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SIGNALING IN THE LIVING SYSTEMS : GENE KEYS AND GENE LOCKS

This article briefly discusses the complex and intricate mechanisms of the functioning of signaling systems between the plasma membrane and the nucleus in the living organisms. An alternative model of DNA packaging is described. The milestone of this model is assumption that DNA winds not around the histone octamer: it is organized between two layers of nucleosomes. The basic structure of chromosomes is a triplet of nucleosomes. A single nucleosome is a parallelepiped in shape (11x11x6 nm). An alternative to protein receptors is proposed receptors composed from nucleic acid (DNA, RNA), called gene keys, that open the code (gene lock) of gene/cluster at the promoter.

Keywords: nucleosome, chromosome, histones, gene keys, gene lock.

I.Protein receptor paradigm: A maze of mechanisms in which it is difficult to navigate.

A paradoxical situation has arisen: researchers are competing in the discoveries of new receptors and, judging by the trend, there is no limit to the number of receptors. Excessive enthusiasm for the discovery of new receptors by researchers did not lead to the creation of the unified theory of biochemical process regulation in the living systems with participation of receptors. Moreover, the relationship between the steric structure and the biological activity of various chemical agents are completely ignored in the protein receptor theory. But it is the elementary chemical reactions that underlie the functioning of living systems. In addition, the fundamental laws of chemistry have not yet been canceled.

In contrast to the theory of protein receptors, I proposed the receptors composed from nucleic acids (DNA or RNA) that work in vivo as gene keys and gene locks [1,2]. The gene key that contains TA (or TATA box) nucleotide pairs is presented *in vivo* by single-stranded DNA or RNA (which must contain adenine nucleotides). The starting point of RNA/DNA synthesis is the formation of non-covalent hydrogen bonds between paired AT bp of the lock and the key at the beginning of double-stranded DNA (TA or TATA) and also between one pair of AT bp that can be disposed at different distance to the end of the lock and the key as we can see, for example, in the case with TTGACA and TATAAT boxes. The hydrogen bond is formed between the hydrogen at N6 of the adenine and the oxygen at C2 of the thymine. This process leads to the separation of double-stranded DNA in the lock of the gene or gene clusters.

II. The steric arrangement of amino acids in nucleosomes remains an unsolved mystery.

Each nucleosome is composed of five histone proteins H2A, H2B, H3, H4, and H1 (or H5). Here the term a nucleosome is used separately without the presence of DNA, i.e. as an intact structure. Histones of the first four classes, also called core histones, interact directly with DNA and consist of a significant amount of acidic and basic amino acids. Histones H3 and H4 are arginine-rich histones, while histones H2A and H2B are moderately lysine-rich. The fifth class H1, called linker histone, is represented by very lysine-rich amino acids. Each complex of the structure consisting of H2A, H2B, H3 and H4 histones is associated with one H1 histone molecule [3, 4]. All histone H1 can be removed without disrupting the nucleosome core [5]. From this it follows that H1 is localized outside the histone cortex.

Each nucleosome has a different amount of DNA (147-200 base pairs, bp), "wound" on a nucleosome (more precisely, on a structure that resembles a "disk", "cylinder" or "ball") [5]. Logically, based on current knowledge of chemistry and physics, this is difficult to understand. One can only rely on some intelligent force to make the DNA so neatly wrap itself around the histone proteins rather than slip off it. One thing is clear: the processes of transcription and replication of DNA when interacting with disk-shaped or spherical nucleosomes is a puzzle that is fertile ground for creating various models.

Also, the structural-spatial interactions of DNA and nucleosomes are still not clear: either

DNA "rests" on nucleosomes, or nucleosomes shelter DNA from the attack of active agents.

There is no clear clarity about the role of each class of amino acids in the functioning of histones. It can be assumed that basic and acidic amino acids in the peptide chain occupy the *cis*- or *trans*-position, which is more favorable for the formation of hydrogen bonds both between amino acids and DNA, and between the histones of the nucleosome itself.

Based on the foregoing, I proposed a model of the structure of eukaryotic nucleosomes, which can have the shape of a parallelepiped [2, 6], in which the histones (H3-H4, H2A-H2B and H1) are arranged in a certain order (Fig. 1).

Based on the phenomenon of the presence in the nucleosome of nine basic histone proteins, differing in amino acid composition, it should be assumed that this is not an accident. This means that each protein must perform a specific function both as part of the nucleosome and in cooperation with DNA. The histones of the nucleosome are linked by hydrogen bonds. A hydrogen bond is a form of bonding between an electronegative atom (such as N or O) and a hydrogen atom H that is covalently bonded to another electronegative atom. The HO-groups of phosphoric acid of two DNA polypeptide chains seem to play an important role in the linkage of the DNA strand to nucleosomes. The strongest hydrogen bond can be formed with the arginine nitrogen atoms in the histone H3-H4 molecule. Consequently, histones H3-H4 form the L1 layer for storing DNA by forming hydrogen bonds between the HO-groups of phosphoric acid and the arginine nitrogen atoms of the peptide chain of histone proteins H3-H4. In this case, a more stable combination will be if in the polypeptide chain of histones H3-H4 the radicals ($–NH_2$ and =NH) of arginine are directed to the DNA chain, and the radicals ($–NH_2$) of lysine to the second layer formed from histones H2A-H2B (Fig. 1).

Similarly, in the second L2 layer formed by histones H2A-H2B, the arginine nitrogen radicals are directed to the L1 layer, and lysine to the L3 layer. In the L3 layer, lysine nitrogen radicals are located on both sides. The relatively easy removal of the L3 layer in the experiment may be due to the weaker hydrogen bond between the radicals of the H_2N- groups.

A characteristic feature of proteins is that the peptide bonds are planar. The *cis*- or *trans*-configuration of flanking radicals can also play an essential role in the interaction of histones. The *trans* isomer of the peptide bond is more stable [7]. There are also steric hindrances for the participation of four bond atoms (C, N, O, and H) in the formation of hydrogen bonds with DNA, since they are located in the same plane. Therefore, the role of amino acid flanking radicals can be decisive in the formation of hydrogen bonds. An important feature of the flanking radicals of histone amino acids: significant differences in the length of the chains, which can also determine the fate of the formation of hydrogen bonds both with DNA and between different histones.

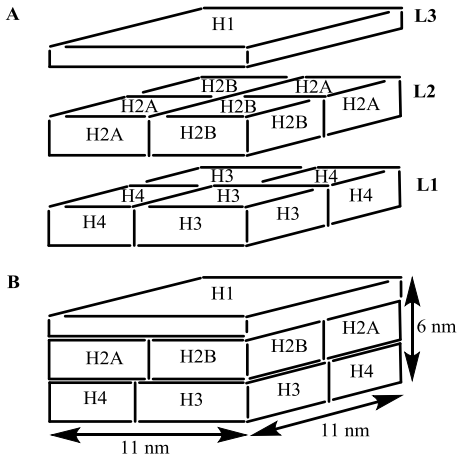

Fig. 1. Proposed schematic structure of the eukaryotic nucleosome (here the term a nucleosome is used separately without the presence of DNA, i.e. as an intact structure). **(A)** Three layers of a nucleosome are composed from different histones: the first layer L1 is formed from H3 and H4 histones, the second layer L2 contains H2A and H2B histones and the third layer L3 is presented by one H1 (or H5) histone. (B) A general view for the complete structure of the nucleosome.

The guanidine group, due to resonant charge delocalization, is strongly basic and is capable of forming multiple hydrogen bonds. Arginine is a basic amino acid bearing two main centers: an amino group at the α-position and a guanidine group at the δ-position. In slightly alkaline and neutral solutions, arginine forms a zwitterion. The ability of arginine to form ionic bonds with phosphate groups of DNA determines the formation of nucleoproteins.

I believe that such a variety of proteins is necessary, most likely, in the formation of the triplet structure of nucleosomes. Therefore, for the formation of a triplet filament, stronger histone bonds are more needed than for the formation of a chromosome as an integral compact structure.

Apparently, histone H1 is necessary for DNA not to bind with it due to the lack of atoms necessary for the formation of hydrogen bonds. For this reason, it prevents the formation of sufficiently strong hydrogen bonds with other chromosomes, which allows the divergence of sister chromosomes during cell division.

An important issue is also the relative position of histones in each of the proposed layers. It is logical that the presence of two classes of histones in each layer is not a random phenomenon. There is nothing superfluous in the nature of the genome. Therefore, the four classes of histone proteins (H2A, H2B, H3 and H4) must have some function. Indeed, for a “disk”, “ball” or “cylinder” on which DNA is “wound”, one class of histones would be enough.

Therefore, the formation of bonds between DNA and histone is possible due to flanking radicals of histones of amino acids. The bond of nitrogen atoms with the HO-group of phosphoric acid DNA will be stronger due to the shorter bond length: the bond length between O-H atoms is 2.67 Å, and between N-H atoms is 2.75 Å [8]. According to other data, the bond length between N-H atoms is 101 pm, and between O-H atoms is 96 pm, respectively, the bond energy will be 391 kj/mol and 467 kj/mol [9]. These data can explain the easiest removal of histone H1 from the L3 and L2 layers of histones by various experimental manipulations.

III. The chromosomes as mysterious structures.

The arrangement of nucleosomes on the chromosome is believed to regulate DNA availability for transcription factors and RNA polymerases [2]. How this happens if the DNA is located outside of the nucleosome is not clear. Moreover, for the initiation of transcription, a small initial part of the gene referred to as a promoter is needed, [3, 4].

However, this point of view can be based on a model of grouping of cylindrical nucleosomes with DNA wrapped around them [3, 4]. How many of these cylinders are located in the thickness of the chromosome is unknown. Chromosome thickness data differ significantly. But how then DNA is protected from attacks by nucleases and reactive agents on the chromosome if it is located outside the nucleosome? Reactive agents include free radicals, reactive oxygen species, and redox systems functioning in the cell. Due to its small size, histone H1 is unlikely to function as a protective umbrella for DNA.

The structure of the chromosome is the key to the functioning of the genome. Even theoretically, it is difficult to simulate the formation of sister chromatids from the proposed models of DNA packaging around nucleosomes in a whole chromosome [3, 4]. In addition, there is no definite answer as to how genes are located on an unbroken chromosome: along or across the chromosome.

In the proposed models in the literature, DNA is wrapped around nucleosomes [3-4]. There is no logical explanation why DNA is so irrationally packed: the distance between nucleosomes is about 15 bp [10], which corresponds to about 4.4 nm. This is much smaller than the size of RNA polymerases.-

In order not to burden the thinking reader with difficult-to-understand questions, I proposed an alternative model of chromosome construction (Fig. 2) [5-6]. The ribbon of DNA is located between the two layers of the nucleosome, which form the chromosome. From the point of view of the synthesis of a new chromosome, this is the most advantageous conformation.

In the model of gene packing on the chromosome proposed here, two options are possible: the promoter can be linked to the next DNA sequence of the gene, i.e. hidden by nucleosomes (Fig. 3) or protrude beyond nucleosomes (Fig. 4). Since chromosomes can nevertheless be attached to the wall of the nucleus, it is possible that some of the gene promoters are open and can be influenced by the pH of the medium and untwist, which is important in stressful situations for the rapid transcription of protective genes without the participation of specific receptors.

Two fundamental processes of redox reactions determine the large extent many biological

processes, including functioning of nucleic acids. In this regard, DNA and RNA must have reliable defense mechanisms. Consequently, nucleosomes are not only the architectural structure for storing DNA, but also an element of DNA protection against reactive agents and enzymes. The side of the nucleosome that contacts with DNA does not contain any reactive groups. Substances such as purine, adenine, guanine, pyrimidine and cytosine have unsaturated bonds that are less reactive than =NH or –OH in deoxyribose, ribose, uracil and thymine.

It should also be emphasized that the processes of methylation and acetylation in this model can only occur on promoters. Methylation or acetylation of the entire gene is possible due to the removal of nucleosomes. In addition, the chemical composition and structure of nucleosomes must be closely related to the structure of chromosomes.

In conclusion, one more feature of chromosome structure should be mentioned. After the removal of nucleosomes remains the protein backbone (scaffold) of the chromosome [11]. The real functions and steric structure of such structure remain to be clarified.

IV. DNA replication: Do in vitro reactions correspond to in vivo reactions?

During DNA replication, both new DNA strands and new nucleosomes are formed. In this case, the chromatin assembly factor (CAF-1) first attaches the tetramer to the DNA of the $H3_2$-$H4_2$ and then adds H2A-H2B dimers [4, 12].

Before considering the process of DNA replication or transcription, it is necessary to clarify the question of the place of DNA in the chromosome. Here I am not considering the data about DNA location on the surface of cylindrical nucleosome. This view needs further investigations.

It is believed that there are 14 contacts (chemical bonds) of DNA with the individual nucleosome [13]. However, the type of chemical bonds between these structures has not yet been elucidated. How and by what these structures are performed the mechanism of precise wounding of DNA from nucleosome to nucleosome on the surface of the nucleosome cylinder is not clear. There is no logical explanation for why DNA should first wind up on nucleosomes and then unwind, spending energy resources.

Based on the foregoing, the proposed mechanism of DNA replication and synthesis of the new chromosome is shown in Fig. 5 as an example of single triplet. Elementary reactions of DNA replication are not considered here. A general mechanism shown here is based on the chromosome model presented in Fig. 2. It is also unknown whether individual primers (gene keys) are needed for the synthesis of each of the genes in the chromosome? If not, the separation of the DNA promoter strands is possible due to changes in the salt concentration in the cytoplasm and the cell nucleus. This is the most energetically advantageous option for both replication and transcription. Also, there is no clarity about the attachment of nucleosomes to the DNA chain on the chromosome: do nucleosomes do this spontaneously, or does DNA polymerase contribute to this?

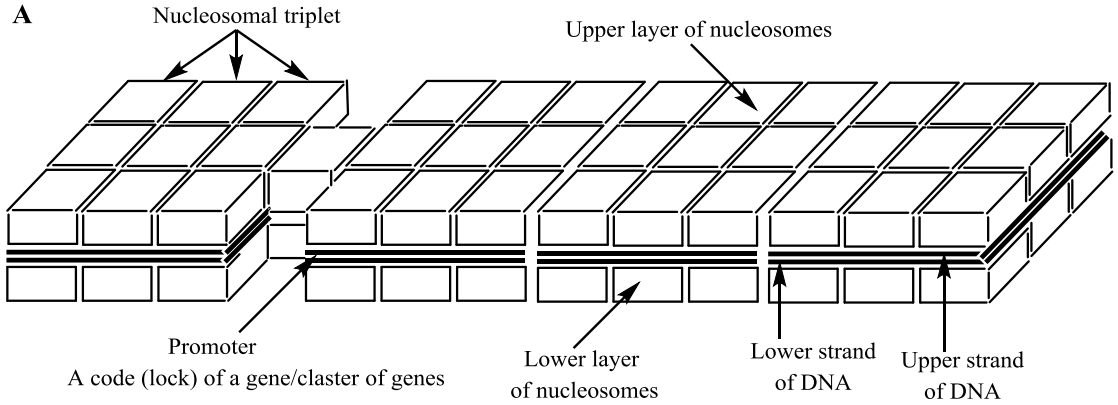

Fig. 2. A simple model for the packaging of DNA and nucleosomes into the chromosome: a general view of the chromosome.

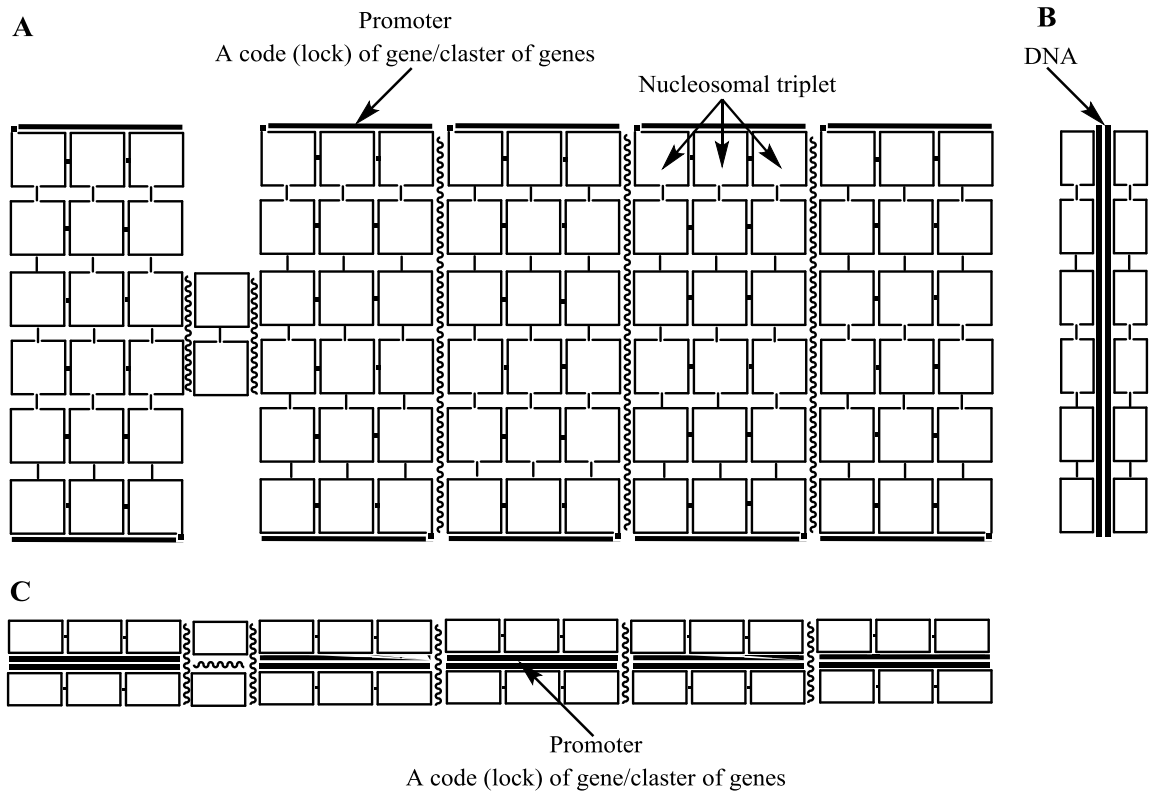

Fig. 3. A simple first model for the chromosome structure. (A) view from the top of chromosome, **(B)** view from the end of chromosome, (**C**) view from the side of chromosome. Here a promoter is designated as "a lock (or code) of gene/claster of genes".

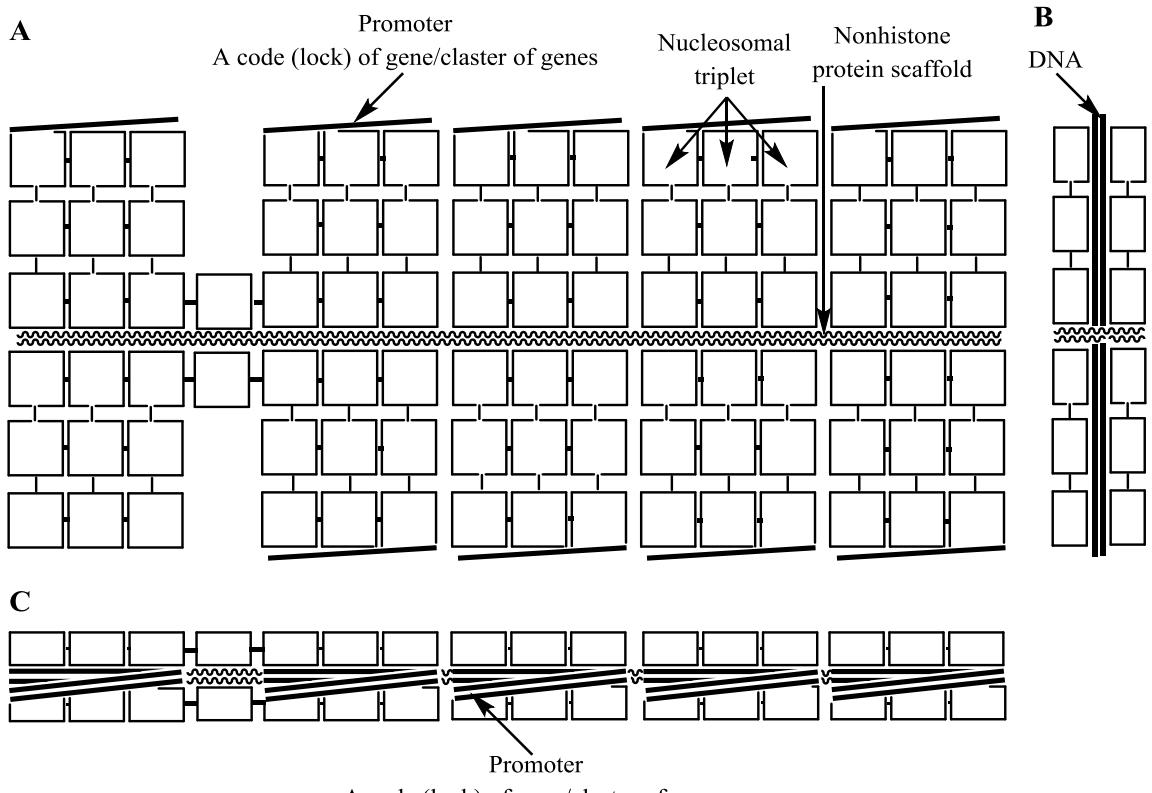

Fig. 4. A simple second model for the chromosome structure. (A) view from the top of chromosome, (B) view from the end of chromosome, (C) view from the side of chromosome.

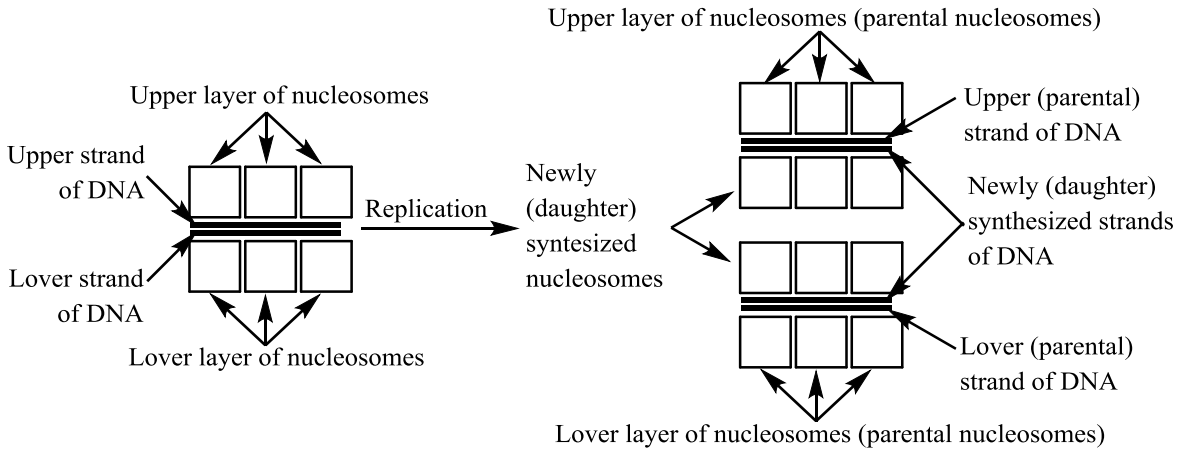

Fig. 5. A model to explain the synthesis of novel chromosomes and DNA replication.

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СИГНАЛІЗАЦІЯ В ЖИВИХ СИСТЕМАХ : ГЕННІ КЛЮЧІ ТА ГЕННІ ЗАМКИ

У цій статті коротко розглядаються складні та заплутані механізми функціонування сигнальних систем між плазматичною мембраною та ядром живих організмів. Описано альтернативну модель упаковки ДНК. Віхою цієї моделі є припущення, що ДНК не обертається навколо октамера гістону: вона організована між двома шарами нуклеосом. Основною структурою хромосом є триплет нуклеосом. Одиночна нуклеосома має форму паралелепіпеда (11x11x6 нм). Альтернативою білковим рецепторам є запропоновані рецептори побудовані із нуклеїнових кислот (ДНК, РНК), які називаються генними ключами, що відкривають код (генний замок) гена/кластера на промоторі.

Ключові слова: нуклеосома, хромосома, гістони, генний ключ, генний замок.