Trends in Biochemical Sciences

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REVIEWS

Opinion

ATP, Mg^{2+}, Nuclear Phase Separation, and Genome Accessibility

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Misregulation of the processes controlling eukaryotic gene expression can result in disease. Gene expression is influenced by the surrounding chromatin; hence the nuclear environment is also of vital importance. Recently, understanding of chromatin hierarchical folding has increased together with the discovery of membrane-less organelles which are distinct, dynamic liquid droplets that merge and expand within the nucleus. These 'sieve'-like regions may compartmentalize and separate functionally distinct regions of chromatin. This article aims to discuss recent studies on nuclear phase within the context of poly (ADP-ribose), ATP, and Mg^{2+} levels, and we propose a combinatorial complex role for these molecules in phase separation and genome regulation. We also discuss the implications of this process for gene regulation and discuss possible strategies to test this.

Highlights

Membrane-less organelles within the eukaryotic nucleus form distinct, dynamic, and functional compartments.

The process of liquid phase separation generates these compartments.

Chromatin and hence the genes contained within can be separated within these 'sieve-like' compartments to fulfill specific biological roles response to external stimuli.

In addition to a concentration of proteins within these regions, ATP and Mg^{2+} may also concentrate within these droplets.

Levels of ATP and Mg^{2+} may regulate the generation, maintenance, and dissolution of dynamic phase separation within the nucleus.

Chromatin 3D Structure

Our understanding of the mechanisms involved in the folding of chromatin fibers within the cell nucleus evolved in recent years owing to the extensive development of **chromosome conformation capture** (3C; see Glossary)-derived techniques based on **proximity ligation** [1]. Genome-wide versions of this approach, such as **Hi-C** and ***in situ* Hi-C**, applied both to cell populations [2,3] and single cells [4], combined with super-resolution microscopy of chromatin [5,6] and computational modeling [7], are providing a wealth of unprecedented and novel information in different biological systems. The present view of nuclear genome structure and topology implies a hierarchical organization. Individual chromosomes occupy independent chromosome territories [8,9] that are divided into active (A) and inactive (B) chromatin compartments. Each compartment contains multiple **topologically associating domains** (TADs), within which subregions with even higher interaction frequencies and chromatin interaction loops occur (Figure 1) [10-12]. Although chromosome territories and TADs are relatively conserved between different cell types and chromatin compartments, sub-TADs and loops are distinct and characteristic of various terminally differentiated somatic cell types. This cell-specific level of genome organization is now well accepted to contribute to the function of the cell type-specific network of **transcription factors** (TFs), and is therefore assumed to play a major role in the gene expression characteristics of differentiated cells [13–15].

In addition to the 3D organization of chromatin, gene expression and therefore disease are also dependent on the nuclear environment. **Membrane-less organelles** within the nucleus, formed by the process of phase separation, are part of this environment. Indeed, the localization, dynamics, and contents of membrane-less organelles have received increasing attention in recent years. This Opinion article aims to discuss recent studies on phase separation within the nucleus, focusing on the potential essentiality of ATP levels in the formation and functionality of the nuclear environment where specific regions of chromatin reside. We propose and discuss a hypothesis, and potential future experimental lines of investigation to test the hypothesis, that

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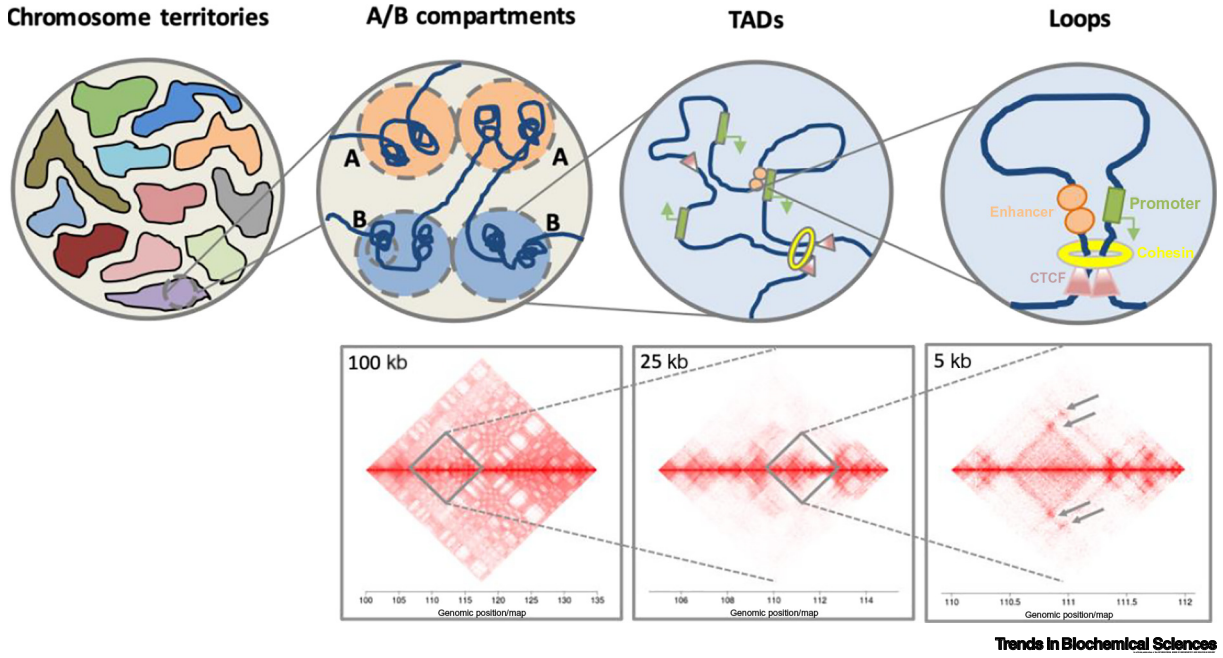


Figure 1. Hierarchical Organization of the Higher Eukaryotic Genome. Individual chromosomes occupy independent chromosome territories [8] that are divided into active (A) and inactive (B) chromatin compartments, yielding a chessboard-like pattern of interaction frequencies in the contact matrix [2,9]. Each compartment region encompasses several topologically associating domains (TADs) that are visible in the contact matrix (below) as red triangles (highlighted by the grey squares) containing high interaction frequencies. The higher the intensity of red coloration the higher the frequency of interaction detected between the two regions. Within the TADs, subregions can be distinguished with even higher interaction frequencies. Often the borders of the TADs or the sub-TADs exhibit punctual very high interaction density, corresponding to the anchoring of the loops (marked by arrows).

ATP levels are a crucial determinant of the dynamics of phase separation and gene expression within the nucleus.

Role of Architectural Proteins and Transcription Factors in Genome Organization

Given increased coverage enabling sub-10 kb resolution [16], *in situ* Hi-C has revealed genomic structural changes in response to external cues [17,18]. During cell differentiation and cell reprogramming, chromosomal domains can switch between the active and the inactive chromatin compartments, and the intra-TAD loop structure can also change before changes in gene expression [15,19]. These and similar results in other model systems suggest that the various levels of chromatin folding play important roles in regulating gene expression.

How the complex folding of chromatin fibers is established and maintained is beginning to be revealed. Establishment appears to depend on so-called architectural proteins [20, 21] and TFs [19], while maintenance depends on epigenetic post-translational modifications of chromatin proteins [22]. Among the architectural proteins, **CCCTC-binding factor** (CTCF) and cohesins play a major role, as comprehensively documented by Rowley [23]. However, **YY1** and **TFIIIC** are also important [21,24,25]. Cohesins catalyze the process of **loop extrusion**, whereby chromatin loops start at an occupied CTCF site and progress until another CTCF bound in the convergent orientation is encountered [3,26,27]. This mechanism establishes the basic loop structure of the genome and contributes to the TAD structure by influencing the insulation strength of TAD boundaries that are enriched in bound CTCF sites. This process of loop extrusion requires ATP for the active transfer of chromatin through the anchor point. Rapid degradation of CTCF and/or cohesins results in loss of both loop and TAD structures, but does not influence the

separation of active and inactive chromatin compartments, indicating that compartment formation is dependent on epigenetic mechanisms but is independent of chromatin looping [3,10,28].

The segregation of chromatin into active and inactive compartments – as well as the **Polycomb** compartment [29] – is cell type-specific and mostly relates to the different epigenetic states of chromatin, which correlate with gene expression activity and favor their global interaction [30]. How these epigenetic states are controlled is partly dependent on the specific subset of signaling proteins, pioneer factors, and other sequence-specific TFs expressed in each cell type. TFs recruit post-translational histone modification complexes and ATP-dependent chromatin-remodeling enzymes that modify the intra-TAD chromatin folding, as well as distant interactions between similarly modified chromatin domains, even before the activation of transcription [19]. In terminally differentiated cells, TFs whose activity is modulated by external cues, for example steroid hormone receptors, are also involved in maintaining a genome structure favorable to gene expression changes. For instance, in tubular epithelial breast cancer cells, hormone-responsive TADs are organized around clusters of bound **estrogen receptors** and **progesterone receptors** (ESRs and PGRs, respectively) that form **hormone control regions** (HCRs). HCRs interact with the promoters within the TAD in a way that is sensitive to hormone exposure. HCR-containing TADs interact with each other over 10 Mb distances even before hormone exposure. These intra- and inter-TAD interactions are not observed in other tumor cells, nor in breast cancer cells of the tubular epithelial type that do not express ESR or PGR (Figure 2), indicating that

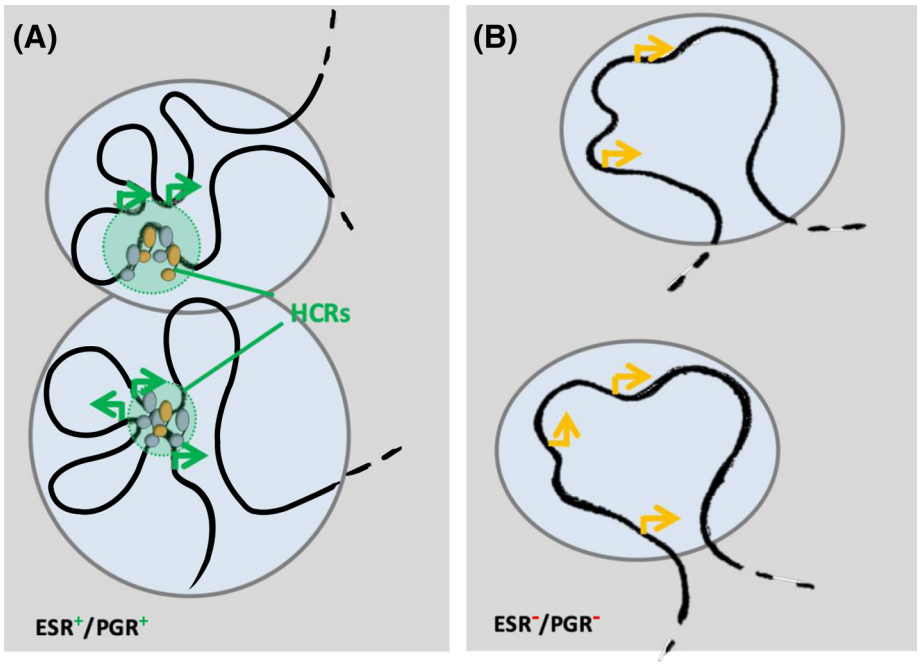


Figure 2. Topologically Associating Domains (TADs) Are Stimulus-Specific Units of Response. In estrogen receptor- and progesterone receptor-positive (ESR^+/PGR^+) cells in the absence of hormone, ESR (yellow ovals) and PGR (grey ovals) steroid receptors bind in a clustered way to large genomic regulatory regions that constitute hormone control regions (HCRs). HCRs organize functional loopings with promoters (green arrows) within the TADs, and HCR-containing TADs establish long-range interactions between them (A). In the absence of receptors, intra-TAD contacts between HCRs and promoters and inter-TADs interactions do not become established (B), and promoters (yellow arrows) are maintained at a distance from enhancers.

Glossary

ARTD1: ADP-ribosyltransferase 1, also known as PARP1. Catalyzes the synthesis of poly(ADP-ribose) from NAD^+.
BRD4: bromodomain and extra terminal domain 4, a chromatin-targeting protein involved in gene transcription and elongation.
CCCTC-binding factor (CTCF): involved in the organization of chromatin and enhancers.
Chromosome conformation capture (3C): molecular biology technique used to analyze the special organization of chromatin based on proximity ligation.
Estrogen receptor (ESR): a nuclear hormone receptor that activates target genes either by direct or indirect binding once activated by bound ligand.
Fluorescence resonance energy transfer (FRET): a microscopy technique where one electronic excited fluorophore is transferred to another acceptor chromophore.
GCN4: general control of amino acid synthesis protein 4, a histone acetyltransferase enzyme.
Heteroprotein 1 (HP1): a group of epigenetic regulators of chromatin that recognize and bind to specific epigenetic post-translational modifications of histone tails.
Hi-C: an extension of the 3C method to identify long-range interactions (>1 Mb) within and between individual chromosomes at the genome-wide scale by using global sequencing.
Hormone control regions (HCRs): genomic regions within TADs that are responsive to hormone exposure and are regulated by hormone receptor binding. Promoters within HCRs show an increased interaction between themselves relative to promoters outside a HCR.
Hydrotrope: a molecule that improves the solubility of hydrophobic compounds.
***In situ* Hi-C:** a Hi-C method used to evaluate DNA–DNA proximity ligation within intact nuclei.
Intrinsically disordered regions (IDRs): protein regions lacking a stable structure.
lncRNAs: long noncoding RNA; transcripts >200 nt in length that are not translated into protein.
Loop extrusion: a mechanism whereby loop-extruding factors maintain a region of DNA at two points, and then

cell-specific TFs can play a role in fine-tuning 3D genome folding [31]. Spatial hubs of tissue-specific regulatory regions are also observed for cytokine-dependent enhancers which control the expression of key genes in hematopoietic progenitor cells [32]. Such hubs may participate in coordinated changes of the gene expression program, which, in addition to the hierarchical organization of chromatin and the epigenetic histone marks, may be influenced by the local environment of the chromatin and its accessibility.

One key component that determines access to the genome in chromatin is the activity of **poly (ADP-ribose)** (PAR)-synthesizing enzymes that constitute the PAR polymerase (PARP) family. The main enzyme of the ADP-ribosyltransferase 1 family [**ARTD1,** also known as PARP1) is essential for DNA repair and for the regulation of transcription [33]. ARTD1 is a very abundant enzyme, and in its inactive form binds to DNA similarly to histone H1. However, when activated in response to DNA damage, ARTD1 becomes heavily auto-PARylated and also PARylates linker and core histones, thereby increasing the accessibility of chromatin by the DNA repair machinery [34]. In breast cancer cells exposed to either estrogens or progesterone, ARTD1 is rapidly activated by phosphorylation via CDK2 [35]. Within 5 minutes the level of PAR increases in the cell nucleus, and remains high for 50–60 minutes before returning to basal levels [35]. The transient increase in nuclear PAR is essential for hormone-induced chromatin remodeling and gene regulation [35]. Intriguingly, the hydrolysis of PAR to its constituent ADP-ribose (ADPR) units by **PAR glycohydrolase** (PARG) is also essential for gene regulation by hormones and for hormone-induced cancer cell proliferation [36]. This finding led us to consider the possibility that ADPR could be the substrate for nuclear ATP synthesis, as previously proposed by Tanuma and colleagues in 1997 and 2000 [37–39].

Using **fluorescence resonance energy transfer** (FRET) detectors of ATP levels in living cells [40], we found an increase in nuclear ATP after 30 minutes of hormone exposure that was not accompanied by changes in the levels of mitochondrial or cytoplasmic ATP. The nuclear levels of ATP remained high for 20–30 minutes and returned to basal levels after 60 minutes. This transient increase in nuclear ATP was blocked by inhibition of CDK2, ARTD1, or PARG, proving that the increase depends on the generation of free ADPR. We subsequently identified nucleoside diphosphate-linked moiety X)-type motif 5 **(NUDIX5**/NUDT5) as the enzyme responsible for the increase in nuclear ATP in response to hormone exposure, and showed that *in vitro* it can use ADPR and pyrophosphate (PPi) to synthesize ATP [36]. Hormone activation of NUDIX5 is accompanied by rapid dephosphorylation at T45 [36]. The phosphorylated enzyme can only hydrolyze ADPR to AMP and ribose-5-phosphate (R5P), whereas the dephosphorylated enzyme can also generate ATP in the presence of PPi. NUDIX5 is also important for DNA damage repair [36] as originally proposed [37].

We originally proposed that nuclear ATP synthesis as described above was required for hormone-induced chromatin remodeling, more specifically for displacement of histone H1 and core histones H2A/H2B [36]. We hypothesized that the high nuclear ATP levels were required during the extensive global changes in chromatin structure induced by hormones, and could not be met by mitochondrial ATP synthesis alone. Given that the synthesis of NAD^+ requires considerable energy that is partly stored in PAR, it seems plausible to use this energy for the ATPases involved in chromatin dynamics, including chaperones [41] and ATP-dependent remodeling enzymes. However, the kinetics of the nucleosome and chromatin changes in response to hormone exposure are very rapid, occurring between 1 and 15 minutes [42], but we were only able to detect the increase in nuclear ATP after 30–40 minutes of hormone exposure [36]. Moreover, if nuclear ATP was used for the ATPases of chromatin-remodeling enzymes, it would not accumulate to the levels required for its detection using FRET sensors. Thus, what we observed with FRET must represent an accumulated excess of ATP that is not degraded by ATPases

move along the chromatin, resulting in extrusion of the loop.
Low-complexity domains (LCD): amino acid repeats.
MED1: Mediator complex unit 1, a cofactor essential for RNA polymerase II (Pol II)-mediated transcription.
Membrane-less organelles: distinct regions that, unlike organelles such as mitochondria and the Golgi, do not contain a lipid bilayer.
NUDIX5: nucleoside diphosphate linked moiety X-type motif 5. Catalyzes the synthesis of AMP or ATP and ribose-5-phosphate from ADP-ribose and or PPi.
Paraspeckles: subnuclear bodies that control gene expression via retention of RNA.
PAR glycohydrolase (PARG): an enzyme responsible for the catabolism of PAR to single ADPR units.
Poly(ADP-ribose) (PAR): a polymeric form of ADP-ribose synthesized by ARDT1.
Poly(ADP-ribose) polymerase 1 (PARP1): see ARDT1
Polycomb: a group of epigenetic silencing proteins important for cellular differentiation and development.
Progesterone receptor (PGR): a nuclear hormone receptor that activates target genes either by direct or indirect binding once activated by bound ligand.
Proximity ligation: an experimental technique whereby protein–protein interactions can be detected based on their spatial close proximity.
Soft X-ray tomography: an imaging technique whereby photons penetrate biological materials deeper than electrons, allowing specimens up to 10 μm in thickness to be imaged.
Superenhancers: regions of the genome that contain multiple enhancers, transcription factors, and histone marks.
TFIIIC: general transcription factor 3C polypeptide 1, a factor required for RNA polymerase III-mediated transcription.
Topologically associating domains (TADs): a self-interacting sublevel of chromatin. Increased interaction within these regions results in a functional 3D chromatin unit.
Transcription factors (TFs): protein factors that bind to sequence-specific DNA motifs to control the transcription of the associated gene.
YY1: a multifunctional transcriptional repressor protein that also plays a role as an architectural protein in the 3D organization of chromatin.

during chromatin remodeling. If this is the case, what could be the function of these high levels of nuclear ATP? Why does it accumulate and indeed persist even after the completion of chromatin remodeling, before ultimately diminishing to basal levels?

In this context, it is intriguing that nuclear PAR has been shown to have a seeding function in the formation of liquid droplets at sites of DNA damage [43], a process that also requires NUDIX5-mediated synthesis of nuclear ATP from PAR [36]. In addition, recent results show that during B cell activation the dynamic appearance of new chromatin loops is strictly dependent on a continuous supply of ATP, and this cannot be explained by the ATP required for cohesin-mediated loop extrusion [44]. Therefore, we consider the possibility that the high levels of nuclear ATP may fulfill a function in the context of nuclear phase separation, which we discuss next.

Phase Separation within the Nucleus

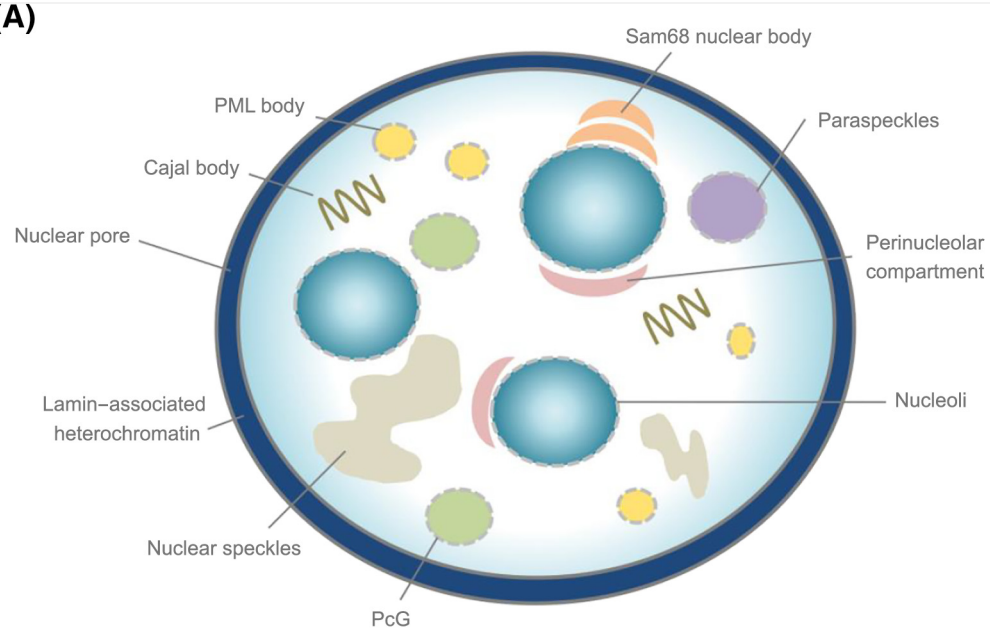
The nucleus is organized around various nuclear membrane-less organelles, sometimes referred to as nuclear bodies, that can merge and expand [45]. These nuclear bodies can serve to concentrate specific factors within a small region, thereby facilitating specific processes ranging from ribosome biogenesis in the nucleolus to RNA splicing within the periphery of speckles and gene regulation in **paraspeckles** (Figure 3). Other nuclear bodies include Cajal bodies, promyelocytic leukemia (PML) bodies, Polycomb group bodies, and P granules (Figure 3) [46].

In addition, using single-molecule tracking in live cells, it has been shown that eukaryotic TFs and other transcription-related proteins that contain **intrinsically disordered regions** (IDRs) or **low-complexity domains** (LCDs) can form local high-concentration interaction hubs that stabilize DNA binding and promote the recruitment of RNA polymerase II (Pol II) and transcriptional activation [47]. The IDRs of transcriptional coactivators enriched on **superenhancers**, such as **GCN4**, **BRD4**, and Mediator (**MED1**), can also form microscopically separated nuclear puncta composed of biomolecular condensates that exhibit the properties of liquid droplets by being able to merge and expand [48,49] (Figure 4, Key Figure). It has also been shown that Mediator and Pol II can form transient and stable clusters in living embryonic stem cells that associate with chromatin, recruit TFs, and exhibit the defining properties of liquid droplets [50]. The main constituents of chromatin, the nucleosome core particle and the linker histones, as well as the C-terminal domain (CTD) of Pol II [51], have intrinsically disordered tail regions rich in charged amino acids that are subject to numerous post-translational modifications. Therefore, chromatin offers the optimal conditions to generate and maintain, for a limited period of time, biomolecular condensates within a specific region [52] (Figure 4). These dynamic chromatin phases could correspond to the active and inactive chromatin compartments observed in Hi-C interaction maps. Indeed, although not a physical barrier *per se* as in the case of nuclear or plasma membranes, these 'sieve-like' distinct phases will favor the concentration of specific factors, permitting access to the enzymes that ensure the shared histone modifications that are characteristic of bound genomic regions in each compartment.

Nuclear ATP as a Hydrotrope and Regulator of Free Mg^{2+} Ions in Dynamic Phase Separation

In a recent report it was proposed that ATP at millimolar concentrations may act as a **hydrotrope** that helps to maintain the solubility of macromolecules in liquid droplets [53]. This model explains the need for high concentrations of ATP in cells (5–10 mM) despite the fact that cellular ATPases have a K_m in the micromolar/nanomolar range. In fact, in *in vitro* experiments nonhydrolyzable analogs of ATP can also fulfill the function of hydrotropes [55]. It is therefore possible that the nuclear ATP that accumulates in the nuclei of breast cancer cells exposed to hormone acts as a hydrotrope and helps to maintain in solution TFs, Pol II, and all the cofactors needed for

(A)



(B)

Organelle	Function	Size	General composition
Nucleoli	Ribosome biogenesis, role in response to stress	0.2–3.5 μM	rRNA, DNA, RNA polymerases I and III, polymerase-associated factors
Promyelocytic leukemia (PML) body	Transcriptional activation, DNA damage response	0.3–1.0 μm	PML protein
Cajal body	snRNP biogenesis, splicing, regulation telomerase	0.3–1.0 μm	Small nuclear ribonucleoproteins (RNPs)
Nuclear speckles	Cotranscriptional splicing, splicing factor storage	20–25 nm	RNPs, various splicing factor proteins
Sam68 nuclear body	Protein concentration	0.3–0.5 μm	RNA, Sam68, HNRNPD
Paraspeckles	Transcriptional regulation, splicing	0.2–1.0 μm	PSP1, p54nrb, PSP2, lncRNA
Perinucleolar compartment (PNC)	Gene regulation	0.25–3 μm	RNPs, RNA polymerase III (Pol III), RNA
Polycomb group (PcG) bodies	Protein concentration, gene silencing	0.2–1.5 μm	PcG proteins

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Figure 3. Membrane-Less Nuclear Bodies. (A), Schematic representation and characteristics of nuclear membrane-less organelles. (B), Roles, size, and composition of nuclear membrane-less organelles.

transcription within PAR-seeded biomolecular condensates (Figure 4). If this is the case, we should be able to mimic the role of nuclear ATP using nonhydrolyzable analogs, although these types of experiments are not trivial in living cells.

Key Figure

Schematic Representation of Dynamic Phase Separation

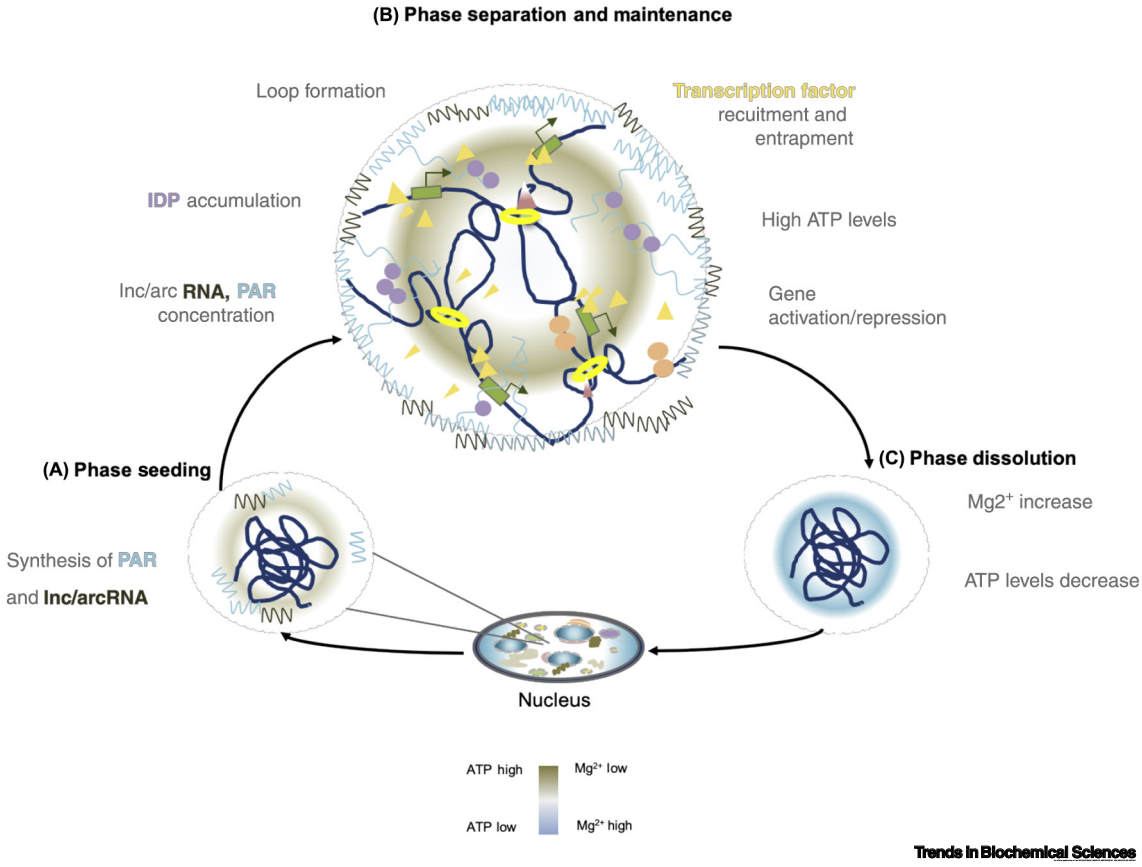

Figure 4. Phase separation can be split into three processes. In the first the phase is 'seeded' by the initial accumulation of poly(ADP-ribose) (PAR) or RNA (A). Second, the phase grows and expands owing to the further accumulation of intrinsically disordered proteins (IDPs), RNA, and PAR, as well as high ATP concentration. This facilitates the entrapment of transcription factors and the formation of loops, resulting in gene expression within the 'sieve-like' temporary compartments (B). Evidence has shown that phase separation is temporary, and the final stage of the process is therefore phase dissolution (C), which we propose to be due to the accumulation of Mg^{2+} ions. Abbreviations, arcRNA, architectural RNA; lncRNA, long noncoding RNA.

However, there is another way in which ATP may function in nuclear phase separation. It is known that ATP is the main cellular regulator of the levels of free Mg^{2+} ions [54]. In addition, Mg^{2+} ions have been known for a long time to influence the solubility of chromatin. In fact, millimolar Mg^{2+} concentrations have been classically used to precipitate chromatin from nuclear extracts [56]. Using a new FRET detector of free Mg^{2+} ions, it has been recently confirmed that the majority of the intracellular Mg^{2+} pool is complexed with ATP, and that a transient rise in free Mg^{2+} released from ATP occurs during mitosis and contributes to mitotic chromosome condensation by charge neutralization [57]. It is therefore plausible and experimentally testable that the transient increase in nuclear ATP observed in hormone-exposed cells contributes to regulating chromatin access by reducing the Mg^{2+} concentration, whereas ATG hydrolysis releases free Mg^{2+} and restores the condensed chromatin phase, thus limiting the period of chromatin

availability for transcriptional control via a process of dynamic phase separation (Figure 4). The availability of FRET indicators of Mg^{2+} concentrations [57], of specific inhibitors of NUDIX5 [58] and of various ATPases, including cohesins [59], may help to answer these questions.

What Forces Are Involved in Liquid Phase Transitions?

From a chemical standpoint it is clear that interactions between macromolecules within different liquid phases are based on weak forces, mostly hydrophobic, but also on electrostatic interactions between the positively charged residues in IDR domains of proteins (arginines, lysines, histidines) and the negatively charged residues in other proteins or the phosphate residues in nucleic acids (DNA, RNA, or PAR), as well as hydrogen bonds between glutamines, histidines, serines, and threonines and complementary residues in proteins or accessible nucleotides. These interactions generate high attraction between biopolymers, but are of low specificity and very sensitive to post-translational modifications of the protein residues (phosphorylation, acetylation, methylation, deimination, and PARylation, among others) as well as to hydrotropes and changes in ionic strength, Mg^{2+} concentration, pH, and temperature. In fact, we have recently observed that in breast cancer cells exposed to moderate osmotic stress there are dramatic changes in genome topology as well as in CTCF and cohesin binding, which are rapidly reverted after restoring isotonicity [60]. These observations are compatible with an effect of ionic strength on chromatin phase transitions.

Biochemistry in the past has focused on studying individual specific interactions between proteins (receptors, enzymes, TFs) and defined nucleic acid sequences or small molecules (substrate or ligands) with affinities in the nanomolar range. The interactions between IDRs of proteins or between these regions and nucleic acids are in the millimolar/micromolar range and their strength derives from their high density and coordination. How this coordination is accomplished is not yet clear and is one of the major challenges in the field. The observed long-distance coordination found in nuclear interaction matrices [30] may result from the rapid mixing of similar liquid phases. In that respect, recent **soft X-ray tomography** studies have shown that the two basic states of chromatin – euchromatin and heterochromatin – form two distinct contiguous entangled webs that fill the whole cell nucleus, and that these two phases experience dynamic changes during cell differentiation [61]. Moreover, very recently it has been demonstrated that the accessibility of chromatin for proteins is determined by the frequency of spontaneous fluctuations that are controlled by acetylation and ATP [62], and that there are long-range correlations extending over several micrometers between coherently moving regions over the entire nucleus [63]. There are various possible mechanisms that could explain this long-distance coherence of various chromatin states, including a role for **heteroprotein 1** (HP1) [64,65], a possible scaffold function of long noncoding RNA (**lncRNAs**) [66–68], and/or the participation of interspersed short DNA repeats [69] as binding sites mediating indirect protein interactions. Each of these mechanisms or a combination of them could create a heavily connected and dynamic macromolecular network. Understanding the various forces and mechanisms used within the cell nucleus to coordinate chromatin dynamics and genome activity in higher eukaryotes will be a major challenge for the next decade.

Concluding Remarks

The abundance of various forms of liquid–liquid phase transitions recently reported in the cell nucleus offers a new logic for interpreting the rapid changes in chromatin topology and accessibility as well as the associated changes in gene expression observed during cell differentiation, DNA damage, or in cells exposed to external stimuli. In fact, this new concept may represent the most significant advance in our views on gene regulation since the discovery of TFs as specific DNA-binding regulators of gene expression. In this article we postulate a dual and complex role for nuclear ATP derived from PAR via ADPR in the transient transition of chromatin states in

Outstanding Questions

Are the chromatin compartments observed in Hi-C interaction matrixes simply a result of insulator proteins such as CTCF, or are these compartments also dynamic nuclear phases?

Does disruption of these phases alter the Hi-C matrices?

High millimolar concentrations of ATP in the nuclei of cells following stimulus may act as a hydrotrope and maintain RNA Pol II and other required factors in solution. If this is the case, can the effect of nuclear ATP be recapitulated using nonhydrolyzable homologs?

If this is the case, what is the consequence of maintaining elevated ATP and low Mg^{2+} concentration with nonhydrolyzable analogs on chromatin structure and gene regulation?

If the long-range interactions within the nucleus (as measured by Hi-C) are controlled by spontaneous fluctuations of ATP, what will be the consequences of selective NUDIX5 inhibition?

cells exposed to hormones or to DNA damage. We suggest that nuclear ATP is not only directly required for ATPases and other enzymes involved in chromatin remodeling, transcription, and repair but in addition contributes to transiently maintaining an accessible chromatin conformation by acting both as an hydrotrope and as a regulator of free Mg^{2+} ion concentration (Figure 4). We have highlighted the available evidence and some possible strategies for testing the hypothesis. One of the more difficult of the remaining issues (see Outstanding Questions) concerns the accurate determination of the actual local concentrations of ATP. Addressing this issue will require the development of appropriate internal standards for measurements of ATP levels in living cells. If this hypothesis is confirmed experimentally, and given that cancer cells are particularly dependent on DNA damage repair for survival, identifying all the contributing factors will provide new targets for cancer management and patient care.

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