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REVIEW

Biomolecular Condensates in the Regulation of Transcription and Chromatin Architecture

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Abstract—Recent studies have highlighted the pivotal role of biomolecular condensates (liquid-like complexes) in gene control. Biomolecular condensates create a specific microenvironment around enhancers and gene promoters, which can activate transcription, repress it, or maintain at an appropriate level. They can also influence the chromatin structure and are important participants in the enhancer–promoter communication. Finally, biomolecular condensates represent promising therapeutic targets, as their dysregulation results in a broad spectrum of pathologies. The review present most recent, as well as fundamental studies establishing the role of condensates in the regulation of gene expression and enhancer–promoter communication.

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INTRODUCTION

The activation of eukaryotic gene transcription requires a coordinated action of transcription factors (TFs), co-activators, and RNA polymerase 2 (RNAP2). These proteins bind to chromatin in the regions of gene promoters and enhancers (regulatory DNA sequences) [1, 2]. Large enhancers that bind the highest number of transcription regulators are referred to as super-enhancers [3, 4]. Enhancers and super-enhancers form spatial contacts with activated genes. This process is facilitated by the ring-shaped ATPase cohesin that extrudes chromatin loops [5]. Another key participant of the enhancer–promoter (EP) communication is the CTCF protein, which acts as a physical barrier preventing the movement of the cohesin complex [6, 7].

Approximately 30 years ago, RNAP2 clusters were observed in the nucleus by electron microscopy. These clusters were often associated with several *cis*-regulatory elements and, therefore, designated as "transcription factories" [8]. The following development of light microscopy and genome editing techniques has made it possible to observe dynamic clusters of protein transcription regulators with the properties of liquid-phase condensates in live cells. Based on the concept of phase separation, they were named transcriptional condensates (TCs) [9] (Fig. 1).

Biomolecular condensates (Fig. 1) are nonstoichiometric complexes formed as a result of multivalent interactions between their components (proteins, RNA, DNA). Although structured protein domains may play an important role in these interactions [10], the latter almost always, to a greater or lesser extent, occur between intrinsically disordered regions (IDRs). Such interactions are highly specific and maintain a constant composition of the condensates despite the absence of membrane envelope [11]. The key factors providing specific interactions between the IDRs are their repetitive short linear motifs (SLiMs) [11-14], typically composed of 4 to 12 amino acid residues (no more than 8 residues in most cases) [11, 13, 15]. Despite their small length, SLiMs are evolutionary conserved sequences. The interactions between SLiMs

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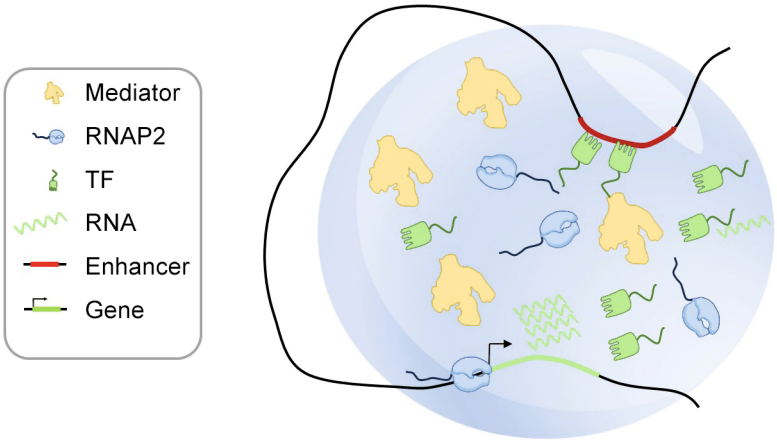

Fig. 1. The model of TC in the region of EP contact. The condensate concentrates transcription factors (TFs), co-activators (Mediator), and RNA polymerase 2 (RNAP2) and promotes transcription. Protein components of the condensate often contain extended unstructured domains (shown in RNAP2 and TF).

are diverse and include hydrophobic, π–π, π–cation, electrostatic, dipole–dipole interactions, and hydrogen bonds [12]. Mutations in these repeats impair phase separation, while their post-translational modification regulates phase formation in cells [12, 13]. At the same time, usually only one amino acid residue within a SLiM is crucial for the phase separation [15]. Hence, in contrast to the interaction between the structured domains, which is based on the mutual recognition of protein fragments that are tens or hundreds amino acids in length, the central role in the interaction between IDRs belongs to individual amino acids [16, 17].

The formation of condensates is finely regulated via numerous mechanisms. The propensity of proteins for the phase separation varies between cell types and cellular compartments [18]. It depends on the protein concentration, post-translational modifications, heterotypic interactions, the presence of multimerization domains, and external conditions (e.g., temperature and pH) [9]. In addition, the clustering of TF-binding sites on chromatin and their spatial proximity create the regions of locally high TF concentration.

In this review, we briefly describe the main experimental approaches used in the studies of TCs, the functions of TCs in the regulation of transcription and chromatin architecture, and their role in the development of various pathologies. Multiple mechanisms have been proposed to explain the formation of TCs, the most discussed of them being liquid–liquid phase separation (LLPS) [19], surface condensation [20], and phase separation coupled to percolation (PSCP) [21]. All these mechanisms describe the formation of non-stoichiometric dynamic complexes driven by weak multivalent interactions. In this review, as well as in some other works [22, 23], such complexes are referred to as TCs.

METHODS FOR TC VISUALIZATION

Since the size of TCs is often at the diffraction limit, their detection required the development of super-resolution microscopy techniques, such as tcPALM (time-correlated photoactivation localization microscopy) [24]. The most common method of TC visualization is immunofluorescence, which allows to visualize focal clusters of transcription proteins and observe their transition from the uniform distribution in the nucleoplasm or cytosol to clusters upon exposure to a stimulus [25-28]. Besides its relative simplicity, the advantages of this method include the possibility to work with endogenous protein concentrations in cells. An obvious disadvantage is that this method requires cell fixation, thus preventing investigation of the dynamic properties of such clusters. In addition, a fixator (usually formaldehyde or paraformaldehyde) can interfere with the process of condensate formation by disturbing or, on the contrary, promoting protein–protein or DNA–protein interactions [29-32].

The use of endogenously labeled proteins helps to bypass these limitations. For example, CRISPR-mediated knock-ins can be employed to add a fluorophore (most often, fluorescent or photoconvertible protein or HaloTag) to the protein reading frame, which allows to study the dynamics of formation/dissolution of TCs and to observe their liquid-like behavior *in vivo* [33-35]. Thus, FRAP (fluorescence recovery after photobleaching) was used to demonstrate both diffusion within the condensates and exchange of molecules between the condensates and their environment [36]. Although expression of a labeled protein from an endogenous promoter makes it possible to avoid its overexpression, some studies have used exogenously expressed labeled proteins, usually,

in cells not expressing such protein under normal conditions [37, 38] or cells where this protein was knocked out/knocked down [39]. In these cases, a doxycycline- or tetracycline-inducible promoter is typically used to adjust protein expression levels to the endogenous ones.

Super-resolution microscopy makes it possible not only to visualize condensates, but also to evaluate their colocalization with the active transcription sites in live and fixed cells. DNA- or RNA-FISH (fluorescence *in situ* hybridization) techniques are used in fixed cells [24, 28, 33, 40, 41], while in live cells, the transcripts can be visualized using modified nucleotides (e.g., 5′-ethynyl uridine) or viral tags (usually MS2/MCP or PP7/PCP systems) [15, 18, 24, 42].

Light microscopy can be helpful in evaluating the size, shape, and dynamics of condensates, as well as in determining the site and time of their emergence. However, it provides little information on the TC composition, because in most studies, the condensates are visualized using only one or two component(s), e.g., Mediator and RNAP2. A more detailed characterization of the TC composition can be achieved by using proteomics-based approaches.

METHODS FOR THE ANALYSIS OF TC COMPOSITION

Characterization of the TC proteome is important for understanding the action mechanisms of condensates. For example, using biotinylated inactive Cas9 nuclease (dead Cas9, dCas9) fused with the FUS protein IDR allowed to identify the key transcription and architecture proteins in TCs [43]. The authors of [44] used inducible delivery of biotin ligase (potentially, any enzyme) in a complex with IDR of the ubiquitous transcriptional co-activator BRD4 for the local biotinylation of all TC components [44]. In both studies [43, 44], RNAP2, Mediator, BRD4, and other transcription proteins were found as the most frequently occurring TC components. Using chemical crosslinking and mass spectrometry, FUS was identified as a key partner of the TAZ TF, required to maintain fluidity and robust transcriptional activity of TAZ condensates [28]. Finally, a similar method helped to identify AMPK (AMP-dependent kinase) as a negative regulator of pathological condensates of the FOXM1 TF and a promising therapeutic target [16].

Combined with modern microscopy techniques, proteomics methods allow detailed characterization of the composition of TCs in live cells. However, it remains unclear whether condensates make any specific contribution to the transcription regulation in addition to that of the soluble complexes.

METHODS FOR EVALUATION OF CONDENSATE FUNCTIONALITY

The attention of researchers is currently focused on the biological functions of condensates compared to soluble protein complexes. There are several ways to resolve this issue.

1) Creation of artificial TCs and analysis of their transcription activation capacity. The condensates are often reconstructed on the studied genes through the targeted recruitment of dCas9–IDR complexes with fluorescent proteins, which enables visualization of condensates in live cells and evaluation of their biophysical properties [43, 45]. Such systems are used for the targeted regulation of both transcription [46] and chromatin architecture [47, 48] (see “Transcriptional condensates as transcriptional activators”). Another frequently used approach is the use of the LacO/LacI- or TetO/TetR-based systems.

2) Genetic complementation [49]. The principle of this method consists in the identification of domains responsible for the phase separation followed by their substitution with functionally analogous domains of other proteins. If such substitution restores both the biological function of the protein and its ability to form condensates, this is indicative of the condensate functionality. However, this does not exclude the contribution of soluble complexes [19, 33, 50-52]. It is important that the replaced domains lack the amino acid sequence similarity (18% on the average [49], but can reach 0% [51]) despite their common role in the phase separation. The substitution of domains does not always restore the protein biological function, because the amino acid composition of IDRs may affect the consistency of condensates or their ability to concentrate co-activators. In particular, the substitution of the IDR of MYOCD (myocardin) by IDRs from FUS, EWS, or DDX4 proteins fully restored the ability of MYOCD to form condensates and activate transcription, while its replacement with the IDR from CDT1 led to the formation of nonfunctional condensates unable to concentrate RNAP2 and Mediator. Another example is histone deacetylase UTX, whose phase separation underlies its chromatin-regulatory activity in tumor suppression. The substitution of its IDR by the IDRs from eIF4G2 and AKAP95 (8 and 18% identity, respectively) restored the condensates and their tumor-suppressing activity. At the same time, replacement with the IDR from its paralog UTY (74% identity) resulted in the formation of more solid, nonfunctional condensates [52]. Interestingly, the catalytic activity of UTX is not necessary for its tumor suppression function, in contrast to the ability to form condensates [52].

3) Point mutagenesis aimed at the uncoupling of protein–protein interaction from the phase separation [53-56]. However, in some cases, these two

activities cannot be separated. For example, in the yeast transcription factor Gcn4, the same amino acid residues are involved in the protein interaction with Med15 and condensate formation [53]. Hence, these proteins exist in live cells as both soluble complexes and phase condensates [53]. At the same time, in the IDR of the chromatin remodeling factor ARID1A/B, the residues responsible for the condensate formation and interaction with other proteins are different, and both activities are essential for the protein binding to chromatin and implementation of its biological functions [54].

4) Recruitment of solubilizing proteins (e.g., fructose- or mannose-binding proteins), which dissolve condensates but do not abolish the protein–protein interactions [57-60], to condensates. A major advantage of this approach is its high selectivity. Moreover, it does not affect the level of synthesis of cellular proteins and does not require introduction of mutations into them. These methods allow identification of genes specifically regulated by the condensates [57-59].

5) Treatment of cells with small organic molecules that dissolve condensates without changing the levels of synthesis of their protein components or protein–protein interactions [27, 34, 61]. Such approaches are effectively used for the regulation of gene expression in both cultured cell and live organisms.

In addition, by using live-cell microscopy, it was shown that condensates form on the regulated genes before the onset of transcription [62]. For example, one of the recent works demonstrated that the emergence of large dynamic clusters of the Nanog TF on the actively expressed *mir430* gene in *Danio rerio* embryos preceded the start of its transcription [63]. Another example is clustering of nonphosphorylated RNAP2 on various genes in mouse embryonic stem cells (mESCs) before the transcription initiation [64]. At least for some proteins, e.g., MYOCD, the formation of condensates and activation of transcription occurred at the same critical threshold concentration. The condensates formed specifically on the activated genes, presumably, due to the MYOCD interaction with TFs [33]. Therefore, available methods demonstrate the contribution of TCs to the regulation of gene expression, including both activation and repression of transcription.

TRANSCRIPTIONAL CONDENSATES AS TRANSCRIPTIONAL ACTIVATORS

The colocalization of condensates and active transcription sites in live cells has been shown for both native and synthetic TCs [18, 24, 26, 28, 33, 40-43, 45, 65, 66]. Early observations demonstrated a direct correlation between the levels of mRNA synthesis and stability of transcriptional factories associated with the β-actin gene in live mouse cells [62]. Later experiments with endogenously labeled proteins showed that the key transcriptional activators BRD4, Mediator, and RNAP2 formed dynamic condensates on active genes in mESCs [40]. Immunofluorescence analysis in combination with RNA- and DNA-FISH revealed that these condensates form on super-enhancers and are in a close proximity to or overlap with the sites of mRNA synthesis [24, 40]. Moreover, TCs colocalized (although episodically) with the MS2-labeled transcripts of the *Esrrb* gene actively expressed in mESCs [24]. Later studies have shown a significant inverse correlation between the levels of gene transcription and distance to the TC [35, 36]. TCs formed in mESCs were over 300 nm in size and contained up to 400 Mediator and RNAP2 molecules [24], suggesting their nonstoichiometric nature. Promoter-associated clusters containing 10 to 90 molecules of RNAP2 phosphorylated at Ser5 at the moment of transcriptional bursting, were identified using super-resolution microscopy [67, 68]. Beside the regulation of transcription initiation, proteins forming condensates with the unstructured C-terminal domain (CTD) of RNAP2 were found to regulate transcription elongation [65, 69], splicing [70] and, in some cases, transcription termination [50].

Optogenetic techniques were used to reveal a direct correlation between the intensity of condensate fluorescence and transcription levels of associated genes [18]. The use of dCas9 in a complex with the CRY2 domain allowed to create TCs at the genomic loci of interest and to analyze their effects on the transcription of individual genes [43, 45, 71, 72]. Such synthetic condensates efficiently concentrated transcriptional co-activators and RNAP2 phosphorylated at Ser2 [46]. In HeLa cells, synthetic light-induced condensates based on dCas9 and guide RNAs to the beta-globin gene *HS2* enhancer and *BCL11A* gene promoter, increased transcription of the target genes 11-23 and 24-35 times, respectively [43]. The complexes of the viral protein VP64 (commonly used transcriptional activator) with IDRs of the phase-forming proteins FUS and NUP98 enhanced transcription much more efficiently than VP64 alone [45, 72, 73]. Finally, the DroprCRISPRa system based on the FUS IDR fused with dCas12a-VP64 activated transcription *in vivo* in mice [46]. Besides providing targeted expression activation, these approaches demonstrate the need for the optimal intensity of multivalent interactions in order to ensure an efficient transcription activation [45, 71, 73]. For example, mutations in the IDR of FUS, causing the dissolution of condensates or changes in their material properties, reduced transcription [46].

These observations are in good agreement with the data obtained for native TFs. For example,

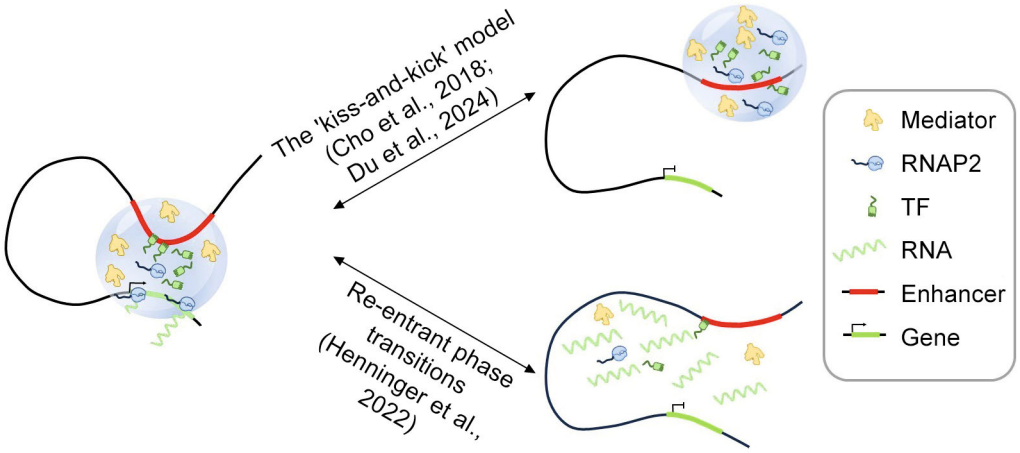

Fig. 2. The "kiss-and-kick" and the re-entrant phase transition models of the transcriptional bursts. In the kiss-and-kick model [24, 35], transcriptional bursts are initiated by the periodic gene convergence with TCs and enhancers. In the model of recurrent phase transitions [81], TCs form on weakly expressed genes, thus enhancing their transcription, and then dissolve at a high local transcript concentration (negative feedback).

in the presence of androgens, diffusely distributed androgen receptor (AR) translocated to the nucleus and formed condensates on enhancers via the IDR–IDR interactions [61]. The treatment of cells with 1,6-hexanediol, which dissolved the AR condensates but did not affect the level of AR biosynthesis, significantly reduced chromatin accessibility and transcription on the androgen-regulated enhancers [61]. At the same time, an increased number of glutamine residues in the IDR of AR in various pathological states results in the formation of stable aggregates and noticeable decrease in the enhancer activity [61]. A similar picture was observed for the excessive stimulation of estrogen enhancers [74]. In general, an increase in the multivalency can inhibit transcription due to alterations in the material properties of condensates or their formation outside of the chromatin body [23, 74-76].

The intensity of multivalent interactions can be influenced by external factors. For example, at temperatures above 27°C, the nucleoplasmic condensates in *Arabidopsis thaliana* cells concentrate the ELF3 protein (negative regulator of flowering time), thus preventing its binding to chromatin and repression of genes involved in flower development [77]. The biomolecular condensates that play a key role in the regulation of flowering are typically temperature-sensitive [78]. The transcription factor Hsf1, an activator of heat shock response genes, uses a similar mechanism to form condensates at elevated temperatures [79]. TCs can also appear in response to pH changes. Thus, acidification of the nucleoplasm in macrophages, which is typical for inflammatory processes, partially dissolved BRD4 and MED1 condensates via protonation of His residues in the IDR of BRD4 [80]. This mechanism most strongly reduces transcription of proinflammatory genes regulated by distant super-enhancers (negative feedback-mechanism) [80]. Hence, TCs can act as sensors of external conditions.

The formation of TCs allows to explain some features of eukaryotic transcription. For example, it is known that transcription of eukaryotic and some prokaryotic genes occurs in bursts, which can be due to the periodic formation of contacts between the genes and TCs [35]. For example, the intensity of the *Sox2* gene transcription in mESCs was proportional to its proximity to the condensate associated with its super-enhancer [35] (Fig. 2). The alternative mechanism is periodic emergence and dissolution of condensates due to the electrostatic repulsion of mRNAs accumulated in the active transcription sites [81] (Fig. 2). This suggestion was confirmed by the fact that the treatment of cells with transcription elongation inhibitors stabilized Mediator-containing initiatory condensates [81]. At the same time, this treatment led to the dissolution of the elongation condensates appearing due to the interaction between proteins and the CTD of RNAP2 phosphorylated at Ser2 [65].

Special attention has been focused on a potential role of condensates in the regulation of TF binding to DNA [22, 82-86]. If the properties of the nucleoplasm were similar to those of a diluted homogeneous solution, then, according to the Smoluchowski equation, it would take days for a TF to find its binding motifs in the volume of the nucleus [82]. However, DNA, proteins, and RNA are intrinsically present in the nucleus at the concentrations that prevent free diffusion and create the effect of macromolecular crowding [87], which could significantly increase the time required for such search. However, some transcriptional responses are observed within several minutes.

Phase separation intensifies the interactions between some TFs and chromatin [16, 19] (see also "Transcriptional Condensates in Pathology" section), while chromatin-associated condensates help some TFs find their binding sites [80, 82]. The role of IDRs in the search of binding motifs on DNA has been well demonstrated. It supplements and, in some cases, even exceeds the significance of DNA-binding domains in the identification of binding sites [79, 83]. Interestingly, TFs diffuse slower inside the condensates than in the nucleoplasm [76]. Analysis of diffusion trajectories of single TF molecules revealed two types of diffusion: rapid free and slow limited [88]. At least in some cases, limited diffusion required the presence of IDRs [89]. Based on these observations, it was suggested that local diffusion does occur inside the condensates, which facilitates the binding of TFs to DNA and slows down their dissociation [84, 89].

However, the situation might be more complicated. For example, in the yeast Msn2 TF, extended IDRs play a key role in the search of binding sites independently of the phase separation, as Msn2 is diffusely distributed in the nucleoplasm [90]. The limited diffusion of RNAP2 can be observed in the absence of evident signs of condensate formation [91]. To summarize, although IDRs and multivalent interactions help TFs find their binding sites, the need for condensates in this process remains debatable.

The role of TCs is not always limited to the transcription activation or repression; sometimes, it is to maintain required levels of gene expression. This phenomenon was observed in mESCs, which stably express differentiation genes at low levels. Recent study has shown an association between these genes and specific dual-activity TFs combining the functions of transcriptional activators and repressors [34]. Using endogenous fluorescence labeling of investigated TFs, the authors were able to observe the formed condensates *in vivo*. The condensates neither colocalized with the markers of active and inactive chromatin, nor associated with the bivalent chromatin. In contrast to classical TCs, the condensates formed by the dual-action TFs concentrated RNAP2 very moderately and were almost entirely depleted of Mediator. Moreover, artificial reconstruction of these condensates on chromatin in HEK293 cells stabilized intermediate expression levels of genes that had been transcribed above or below these levels. Experiments with reporter genes have shown that after reaching a threshold concentration necessary for the phase separation, further increase in the TF concentration did not lead to any significant enhancement of transcription. This fact distinguishes dual-action TFs from the classical activators, which activate transcription proportionally to their recruited amount [34]. Finally, chimeric proteins obtained by fusing these TFs with solubilizing proteins (e.g., mannose-binding protein) did not form condensates and displayed no dual action at relatively low concentrations; however, both effects were restored at the high concentrations. Therefore, the formation of microcompartments with a specific protein composition characterized by the absence of repressors and relatively low content of transcription activators allows to maintain gene expression at a necessary level [34].

Some TCs, in particular, those including components of facultative and constitutive heterochromatin and regulators of the promoter-proximal pausing of RNAP2, specialize in the repression of regulated genes.

TRANSCRIPTIONAL CONDENSATES AS TRANSCRIPTIONAL REPRESSORS

Some molecular condensates are formed by the key components of heterochromatin. One of these components is MeCP2, which binds methylated DNA and histones and represses transcription by either displacing transcription activators or recruiting corepressors (e.g., histone deacetylases). In mESCs, MeCP2 formed condensates that selectively concentrated transcription repressors [92]. MeCP2 droplets actively concentrated HP1-α, but did not fuse with the BRD4 or MED1 droplets even after physical contact *in vitro* [92]. Mutations affecting the ability of MeCP2 to form the condensates reduced its capacity to bind DNA and repress transcription [92].

Repressive condensates can also emerge in response to changing environmental conditions. NELF (negative elongation factor) is homogeneously distributed in the nucleoplasm under normal conditions, but forms condensates under heat stress [25]. The substitution of its IDR by IDRs from FUS or EWSR1 restored both cluster formation and NELF-mediated repression [25].

Other important transcription repressors are proteins from the Polycomb group (in particular, components of the PRC1 and PRC2 complexes) that are necessary for the facultative heterochromatin formation. Polycomb condensates were originally discovered by the super-resolution light microscopy [84, 93]. Recently, their ultrastructure and mechanisms of formation were elucidated by electron tomography. For example, a subunit of the PRC1–CBX8 complex condenses with chromatin due to the multivalent interactions with DNA and nucleosomes [94]. Although chromatin in the CBX8 condensates was more static, there were pores between the nucleosomes [94] which allowed the passage of complexes up to 600 kDa (~8 nm). Hence, RNAP2 (~550 kDa) or CBX8 (~43 kDa) could freely diffuse within such

heterochromatin, and transcription repression was achieved not due to the chromatin compaction but rather to the inability of RNAP2 to stably associate with chromatin [94-97].

Polycomb proteins are also required for the X chromosome inactivation, a mechanism that provides balanced expression of X-linked genes in males and females. The key role in this process in placental mammals belongs to the long noncoding RNA Xist that recruits transcriptional repressors. However, the mechanism of its distribution over inactive X chromosome had remained unclear for a long time. Recently, it was demonstrated that it occurs through the formation of a repressive condensate covering the entire X chromosome [98]. At the same time, it was found that HNRNPK protein condensates concentrate Xist and its protein partners, leading to the increase in their adhesiveness and fluidity *in vitro*, which presumably limits Xist diffusion and facilitates its distribution *in cis in vivo* [98].

Another interesting example of transcriptional repression was observed for the circadian genes of *Drosophila melanogaster* [29], which are periodically activated and inactivated throughout the day. The binding of the PER and CLK TFs in the promoter regions of these genes leads to their repression. Endogenous labeling of PER in *D. melanogaster* live cells showed that during repression, PER forms several large (~300-400 nm) condensates that colocalize with the repressed genes [29] and translocate them to the nuclear lamina by interacting with lamin B [29]. Therefore, condensates are also able to position genomic loci in the nuclear space and to determine chromatin architecture.

The architectural function of condensates is universally observed. In most cases, it involves creation and maintenance of loop contacts [21, 99, 100]. At the same time, the condensates often partially or completely associate with super-enhancers, which are probable sites of their emergence [35, 40, 101].

TRANSCRIPTIONAL CONDENSATES AS MEDIATORS IN THE EP COMMUNICATION

The concept of TCs was originally based on the observations of super-enhancers as structures with an abnormally high level of associated transcriptional IDR-containing proteins [102-104] and emerging as a result of cooperative assembly through a single nucleation event [40, 105]. Super-enhancers form spatial hubs that do not depend on the loop extrusion [106]. Taken together, these observations suggested the existence of TCs [105], which have then been found on super-enhancers in live cells [24, 26, 40, 107]. The ability of super-enhancers to participate in the nucleation of condensates was demonstrated *in vitro* [101].

The idea that condensates can regulate chromatin architecture has originated from the two types of evidence. First, it was found that mutations disrupting phase separation result in the weakening of genome loops, while the overexpression of phase-forming IDRs strengthens certain EP contacts [19, 21, 99]. Second, artificial nucleation of condensates on chromatin can alter its 3D structure [43, 47, 48, 108]. In particular, condensates artificially reconstructed on chromatin were able to form loops and concentrate cohesin [43]. Moreover, many proteins impeding cohesin movement (e.g., RNAP2, Mediator, MAZ, RUNX2, and other TFs) form condensates, although the detailed relationship between these activities requires further elucidation [109]. At the same time, condensates can stop at least some molecular motors on chromatin [50]. There is evidence (although contradictory) [110, 111] that the CTCF protein, the most canonical barrier for the cohesin-dependent loop extrusion, also forms condensates [112, 113]. In some cases, chromatin folding promotes the emergence of condensates. Thus, the chromatin framework formed by CTCF and cohesin is necessary for the appearance of BRD4 and RNAP2 clusters in human HCT116 cells [110], presumably, due to the convergence of actively transcribed genomic elements. Also, a recent study showed that the high local density of piRNA (piwi-interacting RNA) genes in *C. elegans* germ cells is crucial for the emergence of condensates activating expression of these genes [14].

Regardless of whether formation of condensates is a consequence or a mechanism of chromatin loop formation/maintenance, the condensate paradigm explains many observations about EP communication, such as long (comparable to the condensate size) distances separating active enhancers and corresponding promoters [114], coregulation of several genes by a single enhancer [66, 115], co-expression of spatially convergent genes [116], coupling of transcription on enhancers and promoters [115, 117], formation of hubs of multiple enhancers and promoters [118-120], as well as the increase in the local viscosity between them [121]. All the above implies the existence of a common compartment where the enhancer and the promoter can exchange associated regulators [122]. Another important indicator of enhancer activity is the production of enhancer RNAs, which, in many cases, contribute to the condensate formation and can play the crucial role in the EP communication [123].

Therefore, condensates play the key role in the EP communication, chromatin architecture, and gene regulation. Impairments in their formation, localization, or composition can lead to the development of various pathologies.

TRANSCRIPTIONAL CONDENSATES IN PATHOLOGY

An impaired regulation of TC formation and/or composition can result in various pathologies, such as disorders in the development of the nervous system [92] and limbs [55], viral infections, and many types of cancer. Recently, tens of thousands of mutations in condensate-forming proteins have been identified, which can lead to more than 1000 types of genetic disorders and hundreds of types of cancer [124]. These pathologies result from the formation of aberrant condensates and changes in their material properties or localization [124] (Fig. 3a).

Disruptions in the condensate localization as the result of the derepression of endogenous retroviruses led to embryonic lethality in mice [125]. RNA transcribed from the endogenous retroviruses effectively recruited RNAP2 and Mediator, resulting in the TC emergence on retrotransposons and their disappearance on enhancers and promoters [125] (Fig. 3b). The authors also observed the hyperactivation of genes located closely to the derepressed retroviruses [125]. Hence, in this case, the derepressed retroviruses exerted a pathological effect not via retrotransposition, but through the disruption of TC localization. Similarly, the condensates of ICP22 protein of the human herpes virus (HSV-1) successfully competed with the genome of infected cells for active (capable of elongation) RNAP2 phosphorylated at Ser2 [126].

Another example of pathology associated with the disruptions in the condensate localization and material properties is brachyphalangy, polydactyly and tibial aplasia syndrome (BPTAS). It is caused by the frameshift mutations in the IDR of the chromatin protein HMGB1, which alter its amino acid sequence [127]. The role of impaired phase separation in the development of this disorder has been demonstrated in a recent study [127]. Under normal conditions, HMGB1 is localized in the nucleoplasm, where it forms numerous small dynamic (capable of rapid fluorescence recovery) spherical clusters [127]. The frameshift mutations associated with BPTAS lead to the enrichment of the arginine residues in the HMGB1 IDR, which is typical of nucleolar proteins. As a consequence, the protein is redistributed to the nucleolus. The frameshift also results in the appearance of a hydrophobic patch in the IDR, leading to solidification of nucleolar aggregates of HMGB1. The solidified aggregates of the mutant protein are irregularly shaped and much less dynamic. Pathological clusters of HMGB1 in the nucleolus lead to the organelle dysfunction and defects in the limb formation [127]. Although mutant HMGB1 noticeably reduced the survival of U2OS cells, this effect was neutralized by the exogenous expression of HMGB1 variant lacking the hydrophobic patch [127], thus showing the crucial significance of physical nature of HMGB1 clusters for the BPTAS development.

Mutations in IDRs altering the composition of condensates can also result in pathologies. For example, an increased number of alanine residues in the IDR of HOXD13 (regulator of limb development) significantly reduces the ability of condensates containing this TF to concentrate Mediator and to activate transcription, leading to the development of synpolydactyly [55]. A similar situation is observed for the HOXA13, RUNX2 and TBP TFs [55].

The formation of chimeric transcription factors via chromosomal translocations underlies many types of cancer. In these cases, the structured DNA- or nucleosome-binding domain of one protein is fused with the IDR of another protein, which enables the mutant protein to form condensates and affects its protein interactome. Quite often, such aberrant condensates concentrate large amounts of transcriptional co-activators and hyperactivate transcription of associated genes [128, 129]. For example, chimeric TFs generated by the fusion of the DNA-binding domain of HOXA9 and IDR of nucleoporin NUP98, form leukemia-causing condensates on chromatin [19, 128]. The phenylalanine/glycine repeats in the IDR of NUP98 contribute to the concentration of cofactors and promote oncogene expression [128]. Similarly, mutations in the ENL protein recognizing acetylated chromatin, lead to the emergence of condensates excessively concentrating transcription elongation factors, resulting in the cancer development [23]. Interestingly, the mutant TF in this case is formed due to the point mutations in the structured domain and IDR and not as a result of translocation [23]. A recent study has shown that the amino acid sequences of transactivation domains in chimeric oncogenic TFs typically recruit RNAP2 more intensively due to the increased number of aromatic residues and amino acids interacting with them, which promotes overexpression of the target genes [129].

In addition to the hyperactivation of target genes via a higher recruitment of RNAP2, aberrant condensates can form new EP contacts. For example, the chimeric TF NUP98-HOXA9 forms super-enhancers and CTCF-independent spatial contacts with oncogenes as a result of phase separation [19, 119]. The substitution the specific Phe residues in the IDR of NUP98-HOXA9, making it incapable of phase separation, led to the disappearance of EP contacts anchored by this factor and significantly reduced pathogenicity of the chimeric protein [19]. Another example is the Ewing sarcoma, in which aberrant EP contacts are formed due to the chimeric EWS/FLI1 TF and chromatin remodeler ARID1A [39, 76, 130].

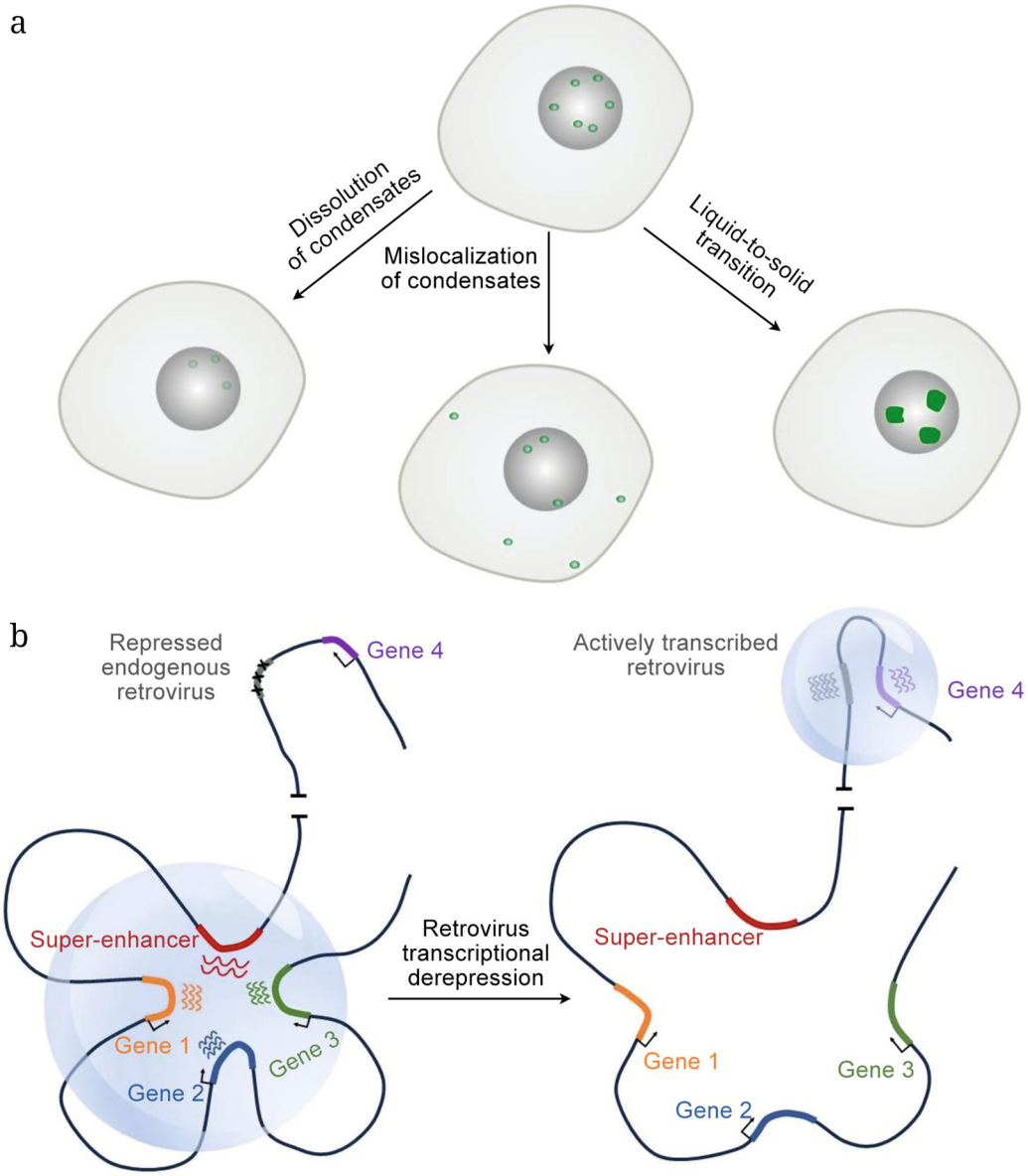

Fig. 3. The role of condensates in the development of pathologies. a) Condensate involvement in the pathology development; b) expression deregulation caused by disruptions in the condensate localization as a result of derepression of endogenous retroviruses competing with the genomic loci for the transcription proteins.

The therapeutic approaches for dissolving aberrant TCs are being actively developed [16, 131]. For example, in breast cancer cells, aberrant TCs are formed by the FOXM1 TF. This promotes extensive FOXM1 binding to chromatin and increases its activating capacity [16]. AMPK, which phosphorylates the IDR of FOXM1 at a single serine residue, acts as an antagonist of this process. The agonists of AMPK and synthetic peptides containing phosphorylated serine facilitate dissolution of FOXM1 condensates in live cells [16]. The treatment of cells with these peptides significantly reduced the proliferative potential of cells. When injected into mice as components of nanoparticles liposomes, such peptides significantly reduced tumor growth and metastasis and activated the immune system [16].

Micropeptides can also produce a therapeutic effect, although via the opposite mechanism, i.e., by causing the solidification of condensates. Recently, a 17-amino acid micropeptide with a high propensity for oligomerization has been obtained [132], whose targeted delivery to condensates (including oncogenic condensates formed by chimeric TFs, viral condensates, and nucleolus) made them much less dynamic and fully stopped exchange of components between the condensates and their environment [132]. At the same time, the interactions between the soluble complexes were not affected, which emphasizes the key

contribution of condensates to the pathogenesis of diseases. The treatment of murine leukemia tumor cells isolated from the bone marrow with this peptide decreased their proliferative potential to nearly zero [132]. Similarly, this peptide significantly reduced production of new viral particles in infected HEK293T cells [132].

CONCLUSION

Recent studies of TCs have resulted in a considerable progress in understanding molecular mechanisms involved in the regulation of gene expression and chromatin architecture. New methods of super-resolution microscopy and genome editing have demonstrated the possibility of transcriptional regulation by dynamic biomolecular condensates emerging due to the multivalent interactions, which determine their liquid nature and nonstoichiometric composition.

Over the past seven years, significant advances have been achieved in understanding the role of TCs in the transcription regulation. TCs are involved in the differentiation of animal and plants cells [40, 41, 77], stress response [79, 118], signaling [27, 38], and many other processes. Due to their selectivity, condensates can create specific microenvironments characterized by a unique composition that can facilitate transcription activation or repression [15, 34, 92]. At least in some cases, the formation of condensates increases the enzymatic activity of chromatin-associated proteins [52] and promotes the activation function of TFs [133]. Moreover, phase separation can be accompanied by the appearance of new material properties. For example, it has been shown recently that in live cells, the condensates of BRD4-NUT and BRD4S TFs behave as a viscous liquid while on chromatin, thus limiting diffusion of these TFs and mobility of nucleosomes associated with them [134]. It is interesting that the zones of TCs corresponded to the A compartments [134].

However, many aspects of TC functioning remain poorly studied. In what situations do condensates become necessary for the transcription activation/repression compared to soluble complexes? It is possible that in most cases, both condensates and soluble complexes are involved in the transcription regulation, although there are genes whose expression strongly depends on phase separation. The mechanisms of TC formation still require detailed investigation. For example, it remains unclear why DNA in some cases facilitates [92, 101] and in other cases prevents [53] the formation of condensates of DNA-associated proteins. Other interesting issues are the relationship between TCs and loop extrusion in the formation of the genome 3D conformation [109] and contribution of phase separation to the maintenance of large loops based on Polycomb proteins.

Recent studies have emphasized the necessity of the optimal level of multivalent interactions for the proper regulation of gene expression and demonstrated that the phase separation can not only activate but also inhibit transcription [75, 76]. Interestingly, phase separation can enhance the activator function of TFs by reducing their binding specificity [133]. The disruption of this fine balance can lead to a wide range of pathologies. Hence, condensates are actively studied as therapeutic targets. Manipulations with TCs have already helped to control gene expression in cultured cells and mice. Elucidation of molecular mechanisms of TC formation and regulation will become an important step in understanding the universal principles of cell nucleus organization and transcription regulation.

Abbreviations

EP	enhancer–promoter
IDR	intrinsically disordered region
mESC	mouse embryonic stem cell
RNAP2	RNA polymerase 2
TC	transcription condensate
TF	transcription factor

Contributions

A.V.S. analyzed the data, wrote and edited the text of the article, and prepared the figures; S.V.U. and S.V.R. edited the manuscript.

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Ethics approval and consent to participate

This work does not contain any studies involving human or animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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