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REVIEW

Structural–Functional Model of the Mitotic Chromosome

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Abstract—In the present review the structural role of noncoding DNA, mechanisms of differential staining of mitotic chromosomes, and structural organization of different levels of DNA compactization are discussed. A structural–functional model of the mitotic chromosome is proposed based on the principle of discreteness of structural levels of DNA compactization.

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Key words: chromatin, chromosome, mitosis, banding, chromomere, chromonema

Impressive progress has been achieved during recent years in studies on organization of mitotic chromosomes. Various proteins that control mitosis have been detected and characterized in detail, including proteins involved in segregation and compactization of mitotic chromosomes. The most of the findings are interpreted in the framework of the popular "radial-loop" model, which postulates the mitotic chromosome to have a special structure, or chromosome scaffold. Nevertheless, the problem of macromolecular organization of different structural subdomains of chromosomes remains open.

In addition to protein-encoding "structural" genes, genomes of eukaryotes contain a large amount of DNA (~90%) that encodes no proteins and plays no noticeable role in the control of gene expression [1, 2]. This fraction of the genome is represented by unique sequences flanking structural genes, or introns, and also repeated sequences which are arranged in tandems or dispersed in the genome. Different types of nucleotide sequences in chromatin of interphase nuclei and mitotic chromosomes form discrete clusters, which are sometimes called isochores [3]. In the cytological literature chromosome loci free of transcribable genes are usually called constitutive heterochromatin, the loci containing both non-transcribable sequences and inactivated structural genes are called optional heterochromatin, and the loci enriched with expressed genes are called euchromatin [4]. These regions can be revealed in mitotic chromosomes by an easy technique of "differential staining" [5-7]. Although the molecular mechanisms of differential staining of mitotic chromosomes are still unknown, this approach shows the distribution of genes lengthwise on the chromosomes, clusterization of definite DNA sequences, and chronology of their replication [5] (Fig. 1).

Functions of noncoding DNA, which forms the bulk of the genome, are still under discussion. Some elements of this DNA fraction probably act as "modulators" changing the local conformation of the expressed chromatin. This hypothesis is supported by experimental detection of a partial or complete inactivation of genes translocated into the region of heterochromatin. The corresponding phenomenon, which was first discovered in experiments on *Drosophila melanogaster*, is called the "position effect" [8]. The position effect is a typical example of the so-called epigenetic regulation of the genome, which is not associated with mutations but based only on changes in the structural state of a certain locus of the chromosome and the status of DNA methylation. Such regulation plays a significant role not only in the gene expression but also in genetic recombination [9].

Another hypothetical function of noncoding DNA (which can be also directly or indirectly associated with the regulation of expression) seems to be its involvement

Abbreviations: DNP) deoxyribonucleoprotein; n.p.) nucleotide pair; BrdU) 5-bromo-2-deoxyuridine; RS) replication sites.

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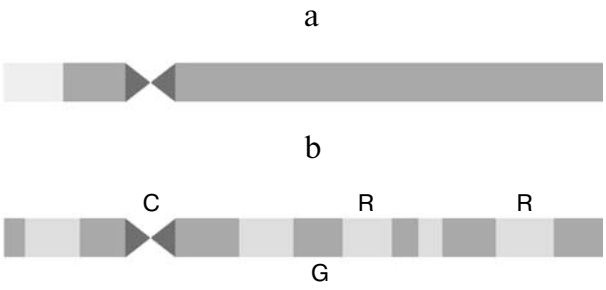

Fig. 1. Lengthwise heterogeneity of mitotic chromosomes revealed by differential staining. a) Fraction of encoding sequences in the eukaryotic genome; b) molecular organization of different type segments. R-segments: 82% of genes, high content of GC-rich sequences, early replication, low level of DNA methylation; G-segments: 18% of genes, high content of AT-rich sequences, late replication, high level of DNA methylation; C-segments: the absence of genes, frequently repeated sequences, late replication, high level of DNA methylation.

in formation of the macromolecular organization of chromosomes [6, 10]. In this case, individual elements of such DNA can serve as "landing fields" for hypothetical "clamp proteins" and ensure different levels of chromatin packaging. Specificity of the distribution of such sequences can be responsible for heterogeneity of the structural lengthwise organization of chromosomes, which can be revealed by different molecular and morphological analytical approaches.

This review considers experimental data on DNA topology in mitotic chromosomes, mechanisms of induction of longitudinal and transversal differentiation of chromosomes, and the possible role of noncoding DNA sequences in the structural–functional organization of chromosomes.

STRUCTURAL LEVELS OF DNA COMPACTIZATION IN INTERPHASE AND MITOTIC CHROMOSOMES

The molecular composition of chromosomes is known in rather good detail. They can be formally described as giant nucleoprotein complexes composed of DNA, histones, some specialized nonhistone proteins, and a small amount of RNA. The most important for the structure are basic proteins, or histones, which are responsible for the "primary" compactization of the genetic material. Histones interact with DNA and produce discrete particles of the same size and structure, or nucleosomes, which are the universal chromatin subunits in all eukaryotes. Each nucleosome contains ~200 nucleotide pairs (n.p.) of DNA coiled on the surface of a histone octamer (H2A, H2B, H3, and H4). Nucleosomal fibrils can be isolated from the nuclei only under low ionic strength or after extraction of the H1 histone. The molecular organization of these fibrils has been rather scrupulously characterized in many works [11].

The next level of compactization is represented by ~30-nm thick deoxyribonucleoprotein (DNP)-fibrils [12-15]. According to the most commonly accepted hypothesis, this 30-nm fibril is a solenoid formed of supercoiled nucleosomal fibril in which nucleosomal octamers are associated with the H1 histone [16]. According to other data, the 30-nm fibril consists of nucleomeres, or "superbeads", which are discrete substructures joining from six to ten nucleosomes [17-20]. Some authors think that the 30-nm fibril lacks a strictly ordered organization [21]. Supposing a single coil of the solenoid spiral (or a single nucleomer) includes six nucleosomes, simple calculations show that the degree of DNA compactization in the 30-nm fibril has to be ~40. However, the compactization in mitotic chromosomes is much higher and reaches ~10,000 [22]. To elucidate how 30-nm DNP-fibrils form the structures of the higher order is still a fundamental problem in studies on mitotic chromosomes.

In many early works, mitotic chromosomes of the majority of plant and animal materials were shown to have a structural base of fibrillar elements, which were called chromonemata because of their ability to absorb some "basic" dyes. Many scrupulous descriptions of cell division resulted in the conclusion that chromonemata are stable "elementary" structural complexes of chromosomes during the cell cycle [23]. It is fundamentally important that chromonema elements have been successfully visualized supravitally in large chromosomes of the lily *Haemanthus katharinae* [24]. The ultrastructure of chromonemata in decondensed telophase chromosomes has been described in some higher plants [25-29] and animals [15].

Macromolecular chromatin complexes were described later as fibrillar chromonemata in interphase nuclei of humans, *D. melanogaster* [30, 31], Chinese hamster [32], and the onion *Allium cepa* [33]. It should be noted that the general term "chromonema" is used for denoting various types of fibrillar chromatin structures, which can vary in their structural organization [31].

The pattern of DNA compactization in chromonemata remains unknown. In particular, chromonemata were supposed to consist of discrete globular complexes, or "elementary" chromomeres [34]. Chromomeres are especially distinctly detected as globular structures of ~100 nm in diameter in nuclei isolated under high concentrations of bivalent cations. Partial deproteinization of chromatin of such nuclei in the presence of polyanions (a mixture of heparin with dextran sulfate) results in releasing of so-called rosette-like complexes which consist of numerous (15-20) loop-like structures with their bases fixed in the dense central core of the rosette [35, 36]. The length of a loop varies from 0.3 to 1 μm, and the total length of the loops in a complex is 15-20 μm (10-60 thou-

sand n.p. (t.n.p.)). The treatment of this fraction with protease completely destroys the rosette-like complexes.

Similar structures have been described in chromatin preparations deproteinized with 2 M NaCl [37] and in mitotic chromosomes treated with ammonium acetate [38]. Numerous arguments supporting the pre-existence of rosette-like complexes in intact chromatin and the lack of their production via nonspecific aggregation of relaxed DNA with nuclear proteins are presented in [38]. The same "radial-loop" principle seems to underlie the organization of hypothetical nuclear subdomains (chromomeres) corresponding to associated replication and transcription factories [39]. According to another hypothesis, the loop-like organization of chromomeres is determined by the exon–intron structure of the genes in these domains. The loops interact with the protein core of rosettes through the intron regions [40].

And what is the chromomere organization based on the known concepts about the structural levels of DNA compactization? The mean length of the "rosette" loop has been stereologically shown to be from 0.3 to 1 μm. Consequently, the relaxed DNA of a single loop can produce 5-17 nucleosomes during compactization; 6-8 nucleosomes form the next level of compactization that is a nucleomere (superbead) or a short hairpin of a solenoid DNP-fibril. Finally, the compactization of all loops results in a globular structure, a chromomere, which contains 20-40 nucleomers. Successive decondensation of this structural complex has to result in unfolding of the chromomere at first to the nucleomeric and then to the nucleosomal DNP-fibril. The discrete organization of the chromomer suggests a possibility of local decompactization of one or more nucleomers without significant disturbance in the integrity of the chromonema complex (Fig. 2).

ROLE OF HISTONES AND NONHISTONE PROTEINS IN THE STRUCTURAL ORGANIZATION OF MITOTIC CHROMOSOMES

It has been established that the mitotic chromosome compactization is accompanied by two important events: phosphorylation of histones H1, H3, and H2A [41-43] and recruitment onto chromosomes of some nonhistone proteins, such as topoisomerase II [44-47], SMC-proteins [48-50], a giant filamentous protein D-titin [51-53], and AKAP95 protein bound with A-kinase [54].

Mechanisms of induction by histones and/or nonhistone proteins of a strictly ordered chromatin condensation during mitosis are still unknown. As supposed in [43], modified histones produce local decondensed regions in chromatin, and this makes it available for other proteins, including SMC-proteins ("condensins"), which are directly involved in the chromosome compactization. By an alternative hypothesis, phosphorylated histones should act as "receptors" for binding of factors responsible for the condensation [55].

According to the radial-loop model, nonhistone proteins (including topoisomerase II and SMC-proteins)

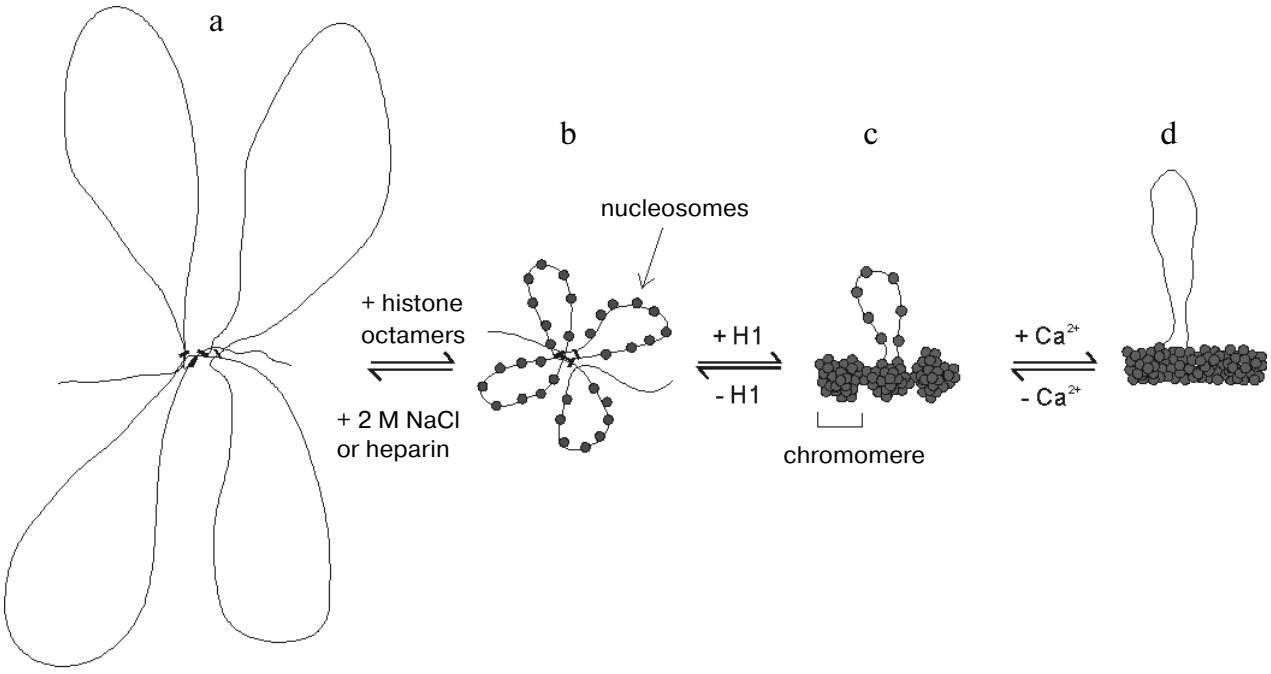

Fig. 2. Hypothetical scheme of elementary chromomere organization. a) Bases of the loops of a decompacted chromomere are associated with structural nonhistone proteins; b-d) successive stages of compactization of DNA loops during its interaction with proteins.

together with some other proteins form the protein scaffold of mitotic chromosomes [56-58]. According to this model, it is postulated that under physiological ionic strength DNA of loop domains is compacted to short rod-like structures with involvement of "nucleosomal" histones and histone H1 [59]. Because the radial-loop model is based on data obtained on chromosomes with extracted histones, it is possible that loosely bound nonhistone proteins that are not scaffold components but can play an important structural role for higher levels of DNA compactization, chromonemata and/or mitotic chromosomes themselves, can be removed from chromatin under these conditions.

Data on photostabilization of isolated nuclei with light in the presence of ethidium bromide indirectly suggest the existence of special "structural" chromatin proteins [60]. In an *in vitro* system, salt extraction of irradiated nuclei extracts histone H1 and decondensed chromatin to nucleosomal fibrils, but does not affect the integrity of DNA macromolecular complexes [61]. Biochemical analysis indicates that the radiation-induced cross-linking between proteins and nonhistone proteins plays an important role during this process [62].

Photostabilization was also exemplified by mitotic chromosomes: after irradiation of cells permeabilized in a solution with low contents of bivalent cations, chromosomes became very resistant to extraction with 2 M NaCl and decompactization [63].

Results of enzymatic digestion indirectly indicated the possible nature of photoinduced cross-linking in mitotic chromosomes. Treatment with DNase I did not abolish the resistance of photostabilized chromosomes to calcium-free solutions; therefore, the radiation was suggested to be unable of producing numerous cross-links between DNA molecules. Conversely, the complete destruction of irradiated chromosomes in decondensing buffer after treatment with protease K indicated the decisive contribution of nonhistone proteins to photostabilization.

The above-presented data allowed us to state some opinions concerning the "nativeness" of nuclear material and/or chromosome scaffold which are believed to play an important role in the structural–functional organization of interphase and mitotic chromosomes [64]. The electron-dense network of the scaffold is reliably visualized in the axial regions of chromatides after exhaustive extraction of histones [56]. Nearly the same conditions are required to obtain the nuclear matrix [65]. After irradiation, which selectively stabilizes chromatin, the extraction of histones does not reveal the matrix in interphase nuclei or the axial scaffold in mitotic chromosomes [66]. This supports the idea that both structures (the nuclear matrix and chromosome scaffold) are produced as a result of the total destruction of chromatin and nonchromatin subdomains of the nucleus which leads to the artifactual redistribution and nonspecific aggregation of nonhistone proteins [67, 68]. Dramatic changes in elastic properties of native metaphase chromosomes caused by a restricted treatment with nuclease [69] also suggest the absence of an uninterrupted rigid protein skeleton of chromosomes. The structural organization of macromolecular complexes of chromatin and mitotic chromosomes is more likely maintained by the system of discrete protein clamps dispersed in the chromosome volume.

ROLE OF Ca^{2+} IN LENGTHWISE DIFFERENTIATION OF MITOTIC CHROMOSOMES

Bivalent cations are known to play a important role in such processes as regulation of the cell cycle, growth, differentiation, cell mobility and division, embryogenesis, intercellular interactions, programmed cell death (apoptosis), etc. [70]. The most important property of bivalent cations, especially Ca^{2+} and Mg^{2+}, is their ability to interact with negatively charged phosphate residues of DNA. The affinity of Ca^{2+} for DNA is the highest [71]. The interaction of bivalent cations with DNA results in chromatin compactization or folding, and because of this Ca^{2+} and/or Mg^{2+} are often used for stabilization of macromolecular DNP complexes [72], including isolated mitotic chromosomes [73]. In contrast, the binding of Ca^{2+} with chelating agents induce serious disorders in the course of mitosis, in particular, it inhibits the separation of sister chromatides in the end of metaphase [74].

In the *in vitro* system the "solubility" of chromatin and, respectively, the degree of its compactization in solutions with low ionic strength directly depends on the concentration of bivalent cations [75]. It is important that the total chromatin isolated from rat liver nuclei contains a minor fraction not precipitated with Ca^{2+} or Mg^{2+}. Biochemical analysis indicates that this fraction contains DNA sequences enriched with GC base pairs and a considerable fraction of a newly produced RNA [76]. This means that "titration" of the total chromatin with bivalent cations can be used as a rather easy approach for preparative separation of the unexpressed heterochromatin from the transcriptionally active euchromatin. Based on the data obtained in the above-cited works, it was proposed to gradually change the concentration of calcium to visualize eu- and heterochromatin regions in mitotic chromosomes. This idea was realized on the isolated mitotic chromosomes from Chinese hamster cells [77, 78].

Gradual decrease in $CaCl_2$ concentration from 3 to 0.5-0.4 mM resulted in appearance in isolated chromosomes of local decondensation zones located symmetrically in the sister chromatides. Routinely stained and differentially decondensed marker chromosomes were compared, and the compact blocks and decondensed regions

were shown to correspond to G- and R-segments, respectively [78]. Decrease in the $CaCl_2$ concentration to 0.4-0.2 mM made the chromosomes twice longer than they were initially and caused the disappearance of compact segments in the chromatide shoulders, whereas the centromeric regions remained condensed. Thus, the resistance to the decondensing exposure directly correlated with the genetic status of the corresponding locus. Zones of so-called constitutive heterochromatin (centromeric heterochromatin) lacking protein-encoding DNA sequences were the most rigid. The optional heterochromatin of G-segments markedly impoverished in structural genes displayed an intermediate resistance. Finally, R-segments containing the majority of protein-encoding sequences were the most sensitive to decondensing exposure (Fig. 3).

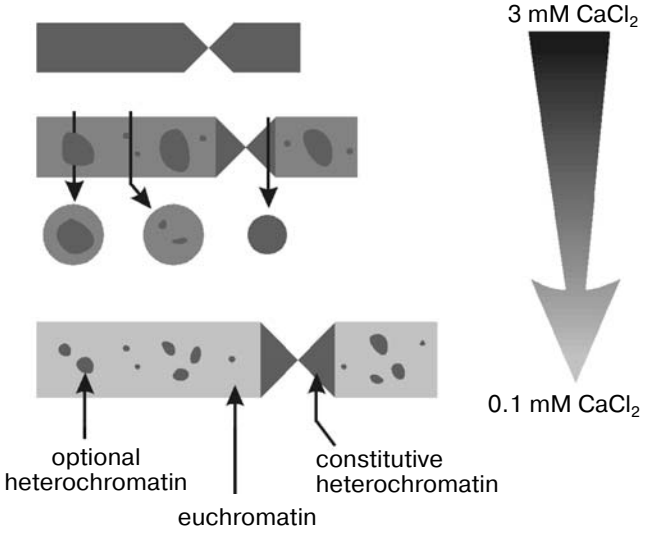

Fig. 3. Differential decondensation of mitotic chromosomes in solutions with gradually decreased calcium ion concentration.

Return of decondensed chromosomes into buffer with the initial concentration of Ca^{2+} is accompanied by their rapid recondensation, and such "recondensed" chromosomes retain the ability for repeated differential decondensation. Note that extraction of nonhistone HMG-proteins completely inhibits the differential decondensation of chromosomes. HMG-I/Y are shown to contain the so-called AT-hook-domains which have a high affinity to AT-rich sequences specific for bases of DNA loops [79]. AT-hook-domains seem to play an important role in chromosome maintenance [80]: their not uniform distribution lengthwise of the chromosomes [81] can directly or more likely indirectly (through special calcium-binding nonhistone proteins) determine the local specific features of structural organization of different segments of chromosomes and their behavior during differential condensation.

Note that the phenomenon of differential staining has been mainly studied during the stages of chromosome treatment after their fixation. In no work the possible influence of hypotonic pretreatment of living cells on the structure of chromosomes was considered, although such treatment is always used to obtain preparations of spread chromosomes.

It is reasonably to suppose that the differential decondensation of chromosomes associated with the genetic status of their individual loci can exist in living cells incubated in a hypotonic medium. The possibility of *in vivo* differential decondensation of mitotic chromosomes was tested on cells subjected to short hypotonic treatment. Hypotonic solutions are known to cause completely reversible decondensation of interphase chromatin and mitotic chromosomes [82, 83]. In particular, such is the reaction of chromosomes on the prefixation treatment of living cells with 0.075 M KCl, which is most often used in the known procedures of differential staining [84].

Electron microscopy shows that even 5-10 sec of treatment with 0.075 M KCl cause manifestations of differential decondensation of mitotic chromosomes. To reveal what segments correspond to compact and decondensed regions, the segmentation pattern of marker nucleolus-forming chromosomes stained by the routine G-technique was compared with topology of differentially decondensed regions of the same chromosomes reconstructed by serial sections. In individual chromosomes identified in such a fashion the zones of condensation strictly corresponded to G-segments. Consequently, the structural heterogeneity of mitotic chromosomes imitating the typical segmentation appears even on the first stage of the cell pretreatment before their subsequent differential staining. As a result, regions with differently exposed chromatin are produced lengthwise of the chromosomes, with respect to enzymatic and extracting treatments used directly before the staining with Giemsa or fluorochromes which seem to "aggravate" the preexistent structure. This finding apparently contradicts numerous data that reveal segmentation in "native" chromosomes. However, in all works demonstrating the segmentation of chromosomes by phase contrast and light microscopy [85], staining by Felgen [86], or by electron microscopy [87-89], living cells were imperatively pretreated with hypotonic 0.075 M KCl for obtaining preparations of chromosomes.

Similarly to the *in vitro* system, differential decondensation of chromosomes under these conditions was induced by changes in the Ca^{2+} concentration in the cytosol of dividing cells [90]. Immediately after hypotonic shock, during decompactization of mitotic chromosomes, the Ca^{2+} concentration in the cytosol decreased; the recondensation of chromosomes correlated with recovery of the initial concentration of free Ca^{2+}. When the Ca^{2+} transport across the plasma membrane was prevented with glutaraldehyde, the recovery of chromosome

structure was also disturbed. This effect could be abolished by addition of a calcium ionophore into the incubation medium. It was important that the ionophore increased the Ca^{2+} concentration in the cytoplasm to the level corresponding to that in the initial cells. That was about two orders of magnitude lower than the concentration of Ca^{2+} used for stabilization of isolated chromosomes. Both Ca^{2+} and Mg^{2+} were detected in mitotic chromosomes by mass-spectrometry of secondary ions in cryosections of tissue culture cells [71]. According to calculations, concentrations of these ions in chromosomes are rather high: one Ca^{2+} atom falls on every 12-20 DNA nucleotides and one atom of Mg^{2+} falls on every 20-30 nucleotides. But it is more likely that bivalent cations display their compacting effect not by direct interaction with DNA but as metalloproteins. Thus, some nonhistone proteins, including topoisomerase II, have been shown to bind Ca^{2+} [71, 91, 92].

Based on the above-presented data, it can be concluded that the structural–functional reconstruction of chromosomes in living cells is regulated by Ca^{2+} (possibly with involvement of Ca^{2+}-binding proteins).

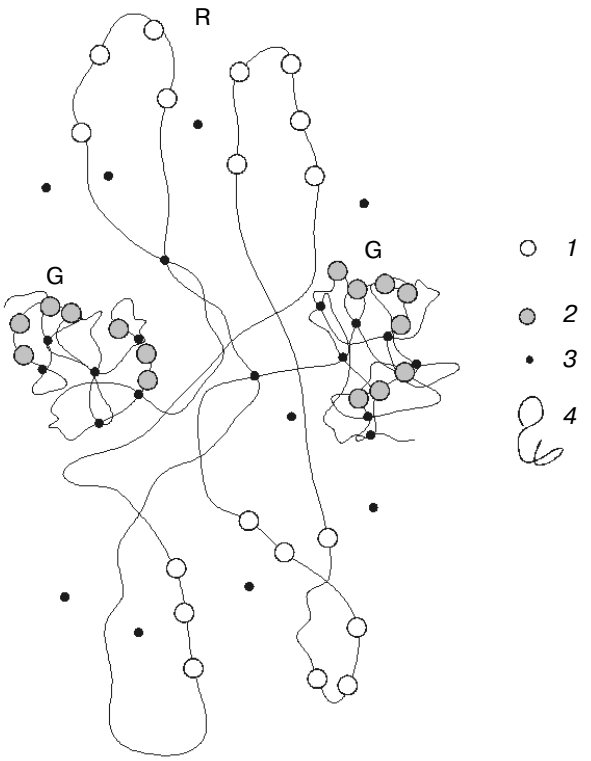

Fig. 4. Topology of early and late replicating chromatin in the G- and R-segments of differentially decondensed mitotic chromosomes. *1*) Early RS; *2*) late RS; *3*) clamp proteins; *4*) chromatin fibril.

LENGTHWISE AND TRANSVERSE HETEROGENEITY OF MITOTIC CHROMOSOMES IS A CONSEQUENCE OF DIFFERENT RESISTANCE OF CHROMATIN CONTAINING STRUCTURAL GENES (EUCHROMATIN) AND NONCODING DNA (HETEROCHROMATIN) TO DECOMPACTING TREATMENTS

The lengthwise and transverse heterogeneity of chromosomes can be studied in particular using the approach of so-called replication banding, which is based on differences in the time of replication of the R- and G-segment materials [93-95]. In contrast to differential staining, the replication, or "dynamic" banding rests on incorporation into DNA of 5-bromo-2-deoxyuridine (BrdU) and its subsequent immunocytochemical detection in fixed preparations. By immunofluorescent microscopy, BrdU can be revealed in the interphase nuclei as multiple small foci or spots, which have been called replication sites (RS) [96-98]. In cytological preparations, RS appear as small fluorescent globules located in the nucleus depending on chronology of the DNA replication [99-105]. RS, which are tested with antibodies to BrdU, are not transitory complexes but are retained as discrete structures during all stages of the cell cycle, including mitosis, and even during some generations of dividing cells [64, 95, 103-105]. Thus, RS can be used as reliable markers in studies on the structural organization of R- and G-bands in decondensed chromosomes.

Spatial changes in RS in differentially decondensed mitotic chromosomes of PK cells have been studied [106]. For decompactization of mitotic chromosomes an ingenious procedure of double hypotonic treatment of cells was developed which combined two approaches: the primary long-term exposure which resulted in compact axial structures in sister chromatides [107] and the short-term exposure of cells readapted to a normotonic medium, which allowed us to visualize the G-segments [84]. These exposures were reversible and not lethal for the cells but provided clearly pronounced differential decondensation of chromatides into two components, globular axial structures and peripheral DNA-containing halo. The axial granules corresponded to G-segments. As a result, in accordance with previously published data [84, 88], the procedure of double hypotonic shock selectively modified the material of R-bands. It seems that the discrete axial chromatide complexes corresponding to G-bands are a fundamental feature of the supramolecular organization of mitotic chromosomes and their structural–functional organization is likely to be equivalent to that of chromomeres of meiotic chromosomes [108, 109].

It is especially interesting that in differentially decondensed chromosomes the behaviors of RS corresponding to the early and late replicating chromatin are fundamentally different: the RS labeled during the early S-phase are detected in the peripheral halo, whereas the RS labeled during the late S-phase remain in the axial

granules in the axial region of chromatides. This phenomenon can be caused by the different macromolecular composition of the R- and G-segments. These data do not contradict the hypothesis that the differentially decondensed chromosomes contain two types of loops: short ones in the G-segments and long ones in the R-segments [81] (Fig. 4). Considering the distribution pattern of condensins and topoisomerase II (basic proteins of the chromosome scaffold) with their maximum concentrations alternating lengthwise of the metaphase chromosome [110], these proteins are supposed to stabilize the loops in the R- and G-segments, respectively. However, a high mobility of condensins and topoisomerase II during mitosis along with retention of the compact chromosome structure [111-114] does not allow us to unrestrictedly consider them as "clamps" maintaining the supramolecular organization of chromosomes.

THE MODEL

The proposed model is based on the principle of "discreteness", which considers mitotic and interphase chromosomes to represent a hierarchy of interrelated structural–functional domains: nucleosomes, nucleomeres (superbeads), chromomeres, and chromonemata (Fig. 5).

In the general architectonics of mitotic chromosomes the key role belongs to loci that contain noncoding DNA, presumably consisting of frequently repeated sequences of satellite DNA. These loci interact with specific structural nonhistone proteins (X-"clamps") (Fig. 5a). The nontranscribable (constitutive) heterochromatin, which is usually located in the centromeric regions of chromosomes (C-segments), is represented by homogenous repeated sequences of satellite DNA. Respectively, numerous structural proteins are exposed on these loci of the chromosomes. The optional heterochromatin of the G-segments, in addition to frequently repeated sequences associated with structural proteins, contains fragments of protein-encoding DNA. This DNA seems to be transcriptionally inactive [5, 7]. The R-segments (euchromatin loci) have an increased content of genes, especially housekeeping genes, with intercalated noncoding DNA associated with the structural proteins.

The proposed scheme leads to two important conclusions. First, frequently repeated sequences of noncoding DNA and, respectively, associated with them structural proteins (X-clamps) do not produce an uninterrupted rigid axial structure of chromatides but are dispersed in the whole volume of chromosomes. Second, the frequency of X-cross-links per unit of the chromosome volume is not uniform and seems to be associated with the genetic status of the chromatin: the highest density in the location of clamp proteins is inherent in the loci of constitutive heterochromatin (C-segments), the lower number of

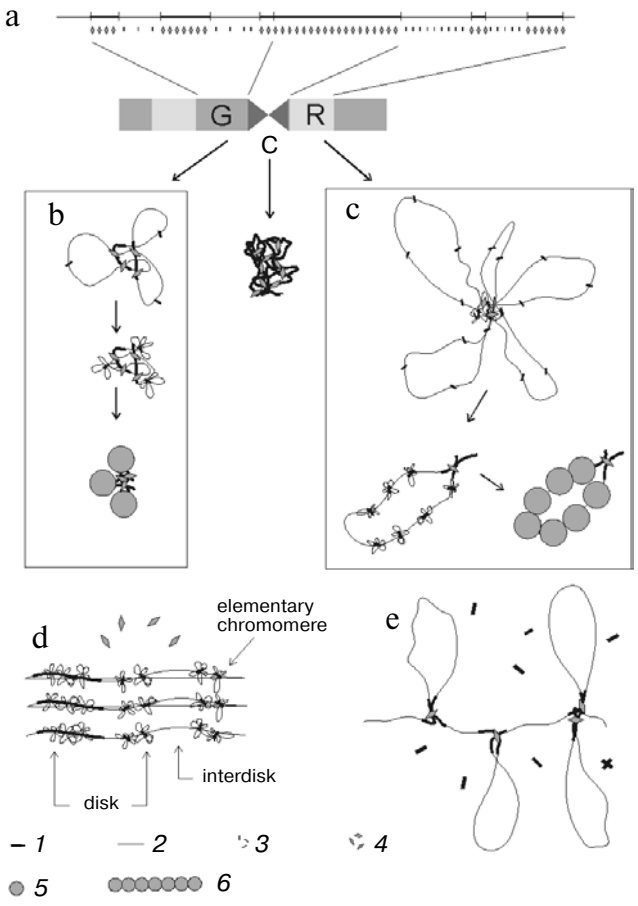

Fig. 5. Distribution of hypothetical clamp proteins in euchromatin and in optional and constitutive heterochromatin. a) The genome fragment located near the centromeric region; b-e) schemes illustrating the role of clamp proteins in organization of the higher levels of DNA compactization (chromomeres and chromonemata) in G- (b) and R-segments (c), and in formation of polytene chromosomes (d) and lamp-brush chromosomes (e). *1*) Frequently repeated protein-noncoding DNA; *2*) protein-encoding DNA; *3*) clamp proteins Z; *4*) clamp proteins X; *5*) elementary chromomere; *6*) chromonema.

cross-links is present in the intercalary heterochromatin of the G-segments, and the euchromatin loci of the R-segments are strongly impoverished in clamp proteins (Fig. 5, a and c).

Compactization of DNA in chromosomes occurs via its interaction with histones and nonhistone proteins.

The chromomere, a compact macromolecular complex that includes a number of loop DNA domains, is an "elementary" structural–functional unit of the remaining chromatin (Fig. 1).

The structural integrity of the chromomere is ensured by nonhistone proteins, which produce the specific second order clamps (Z-clamps) (Fig. 5b). Z-Clamps are supposed to be labile Ca^{2+}-binding nonhistone proteins.

At the cost of additional compactization of linkers, the adjacent chromomeres form the next, fibrillar, level of

DNA packaging, or chromonema [115]. The packaging of chromonemata in mitotic chromosomes is controlled by SMC-type proteins and/or Ca^{2+}.

The structural rearrangements of chromosomes during ontogeny of some eukaryotes can be easily explained in the framework of the proposed model (Fig. 5, d and e): the selective elimination of X-cross-links can result in the unfolding of chromosomes into elongated structures, which after repeated replications produce polytene chromosomes in the cells of *Diptera* larvae or giant (polytene?) chromosomes of plants. After removal of Z-cross-links, lateral loops are produced in chromatides, which are specific for lamp-brush chromosomes.

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