The role of physicochemical processes in the formation of the 3D genome and compartmentalization of the cell nucleus

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Abbreviations: BRD – bromodomain motif in amino acid sequence; LLPS – liquid-liquid phase separation; PML – promyelocytic leukemia protein (PML nuclear bodies are also known as ND10 nuclear bodies); PPPS – polymer-polymer phase separation

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DEDICATION

Dedicated to the memory of Ron Hancock, a scientist who was always interested in science as such, and not in a scientific career.

ABSTRACT

The review analyzes the role of physicochemical processes in the formation of the function-dependent architecture of the cell nucleus, built on the platform of a folded genome. The main attention is paid to various forms of the phase separation process, primarily the processes of liquid-liquid phase separation and polymer-polymer phase separation. The role of these processes in the formation of chromatin compartments and maintenance of three-dimensional genome architecture is discussed in detail. The relationship between genome activity and the creation of functional compartments in the cell nucleus is also analyzed.

INTRODUCTION

Seventy years ago, Watson and Crick proposed the double helix model of DNA [1]. Over the next few years, the genetic code was deciphered, and the central dogma of molecular biology was formulated, reflecting the transfer of information from DNA to RNA and then to proteins [2]. Rapid progress in the field of molecular biology has led to the belief that the molecular mechanisms underlying life will be quickly disclosed. These hopes did not come true. Quite soon it became clear that living matter is much more complex than was imagined at the time of the emergence of molecular biology. With the sequencing of the human genome [3] and many other genomes, it became clear that in multicellular organisms, protein-coding sequences occupy only a few percent of the total genome size [3-5]. At the same time, it was demonstrated that in addition to ribosomal RNAs, there are many other non-coding RNAs that perform various functions in the cell, including enzymatic, regulatory, and architectural functions [6]. Already in the 21st century, so-called 3D genomics appeared [7-10]. Of course, the genome, like DNA, is linear, or, in some cases, circular. However, in the cell nucleus, the genome is packaged in such a way that distant parts of it can contact each other. It turned out that spatial contacts between genes and distant regulatory elements play an important role in the control of transcription [11]. It was this observation that laid the foundation for 3D genomics. The packaging and operation of the genome is closely related to the functional compartmentalization of the cell nucleus [12]. In fact, the cell nucleus is assembled on the platform of a packaged genome and, in a certain sense, can be regarded as the exoskeleton of the folded genome [13]. In this review, I will analyze the role of simple physicochemical processes in the formation of the 3D genome and compartmentalization of the cell nucleus.

Compartmentalization, i.e., the creation of relatively isolated locations characterized by an increased concentration of certain components, is a basic property of living cells. The living cell itself and some cellular compartments, such as the cell nucleus or mitochondria, are separated from the surrounding space by lipid membranes. However, in the cell nucleus there are many functional compartments that are not surrounded by membranes [14]. Recent studies indicate that most of these compartments arise in connection with one or another activity of the genome: transcription, replication, and repair of DNA damage [15-17]. The question of how the intranuclear functional compartments, which include the nucleolus, speckles, paraspeckles, and the Cajal and promyelocytic leukemia protein (PML) bodies, are formed, has long been debatable. At present, convincing evidence has been obtained that the formation of all these compartments is provided by simple physicochemical processes, including the process of liquid-liquid phases separation and the forces arising under conditions of macromolecular crowding [14,18-22]. These processes, however, are modulated by genome activity. In this review, we first briefly describe the physicochemical processes mentioned above and then consider how they are involved in the compartmentalization of the cell nucleus and the formation of the three-dimensional organization of the genome.

LIQUID-LIQUID PHASE SEPARATION

Liquid-liquid phase separation (LLPS) occurs when the macromolecules present in the solution interact with each other better than with the solvent molecules. In this case, upon reaching a certain concentration of the dissolved substance, it is demixed into a separate phase. With further addition of this substance to the solution, its concentrations in the solution and in the separated phase remain constant, while the volume of the separated phase increases. A typical example of LLPS is the formation of oil droplets in an aqueous solution. The process of LLPS is described by a phase diagram (Fig. 1). This diagram shows that to initiate phase separation, a certain threshold solute concentration must be reached, the value of which depends on additional conditions, such as temperature [23,24]. The demixing of macromolecules into a separate phase is directed by multivalent unstable interactions between these macromolecules. The nature of the interactions may vary. They can be, for example, electrostatic or hydrophobic [25,26]. In biological systems, proteins possessing intrinsically disordered domains (IDRs) are known to form liquid droplets *via* LLPS [27]. Many nuclear proteins possess such IDRs and hence can form phase-separated condensates in overcrowded nuclear milieu [28-35]. Partially complementary RNA molecules can also form liquid phase condensates [36-38]. Phase condensates can be formed by several different macromolecules if they can establish multivalent interactions with each other [39-41]. An important characteristic of a liquid phase condensate is that the environment inside this condensate differs from the solution in which the phase condensate was formed. After phase separation, the macromolecules present in the solution are distributed between the phases in accordance with their ability to interact with the solvent and with the macromolecules released into a separate phase. Macromolecules that have passed from solution to the phase condensate, but do not participate in its formation, are called client molecules [42]. For compartmentalization of the cell nucleus, the localization of phase condensates, i.e., their formation in certain areas of the cell nucleus, is fundamentally important. This is ensured by an increase to the threshold value of the local concentration of macromolecules capable of separating into a separate phase. As a rule, this is achieved by attracting such macromolecules to a certain platform which may be a protein [43,44], RNA [45,46], polyADP-ribose [47], DNA [48,49] or chromatin fibril [48]. The liquid phase condensates are expected to possess round shape, fuse upon coalescence, and quickly exchange components with the external milieu [24,50]. The interacting macromolecules gathered in a certain location within the cell nucleus are additionally held together by the entropy forces that arise under the conditions of macromolecular crowding, which will be discussed in the next section. The ability of certain proteins to establish contacts leading to the formation of phase condensate, can be modulated by post-translational modifications [51-53]. Post-translational modifications can also affect the ability of client proteins to be retained in the phase condensate [54,55].

It is worth saying that in addition to the most frequently discussed LLPS, other phase separation processes are also involved in the organization of intranuclear space: liquid-gel-phase separation; liquid-solid phase separation and polymer-polymer phase separation [56-58]. The first two of the processes mentioned are similar in principle to LLPS. The only difference is whether the resulting phase condensate is gel-like or solid. Polymer-polymer phase separation (PPPS) is the process of physically separating long polymers, each of which has a certain number of cross-links between distant regions. PPPS leads to the formation of a polymer globule (Fig. 2) [56]. A significant difference between such a globule and a liquid-phase condensate is that the medium inside the

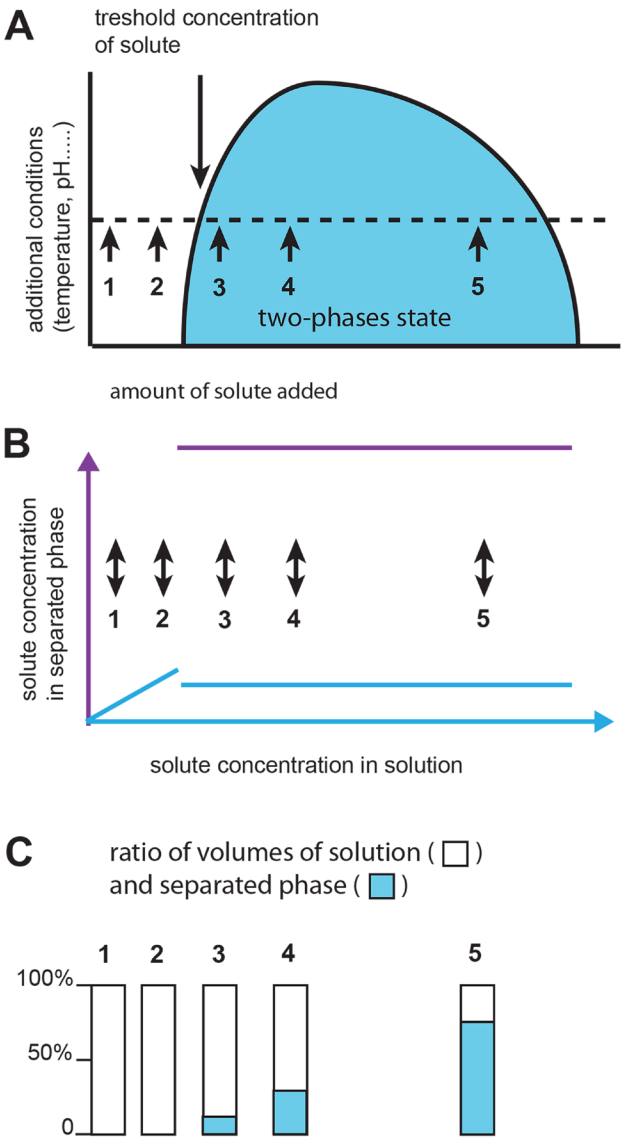

Figure 1. Phase diagram. (**A**) The process of separation of liquid phases is described by a phase diagram. As a substance capable of forming a separate phase is added to the solution, the concentration of this substance in the solution gradually increases (points 1, 2) and reaches a threshold value, after which phase separation occurs (points 3–5). The threshold value depends on additional conditions, such as temperature, pH, etc. (**B**) Concentrations of the solute in the solution (blue line) and the separated phase (purple line) when the solute is added to the solution. Note that after phase separation, further addition of the substance that formed the separated phase does not lead to a change in its concentration either in the solution or in the separated liquid phase. In this case, the volume of the separated phase increases and the volume of the solution decreases accordingly, as shown schematically in section **C**.

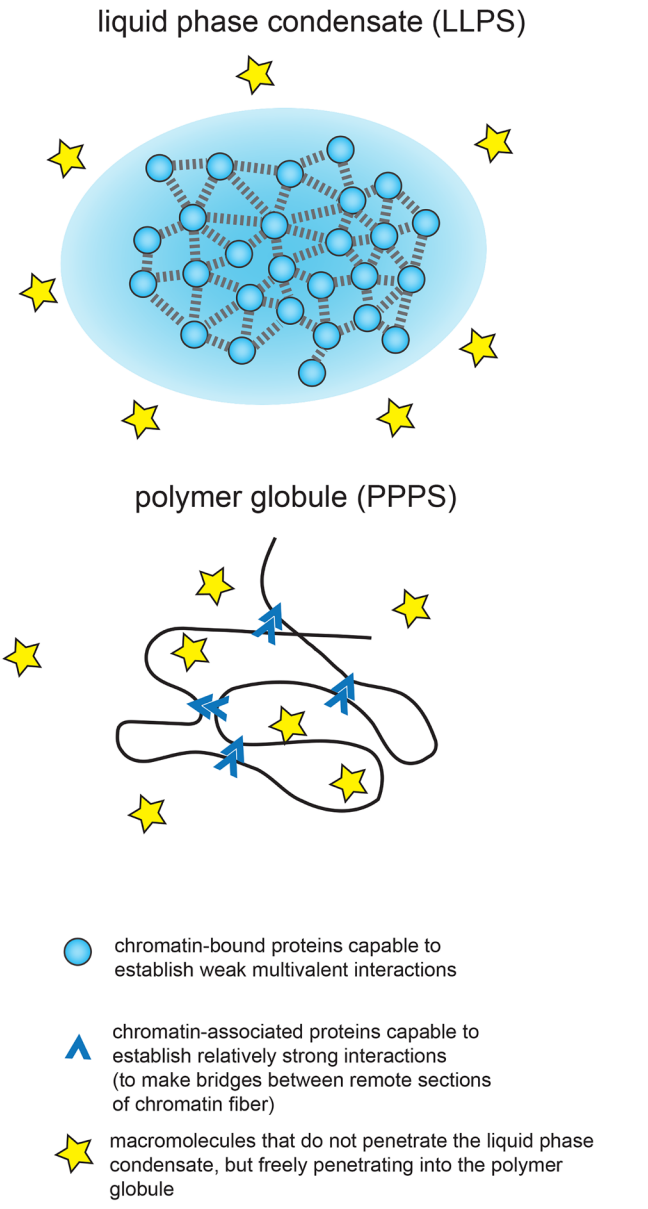

Figure 2. Liquid-Liquid Phase Separation (LLPS) and Polymer-Polymer phase separation (PPPS). Note that the medium within a polymer globule does not differ from surrounding solution.

globule does not differ from the surrounding solution [56] (Fig. 2).

ENTROPIC FORCES ARISING UNDER CONDITIONS OF MACROMOLECULAR CROWDING

Entropic forces (depletion attraction force) arise in conditions of a very high concentration of macromolecules (macromolecular crowding), when the opportunity for free movement of macromolecules in the solution is limited [59-61]. Depletion attraction force promotes the association of macromolecules into aggregates. Moving solvent molecules bombard macromolecules from various directions. However, when macromolecules happen to be nearby, bombardment from the contact surface will be impossible. Accordingly, there will be no forces capable of pushing the macromolecules apart, while the forces that hold them in the complex

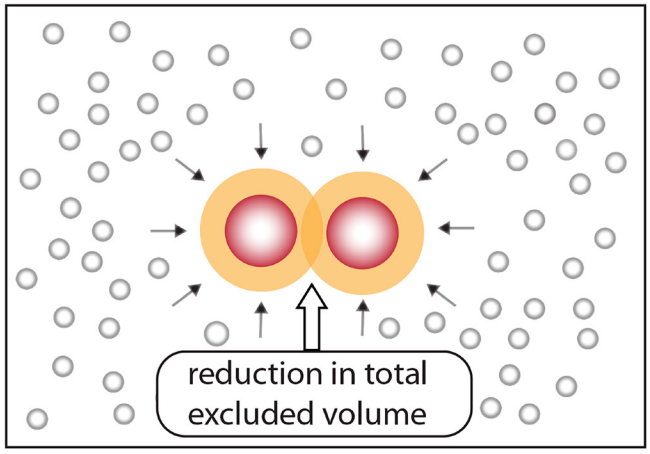

Figure 3. Aggregation of large macromolecules (macromolecular complexes) under conditions of macromolecular crowding. Under macromolecular crowding conditions, macromolecules (the big red balls) often collide. In this situation, solvent molecules (small gray balls) cannot bombard macromolecules from the contacting surfaces, which leads to stabilization of macromolecular complexes. When macromolecules aggregate, their excluded volumes (orange rings around red balls) are partially combined, which gives a gain in entropy.

will remain. The formation of the complex also leads to a decrease in the space occupied by macromolecules in the case when the surfaces of macromolecules have spatial complementarity, which is typical for macromolecules capable of dimerization and multimerization. This provides more space for the movement of solvent molecules, i.e., it gives a gain in entropy (Fig. 3). In the pioneering work of R. Hancock, it was demonstrated that the depletion attraction force plays a significant role in the formation of the nucleolus, PML bodies and Cajal bodies. The decrease in the level of macromolecular crowding in the nuclei upon placement of cells to a hypotonic solution, caused disintegration of these compartments. The addition of an inert crowding agent (polyethylene glycol) to the medium ensured the restoration of compartments [18,19]. The entropic forces that arise under conditions of macromolecular crowding help maintain the integrity of various biological structures. It has been shown, for example, that ensuring a high level of macromolecular crowding makes it possible to isolate compact metaphase chromosomes even without the use of high concentrations of divalent cations [62].

Depletion-attraction force does not have any specificity, contributing to the stabilization of any macromolecular complexes. Thus, it was shown that entropic forces support self-association of polynucleosomal chains [63]. As another example, I can mention active chromatin hubs, which are disassembled when the level of macromolecular accumulation decreases and reassembled when it increases [64]. Obviously, the specificity of the interaction between enhancers and promoters is ensured by other interactions. However, these interactions themselves are quite weak, and the complexes assembled through these interactions turn out to be stable only under conditions of macromolecular crowding. In model experiments, it was shown that a high level of macromolecular crowding promotes more stable transcription, possibly due to stabilizing the binding of the transcription complex to DNA and limiting diffusion [65]. The effects of macromolecular crowding on chromatin fol-

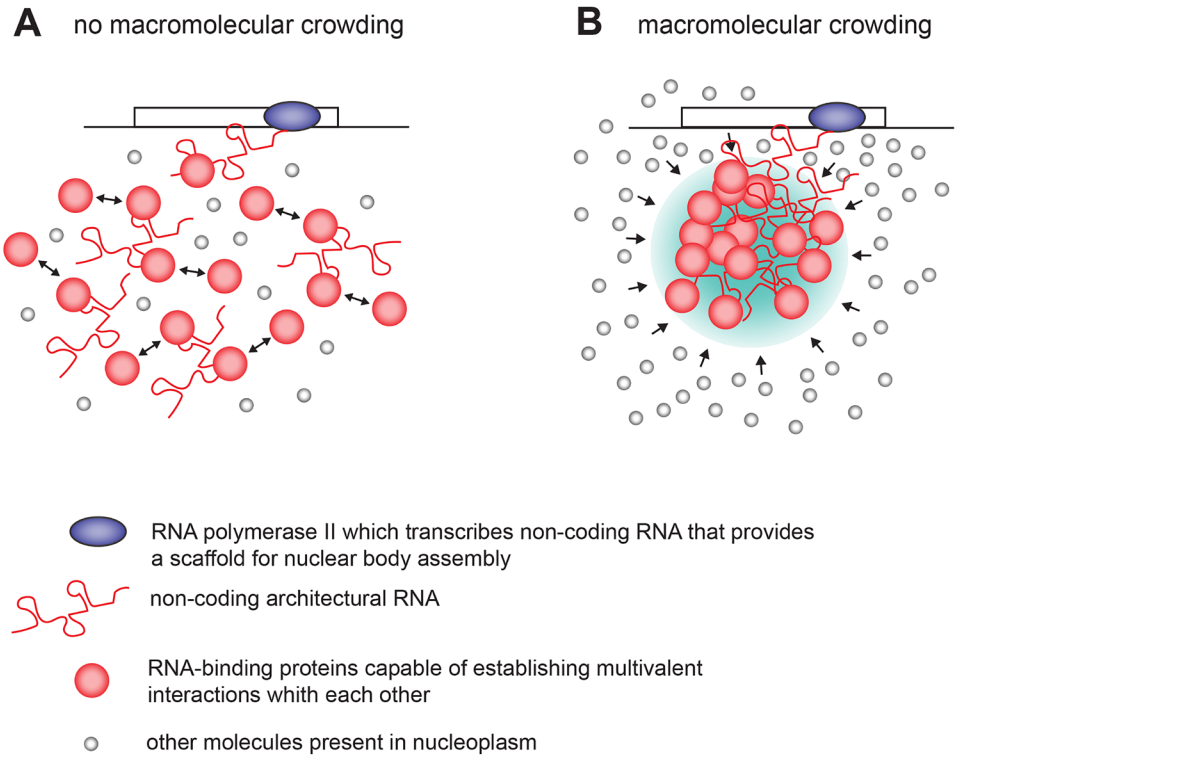

Figure 4. Assembly of a nuclear body near the site of scaffolding RNA synthesis. (**A**) RNA-binding proteins, including those capable of multivalent interactions, bind to scaffold RNA as soon as it is transcribed. At a low level of macromolecule crowding, RNP complexes diffuse into the nucleoplasm. (**B**) Under macromolecular crowding conditions, diffusion of RNP complexes assembled on scaffold RNA is limited in part by the aggregation of these RNP complexes. As a result, a threshold concentration of proteins capable of establishing multivalent interactions is reached, and a liquid-phase condensate is formed.

ding have been analyzed using computer simulations. The results of the simulations suggest that increasing the level of macromolecular crowding causes aggregation of nucleosome clutches with high nucleosome density but has little effect on nucleosome clutches with low nucleosome density. Based on these results, the authors hypothesized that macromolecular crowding contributes to the formation of compact heterochromatin [66].

THE ROLE OF LIQUID-LIQUID PHASE SEPARATION IN THE ASSEMBLY OF INTRANUCLEAR COMPARTMENTS – NUCLEAR BODIES

The role of LLPS in the assembly of the so-called nuclear bodies, such as nucleolus, nuclear speckles, Cajal bodies, PML bodies et cetera is well documented and has been extensively reviewed [21,22,67,68]. Many nuclear bodies possess expected characteristics of phase-separated condensates [69]. They have spherical shape, are not surrounded by membranes, and are composed of proteins that are quickly exchanged with a nucleoplasmic pull. The sets of proteins present in various nuclear bodies can partially overlap but still are specific [70]. This specificity is imposed by nucleation of liquid condensate assembly at a specific interaction platform which may be non-coding RNA as in paraspeckles [71] or protein as in PML-bodies [72]. At the same time, not all nuclear bodies have a spherical shape typical of classical phase condensates. The shape of nuclear bodies is largely determined by the features of the platform on which they are assembled. Thus, in the case of paraspeckles, NEAT1_2 RNA forms copolymers with RNA-binding proteins, followed by the assembly of micelles, which, depending on additional conditions, can have different shapes: spherical, cylindrical, lamellar, and vesicular [73,74]. Microphase separation plays an important role in the formation of some nuclear compartments. [75,76]. Many nuclear compartments are scaffolded by non-coding RNAs [16,73,77]. These RNAs contain binding sites for various proteins, including proteins capable of establishing multivalent interactions. In a crowded nuclear environment, large RNP complexes tend to aggregate, resulting in an increase in the concentration of interacting proteins above the phase separation threshold value. As a result, nuclear bodies assemble near sites of transcription of scaffolding RNAs (Fig. 4). Here, the connection between the compartmentalization of the cell nucleus and the activity of the genome, as well as its packaging in three-dimensional space, is easily traced [16). Another type of liquid-phase functional compartments arises in the process of DNA damage repair [78-80]. Poly(ADP-ribose) (PAR) [47,81,82] or RNA, the synthesis of which is initiated near DNA breaks, can scaffold the assembly of such compartments. It has been shown that Pol II and other components of the transcriptional machinery are recruited at DNA break sites, where the synthesis of a non-coding RNA called damage-induced long non-coding RNA (dilncRNA) is initiated. This RNA scaffolds the assembly of liquid condensates containing 53BP and other DNA Damage Response proteins [83,84].

A special type of phase condensate is the nucleolus. In mammalian cells, the nucleolus is three-component. The fibrillar centers (Pol I transcription factories) surrounded by a dense fibrillar compartment are immersed into huge

granular layer. Fibrillar centers assembled on the scaffold provided by multifunctional protein Treacle exhibit typical properties of phase condensates. However, the nature of the forces directing the formation of these condensates remains unclear, since they are not destroyed by agents that suppress hydrophobic interactions and high ionic strength, which weakens electrostatic interactions [85]. Primary transcripts are released into a dense fibrillar compartment, where they interact with fibrillarin. The latter forms a second phase condensate [86]. After removal of introns, the processed rRNA loses its affinity for fibrillarin and moves out of the dense fibrillar compartment, providing now a scaffold for the assembly of nucleophosmin phase condensate [86]. As ribosomal particles assemble, nucleophosmin is displaced from rRNA and precursor ribosomal subunits diffuse into the nucleoplasm. It is easy to see that the nucleolus is a nonequilibrium structure that can exist only as long as rRNA is synthesized and processed. Indeed, treatment of cells with agents that suppress Pol I causes disruption of nucleoli [87,88].

In the cell nucleus, various functional compartments are located in the interchromatin domain, which serves for the transport of various precursors to the places of their utilization and export of mature mRNA into cytoplasm. Although the existence of this relatively chromatin-free compartment was demonstrated quite a long time ago [89-91], the mechanism of its formation is not entirely clear. Now it seems very likely that this entire compartment is a phase condensate formed on the platform of RNA and RNP particles present there [77]. Multi-bromodomain (multi-BRD) proteins associated with the perichromatin layer (transcriptionally active chromatin), localized at the border of the chromatin and interchromatin domain, may also take part in the formation of this phase condensate [92].

THE ROLE OF PHYSICOCHEMICAL PROCESSES IN THE FORMATION OF A 3D GENOME

It is well known that interphase chromosomes occupy relatively isolated non-overlapping spaces in the nucleus, called chromosomal territories [90,93]. Separation of chromosomal territories is achieved by the process of polymer-polymer phase separation (PPPS) [94-96]. The parameters of chromosomal territories are determined by the presence of DNA loops generated by cohesin extrusion complexes and other contacts between distant regions of the chromosome. Computer modeling shows that to approach the experimentally observed parameters of chromosomal territories, the size of the loops must be comparable to the sizes of topologically associated domains (TADs) [97]. The organization of the cell nucleus can be modeled quite well by adding contacts of active chromosome regions with speckles and inactive ones – with nuclear lamina [98].

In recent years, the role of LLPS in the compartmentalization of chromatin, i.e. in the spatial segregation of active and inactive chromatin, has been actively discussed [99-102]. The presence of such segregation is evidenced by the analysis of Hi-C maps, demonstrating that within the chromosomal territory, active genome segments preferentially contact each other. The same can be said about inactive segments. But spatial contacts between active and inactive genome segments occur much less frequently. These observations were formalized in terms of the spatial segregation of active (A) and inactive (B) chromatin compartments [103]. Analysis of high-resolution Hi-C maps made it possible to identify several sub-compartments in each of these compartments [104]. It is obvious that certain forces must exist in the cell nucleus that promote the establishment of preferential contacts between chromatin segments that carry similar spectra of epigenetic marks. According to one of the models, the process of formation of phase condensates can play an important role here [56,101,102,105,106]. It has been experimentally shown that the architectural proteins involved in the formation of constitutive and facultative heterochromatin (HP1, H1, MeCP2, CBX) contain unstructured domains and, under certain concentrations, form liquid-phase condensates, both on their own and together with DNA or nucleosomes [30,48,56,107,108]. Oligonucleosomal fragments with H1 or without it also can form phase condensates *in vitro* [92,109]. In model experiments, the formation of liquid condensates by various heterochromatin proteins was also demonstrated inside the nucleus [30,108]. However, analysis of actual pericentromeric chromatin blocks has not provided evidence that they are typical liquid-phase condensates formed on a platform of densely packed DNA. The main characteristic of liquid-phase condensate is that the environment inside the condensate is different from the rest of the solution. The molecules inside the condensate preferentially interact with each other, avoiding contact with the solvent. However, experiments analyzing the recovery after laser beam bleaching of luminescence of HP1 and MeCP2 conjugated to fluorescent proteins showed that the movement of HP1 and MeCP2 within pricentromeric heterochromatin and between heterochromatin and the nucleoplasm is equally probable [110]. In addition, the concentration of HP1 inside heterochromatic clumps turned out to be significantly lower than the threshold required for the formation of liquid-phase condensate *in vitro* [110].

An alternative model suggests that constitutive heterochromatin is formed *via* polymer-polymer phase separation (PPPS) [99,111]. The result of PPPS is the formation of a polymer globule [56,112]. A significant difference between such a globule and a phase condensate is that the medium inside the globule does not differ from the surrounding solution (in the case of a heterochromatic globule, from the nucleoplasm (see Fig. 3) [56]. Accordingly, the accessibility of DNA in heterochromatin should be regulated by purely physical restrictions (packing density). Currently, this model fits most of the available experimental data. However, the nature of the crosslinks underlying PPPS remains unclear. It was experimentally shown that HP1 is required for transcriptional repression, but not for maintaining the compact organization of pericentromeric heterochromatin. Pericentromeric heterochromatin maintains a compact organization in mice with knockouts of various forms of HP1 [113]. In addition, delocalization of HP1 from pericentric heterochromatin does not lead to its decompaction [114,115]. Finally, partial exclusion of inert proteins from chromocenters does not depend on the presence of HP1 [110]. MeCP2 (in organisms with CpG methylation) seem to be more important for maintaining this compact organization of peri-

centric heterochromatin [116-118]. Also, certain proteins that bind to satellite DNA may participate in the formation of crosslinks [116]. The blocks of constitutive heterochromatin in the chromosome arms (constitutive heterochromatin that ensures the inactivation of transposons) are quite small, which is why modern methods do not allow us to directly analyze the question of whether they are liquid phase condensates. As for facultative heterochromatin, several results indicate that chromatin segments inactivated by Polycomb complexes are phase condensates [33,119]. In agreement with this, treatment of cells with 2,6-hexanediol, an agent that destroys liquid-phase condensates, leads to the disintegration of Polycomb bodies [120].

It is worth noting that distinguishing between LLPS and PPPS is not always easy. In the classic version, PPPS bridging factor molecules should not interact between themselves. The establishment of cross-links between distant regions of the polymer is sufficient to initiate PPPS. However, HP1a molecules can act as a bridging factor and simultaneously interact with each other, forming phase condensates. Accordingly, both PPPS and LLPS may contribute to the formation of constitutive heterochromatin. Another noteworthy detail is that the chromatin fibril is a block copolymer consisting of alternating regions of active and inactive chromatin. Such copolymers display microphase separation, rather than macrophase separation [121]. Accordingly, one cannot expect that heterochromatin clusters will have the spherical shape characteristic of classical liquid phase condensates [122]. Theoretical analysis shows that the formation of phase condensates by HP1a molecules on a chromatin platform containing H3me2/3 will strongly depend on the genomic distribution of modified histones and the result of this process will be the formation of multiple long-lived microcondensates [122].

For some time, it was believed that repressed chromatin tends to stick together, while active chromatin occupies a volume free of heterochromatin, due to which preferential contacts are realized also within the active chromatin compartment. It is now obvious that contacts within the active chromatin compartment are established through special mechanisms. Here we can mention the attraction of active genes to speckles and common transcription factories [123-129]. In some cases, it has been directly shown that the process of liquid phase separation plays an important role in establishing contacts between active genes. Thus, in yeast cells under heat shock conditions, liquid condensates containing Pol II, mediator, and heat shock factor 1 (Hsf1) are formed. These condensates attract genes activated by the HSF1 factor, including genes located on different chromosomes [130]. The formation of such condensates is regulated by phosphorylation of HSF1. HSP70 initiates the dissociation of HSF1 condensates after returning to normal yeast cultivation conditions [131]. If we talk about transcription factories in the cells of multicellular organisms [132-134], then in this case too there is good reason to believe that they are formed through a process of liquid phase separation [28,135-137].

In the cell nucleus, euchromatin is located on the surface of 1 Mb chromatin globules, being exposed in the interchromatin domain [138-140]. Active chromatin is characterized by a high level of H3K27 and H3K9 acetylation. These modifications attract proteins containing bromodomain, including BRD4, the short isoform of which forms phase condensates [141]. Active chromatin exposed on the surface of chromatin globules can also participate in the formation of mixed phase condensates with RNA and RNA-binding proteins filling the interchromatin domain [77].

The role of liquid phase separation in transcriptional regulation is best documented. It has been demonstrated that activator compartments, which include RNA polymerase II, Mediator, histone acetylase P300, various transcription factors and other components of the transcription apparatus, are formed on enhancers and promoters by LLPS [28,49,142,143]. For some transcription factors it was demonstrated that activation of transcription by these factors is directly related to their ability to form liquid phase condensates which attract RNA polymerase II, Mediator and other components of transcription apparatus [144]. Enhancer RNA can serve as a platform for the formation of phase condensates on active enhancers [77,145-147]. The fusion of phase condensates assembled at enhancers and promoters keeps promoters and enhancers, as well as individual blocks of superenhancers, close to each other, which plays an important role in the formation of function-dependent spatial organization of the genome [142,148-150]. Treatment of cells with an agent that destroys phase condensates leads to the loss or weakening of a significant number of enhancer-promoter loops [151]. The importance of phase condensates in the organization of transcription is not limited to the creation of activator compartments at enhancers and the establishment of communication between enhancers and promoters. Recent observations suggest that distinct liquid condensates are formed at gene bodies in the course of transcription [54,152]. Assembly of these condensates is likely scaffolded by nascent RNA which interacts with various proteins, including the splicing machinery components [54]. Of note, release of Pol II from activating compartment assembled on promoter is regulated by phosphorylation of C-terminal domain of Pol II large subunit (CTD) [54,55,136,153]. Hypophosphorylated Pol II CTD is retained in mediator condensates whereas hyperphosphorylated CTD is preferentially incorporated into condensates that are formed by splicing factors [54]. Phosphorylation of Pol II CTD is mediated by the kinase CDK9 which is a subunit of positive transcription elongation factor b (P-TEFb) [154]. Active form of P-TEFb constitutes a part of a super elongation complex (SEC) [155, 156] which, under certain conditions, is also recruited to heterotypic phase-separated complex with ELL and AFF4 [157].

CONCLUDING REMARKS AND PERSPECTIVES

Understanding the role of LLPS in the organization of various processes in the cell nucleus allows us to take a new approach to answering long-debated questions. These include the question of why Xist RNA does not diffuse into the nucleoplasm, but envelops the chromosome on which it is synthesized, or why the MSL complex is present and works only on the X chromosome of Drosophila males, although binding sites for this complex are also present on autosomes. The formation of relatively low-mobility phase

condensates on the platforms of synthesized Xist and RoX1/2 RNAs provides an answer to these questions [158, 159]. It is possible that similar mechanisms work in other situations when it is necessary to limit the diffusion of certain macromolecules. For example, retention of histone dimers and tetramers removed from DNA during transcription in the phase condensate associated with the transcription complex could ensure their preferential relocation to free DNA behind the transcription complex. The same can be said about histones removed from DNA as the replication fork progresses. These possibilities deserve experimental testing. Another long-standing question concerns the platform for the cell nucleus compartmentalization. In the late sixties and early seventies of the last century the high-salt resistant proteinous structure termed the nuclear matrix was described [160]. Subsequent studies made it possible to suggest that this structure represents a structural milieu for nuclear compartmentalization [161]. However, numerous attempts to visualize nuclear matrix in living cells failed. Now it is evident that what was thought to be a nuclear matrix is in fact a liquid condensate filling the interchromatin compartment, which (the condensate) solidifies as a result of dehydration caused by high-salt extraction [77,162]. As for the platform for assembly and compartmentalization of the cell nucleus, it is provided by the folded genome [13].

An important property of liquid-phase condensates, which explains their almost universal role in the organization of the cell nucleus, is the possibility of rapid and regulated (for example, through post-translational modifications of the constitutive components of the condensate) assembly and disassembly, as well as the possibility of concentrating various sets of client components in these condensates. Moreover, the accumulation of certain macromolecules, including proteins and RNA, in the condensate can also be regulated by various modifications. This property is realized, in particular, if it is necessary to temporarily remove certain proteins or micro-RNAs from the nucleoplasm (the function of a molecular sponge) [163, 164]. In another scenario, condensates act as reaction centers in which enzymes and auxiliary components necessary for carrying out a particular process, for example, repairing DNA damage, accumulate [20]. Finally, the assembly of liquid-phase condensates can limit the diffusion of various macromolecules, keeping them in a specific location. It is significant that relatively slow-moving condensates can be anchored on a folded genome through their assembly on a platform of transcribed non-coding RNAs [16]. The number of works demonstrating the role of the process of liquid-liquid phase separation in the implementation of various functional processes inside the cell nucleus is increasing every year. It seems that we still have a lot to learn in this area of research.

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