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REVIEWS

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Nucleosomal packaging of eukaryotic DNA and regulation of transcription

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The eukaryotic nucleus harbors genomic DNA, which is tens of thousands of times greater in linear size than the nuclear diameter. Its high condensation is due to DNA packaging in chromatin, and DNA wrapping around nucleosomal globules is a key step in the process. A histone octamer, which forms the nucleosomal globule, interacts with DNA via electrostatic contacts. DNA–histone interactions are rather tight and prevent nucleosomal DNA from being accessed by various enzymes and transcription factors. At the same time, nucleosomes do not prevent transcription and other processes related to the genetic function of DNA. The review considers the structure and diversity of nucleosomes and the central role they play in regulating transcription. Special emphasis is placed on how internucleosomal interactions contribute to genome accessibility to transcription machinery and how nucleosomes are removed from regulatory elements and transcription units in a controlled manner during transcription elongation.

Keywords: chromatin, histone modifications, nucleosome, transcription.

Introduction. Histones are among the most conserved eukaryotic proteins [1]. The mere fact points to an extremely important role they play in the eukaryotic cell. For a long time, studies of histones and chromatin focused primarily on the structural aspect, elucidating how DNA is compactly packaged in the nucleus [2]. However, the role nucleosomes may play in regulating differential gene expression and other processes related to the genome function came into consideration almost as soon as nucleosomes were discovered [3]. It is beyond doubt now that several regulatory mechanisms work at the level of DNA packaging in chromatin to control various aspects of genome function, including the so-called epigenetic memory mechanisms, which play a key role in establishing the identity of differentiated cells. The transcription-regulating role of nucleosomes is a main focus of this review. Special emphasis is placed on how the nucleosome structure and positioning on DNA are associated with the regulation of transcription. A limited number of model loci – such as the beta-globin genes of vertebrates or *PHO5*, *GAL1-10*, and *HIS3* of yeasts – were used for many years to study functional activity of the genome organized in chromatin. The results obtained with the model systems were extrapolated to the whole genome. High-throughput sequencing technology developed in the past decade allowed a number of methods, such as ChIP-seq, Dnase-seq, and others, to be used to verify the structural–functional correlations at the whole genome level. We have tried to involve these new data wherever possible.

First, the structures of the basic nucleosomal particle and 10-nm chromatin fiber, which is composed of nucleosomes, are briefly considered in the review. Then we discuss the modern data that indicate a lack of regular interactions between nucleosomal particles in the eukaryotic nucleus. Emphasis is placed on the specifics of nucleosome positioning on DNA and primarily on nucleosome-free regions, which usually harbor

various regulatory elements of the genome. Transcription of nucleosomal DNA is also considered. The final part describes the current views of the modulation of internucleosomal interactions and its role in regulating transcription.

Nucleosome fiber is a basic structure of chromatin. The 10-nm nucleosome fiber is the level of DNA packaging in chromatin that is best understood now [4, 5]. The fiber is a DNA molecule interacting regularly with protein globules known as the nucleosome cores. A DNA region of 145–147 bp is wrapped around each globule. The DNA region forms 1.65 left-handed superhelical turns. The globule consists of eight core histones. Having a modular organization, the globule is a complex of an $(H3–H4)_2$ tetramer and two H2A–H2B dimers [6]. The structure of a nucleosomal particle (a core with DNA wrapped around it) was solved to 1.9 Å by X-ray analysis [7]. The histones of the octamer are organized in a left-handed helix, which sterically matches the superhelical turns of the wrapping DNA fragment. The histone arrangement along the DNA molecule is as follows: the H2A–H2B dimers contact DNA at the entry and exit of the nucleosomal particle, while the $(H3–H4)_2$ tetramer contacts the central part of the DNA region wrapped around the nucleosomal globule. The nucleotide sequence-independent interaction of the nucleosome core with DNA is due to ionic, hydrogen, and hydrophobic bonding of the proteins with the DNA sugar–phosphate backbone. Two structural and functional domains are recognized in the core histones. The domains are a histone tail (~ 20–35 nonstructured N-terminal amino acid residues) and a histone fold (the other ~ 80–100 residues), which consists of three α-helical regions linked by small loops. Two short (10–14 residues each) helices of the histone fold flank a longer helix, which consists of 28 residues. Along with additional secondary structure elements unique to each of the core histones, the histone fold ensures the majority of histone interactions with nucleosomal DNA and other histones. A DNA region between two neighbor nucleosomes is known as the linker and varies from 10 to 90 bp among different organisms, different cells, and different genome regions [8]. Histone H1, which substantially differs in both size and structure from the core histones, can bind to the linker at the nucleosome entry–exit sites, thus closing two full superhelical turns. Histone H1 is presumably involved in maintaining the supranucleosomal packaging levels [9, 10]. The nucleosome fiber is a basic structure of eukaryotic chromatin. The only exceptions are dinoflagellate chromatin [11] and male gamete chromatin in many eukaryotic groups, including mammals [12].

A conserved character was emphasized for nucleosomal particles over many years. Now it is clear that nucleosomes are not all identical. Modified nucleosome forms occur along with canonical nucleosomes in chromatin. To produce these forms, variant histones are incorporated in nucleosomes and posttranslational modifications made to histones of the nucleosomal globule. More than one hundred of posttranslational modifications have been observed in histones to date [13, 14], of which the best known are acetylation (at lysines), methylation (at lysines, arginines, and histidines), phosphorylation (at serines), poly-ADP-ribosylation (at glutamates), ubiquitination, and SUMOylation (at lysines). Proline *cis–trans* isomerization is also possible. The development of new methods, especially those based on mass spectrometry [15], allowed the identification of new histone posttranslational modifications, such as *O*-glycosylation at serine and threonine [16], formylation and crotonylation at lysine, and hydroxylation at serine [17].

The main targets of posttranslational modification occur in the nonstructured N-terminal tail domains of histones [13, 14], although exceptions are possible; *i. e.*, several residues acting as targets for functionally important modification are in the globular histone regions [18, 19]. As already mentioned, many variant histones exist along with the canonical one; they are encoded by separate genes and can be incorporated in a nucleosome in place of their canonical counterparts (via a replication-independent mechanism, while canonical nucleosomes are assembled on newly synthesized DNA molecules). The nucleosomes that incorporate variant histones often differ from canonical nucleosomes to a substantial extent and perform special functions, for example modulate transcriptional activity [20–23] The variant histones characterized most comprehensively include CENP-A (centromeric H3), H3.3, macroH2A, H2A.Bbd, H2A.Z, H2A.X, and H5 (variant H1) [24].

Lateral internucleosomal interactions and the 30-nm fiber. It was believed until recently that a nucleoso-

mal thread folds in vivo to produce a regular structure of 30 nm in diameter, which is known as the 30-nm fiber. *In vitro*, these structures form in the presence of histone H1 or high concentrations of divalent cations [25, 26]. Two main models were advanced for the nucleosome thread folding in the 30-nm fiber. One suggests that the 10-nm fiber folds into a solenoid containing 6 nucleosomes per turn (one-start helix) [25]. According to the other model, a nucleosome thread forms a zigzag structure (two-start helix) [27–29]. Several other, less common models were discussed along with the above ones [30]. While the fine organization of the 30-nm fiber was a matter of dispute, it seemed unquestionable until recently that 30-nm fibers occur in the eukaryotic nucleus. As experimental methods improved and the interpretation of experimental findings was refined, the question arose as to whether 30-nm chromatin fibers actually exist *in vivo* in both interphase nuclei and metaphase chromosomes [31–34]. A molten polymer model was proposed on the basis of new findings to describe the folding of the 10-nm fiber in the interphase nucleus [31–33]. The model postulates that the 10-nm fiber produces an irregular dynamic structure via internucleosomal interactions between its distant regions «in trans». This fold is thought to provide for a more plastic chromatin packaging as compared with the 30-nm fiber, thus eventually facilitating all chromatin-related processes [31–33]. The molten polymer model allows spatially close nucleosomes to form the same internucleosomal interactions that were observed for structures like the 30-nm fiber, but the interactions are not regular in the molten polymer, arising and breaking down in a stochastic manner. Indeed, one of the key interactions in the molten polymer is a contact of the N-terminal domain of histone H4 with an acidic patch of the H2A–H2B dimer belonging to another nucleosome, that was detected in an X-ray analysis of tetranucleosomes producing a zigzag structure (two-start helix) [28, 35].

Nucleosome depletion is characteristic of active regulatory elements. In spite of their dynamic character [5], nucleosomes prevent, to a certain extent, a free access to DNA for various protein factors [36, 37]. To bind to DNA, the majority of general and specific transcription factors require that the regular nucleosome arrangement on a DNA thread be locally disrupted to generate a nucleosome-free region (NFR) or a nucleosome-depleted region (NDR) [38, 39]. The regions are several hundreds of base pairs in size and can be mapped as DNase I-hypersensitive regions [40–42]. Various regulatory elements of the genome usually occur in NFRs and NDRs [43–47]. It is possible to say that, compared with the prokaryotic genome, the eukaryotic genome is repressed on default and that transcription is regulated largely by modulating the genome accessibility to transcription machinery [48, 49].

First, the generation of NFRs and NDRs is necessary for assembly of the preinitiation complex on a promoter [50]; *i. e.*, active promoters are always NDRs [39, 51]. It is typical of higher eukaryotes that chromatin remodeling complexes work to release the promoters from nucleosomes [52, 53], as is considered below. Another strategy is used in the case of *Saccharomyces cerevisiae* constitutive promoters, where nucleosome occupancy depends to a substantial extent on the DNA sequence [50, 54]. Although the binding of the nucleosome core to DNA is not sequence specific, there are sequences that more or less preferentially interact with the histone octamer and those where the octamer is usually not assembled. The probability for a nucleosome to land on a particular DNA sequence depends to a great extent on the DNA flexibility, that is, the capability of wrapping around the nucleosomal globule. A poly(dA:dT) tract is one of the sequences that poorly bind with the nucleosome core [55]. A typical constitutive yeast promoter contains a poly(dA:dT) tract flanked by two sequences that preferentially bind nucleosomes and are known as the nucleosome positioning sequences (NPSs) [51, 56]. The nucleosome-free region usually harbors binding sites for transcription factors, which recruit transcription initiation proteins to the promoter [57].

A chromatin remodeling strategy is commonly utilized to establish and maintain the NDR in inducible *S. cerevisiae* promoters (TATA-containing promoters) and gene promoters of other eukaryotes examined [50]. A key role is played in this case by active ATP-dependent nucleosome displacement involving chromatin remodeling complexes [52, 53]. Various chromatin remodeling complexes move the nucleosome cores along a DNA molecule, remove them from DNA, replace canonical histones with variant ones, and perform several other functions. Chromatin remodeling complexes of the SWI/SNF and ISWI families play a main role in es-

tablishing and maintaining NDRs [58, 59]. NDRs are partly occupied by nucleosomes in *S. cerevisiae* upon depletion of the RSC complex, which belongs to the SWI/SNF family [60]. Transcription factors known as the pioneering factors are the first to initially recruit the chromatin remodeling complexes to *cis*-regulatory elements to establish an NDR [57, 61]. The pioneering factors differ from the majority of transcription factors in being capable of recognizing their sites on nucleosomal DNA. A small site for a pioneering factor can occur in the linker between two positioned nucleosomes [62, 63]. Other pioneering factors are capable of competing with nucleosomes for binding to DNA [61]. The pioneering factors recruit either chromatin-remodeling complexes or the enzymes that introduce certain posttranslational modifications acting to recruit chromatin remodeling complexes. A primary remodeling of the promoter region can open DNA to the binding of other transcription factors, which similarly facilitate the NDR maintenance and extension [64].

An association between the presence of NDRs and the enrichment of chromatin regions with certain histone marks was demonstrated at the whole-genome level in many studies [39, 65–68]. Among the histone posttranslational modifications that serve to recruit chromatin remodeling complexes, lysine acetylation in the tail domains of histones H3 and H4 plays an essential role and is high in active promoters [39, 65–68]. Nucleosomes that incorporate histone H3 acetylated at K9 and/or K27 recruit the remodeling complexes with a bromodomain, which recognizes these modifications [69, 70]. Acetylation additionally acts to increase activity of the complexes recruited [71, 72]. Histone acetyltransferase activity is inherent in many conserved coactivator complexes, including SAGA, p300/CBP, and TAF1 [73–75].

Along with high-level acetylation, the incorporation of variant histones H2A.Z and H3.3 in the vicinity of an NDR seems to contribute substantially to nucleosome depletion from active promoters [65, 76]. Nucleosomes with H2A.Z and H3.3 are less stable [77] and facilitate the NDR maintenance by chromatin remodeling complexes [78]. According to recent data, such nucleosomes are almost always present within NDRs as well, being easily displaced from DNA by certain protein factors [21]. The H2A–H2B dimers are replaced with the H2A.Z–H2B dimers by the Swr1 complex of the SWI/SNF family in yeasts (and by its orthologs SRCAP and p400 in Metazoa) [79, 80]. Swr1 is recruited to acetyla ted nucleosomes and has affinity for nucleosome-free DNA [81].

Enrichment in H3K4me3 is one of the most distinct features of active promoters [39, 65–68]. The modification probably maintains NDRs apart from its other putative functions [82]. A characteristic location of H3K4me3 in the 5' regions of genes is related to the mechanism of this modification. Histone methyltransferase Set1, which is conserved among all eukaryotes and is responsible for H3K4 trimethylation, binds to the Ser5-phosphorylated C-terminal domain of initiating RNA polymerase [83, 84]. As the polymerase starts elongation and the posttranslational modification profile of its C-terminal domain changes (phosphorylation at Ser2 rather than at Ser5), Set1 dissociates, and the level of H3K4 methylation grows lower [36, 85]. A transcription-independent mechanism is also possible for H3K4 methylation in vertebrates. In vertebrates, Set1 is recruited to the promoters of housekeeping genes and master regulators of cell differentiation by Cfp1: the promoters occur in CpG islands, Cfp1 is capable of recognizing nonmethylated CpG dinucleotides, and both Cfp1 and Set1 are components of one complex, COMPASS [86, 87]. Many chromatin remodeling complexes have protein components that interact with H3K4me3 (this modification is recognized by the PDH, Chromo, Tudor, MBT, and Zf-CW domains of various proteins [88]). For instance, H3K4me3-binding domains are responsible for the recruitment to promoters of human proteins CHD1 and BPTF, which are components of chromatin remodeling complexes and have homologs in many eukaryotes [89]. Histone acetyltransferases (HATs) contained in the SAGA and NuA3 complexes are similarly recruited to promoters as other components of the complexes interact with H3K4me3 [90].

In higher eukaryotes, NDRs are associated not only with promoters, but also with transcription factor-binding sites located in distant regulatory DNA elements, of which enhancers and insulators are two main classes. Distant regulatory elements, rather than promoters, account for the vast majority of regions where NDRs are established in some or other cells in Metazoa [39, 66, 91]. Enhancers are sequences of several hundreds of ba-

se pairs in length and harbor binding sites for several transcription factors, which are responsible for specific activation of enhancer-regulated genes [92, 93]. Enhancers can be up to tens or hundreds of kilobases away from their target promoters [39, 66, 94]. The distance is even greater than 1 Mb in exceptional cases [95]. Enhancers can occur both upstream and downstream of the target promoters, in both intergenic regions and introns [96, 97]. Cases were documented where enhancers are in coding gene regions [98].

Enhancer NDRs are far more tissue specific than promoter-associated NDRs [39, 66, 99]. A close relationship is assumed for the establishment of NDR and the establishment of the enhancer-associated H3K4me1 mark at enhancers [39, 65, 66, 68]. Pioneering factors recruit histone methyltransferases, which establish an H3K4me1-enriched region at the enhancer [100]. In turn, H3K4me1 recruits the p400 remodeling complex, which incorporates H2A.Z in nucleosomes [101, 102]. H2A.Z-containing nucleosomes are unstable, and a small NDR consequently forms at the so-called poised enhancers [99, 103, 104]. Differentiation signals activate the poised enhancers by targeting additional tissue-specific transcription factors and signaling pathway effectors to them, and the factors expand the NDR by recruiting and activating the chromatin remodeling and coactivator complexes possessing HAT activity, including p300/CBP as a main one [93, 105]. A main target of p300/CBP is H3K27, and its acetylation is thought to provide a mark associated with active enhancers [99, 103].

Along with enhancers, *cis*-regulatory elements known as the insulators colocalize with nonpromoter NDRs. Insulators are thought to perform a broad range of functions, the main of which are to prevent the extension of repressive chromatin marks (barrier activity) and to block the action of an enhancer on a promoter when interposed between them (enhancer-blocking activity) [106–108]. Insulators can display either both activities or exclusively enhancer-blocking activity in a transgenic reporter assay. Enhancer-blocking activity is due to binding sites for a special protein group known as the insulator proteins. TFIIIC is one of the most conserved insulator proteins, acting additionally as a general transcription factor to facilitate RNA polymerase III landing on DNA [109, 110]. CTCF also performs the insulator function in vertebrates [111, 112]. Drosophila has not only TFIIIC and a homolog of vertebrate CTCF (dCTCF) to sustain enhancer-blocking activity of insulators, but also a number of other proteins: Su(Hw), GAF, BEAF-32, and Zw5 [113, 114].

Insulator NDRs are enriched in H3K4me1 and the H2A.Z variant [68, 115]. The mechanism that establishes and maintains NDRs at insulators is most likely similar to that of enhancers. It should be noted that vertebrate insulators are less variable than enhancers and that their positions are more or less constant in different cells [66]. This is possibly related to the fact that the main vertebrate insulator protein CTCF occurs in all cells and acts as a pioneering factor, autonomously binding to its sites in chromatin regardless of whether or not they are free of nucleosomes [61, 116].

Several insulators and enhancers display RNA polymerase II binding and enrichment in H3K4me3, thus being functionally similar to promoters [117–120]. The appearance of these features correlates with enhancer activation in certain cells [119, 121, 122]. Moreover, such enhancers and insulators can be transcribed to yield unstable noncoding RNAs. The functional significance of their transcription is a matter of discussion [119, 122, 123].

Remodeling of nucleosomal particles during transcription elongation. Nucleosomal particles provide an obstacle for elongating RNA polymerase II *in vitro* [124, 125]. *In vivo*, histone chaperones and chromatin remodeling complexes improve the efficiency of elongation [126, 127], facilitating local partial nucleosome disassembly in front of the polymerase. Active transcription alters the regular nucleosome arrangement along the transcription unit, and the alteration may have adverse consequences for the cell, *e. g*., activating cryptic promoters (see below) [128]. Special mechanisms work to ensure correct chromatin assembly behind the passing elongation complexes [127, 128]. As the elongating RNA polymerase II complex progresses along nucleosomal DNA, one of the H2A–H2B dimers dissociates, while the residual histone hexamer remains associated with DNA [124, 129]. This mechanism accounts for a higher exchange rate of H2A–H2B dimers on transcribed genes [130, 131]. The exchange rate of the total nucleosome core increases with increasing transcription intensity, indicating that $(H3–H4)_2$ tetra-

mers can also dissociate when elongating complexes pass frequently [130, 132, 133]. H2A–H2B dimer exchange probably involves the Asf1, Nap1, Spt6, and FACT histone chaperones, which act together with the SWI/SNF and RSC chromatin remodeling complexes [134-139]. Histone acetyltransferases PCAF and Elp3, which stimulate the function of chromatin remodeling complexes, specifically interact with elongating RNA polymerase II [140, 141]. SAGA and NuA4 are also recruited to transcription units along with the elongating complex to stimulate nucleosome displacement [142, 143].

On the other hand, nucleosome destabilization in transcribed regions increases probabilities of spontaneous formation of NDRs. Some of them may happen in DNA regions allowing transcription initiation [144–147]. These regions are known as the cryptic promoters, and several mechanisms work to repress their activity. An important role is played by the Chd1 and Isw1 chromatin remodeling complexes, which maintain regular nucleosome spacing in transcribed regions [148–151]. The interaction of H2A–H2B dimers with the Asf1, Nap1, Spt6, and FACT chaperones facilitates the restoration of a nucleosomal octamer as soon as the polymerase has passed. In addition, dynamic acetylated nucleosomes are stabilized as Rpd3, Hos2, and Hda1 histone deacetylases are recruited to transcribed regions [152, 153]. The Rpd3S deacetylation complex plays a key role in the process. RpdS3 is recruited by the Ser2-phosphorylated C-terminal region of elongating RNA polymerase II [152, 153]. Rpd3S activity is higher on H3K36me3-containing nucleosomes, which interact with the Eaf3 and Rco1 subunits of the complex via the Chromo and PHD domains [152]. H3K36 trimethylation, which recruits histone deacetylases, is catalyzed by Set2 histone methyltransferase, which also interacts with the Ser2-phosphorylated C-terminal domain of RNA polymerase II [154, 155]. Thus, the modification provides a specific mark for the bodies of actively transcribed eukaryotic genes [156, 157] and ensures that low-level histone acetylation is restored in gene bodies as soon as the transcription complex has passed [128].

Internucleosomal interactions and the regulation of transcription. A number of modifications occurring in canonical histones and the presence of some variant histones affect, to a certain extent, the strength of internucleosomal interactions. The modifications modulate the chromatin packaging and thereby act as an important factor regulating gene expression. When nucleosomal particles that strengthen the internucleosomal contacts are incorporated in chromatin, chromatin is condensed and DNA becomes less accessible to transcription machinery, while nucleosome modifications that hinder the internucleosomal interactions facilitate a loosening of chromatin and activation of its genes. The latter group of modifications includes H4K16 acetylation, which prevents the N-terminal domain of histone H4 from interacting with the acidic patch of the neighbor nucleosome. Chromatin composed of H4K16ac-containing nucleosomes cannot produce 30-nm fibers *in vitro* [158–160] and is probably depleted of lateral interactions with other nucleosomal fibers *in vivo*. Nucleosome acetylation at other lysines can also affect in part the stability of internucleosomal interactions [161]. Local decondensation is possibly a mechanism that sustains the activator effect of acetylation on regulatory DNA elements. A similar effect is known for the incorporation of variant histone H2A.Bbd. This variant histone lacks the amino acid residues that are involved in the formation of the negatively charged surface (acidic patch) to interact with H4K16 [162]. Paradoxically, variant histone H2A.Z, which usually colocalizes with NDRs, allows a greater acidic patch area as compared with canonical histone H2A, thus strengthening the internucleosomal contacts [163, 164].

A special group of histone modifications includes H3K9me3 and H3K27me3. Nucleosomes with these modifications recruit specific architecture proteins, which facilitate a denser chromatin packaging. The resulting condensed chromatin clusters at the periphery of the nucleus, in the perinucleolar region, and nucleoplasmic foci known as the chromocenters. Chromatin of denser regions was termed heterochromatin as opposed to less compact euchromatin [165].

H3K9me3 binds with heterochromatin protein 1 (HP1). HP1 is highly conserved, and its homologs are found in the majority of eukaryotes with the exception of budding yeasts [166], where a similar function is performed by the SIR proteins [167]. HP1 binds to H3K9me3 via its chromodomain, which is in the N-terminal region of the protein. The C-terminal region of HP1 harbors the so-called chromoshadow domain,

which provides for HP1 oligomerization [168]. Thus, HP1-mediated lateral interactions between H3K9me3-containing nucleosomes lead to chromatin condensation [169]. In addition, HP1 is capable of recruiting histone methyltransferases Suv39h1/2 and SETDB1, which are responsible for H3K9 trimethylation [170]. The resulting positive feedback is one of the mechanisms spreading the «histone code signal» to produce extended H3K9me3-enriched domains [171, 172]. Heterochromatin, which contains highly repetitive DNA and is enriched in H3K9me3 and HP1, occurs in pericentric and subtelomeric regions in the majority of eukaryotes. However, it should be noted that neither HP1 [173, 174] nor H3K9 trimethylation [175] is essential for maintaining the heterochromatic chromocenters containing pericentric DNA. In addition, H3K9me3 domains that usually correspond to individual silent genes occur in chromosome arms. For instance, more than 10,000 H3K9me3-enriched domains with a median size of approximately 7 kb were observed in human embryonic stem cells (hESCs). Similar domains are about twice as large in fibroblasts [176]. Genome-wide studies identified the so-called LOCK (large organized chromatin K9 modification) domains, which are extended (~ 100 kb) genome segments enriched in histone H3 di- or trimethylated at K9 [177].

H3K27me3 is another conserved histone modification characteristic of eukaryotic heterochromatin [178]. The modification is often associated with facultative heterochromatin on genes – master regulators of development [178, 179]. The H3K27me3 establishment and mechanism of action are closely associated with Polycomb group (PcG) proteins. PcG proteins are components of several complexes, of which PRC1 and PRC2 are best understood. PRC2 uses its component histone methyltransferase EZH2 to trimethylate histone H3 at K27. PRC1 binds to H3K27me3 and is thereby associated with sites of PRC2 activity [180]. In Drosophila, PRC2 is recruited to target genes by PRE elements (Polycomb response elements) which harbor consensus binding sites for several repressor factors interacting with PRC2 [181–183]. In vertebrates, the mechanism recruiting PRC2 to target genes is not fully understood [180]. An important role in the process is most likely played by CpG islands, where the promoters of genes targeted by PcG complexes mostly occur in vertebrates [184, 185]. These are usually the promoters of genes involved in maintaining pluripotency and master regulators of differentiation.

In embryonic stem cells, H3K27me3 colocalizes with the activating mark H3K4me3 in the promoters of master regulators of differentiation to produce the so-called bivalent promoters [157]. Depending on the cell lineage, one of the marks is removed during differentiation, and if it is H3K27me3 the gene is activated [157, 186]. A specific recruitment of PRC2 to target promoters was observed in plants, *Arabidopsis thaliana* in particular, but a consensus similar to *Drosophila* PRE was not identified [187].

The mechanism of promoter repression via H3K27me3 and the PRC complexes is presumably related to the fact that, like HP1, PRC1 causes chromatin compaction to prevent free access of transcription factors to the genes involved [188–190]. According to classical views, heterochromatin is a more compact form of chromatin, and its compaction prevents heterochromatin DNA from being accessed by transcription machinery and thereby facilitates repression of heterochromatic genes. However, there is evidence that accessibility to large protein factors is similar between euchromatin and heterochromatin. For instance, the genome is more or less uniformly accessible to Dam methylation regardless of the heterochromatin nature of particular regions in *Caenorhabditis elegans* and *S. cerevisiae* [191, 192]. Transcription factors expressed artificially display no preference in binding to their sites in heterochromatin or euchromatin [191, 193]. Only molecular complexes of more than 1 MDa are specifically excluded from heterochromatic regions according to microscopic studies [194–197].

Accessibility of heterochromatin or even more compact chromatin of metaphase chromosomes to diffusion of large protein complexes is probably related to the dynamic character of internucleosomal interactions, as assumed in the molten polymer model (see above). Owing to this dynamic character, individual nucleosomal particles can locally move relative to each other in the three-dimensional nuclear space and periodically create channels to allow migration of protein complexes within compact chromatin domains [33, 197].

Transcriptional activity was recently demonstrated for the majority of *Drosophila* genes located in HP1-

enriched pericentric heterochromatin [193, 198, 199]. As for genes repressed by the Polycomb complexes, it was found that a preinitiation complex is assembled and transcription initiated on their promoters in both Drosophila and mammalian cells, but elongation is blocked [200–202]. Thus, none of the most important types of eukaryotic heterochromatin prevents access to chromatin for transcription machinery. Then what is the role of chromatin compaction? The role is explained by the model that architecture proteins, such as SIR and HP1, and the PRC1 complex do not act to restrict access of activator factors to DNA, but rather function to create nuclear compartments with a high concentration of inhibitory factors, which ensure repression via other mechanisms [203, 204]. In the case of Polycomb-dependent repression, the mechanism possibly consists in PRC1-mediated recruitment of RING1b ubiquitin ligase, which ubuquitinates histone H2A at K119, to promoters. The modification stabilizes the interaction of H2A–H2B dimers with $(H3–H4)_2$ tetramers, and the elongating RNA polymerase complex cannot pass through these nucleosomes [18, 205]. In addition, a compact arrangement of repressed genome regions in the nucleus makes it possible to limit free diffusion of inhibitory factors in the nuclear space, preventing their nonspecific activity [206]. Well-known examples of such compact regions are provided by peripheral and perinucleolar heterochromatin, chromocenters, and PcG bodies [190, 204, 207, 208].

Conclusions. The structure of nucleosomal particles and its changes that accompany transcriptional activation or repression have been studied for almost half a century. This level of chromatin packaging is the most fully understood. However, several basic shifts occurred in the apparently firm views of nucleosomes and internucleosomal interactions in the past decade. Among these mini revolutions, the 30-nm fiber as an important level of chromatin packaging was rejected and changes were made to the classical views of the heterochromatin structure and the mechanisms of heterochromatic gene silencing. A drift from focusing on one or a few model loci to probing the chromatin organization on a genome-wide scale is one of the main trends in recent studies of the lower levels of eukaryotic DNA packaging. Another trend is collating the genome-wide maps of several epigenetic features, primarily the distributions of histone modifications, variant histones, and NDRs. Both of the trends are implemented in large-scale collaboration projects, of which ENCODE and modENCODE are the best known. A combination of the resulting data sets with information obtained by «C» methods for the spatial organization of chromatin [209–211] and high-resolution microscopy findings will probably yield a comprehensive picture of DNA packaging in the nearest future and will help to better understand how the packaging mode is related to functional processes occurring in the cell nucleus.

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Нуклеосомна упаковка евкаріотної ДНК і регуляція транскрипції

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Резюме

Ядра евкаріотних клітин містять геномну ДНК, лінійні розміри якої у десятки тисяч разів перевищують їхній діаметр. Багато в чому такий високий ступінь компактизації забезпечується упаковкою ДНК у хроматин, ключовим етапом якої є намотування ДНК на нуклеосомні глобули. Октамер гістонів, які складають нуклеосомну глобулу, взаємодіє з ДНК за посередництвом електростатичних контактів. ДНК-гістонові взаємодії достатньо міцні і утруднюють доступ до нуклеосомної ДНК багатьох ферментів і транскрипційних факторів. У той же час наявність нуклеосом не перешкоджає проходженню транскрипції та інших процесів, пов'язаних з реалізацією генетичних функцій ДНК. В огляді розглянуто структуру і розмаїття нуклеосом та їхню центральну роль у регуляції транскрипції. Особливу увагу приділено значенню міжнуклеосомних взаємодій у забезпеченні доступності геному для транскрипційної машинерії, а також регульованому видаленню нуклеосом з регуляторних елементів і транскрипційних одиниць в процесі елонгації транскрипції.

Ключові слова: хроматин, модифікації гістонів, нуклеосома, транскрипція.

Нуклеосомная упаковка эукариотической ДНК и регуляция транскрипции

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Резюме

Ядра эукариотических клеток содержат геномную ДНК, линейные размеры которой в десятки тысяч раз превышают их диаметр. Во многом такая высокая степень компактизации обеспечивается упаковкой ДНК в хроматин, ключевым этапом которой является наматывание ДНК на нуклеосомные глобулы. Октамер гистонов, составляющих нуклеосомную глобулу, взаимодействует с ДНК посредством электростатических контактов. ДНК-гистоновые взаимодействия достаточно прочны и затрудняют доступ к нуклеосомной ДНК многих ферментов и транскрипционных факторов. В то же время наличие нуклеосом не препятствует прохождению транскрипции и других процессов, связанных с реализацией генетических функций ДНК. В настоящем обзоре рассмотрены структура и многообразие нуклеосом и их централь-

ная роль в регуляции транскрипции. Особое внимание уделено значению межнуклеосомных взаимодействий в обеспечении доступности генома для транскрипционной машинерии и регулируемому удалению нуклеосом с регуляторных элементов и транскрипционных единиц в процессе элонгации транскрипции.

Ключевые слова: хроматин, модификации гистонов, нуклеосома, транскрипция.

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