Cell Research (1993), **3**, 1–9

FEATURE

The chromosome as a dynamic structure of the cell nucleus

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Our view of eukaryotic chromosomes is still very much dictated by the classic ideas of geneticists and cytologists considering the chromosome just as a vehicle for genes. This one-sided view of chromosomes may have been strongly influenced by the many cytological observations made on polytene chromosomes. They, in particular, revealed that the activation of genes for transcription is accompanied by local decondensation of the chromosome as it becomes evident in the formation of puffs in polytene chromosomes[1]. The studies of chromosomes have for many years been focused on the relationship between genes and bands or interbands as they are seen in polytene chromosomes (or in the supposed equivalents, the chromomeres, of meiotic prophase chromosomes). This has yielded much fruitful informations such as the first direct evidence for the relationship between differential gene activity and cellular differentiation by the demonstration by Clever[2] that steroid hormones directly regulate gene activity at the chromosomal level.

Studies on polytene chromosomes, however, do have the disadvantage that certain chromosomal elements, in particular centromeres and telomeres, as well as major heterochromatic regions of the genome, are usually not easily recognized or are even not detectable by microscopy. Thus polytene chromosomes have not been particularly useful in studies of these important chromosomal components. Only more recently, attention has been paid to the telomeric regions in polytene chromosomes (for review see [3-5]). Centro meric regions have mainly been studied in diploid cells[6], since in polytene chromosomes these are often underreplicated. This is even more true for other heterochromatic chromosome regions, which often are not replicated at all during polytenization and may, in fact, be partially discarded from the chromosome as recent studies by Spradling and his coworkers[7] indicated. Polytene chromosomes may also detract attention from important aspects of chromosome structure and function if it is not sufficiently realized that they represent interphase chromosomes of cells specialized to produce large amounts of certain gene products. Chromosome

function, however, in reality, includes other essential tasks within the cell during mitosis and meiosis as well.

Indeed, recent research has exposed that the chromosome, apart from its carrier functions for genes, also has to serve for other cellular functions. One example is displayed by the molecular compositionof the centromere region. This region is responsible for the specific control of the distribution of chromatids or homologues chromosomes, respectively, during mitosis and meiosis [6, 8]. The centromere region of each chromosome has a highly specialized structure, at the DNA level as well as at the level of the associated chromosomal proteins. We are still not very advanced in our knowledge concerning the particular meaning of the specific structural composition of the DNA or of the associated proteins. However, in considering the fundamental requirements, which must be inherent to functional centromeric regions, we must not only think of their target functions as attachment sites for the microtubules of the spindle, but also of their possible functions in keeping chromatids together until the anaphase to allow correct distribution of the genome to the daughter cells. The pairing of the homologous chromosomes, in contrast, is based on other chromosomal properties which are yet unknown but might also find an equivalent in a distinct structural property of the chromosome.

The general structure of centromeric regions

The ultrastructural analysis of centromere regions has shown that during pro- and metaphase specific morphological structures are formed in association with the centromere regions of the chromosomes. In vertebrates, usually plate-like structures are found in the centromeric regions, which consist of three layers of proteins, an outer and an inner 40-60 nm thick layer and a middle layer, 25-30 nm thick. Each of these plates is constructed of specific proteins. They serve, in a so far unknown way, for the attachment of the spindle microtubules to the chromosome.

Several proteins associated with centromeric chromosome regions have recently been identified. This was possible with the aid of antisera derived from patients with autoimmune diseases. Some of these proteins (called INCENPs or "inner centromere proteins") are freely distributed in the nucleoplasm, while during prophase and metaphase they become assembled within the centromere regions. During the anaphase they are left behind in the midsection formed between the separating chromatids. Hence, these, and other proteins (e.g. the CENPs or "centromere proteins"), are therefore only transiently associated with the chromosomes[9]. This observation has led Earnshaw to assigne the term "chromosomal passenger proteins" to them to characterize their transient association with the chromosome.

What causes these proteins to assemble in the centromere regions at the beginning of mitotic prophase? What is their function during pro- and metaphase? We have had no answers to these questions yet. It is even unknown whether they behave in the same way during meiosis. However, not. all proteins of the centromere region

behave in this way. Other chromosomal proteins specifically associated with the centromere regions, such as the CREST proteins, seem also to remain associated with this region during the mitotic interphase as immunocytochemistry indicates. This implies that the basic features of the centromeric chromosome regions must remain unchanged throughout the cell cycle, not unexpectedly as the structural properties of the DNA of the centromere regions have already indicated a special structural character which was probably the basis for the association of this DNA with specific chromosomal proteins.

For practical reasons, the DNA of centromere regions has so far been studied mostly in yeast chromosomes, although the number of studies of centromere regions in higher eukaryotes has increased steadily. In yeast chromosomes, a highly specific structure of the DNA has been recognized which has also been demonstrated to be functional as centromere region in yeast artificial chromosomes. It is composed of chromosome-specific central AT-rich sequences which are accompanied by blocks of repetitive DNA sequences. These regions include only 130 nucleotides in yeast and flanking repetitive DNA regions are almost identical in different chromosomes. In higher eukaryotes, the DNA of centromeric regions is usually much more complex and hence much more difficult to analyze. However, for a long time it has been known that the centromeric regions contain blocks of repetitive DNAs, as it was first demonstrated in the mouse major satellite DNA[10]. Later, it was recognized that an additional minor DNA component is present, which is arranged in a defined pattern as was recently shown by Narayanswami et al.[11]. Studies of the distribution of other satellite DNAs rendered it most likely that each chromosome of a species has a very specific pattern of repetitive DNA sequences in its centromere-associated DNA. This could obviously serve to support the specific recognition between homologues during meiotic pairing and to guide a chromosome-specific and site-specific assembly with chromosomal proteins.

The general structure of telomeres

Just as the centromere regions, also telomeric chromosome regions display very specific molecular features. Again, studies of yeast chromosomes have had, and still have, a prominent role in experimental approaches to understand the molecular structure of telomeres, but also chromosomes of ciliated protozoa have been most informative[12]. Chromosomes of ciliates are very particular. These unicellular organisms are characterized by the possession of two nuclei-one micronucleus, as the generative nucleus essentially representing the germ line, and another the macronucleus, representing the metabollically active nucleus in the cell. After a meiotic division of the micronucleus and a sexual process accompanied by an exchange of haploid micronuclei between two cells of opposite "conjugation types", the macronucleus develops from the newly formed diploid micronucleus of the exconjugates. During the macronuclear development, the chromosomes disintegrate

and form linear gene-sized pieces of DNA which are the functional genome structures in the final macronucleus. Since these cells can divide, their nuclei also must be capable of undergoing division and hence DNA replication must have taken place. This has all the molecular consequences connected with the replication of linear DNA double helices as they are supposed to be the constituents of any eukaryotic chromosome. The small gene-sized fragments have therefore been excellent models for studies of the terminal regions of a chromosome during DNA replication.

The problem in the replication of linear DNA resides in the properties of DNA polymerases. Their need for a primer sequence and their exclusive polymerizing activity in a 3' - 5' direction cause incomplete replication of the 5' ends of linear DNA double strands. This leads to a continuous shortening of the chromosome ends in successive replication cycles (which has actually been demonstrated to occur cf. for example[13]). The investigation of the ciliate macronucleax DNA fragments have provided evidence how the cell can solve this problem. It was recognized that the ends of the gene-sized DNA-molecules are Constructed of simple short tandem repeats of the composition $(T_2G_4)_n$. These sequences are occasionally added to the chromosome by a specific enzyme called telomerase. It contains a single-stranded RNA molecule as structural component which is used as a template for the (T_2G_4)-repeats which are added to the ends of the chromosomes. In the extended terminal regions of the chromosomes, RNA primers axe synthesized allow the replication of the chromosomal DNA and compensate for the progressive shortening. Similar mechanisms have been found for yeast chromosomes. They are also based on the addition of short G-rich DNA-repeats to the 5'-ends of the DNA strands[3].

The growth of chromosome ends as a mechanism to prevent the Shortening of the chromatids during replication has also been observed in higher eukaryotes. The presence of repetitive DNA in the telomeric regions of the chromosomes has already been known since in situ hybridization techniques became available. However, the structure of the telomeres of higher eukaxyotes may be more complex than that of yeast or ciliates. In *Drosophila melanogaster* it has been found that in chromosome length is caused by an occasional, but relatively frequent insertion of distinct transposable elements into the telomeric regions [13].

Other chromosomal domains

We have so far discussed two types of chromosomal domains that are closely related to the essential structural requirements connected with the existence of chromosomes. One of these domains, the telomeric domain, is necessary to maintain the integrity of the chromosome over all its length throughout successive cycles of replication. The other domain, the centromere region, is required to guarantee the correct distribution of the chromatids during mitosis and meiosis. However, on a closer look chromosomes also display other properties not at all understood, although they remain remarkably stable throughout evolution. This refers to the

specific staining patterns displayed by pro- or metaphase chromosomes after certain pretreatments before staining. Such "banding patterns" are not simple, artificial subdivisions obtained after a partial distortion of the normal chromosome structure by certain experimental treatments, but they rather display a relationship with a distinct pattern of organization of the chromatids at an intermediate level of chromosomes structure. This is documented by the close relationship of certain types of bands to the time of replication of the respective chromosome region (G- and R-banding: displaying chromosome sections replicating late or early during the S phase) [14]. It has recently been demonstrated that a change at the time of replication during the S-phase of a banded chromosome section results in a change of its banding properties: A chromosome region originally replicating late in S-phase and representing a G-band is converted into a R-band if its replication takes place early[15]. Thus, we can assume that the banding patterns are caused by a specific underlying structural organization of the chromosome which is related to functional requirements. It is tempting to consider that such domains as discovered by banding techniques might include larger units for regulation of chromosome activity at a higher level of the chromatin structure. In this context it should be considered that the constitution of chromatin at quite a distance from a gene can influence its transcription: In several cases the regulatory elements have been identified to be located at a considerable distance from the genes controlled by them. One might also consider that gene clusters which are subjected to a common regulation, such as the globin gene clusters, might constitute such domains. Our knowledge on DNA sequences at a distance more than 1 to 2 kb from genes and their effects on gene expression is very limited and does not help to assess the significance of structural domains as they are identified by chromosome banding techniques at molecular level.

Inspecting the size of such domains we have to accept that they vary widely in their dimensions. In most cases they must accommodate thousands of kilobases of DNA although the smallest detectable bands may include even less than 100 kb. At present it is difficult to give any idea what may be the underlying feature of such domains determining the uniform staining properties of a band, whether there exist border segments between domains[16] and why such regions are created. The answer might come from studies of intrachromosomally amplified regions, known as HSRs (homogeneously staining regions). As their name indicates such regions display a high degree of uniformity in their staining behavior throughout the entire amplified region, quite similar to bands normally seen in chromosomes. This implies that their underlying organization must define both of them as particular domains within the chromosome. Our knowledge on how DNA sequences are selected for amplification and how HSRs are created remains very limited. The uniformity of the staining properties of a band undoubtedly must be based on a distinct structural organization of such regions.

If we try to relate such structural domains to chromosome structure more in general we do realize the fragmentary character of our understanding of chromosome

structure at all levels. Though probably not far from the truth, it might appear as an extreme point of view to conclude that our knowledge on chromosome structure hardly exceeds that what is known about the structure of the nucleosomal chain. Even there, uncertainties exist as to whether - or to which extent - a nucleosomal configuration is maintained throughout the chromatid and during different functional states of genes. Clearly, physicochemical considerations certify that the normal structure of a nucleosome cannot be maintained during the passage of the RNA poly/nerase simply for sterical reasons. At least a transient disassembly of the nucleosomes is needed to permit the passage of the RNA polymerase during transcription[17].

Models for chromosome structure above the nucleosome level are still under discussion. A solenoid structure of nucleosomal DNA as the next step in packaging is widely accepted although the evidence for the existence of solenoids is still circumstantial. Alternative models, such as a superbead structure, are presently not much favored although they cannot be entirely excluded. A more remote view might accept the existence of both such structures as possible transient states in the chromosome which might in some locations be used during limited periods of the cell cycle.

To what extent signals in the DNA sequence itself contribute to the specific packaging of the chromosome fibers at higher levels is totally unexplored. Obviously, selective binding of chromosomal proteins must be related to some extent to the kind of DNA sequences which are found in a particular region of the chromosomes. This has been demonstrated by the specific and exclusive binding of chromosomal proteins to heterochromatic sections of the genome[18]. The high content in repetitive DNA of heterochromatic chromosome regions in general might be responsible for this selective binding of specific proteins, but it can not be excluded that more defined DNA sequences may guide the association of these proteins with the DNA.

A better understanding of the character and the protein/ DNA-interaction of proteins specifically associated with heterochromatin might be an important step forward to a better understanding of the higher order of chromosome structure in general. It might display some of the flmdamental parameters important for the binding of chromosomal proteins to extended chromosome domains.

In summary, it is evident that chromosomes are organized into structural domains extending far beyond the borders of single genes. Such domains are the centromeric and telomric regions, both important for the distribution of chromosomes and for the maintenance of the chromosomal integrity. Other functional regions are present as seen from staining properties, which reveal a banded chromosome structure in pro- and metaphase chromosomes. These staining properties find an equivalent in the uniform behavior of such chromosome sections during replication, in their heterochromatic character, or in a common origin due to amplification of a particular DNA sequence. Up to the present we have no idea on the molecular parameters defining such domains and on the consequences of the existence of bands at a higher

structural level of a chromosome. Whether any relations exists between such bands and the isochores in mammalian DNAs[19] is also open to question.

Other functions of chromosomes

In our prior discussion we have concentrated our attention on the formation of domains within chromosomes, some of them clearly being dedicated to specific chromosomal functions different from protein coding. Recent studies have provided us with an even more intriguing new aspect of chromosome function. Various authors have shown that chromosomes are involved in the reconstitution of the nuclear interphase structure after a mitotic division by transiently binding proteins important for this process. During the telophase, the nuclear membrane is, in part, reconstructed from preformed constituents which assemble along the chromosomes during pro- and metaphase[20, 21]. Simultaneously with this reassembly of the nuclear membrane, most macromolecules are excluded from the nucleus. Subsequently, they are, in a controlled and selective fashion, imported into the nucleus through the nuclear pores[22]. An exception in this respect is the protein lamin B, which together with the lamin A and A1 forms the inner layer of protein around the nuclear membrane. This lamin layer might help to anchor the chromosomes at the membrane. During the telophase, lamin protein are in part imported into the nucleus, but some of the them remain bound to the surface of the chromosomes by forming vesicles. Therefore, they remain directly available within the nuclear compartment while the new nuclear membrane is constructed[21]. The ability of the attachment of lamin to the chromosomal surface during the mitosis is not surprising if one considers that the lamin layer itself seem to serve for an attachment of at least certain chromosomal regions to the nuclear membrane during the inter- and prophase. The important message of these observation is that chromosomes serve during the mitosis as a transport vehicle for molecular components essential for the structural control for their nuclear environment and that they are actively involved in the control of the cell structure.

The function of the chromosomes as nuclear target structures for proteins, which are not directly important for chromosome structure itself, reminds us closely to the properties of the INCENPs mentioned before. Such proteins have been designated as "chromosomal passenger proteins" by Earnshaw. Also these proteins bind transiently to the chromosomes during pro- and metaphase but are released as the chromosomes begin their anaphase movements. Just like lamin they also serve for important intranuclear functions related to processes essential to the cell cycle . One might consequently designate lamin B a "chromosomal passenger" protein as it displays a similar transient binding behavior to the chromosomes as the INCENPs. It will be not unexpected if future research reveals other nuclear proteins with a similar behavior.

These two examples expose a fundamentally new aspect of chromosome function,

which has only recently become evident: Chromosomes can serve as target structures for nuclear proteins which fulfil functions during the cell cycle and are quite distinct from proteins building up the chromosomal structure itself. Chromosomes, therefore, begin to appear as integrated elements of the cell which participate in the morphological structuring of the intracellular and intranuclear environment.

Conclusions

In this short account of some recent aspects on chromosome structure and function, it has become evident that we have to revise our ideas on the properties of chromosomes. In a classical view they essentially represent chains of genes, arranged in a nicely ordered fashion. It had also, since a long time, been recognized that chromosomes have to control their condensation and decondensation according to the respective requirements of the cell cycle. More recent experiments induced us to convert this rather static picture towards a more dynamic view of the chromosome: It has to fulfil duties during the cell cycle such as transiently accommodating proteins (or even other molecules) which are not related to maintaining or changing the chromosome structure itself or to regulate the expression of genes but are important elements of the nucleus and in its metabolism. In such a view the chromosomes will appear as integrated dynamic elements of the cellular architecture, functionally as well as structurally.

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Received 12-10-1992. Accepted 18-11-1992.