where all components are concentrated in a limited space, making formation of the transcriptional condensates redundant [72].

Significant insights have been generated using an optogenetic system designed to induce biomolecular condensates based on the IDRs of FET family transcriptional regulators [such as Fused in sarcoma (FUS), Ewings sarcoma (EWS), and TAF15] [73]. These IDRs can initiate phase separation process in the living cells with TAF15 demonstrating capacity to interact with the RNAPII CTDs and attract RNAPII molecules to form condensates. Notably, nascent CTD clusters at primed genomic loci lower the energy barrier for growth of the TAF15 condensates, which, in turn, further recruit RNAPII to initiate transcription. Thus, the authors have identified a positive feedback mechanism that enhances RNAPII accumulation at transcription sites, leading to improved transcription efficiency. At the same time, the data suggest that elongation occurs outside of the induced condensates, as the RNAPII molecules with unmodified CTD colocalize with the condensates consisting of IDRs of TAF15, and phosphorylated CTD is released into the surrounding region.

Therefore, the existing data demonstrate that nonspecific interactions involving the disordered CTD of RNAPII can enhance transcription efficiency. It is crucial to note, however, that many of the data providing information on the mechanisms underlying formation of transcriptional condensates and role of IDRs are derived from the *in vitro* experiments or experiments with isolated IDRs, rather than the whole proteins. Within the intact proteins, IDRs may exhibit different behavior. For example, recent findings suggest that the IDRs alone could be insufficient for inducing transcription factor clustering [74], so this issue needs further investigation.

TWO TYPES OF TRANSCRIPTIONAL CONDENSATES PROVIDE SEPARATION OF INITIATION AND ELONGATION PROCESSES

Recent advancements in super-resolution microscopy techniques enable estimation of the distances between transcription factors and nascent transcripts. For example, in the case of the *Pou5f1* and *Nanog* genes, nascent transcripts were found to be in close proximity to the elongating RNAPII molecules but distant from the loci enriched with the SOX2 and BRD4 transcription factors [75]. These findings imply spatial separation between the elongation and initiation complexes.

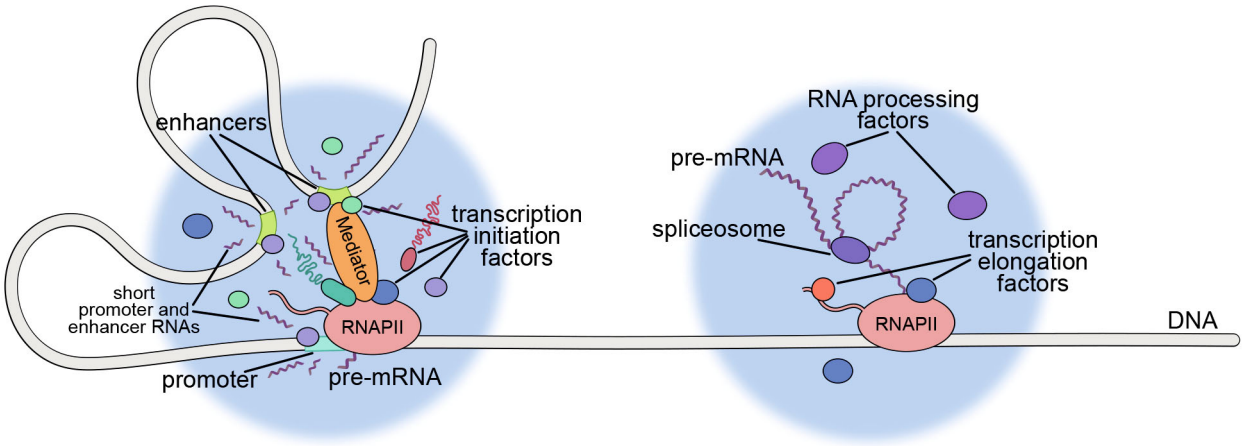

Fig. 2. Models of initiation and elongation condensates that provide regulation of transcription by RNA polymerase II (RNAPII).

Intriguingly, results obtained with the HIV-1 reporter gene indicate that the initiation complex (initiation condensate) exists for a relatively short time (~1 min), during which RNAPII molecules are recruited to the promoter region [76]. Apparently, during transcription and subsequent processes such as splicing and nuclear transport, the molecules travel through several condensates, ensuring high efficiency at each stage of the RNA molecule life cycle [77].

To date, sufficiently compelling data confirming existence of condensates where the components involved in transcription initiation are concentrated have been obtained (Fig. 2). The situation regarding existence of the elongation condensates is somewhat more controversial. As mentioned earlier, long genes form extended loops along which RNAPII molecules move during transcription [35, 36]. It is likely that the described patterns could be extended to all other genes, but this requires experimental confirmation, focusing on the key aspects. Nevertheless, these morphological observations do not align well with the idea of isolated elongation condensates. Further experiments will be able to provide clarity on these findings and reconcile the existing contradictions.

ROLE OF NONSPECIFIC INTERACTIONS IN THE ORGANIZATION OF THE PRE-rRNA TRANSCRIPTION

Nucleolus is the product of transcription from the relatively small regions of the genome containing numerous rRNA genes, referred to as nucleolus organizer regions [78]. Around 80 ribosomal proteins and over 200 pre-ribosome assembly factors accumulate in the nucleoli [79]. Additionally, nucleolus harbors a substantial number of other proteins unrelated or only indirectly related to its primary function – production of pre-ribosomes [80].

Unlike most of the nuclear bodies, nucleoli exhibit relatively complex internal organization. Active nucleoli in mammals, birds, and some reptiles consist of three distinct subcompartments: fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC), clearly visible in electron microscopy images [81, 82]. The DFC and GC form during ribosome biogenesis.

Transcription of rDNA occurs at the boundary of FC and DFC; consequently, DFC represents a cluster of proteins involved in the early pre-rRNA processing. Central role in maintenance of structural integrity of this nucleolar subcompartment is played by fibrillarin (FBL), which binds to the pre-rRNA molecules during transcription [83]. Thus, the transcribed pre-rRNA molecules nucleate assembly of DFC, where both transcription and initial steps of pre-rRNA processing take place simultaneously. It must be noted that this process relies on the disordered glycine and arginine-rich N-terminal region of FBL, known as GAR domain [83]. FBL, a highly conserved methyltransferase, possesses an amino acid sequence and 3D structure that have undergone minimal changes from archaea to higher eukaryotes [84]. Emergence of the GAR domain has enabled FBL to acquire additional functions essential for its role in the significantly more complex eukaryotic cells. Specifically, this domain serves as a nuclear localization signal [85]. Another potential function is involvement of the GAR domain in organization of the DFC [83].

GC is traditionally viewed as a compartment dedicated to pre-rRNA processing and pre-ribosome assembly. Structural integrity of the GC relies on dynamic interactions between the pre-ribosomes with NPM1 playing a pivotal role in this process [86]. During maturation, properties of the GC change to facilitate directed transport of pre-ribosomes from the DFC to the nucleolus periphery [87].

Similar to the RNAPII transcriptional condensates, multiple condensates are formed inside the nucleolus,

determining directional movement of the synthesized pre-rRNA molecules.

ROLE OF THE SYNTHESIZED TRANSCRIPTS IN THE REGULATION OF GENE EXPRESSION

Initiation of phase separation processes during the biomolecular condensate formation often relies on the RNA molecules serving as successful substrates for multivalent interactions. The subsequent discussion will highlight key studies suggesting involvement of RNA molecules in the low-specificity interactions crucial for transcription regulation.

Variety of RNAs and transcription process itself influence three-dimensional structure of chromatin and gene expression mediated by the chromatin state [88-92]. Active transcription and accumulation of the chromatin-associated transcripts at the gene locus, including pre-mRNAs and other nascent RNAs, maintain open state of the chromatin through a positive feedback mechanism. Transcripts regulate chromatin state and associated gene expression through electrostatic interactions with the similarly charged DNA and oppositely charged histone proteins [93]. Additionally, they bind to various protein factors comprising chromatin [94].

Transcription and chromatin remodeling factors YY1, CTCF, DNMT1, and DNMT3A exemplify DNA-binding factors whose association with chromatin can be regulated by nascent transcripts [95]. For the ubiquitously expressed transcription factor YY1, interaction with the nascent RNAs stabilizes its binding with chromatin, predominantly at promoter sites [96]. In contrast, nascent RNAs have an inhibitory effect on association of the DNMT1 and DNMT3A methyltransferases with chromatin [97, 98]. For the DNMT1, which performs maintenance methylation of CpG islands, interaction with various nascent transcripts [99], including of its own mRNA [100], has been demonstrated using the whole-genome RNA–protein interactome analysis methods (fRIP-seq). Interestingly, DNMT1 prefers binding to the non-canonical G-quadruplex structure called pUG-fold, with binding affinity appearing proportional to the length of the GU repeat [100]. Interaction with the RNA pUG-fold prevents DNMT1 from binding to DNA, thereby inhibiting its enzyme activity. Conversely, certain long non-coding RNAs attract methyltransferase to the specific chromatin regions without inhibiting its activity [101-105], and could even determine its cellular localization [106].

Antagonistic relationships with the nascent transcripts are also observed for the Polycomb repressive complex 2 (PRC2) [107]. PRC2, an epigenetic transcription regulation complex, methylates lysine 27 of the histone H3, participating in the repression of gene expression. Due to its function, PRC2 is primarily associated with CpG islands in the repressed genes. High-throughput sequencing of the RNAs interacting with PRC2 components (CLIP/iCLIP-seq) revealed PRC2 interactions with the pre-mRNAs of the majority of active genes [107, 108]. Inhibition of RNAPII or total RNA degradation in the cell led to the recruitment of PRC2 to active genes [109-111], with this effect being reversible [112, 113]. *In vitro* experiments demonstrated RNA competition with nucleosomes for PRC2 binding and inhibition of PRC2 catalytic activity [107, 114-116]. Interestingly, higher affinity was shown for the RNAs forming G-quadruplex structures [112, 117]. By titrating PRC2 complexes with themselves, nascent transcripts reduce likelihood of these repressive complexes binding to chromatin, thereby preventing gene silencing. It has been hypothesized that all PRC2–chromatin interactions are mediated by the RNA “bridge” [118]. However, evidence from the rChIP-seq experiments indicating necessity of RNA presence in the complex with PRC2 for chromatin localization seems to be an artifact of the experiment and normalization. Later studies demonstrated that treating the immunoprecipitated chromatin with RNase A and sonication in a low ionic strength solution reduced specificity of immunoprecipitation and increased background signal of nonspecific chromatin precipitation [119, 120]. Therefore, active transcription leading to formation of new transcripts that bind and immobilize repressive epigenetic regulators maintains itself.

Involvement of the nascent transcripts in regulation of the binding of chromatin-associated protein factors to chromatin seems to be a more widespread mechanism for controlling the structure and state of chromatin than previously thought. For example, experiments involving inhibition of transcription and RNA degradation in the cells have demonstrated that disappearance of RNA from chromatin coincides with the changes in the chromatin-associated proteome [121, 122]. Transcriptional inhibition and concurrent decrease in the number of nascent transcripts result in the recruitment of chromatin modifier proteins and chromatin remodeling factors (DNA methyltransferases of the DNMT family, EHMT1/2, MLL2/SET1A, HUSH, NuRD, NURF, NoRC, CHRAC, NuA4, INO80, BAF, ATRX/DAXX, cohesin, CTCF, SMCHD1, SAFB) and transcription factors (POU5F1, ZFP57, UBTF, TP53, MYBL2, and UTF1) to chromatin. Meanwhile the number of chromatin-associated RNA processing factors were lost from the chromatin during RNAPII inhibition. A similar qualitative change in the chromatin-associated proteome occurs in the case of RNA degradation [121]. Furthermore, significant proportion of the chromatin-associated proteins are known as RNA-binding proteins [122]. These proteins, often have IDRs or low complexity sequences, and can, through binding

to nascent transcripts in close proximity to RNAPII, facilitate formation of biomolecular condensates via the phase separation mechanism, promoting concentration of the factors necessary for gene expression at the transcription site.

ROLE OF THE SYNTHESIZED TRANSCRIPTS IN THE FORMATION OF TRANSCRIPTIONAL CONDENSATES

Numerous transcription factors interacting with chromatin at regulatory sites (promoters, enhancers) possess, along with DNA-binding and effector domains, a conserved RNA-binding domain resembling the arginine-rich motif (ARM) of the HIV-1 Tat protein [123]. Integrated analysis of the DNA–protein interactions (ChIP-seq) and RNA–protein interactomes (CLIP-seq) of several transcription factors (GATA1, YY1, and CTCF) revealed interactions of these factors with the RNA originating from the loci near the factor binding sites on chromatin (enhancer, promoter, and nascent transcripts). Mutations in the ARM-like domain led to the decreased expression of target genes, while its deletion increased proportion of the freely diffusing transcription factors in the nucleus. These findings suggest that interactions of transcription factors with the nascent RNAs via the ARM-like domain may contribute to formation of the transcriptional condensates and provide precise regulation of gene expression.

In line with the aforementioned observations, a model for transcriptional condensate formation mediated by interaction of the nascent RNAs and protein factors possessing RNA-binding domains and unstructured regions that promote formation of biomolecular condensates (RNA-mediated feedback model) appears attractive [122, 124]. In the experiments utilizing *in vitro* and *in silico* systems, it was demonstrated that the RNA molecules, both from promoters or enhancers, irrespective of their sequence, promoted formation of transcriptional condensates involving the Mediator complex [125]. However, this effect was observed only within a limited range of RNA concentrations, with the fixed concentration of the Mediator complex. At such RNA concentrations, the system reached an equilibrium state between the negative charge of nucleic acid, proportional to its length, and the positive charge of protein factors. Increase in the amount of RNA carrying a negative charge disrupted the equilibrium and led to the condensate dissolution. Validity of the RNA-mediated feedback model proposed by the authors was also demonstrated in the *in vivo* experiments. Transcription initiation and production of the short nascent transcripts led to formation of the transcriptional condensate and simultaneous increase in the reporter gene expression. However, when an excessive level of transcription and a threshold concentration of the nascent RNAs were reached, expression of the reporter gene decreased, and the temporarily formed transcriptional condensates resulting from nonspecific electrostatic interactions between the synthesized transcripts and protein factors were dissolved [124-126]. This mechanism of gene expression regulation is realized in enhancers through the short-lived enhancer RNAs [125, 126]. This mechanism can explain transcriptional bursts observed for many genes in the cases when the enhancer- and promoter-associated condensates interact or fuse [126, 127].

CONCLUSION

The data accumulated in recent years indicate that transcription is accompanied by formation of the molecular complexes with non-stoichiometric composition – transcriptional condensates. Apparently, formation of such condensates is determined by combination of the high-affinity specific interactions and nonspecific interactions between the proteins, RNA, and DNA sites that are part of these condensates. As with other described biomolecular condensates, transcriptional condensates that contain RNAPII are extremely dynamic. In contrast, transcriptional condensates formed as a result of RNAPI activity form stable membraneless structures – nucleoli. Nevertheless, in many respects, formation of the transcriptional condensates is similar in the cases of RNAPI and RNAPII.

The IDRs of proteins engaged in transcription play a pivotal role in formation of the transcriptional condensates. Although these domains participate in nonspecific interactions, they significantly enhance efficiency and specificity of the condensate formation during transcription. This applies to various processes, including both search for the target sites in the nucleus and interaction between the individual components. Notably, positive feedback mechanisms may be at play, wherein molecular interactions lead to formation of the transcriptional condensate facilitating attraction of additional molecules of the same proteins and potentially other molecules involved in the process. This phenomenon is particularly evident in the nucleolus, which attracts a diverse array of molecules [80] contributing to its multifunctionality [128].

Nascent transcripts play a crucial role in the transcription regulation. Transcripts still bound to polymerase or RNAs in the transcriptional condensate due to the large negative charge of their sugar-phosphate backbone, can attract RNA-binding proteins to the transcription site through nonspecific interactions with the positively charged IDRs, along with specific interactions facilitated by the RNA secondary structures. Varying length of RNA, ranging from a few tens

to several thousand nucleotides for different RNA types, enables multivalent interactions with proteins. Ability of the nascent transcripts to attract protein factors to the transcription site can be used to develop tools for gene expression regulation involving CRISPR-Cas9 systems with activator domains of the transcription factors [129].

However, it should be noted that many concepts lack complete experimental validation. For example, there is only limited data available on the structure of elongation transcriptional condensates. Some morphological observations suggest that elongation of some genes occurs on the extended DNA strands resembling loops of the lampbrush chromosomes [35, 36]. While these observations do not definitively contradict the idea of the existence of elongation condensates, they introduce some uncertainty. Moreover, consideration should be given to the data on the transcriptional condensate dissolution promoted by high levels of the transcribed RNA molecules [124-126]. Disassembly of such condensate may facilitate movement of the RNAPII molecules along the DNA strand. These questions require further investigation.

Thus, high efficiency and flexible regulation of transcription largely stem from the weak, non-specific, yet multiple interactions that give rise to formation of the highly dynamic at the molecular level transcriptional condensates.

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