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The three-dimensional architecture of eukaryotic chromatin: zigzag-shaped band of DNA is disposed between two layers of nucleosomes forming a chromosome

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Abstract. Three-layered structure of individual eukaryotic nucleosomes and the DNA packaging in the form of zigzag-shaped band are proposed. Nucleosome histones are grouped into three layers: L1 (H3-H4), L2 (H2A-H2B), and L3 (H1 or H5). Each layer realizes different functions. The L1 layer is conjugated with DNA ribbon by hydrogen bonds between amino acids of nucleosomes and a phosphate group of DNA. The L2 layer glues all nucleosomes forming the nucleosomal layer of the chromosome. The L3 layer is functioning as a protective shell for L1 and L2 histone layers. The eukaryotic chromosome is proposed to compose of two nucleosome layers, the DNA being disposed between them in a «zigzag folding» band. Three nucleosomes are arranged into a row along the long axis of the chromosome and form a nucleosomal triplet, a basic structural subunit of the chromosome. Parental nucleosomes do not undergo disruption processes during replication and transcription.

Keywords: chromatin, histones, nucleosome, nucleosomal triplet, snaky model of DNA packaging, «zigzag folding» ribbon of DNA.

Introduction. It is suggested [11] that individual nucleosome particle is a flattened cylinder (10.5 nm in diameter and 5.7 nm in height); therefore, the sizes of a disk-shaped nucleosome form are approximately 6 nm x 11 nm x 11 nm. Because the eukaryotic histone octamer contains five different histones — H2A, H2B, H3, H4 and H1 (or H5), it is difficult to interpret such nucleosome positioning sequences in terms of individual histone-DNA interactions. The function of four different core histones (H2A, H2B, H3, and H4) is to pack the DNA into the cell nucleus; however, little is known about the functional roles of each histone in this eukaryotic histone octamer. The DNA packaging into the nucleosomes exerts negative effects on all DNA functions including replication, recombination, repair, and transcription [9, 14, 15].

Although it is not understood why the DNA should be wrapped around the nucleosome, a large number of models to explain DNA disposition on the nucleosome have been proposed [see ref. 4, 5, 9, 17].

Unfortunately, we do not know the cause of DNA packaging by the way leading to nucleosome wrapping. Why is each individual nucleosome composed of different (mostly 5) proteins? It seems that a usual canonical nucleosome must not disassemble and then reassemble itself during chromatin functioning and lose tremendous energy resources. If DNA is disposed on the nucleosome surface it should be understood how nucleosomes inhibit the transcription being grabbed by the DNA.

It is evident the eukaryotic nucleosome is composed of a H3-H4 tetramer, two H2A-H2B dimers, and a histone H1 (mostly frequently presented in eukaryotic nucleosomes).

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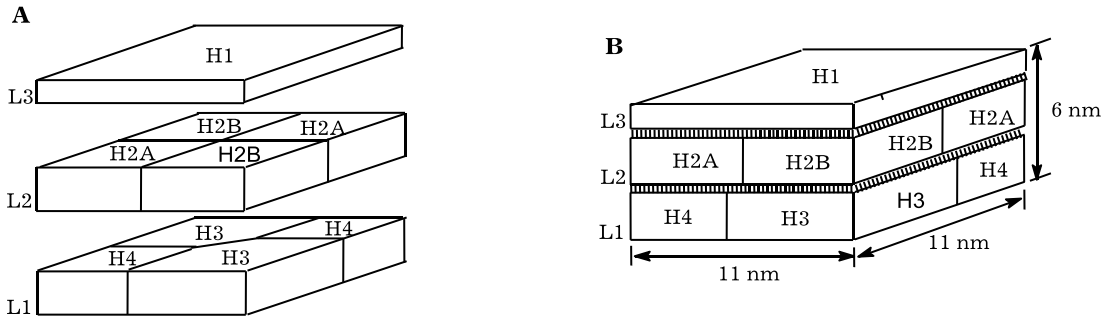

Fig. 1. Proposed structure of an eukaryotic nucleosome. Three layers of the nucleosome are composed of different histones: the first layer, L1, is formed by H3 and H4 histones, the second layer, L2, contains H2A and H2B histones and the third layer, L3, is presented by a H1 (or H5) histone and serves to safeguard both L1 and L2 layers and DNA (A). A general view for the complete nucleosomal structure (B).

Why is the histone H1 found within the nucleosomal core, but not with solitary DNA? What is a mechanism protecting the DNA from the oxidative agents, the H1 bonds with the nucleosomal core being stronger than its bonds with the DNA? We could not find satisfactory explanations for these and many other questions in the literature, so we are going to propose our model concerning the chromatin packaging and its functioning in eukaryotic cells.

Results and discussion. We propose [10] that each individual nucleosome is a parallelepiped (6 nm x 11 nm x 11 nm) consisting of three histone layers (stacked one on another): L1 (composed of H3-H4-H3-H4-histones), L2 (containing H2A-H2B-H2A-H2B-histones), and L3 (including H1 or H5 histones) (Fig. 1A); these layers are interlinked by hydrogen bonds (Fig. 1B). L1 histone proteins rich in positively charged basic amino acids form the bonds with negatively charged DNA phosphate groups.

There are two possibilities concerning the formation of internucleosome linkages: the nucleosomes can be bound through the H2A and H2B (Fig. 2A) or through H4 and H3 histones (Fig. 2B). We believe that a position presented in the Fig. 2A is more preferable. Each L1 layer in the nucleosomal triplet and each triplet are also interconnected.

Because the histone H1 hinders the access of transcriptional co-activators to the DNA [3, 7], it acts as a common transcription repressor [18] and at last stabilizes the chromatin structure [14]. That is why we believe that it occupies the external position in the chromosome, contrary to H3-H4 histones contacting with the DNA. Thus, both the core histone tail domains and linker histones (e.g. H1, H5) are required to form strongly condensed chromatin states.

The milestone of this model is a notion that DNA possessing a «zigzag folding» ribbon is disposed between two nucleosome layers forming the chromosome. No naked DNA exists if chromosomes or nucleosomes are undamaged and there is no impair of histone-DNA interactions (occurring, for example, during *in vitro* manipulations). We propose that the eukaryotic chro-

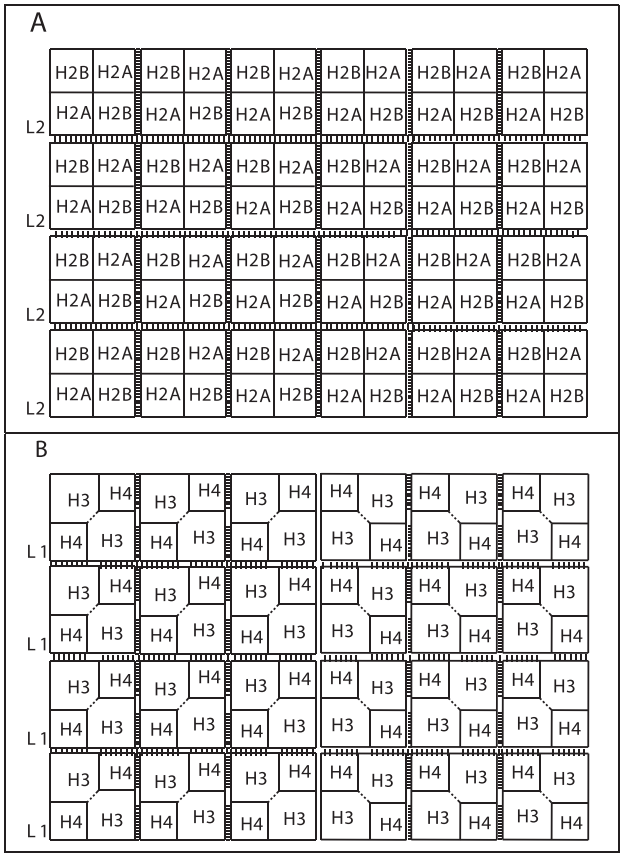

Fig. 2. The formation of linkages between nucleosomes: individual nucleosomes can be bound through H3 and H4 (A) or H2A and H2B histones (B).

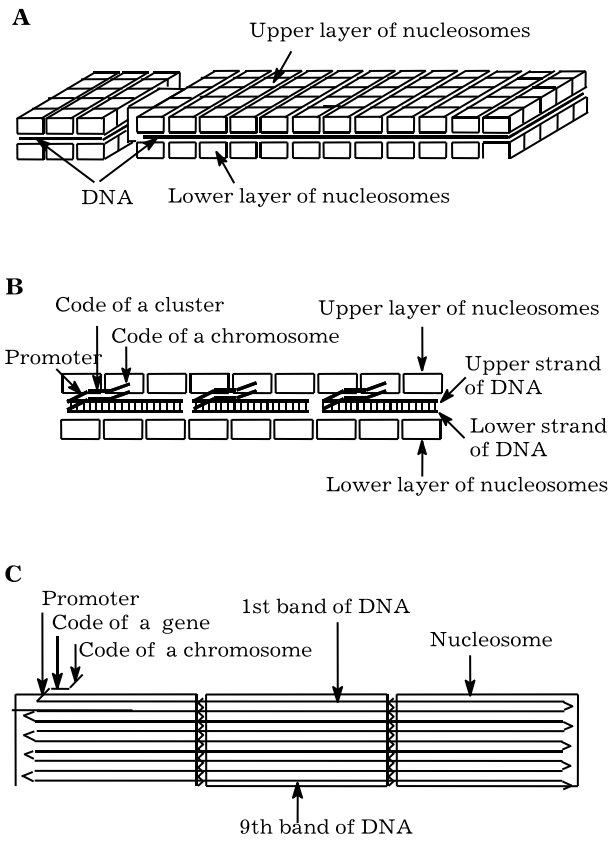

Fig. 3. A model for the DNA packaging and nucleosome assembly in the chromosome: a general view of the chromosome (A) composed of nucleosome layers and DNA between them, a frontal position (B). DNA is disposed onto the nucleosomal triplet (the upper row of nucleosomes is absent) in a «zigzag folding» ribbon, a top view (C).

mosome is composed of two nucleosome layers, and the DNA is disposed between them in a «zigzag-shaped» band (Fig. 3A). Three nucleosomes are grouped into a row by the formation of bonds between L1 layers (Fig. 3B). All three nucleosomes in each row are interconnected and form a column crosswise to the chromosome. We believe that linear sizes of three nucleosomes are adequate to the sizes of DNA and RNA polymerases (especially to their length).

Thus, nucleosomes carry out their dual function in the eukaryotic cell; they protect DNA from damaging agents and provide regulated access to the information contained in chromosomes. In this connection we believe that bilaterality of alive essences is caused by function of «left» or «right» genes in different chromosomes.

Two fundamental chemical processes, such as oxidation and reduction reactions, can determine many biological events including also nucleic acids functioning. That is why both DNA and RNA should have a reliable defense systems preventing their damage by oxidative agents and cleaving enzymes. Thus, nucleosomes are not only architectural units, they protect DNA from damage, i.e. they prevent the access of highly reactive substances (e.g. free radicals) to DNA. A side of the nucleosome contacting with the DNA ribbon does not contain any reactive groups. Such substances as purine, adenine, guanine, pyrimidine, and cytosine contain unsaturated bonds less reactive comparing to 2-deoxyribose, ribose, uracil and thymine possessing high reactivity because of the presence of non-hindered functional reactive groups, such as

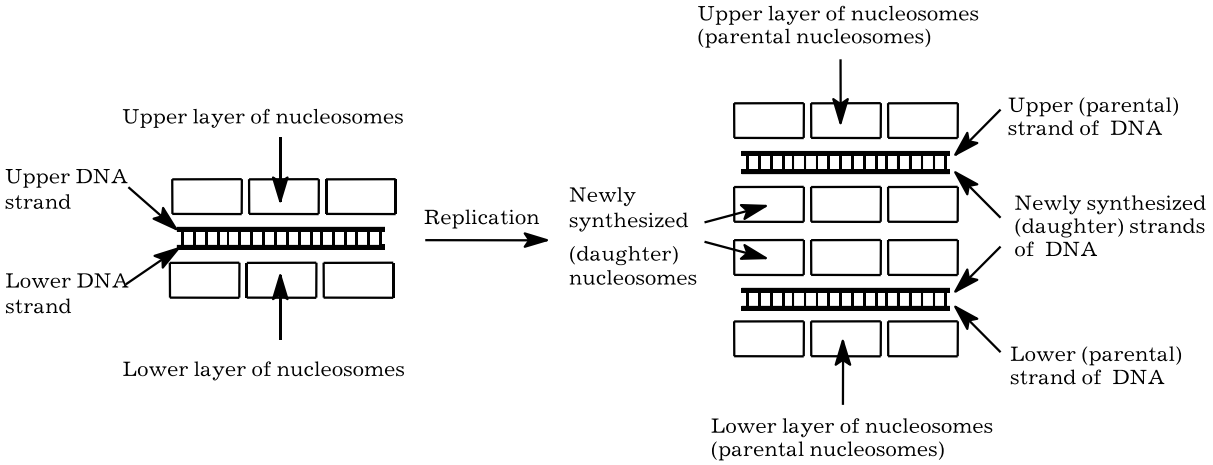

Fig. 4. A model explaining the formation of novel chromosomes. The nucleosomal triplet of the chromosome (frontal view from the side of a promoter). A mechanism of the chromosome duplication: DNA replication machinery displaces all parental nucleosomes from the external sides of the sister chromosomes, and newly synthesized nucleosomes are displaced into «the cavity» formed by parental layers of nucleosomes.

=NH or -OH. The DNA can be linked with NH_2-groups of amino acids forming the nucleosome, and thus the DNA-nucleosome complex can be formed by hydrogen bonds. To prevent the chaotic moving within the nucleus, the chromosomes can be fastened to nuclear membrane for example through the telomer and should be liberated in the period of DNA replication.

It is noteworthy such remodeling occurs without large changes in either conformation or configuration of the core histone octamer [2].

Also, RSC, the chromatin-remodeling complex, exposes the nucleosomal DNA to attack by nucleases, and this exposure occurs without any loss of histones; this is a paradoxical situation, the DNA behaving as a structure free and bound at the same time [1]. Our model is able to explain this central paradox of the remodels of chromatin structure: the DNA in the activated nucleosome state is exposed along its entire length, and yet the nucleosome remains intact. As you can see in the Figs. 4—6, the replication and transcription events occur without disruption of bonds between one strand of the DNA and nucleosomes.

The chromatin is known to be packaged into compact 30-nm fibers which can be formed by three neighboring nucleosomes (a nucleosomal triplet). The nucleosome triplet being a parallelepiped-like structure (33 nm x 11 nm x 6 nm) can present a basic chromosome subunit. The rows of these nucleosomes are assembled into a column the length of which is the same as the width of the chromosome. Each such column begins with the codes (i.e. passwords) of the chromosome, gene (cluster) and promoter and ends by the terminator. All codes, promoters and terminators can be double strands of DNA. The DNA is disposed in the «zigzag folding» band on the plane of such nucleosome triplet. The ribbon of DNA is situated on the plane of the nucleosome; its length varies from 146 bps to 260 bps. Hence, on the plane of «such triplet of nucleosomes» 876-1560 DNA bps can be disposed. The DNA as long as 33 nm contains about 97 bps or 88 bps linear molecules for the 30-nm string of beads (i.e. nucleosomes). Consequently, 876 bps and 1560 bps of DNA can be arranged into 9 or 16 rows. Each row as long as 88 bps or 97 bps (the ladder DNA) may correspond to Okazaki fragment. Then the ribbon of DNA turns back to the beginning of the nucleosomal triplet forming two rows of DNA ribbon. By the next turn on 180 ˚C, a new cycle starts forming two next rows (Fig. 1C). The «zygzag-like» ribbon of DNA proceeds to the next edge of the chromosome and is ended by the terminator. Genes shorter than the chromosome width can be completed by introns. Two or more genes in the column can be also separated by the intron.

Thus, our model supposes the DNA to remain straight in its condensed fiber as it has been earlier proposed for linker DNA [19] and supported in another review [16]; but here there is a contradiction to the well-known solenoid model where the linker DNA is bent [4, 6]. Nevertheless, the data mentioned above agree with steric configuration of naked DNA in salt solutions. It follows from our model that during the S-phase, both DNA and nucleosomes are duplicated concomitantly and the parental nucleosomes do not become transiently disrupted during the passage of the replication fork and become subsequently reassembled forming two daughter DNA strands.

The DNA synthesis can occur by the next way. The DNA is completely surrounded by nucleosomes. If there are some hindrance for the approach of DNA polymerases from button side (for example, the chromosome is linked with the nuclear membrane), the top strand of DNA will be removed from native chromosome. Only DNA polymerases have contacts with DNA within the chromosome. When the DNA synthesis is finished the new nucleosomes are bound to the bottom DNA strand from top and to the top DNA strand from bottom. These newly synthesized structures are disposed within the chromosome (Fig. 3). The parental layers of nucleosomes are completed by two identical daughter layers, each of them includes a parental DNA strand and a newly synthesized one. Hence, the mode of nucleosome formation *de novo* is semiconservative and resembles the DNA synthesis. In such structures, the DNA is constantly safeguarded of accidental attacks by various substances (i.g. free radicals and some enzymes). It also follows from this scenario that, during DNA replication both parental DNA strands are constantly adjacent to parental nucleosomes.

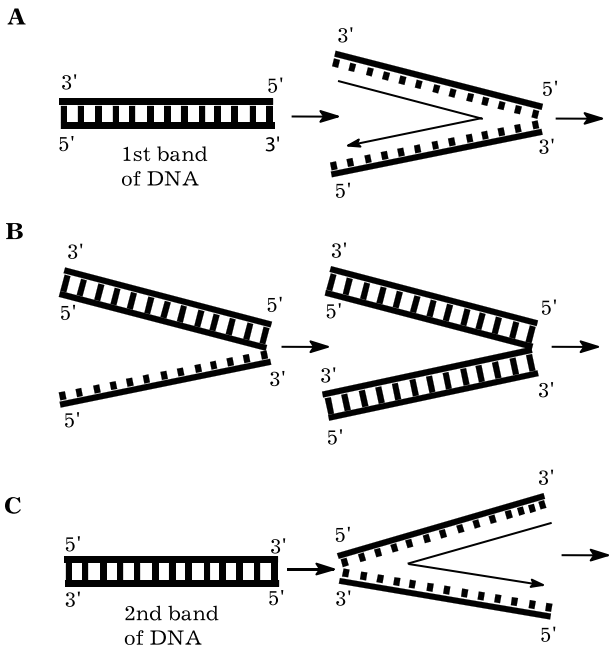

Fig. 5. The scenario for the putative mechanism of DNA synthesis (i.e. replication process). A general view of the first row of two DNA strands from the side of a promoter (A). A replicative fork: the upper and lower DNA strands (corresponding to Okazaki fragments with a total number about 100 bps of DNA) are complemented by novel DNA strands (B). The new cycle of DNA synthesis in the second band continues in the opposite direction (C).

It is believed that the DNA-mediated processes can function in the presence of at least two groups of chromatin remodeling enzymes. One of them includes enzymes changing the structure of nucleosomal histones [13] and destabilizing the folding of nucleosomal arrays; these enzymes promote DNA replication or RNA transcription. Other enzyme group disrupts histone-DNA interaction [8]. As you can see from our model, histones are not disrupted during transcription or replication. These processes do not also require any enzymes to separate DNA from histones core.

We believe that during replication, transcription and repair reactions the only enzymes contacting with matrix DNA within the chromosome are DNA-polymerases (their sizes are the same as the DNA size consisting, for example, of 97 bps). The formation of all RNAs occurs as a two-step process. The first stage is the DNA synthesis; a single gene surrounded by two layers of nucleosomes is transcribed into RNAs by RNA-polymerases within the nucleus, i.e. outside of the chromosome. Such synthesis excludes the accidental incorporation of ribonucleotides into the matrix DNA strand (gene). Thus, the full replication fork can include about 200 bps of DNA, the leading and lagging strand consisting of 100 bps.

It is therefore likely that the DNA replication does not require any codes in contrast to transcription. If the transcription occurs on many genes of the same cluster simultaneously, the individual code of genes can be absent but individual codes of the chromosome and the cluster

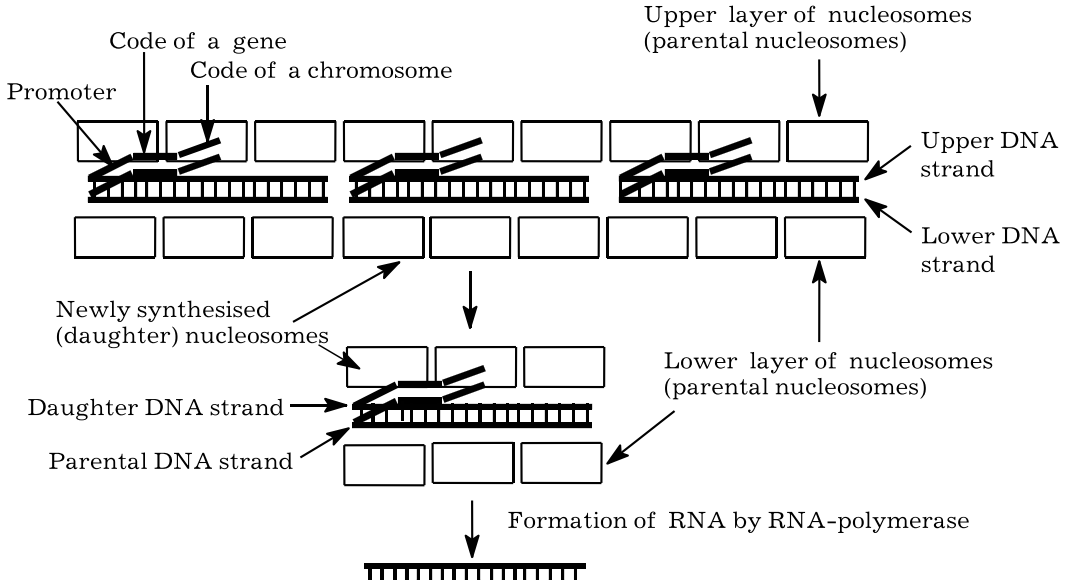

Fig. 6. Schematic representation of a possible DNA synthesis mechanism before the RNA formation (i.e. transcription). As in the case of replication, only DNA-polymerases contact with matrix DNA. Synthesized DNA (gene/s) for RNA formation undergoes to RNA-polymerase action outside the chromosome.

should be presented. A so-called enhancer can present the code of the cluster.

Because the DNA wraps around the histone octamer, it is accompanied by many noncovalent interactions. During the transcription only DNA-polymerases contact with matrix DNA within the chromosome. The formation of all RNAs occurs in two steps: at first the DNA synthesis carries out; a gene or several genes surrounded by two layers of nucleosomes express their RNAs by RNA-polymerases outside of the chromosome during the second steps (Fig. 6).

Hence, as it follows from our model, there is no necessity in the sliding of the histone octamer in *cis* along DNA by the hSWI/SNF-dependent nucleosome sliding complex and other chromatin remodeling complexes. Moreover, some recent data suggest that the hSWI/SNF causes both sliding and disruption of nucleosome structure on nucleosome arrays [12].

Conclusions. A simplified model of chromatin remodeling proposed here does not assume the DNA wrapping around the nucleosome, condensation and decondensation of nucleosomes during replication, transcription and repair processes. The DNA is stored between two nucleosome layers in the «zigzag folding» band. As mentioned above, nucleosomes are not structurally inert entities: they play an outstanding role in the chromatin function *in vivo*. First of all, they protect the DNA from oxidation by diverse reactive agents and from enzyme action. Secondly, it is important to point out that only DNA-polymerases contact with the native DNA within the chromosome. The RNA synthesis occurs outside the chromosome, separate DNA sequences are transcribed by RNA-polymerases forming different RNA types. Such synthesis excludes the accidental incorporation of ribonucleotides into the matrix DNA strand (gene).

We also would like to note, however, that we propose here a working model which does not encompass all the known phenomena of chromatin packaging and function in eukaryotic cells. We understand this model to need experimental verifications and hence cannot be considered as a final one. Nevertheless, we hope that it might be useful for many specialists in the field of molecular biology.

Тривимірна будова хроматину: петлиста стрічка ДНК розміщена між двома шарами нуклеосом, що утворюють хромосому

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Резюме. У статті описано тришарову структуру індивідуальних хромосом та упаковку ДНК у формі петлистої стрічки. Показано, що гістони нуклеосом згруповано в три шари: L1 (H3-H4), L2 (H2A-H2B), L3 (H1 or H5), кожен з яких має різні функції. Шар L1 з'єднаний зі стрічкою ДНК за допомогою водневих зв'язків між амінокислотами нуклеосом і фосфорною кислотою ДНК. Шар L2 служить для з'єднування всіх нуклеосом в один шар хромосоми, а шар L3 є захисною оболонкою для L1 і L2. Хромосома еукаріот складається з двох шарів нуклеосом, і ДНК розміщена між ними у вигляді петлистої стрічки. Три нуклеосоми згруповано на повздовжній осі хромосоми в ряди (триплет нуклеосом), і таким чином утворюється базова (елементарна) структура хромосоми. Виявлено, що батьківські хромосоми не руйнуються під час реплікації і транскрипції.

Ключові слова: нуклеосома, хроматин, петлиста модель упаковки ДНК, гістони, нуклеосомальний триплет, зигзагоподібна стрічка ДНК.

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