

Chromosomes and Chromatin

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DNA, the hereditary material of the cell, is folded into protein-containing fibers in the nucleus of eukaryotes. The structure and location of these fibers is thought to be important in determining whether molecular machines can access the DNA for important cellular processes such as gene activation.

Article contents

- Introduction
- Chromatin Structure
- Does Chromosome Structure Affect the Accessibility of DNA to Molecular Machines?
- Organization of Chromosomal DNA in the Nucleus

Introduction

Each human cell nucleus contains approximately 6 billion base pairs (bp) of deoxyribonucleic acid (DNA), with a total length of around 2 m, divided among 23 chromosome pairs. This 2 m of DNA must be organized within a nucleus that is approximately 10 μm in diameter so that it can be separated easily during cell division and replicated rapidly. In addition, selected regions must be identified and read quickly by the transcriptional machinery, all the time repairing whatever breaks, knots, supercoiling or tangles that might occur during these processes.

To place this in perspective, consider the following analogy: imagine a million-fold magnification, where the 10 μm diameter cell nucleus becomes a 10 m long boat. The persistence length of DNA, corresponding to the distance over which it behaves essentially as a stiff rod, is about 140 bp, roughly 5 cm in our analogy. Therefore the DNA would behave as a fairly stiff, 2 mm diameter wire approximately 2000 km long. This wire must be folded within our 10 m boat in such a way that specific regions of the 2000 km DNA can be found and readily accessed within seconds, and neatly so that at other times the 46 chromosomes can be easily removed from the boat.

With this perspective, the mechanisms by which DNA is organized within the cell nucleus now appear impressive and complex. Abundant, structural chromosomal proteins must bind over large regions of DNA simply to counteract its high negative DNA charge and facilitate DNA bending. A more diverse group of proteins must bind to specific regions of DNA in order to carry out basic DNA functions, including transcription, DNA replication and recombination. Folding of DNA into more compact structures allows chromosomes to fit within the nucleus and to be segregated easily during cell division. However, as the DNA is highly compacted, to as much as one ten-thousandth its original length during

mitosis, how does it remain accessible to the large, macromolecular protein assemblies that act upon it to carry out basic cellular processes? If certain regions of the DNA must remain accessible to protein binding to allow these processes to occur, are other regions made inaccessible? Furthermore, are chromosome regions randomly placed within the nucleus, or is the nucleus compartmentalized with specific chromosome regions targeted to different compartments depending on their functional state?

Chromatin Structure

Local chromatin structure – the nucleosome

The structure of the DNA double helix is known at atomic resolution (**Figure 1a**). In the cell, nearly all DNA undergoes a first level of compaction via the highly positively charged histone class of proteins. Two copies of each of the four core histone proteins, H2A, H2B, H3 and H4, form a discrete, bead-like particle called the histone octamer, around which 146 bp of the DNA double helix wraps 1.6 times. These histone–DNA assemblies are called nucleosome core particles and are spaced fairly regularly, roughly every 200 bp. A fifth class of histone proteins, called linker histones, interacts with the core particle and the DNA between nucleosome core particles, called linker DNA. Within interphase nuclei, approximately equal weights of histone proteins, nonhistone chromosomal proteins and DNA associate to form a complex DNA–protein assembly called chromatin. Packaging of DNA and histones into nucleosomes, also known as local chromatin structure, results in a roughly sixfold compaction in length, forming a 10 nm diameter fiber (nucleofilament).

Basic features of how DNA folds on the nucleosome surface have now been determined by X-ray crystallography (**Figure 1b**). The wrapping of DNA close to

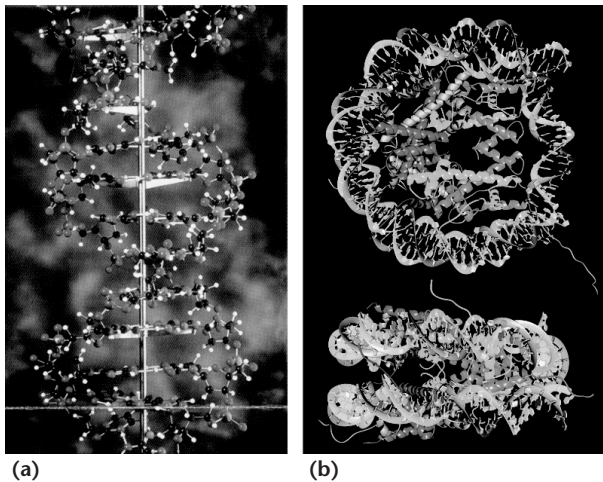


Figure 1 Structure of the DNA double helix and that of the nucleosome have been determined by X-ray crystallography. (a) Two strands of single-strand DNA twist to form an antiparallel DNA double helix. (From *Nature* (2001) **409** (poster supplement).) (b) The nucleosome consists of the double helical DNA strand wrapped 1.6 times around the core histone protein octamer (center). The structure of higher levels of chromatin folding is still unknown. (From Luger K, Mader AW, Richmond RK, Sargent DF and Richmond TJ (1997) *Nature* **398**: 252.)

the octamer surface significantly decreases the binding of certain proteins due to steric hindrance. This provides an inherent regulatory function; by positioning nucleosomes relative to the underlying DNA sequence, the cell can block the binding of certain regulatory proteins to their DNA sequences. In contrast, other proteins are able to bind specific DNA sequences even with DNA packed into nucleosomes.

Histones are found with various chemical groups attached to their flexible tail domains; these include acetyl, methyl and phosphate groups. It has long been assumed that different histone modifications act to physically alter local chromatin structure by modulating histone–DNA interactions. More recently a quite different paradigm has been proposed in which histone modifications form a combinatorial ‘histone code’, directing the recruitment of different proteins to local chromatin regions as a function of which histone modifications are present.

Higher-order chromatin structure – the 30 nm fiber

DNA is highly negatively charged, owing to its phosphate groups. These charges repel each other, contributing to the high persistence length (stiffness) of DNA. Bending of DNA requires neutralization of this negative charge, which occurs through interaction with positively charged histone proteins and positive ions in solution. Reducing the salt concentration of

nuclear isolation buffers reduces the ionic concentration. This causes unfolding of chromatin and the swelling of nuclei. Reducing salt concentrations to very low levels makes nuclei swell and then explode, releasing extended nucleofilaments. Under the electron microscope these filaments look like beads on a string, in which the beads are the nucleosomes and the fiber between the beads is linker DNA. Using nucleases that cut only between nucleosomes in the linker DNA, it is possible to isolate nucleosome oligomers, which can be examined similarly.

Increasing the salt concentration causes these extended nucleosome arrays to fold, forming thicker fibers. At physiological salt concentrations these nucleosome arrays reach maximum condensation, forming fibers of around 30 nm in diameter. This condensation is dependent on the highly positively charged tails of the core and linker histones.

Although there is general agreement that most of the genome is packaged within 30 nm chromatin fibers *in vivo*, there remains no agreement on the mechanism of folding by which the nucleofilament compacts to form these higher-order, 30 nm chromatin fibers. Models of 30 nm chromatin fiber structure have been proposed that vary from precise helical folding of nucleosomes mediated by specific interactions between histone proteins, to irregular folding of nucleosomes, dictated by the local geometry of DNA entering and exiting the nucleosome core particles and statistical variations in linker length.

Methods of studying large-scale chromatin structure

Even with DNA wrapped into nucleosomes, the average human chromosome would still be about 7000 μm long. Adding the additional six- to sevenfold compaction from folding nucleosomes into 30 nm fibers, the average chromosome would remain over 1000 μm in length, much larger than the nuclear diameter of approximately 10 μm . Most regions of the genome must therefore undergo additional levels of compaction.

Higher levels of chromatin folding above the 30 nm chromatin fiber, called large-scale chromatin structure, are very difficult to study. Most experimental approaches to investigating nucleosomes and 30 nm chromatin fibers rely on biochemical and biophysical approaches dependent on isolating small chromatin pieces in soluble form. However, the stability of large-scale chromatin organization requires ionic conditions that cause chromatin to precipitate. Moreover, it is very difficult to isolate chromatin that is sufficiently long enough to fold into large-scale chromatin structure without first breaking the chromatin into pieces.

This leaves light and electron microscopy as the major methods capable of directly observing large-scale

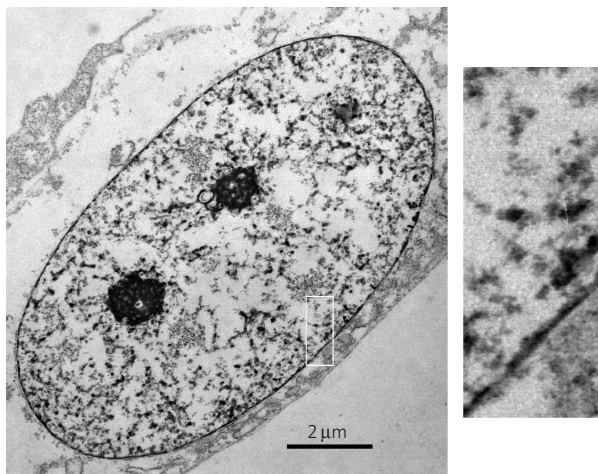


Figure 2 Electron microscopy of a mammalian cultured cell. (Left) The dark oval is the edge of the nucleus, and the three dark masses are nucleoli. In this preparation, the cell is treated with detergent and the chromatin is visible as fibrillar masses much larger than the 30 nm diameter, although the internal structure of these fibers is not readily discernible. Without detergent treatment, no distinct fibers of any kind would be visible with conventional staining methods. (Right) Higher magnification view of the box in the left image. (Original photographs from the authors' laboratory.)

chromatin folding within intact nuclei and chromosomes. Unfortunately, the resolution of currently available microscopes does not allow unambiguous determination of large-scale chromatin folding motifs.

The simplest approach involves observing chromatin with an electron microscope, the highest-resolution microscope available. Even with an electron microscope, however, chromatin appears as compact masses in which individual fibers are not easily discernible (**Figure 2**). This is a result of the high packing density of large-scale chromatin folding, the lack of convenient DNA-specific stains and the limited three-dimensional capabilities of transmission electron microscopy, in which the image represents a projection through the sample. Moreover, because electrons cannot pass through thick samples, biological samples must be embedded in plastic and cut into thin sections, typically 0.1 μm thick, before examination. Given these limitations, progress has been slow due to the difficulty of visualizing the path of tightly packed 30 nm chromatin fibers, with adjacent fibers separated by only several nanometers, as they fold within larger twisted, coiled interphase chromosomes over a radius of several micrometers. Therefore, other approaches have also been developed.

Studying unusual chromosomes

Since the 1980s scientists have learned that many eukaryotic organisms that appear completely different

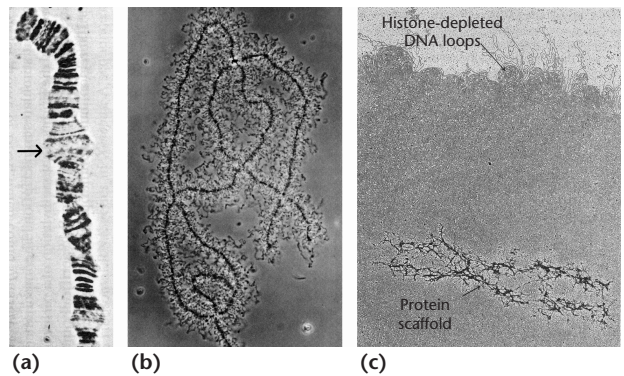


Figure 3 Experiments on unusual chromosomes have contributed to current models of higher-order and large-scale chromatin structure. (a) Polytene chromosomes from the fruitfly (*Drosophila melanogaster*) display a banded pattern. The arrow marks a puff containing decondensed, highly transcriptionally active chromatin. (b) Highly transcriptionally active lampbrush chromosomes from an amphibian (*Chironomus*) show DNA loops stemming from a central axis of condensed chromatin. Transcription occurs largely in the extended loops. (c) Mammalian mitotic chromosomes extracted with a high salt concentration to remove histone proteins show naked DNA extending out from a residual protein scaffold that roughly retains the original chromosome shape. Under certain preparation conditions, individual loops of DNA can be traced as extending outward from the edge of the chromosome scaffold. (From Stryer (1995) *Biochemistry*, 4th edn. New York: Freeman.)

from each other physically have remarkable similarities at the molecular level. For this reason, one approach has been to exploit organisms that have unusual chromosomes, with the expectation that the lessons learned will apply to chromosome structure in other species. For example, many insects have giant larval cells in which the chromosomes have replicated many times without cell division. In some of these cells, hundreds to thousands of identical copies align side by side next to each other in nearly exact register, forming giant interphase polytene chromosomes. These polytene chromosomes reveal a banded pattern when stained (**Figure 3a**). The darker bands contain more compact chromatin, indicating that different portions of the genome are compacted differently. Lampbrush chromosomes in amphibian oocytes are also peculiar because many of their genes are expressed at extremely high levels. These chromosomes show loops of chromatin extending from a central axis (**Figure 3b**). Transcription occurs in these decondensed looped chromatin domains.

In vitro extraction and manipulation of chromosomes

An alternative way of dealing with the high packing density and complexity of large-scale chromatin structure has been to unfold chromatin progressively from isolated mitotic chromosomes or interphase

nuclei. One method used has been to extract histones, and most other nonhistone chromosomal proteins, by exposure to increasing salt concentrations. Removal of these chromosomal proteins results in the unfolding and straightening of DNA, forming extended DNA loops emanating from a residual, protein scaffold (**Figure 3c**). Isolated mitotic chromosomes extracted in this way are called chromosome haloes; their appearance has motivated proposal of the radial loop model of mitotic chromosome structure (see below). Nuclei can be treated similarly, producing a 'nuclear halo' also showing loops of DNA, now extending from a residual nuclear scaffold or matrix. (*See Chromosome Structures: Visualization.*)

Fluorescent protein tags

Although light microscopy has a lower resolution than electron microscopy, its ability to examine intact, even living cells and to localize different proteins within the cell using multicolor fluorescently tagged probes makes it an extremely powerful research tool. To visualize individual chromosomes, or chromosome regions, fluorescence *in situ* hybridization (FISH) methods can be used which light up selected DNA sequences. However, this method requires melting of DNA, exposing specimens to harsh conditions that might change chromosome structure. Alternative methods now allow chromosome regions to be directly observed in living cells. In one approach, binding sites for a DNA-binding protein can be inserted into a selected chromosome region. Adding a fluorescently tagged version of that protein to living cells allows this specific chromosome region to be visualized. Another, more general, approach is to incorporate fluorescent nucleotides or fluorescent histones into DNA or chromatin. These techniques have yielded information about the chromatin fibers present in mammalian interphase nuclei.

Biochemical approaches

Given the limitations of the microscopic approaches described above, molecular and biochemical methods have been invaluable in probing large-scale chromatin structure. These methods provide tremendously sensitive and high-resolution, although only indirect, mapping of variations in chromatin structure. One particularly informative and widely used approach has been to treat nuclei with enzymes that cut or mark the DNA. By analyzing which DNA sequences have more cuts or marks, DNA regions more or less accessible to regulatory proteins can be mapped. Variations in accessibility reflect variations in the chromatin structure overlying these sequences. Unfortunately, this

method does not provide direct structural information or indicate even which level of chromatin structure is responsible for the variations in accessibility. (*See Chromatin Structure and Domains.*)

Models of large-scale chromatin structure (> 30 nm fiber)

The original radial loop model proposed that 30 nm fibers fold into loops of 30–100 kilobase pairs (kbp) anchored by a central scaffold, producing a metaphase chromosome with a diameter of roughly 700 nm. This has now evolved into a radial loop, helical coil model of mitotic chromosome structure, in which a radial loop chromatid of approximately 300–400 nm in diameter is helically coiled to form the final metaphase chromosome. Sequences where DNA is attached to the scaffold are known as scaffold attachment regions or matrix attachment regions (SARs/MARs). Biochemical experiments have suggested that some of the same scaffold attachment sites act as loop attachment sites in nuclear haloes, and the radial loop model has been extended to describe interphase chromosome structure as well. Organization of genomic DNA into such loops has been proposed as a fundamental mechanism for independent gene regulation, with loop attachment sites acting as boundaries to isolate regulatory sequences from neighboring genes. (*See Chromosome Structures: Visualization.*)

A giant-loop, random walk model was proposed for interphase chromosome structure based on statistical analysis of the average distance between two chromosome sites, observed in nuclei by FISH, as a function of genomic distance. In this model, 30 nm chromatin fibers are arranged in random trajectories over lengths of 100–1500 kbp. Because chromosome sites separated by 2000–20 000 kbp are closer together on average than expected for a random trajectory, this model proposed the organization of 30 nm chromatin fibers into giant loops of several thousand kilobase pairs.

Finally, a folded chromonema model has been proposed, based on *in vivo* light microscopy combined with transmission electron microscopy analysis of chromosome folding intermediates observed during the transition into and out of mitosis. In this model, chromonema fibers about 100 nm in diameter, formed by the folding of 10 and 30 nm chromatin fibers, fold into a 200–300 nm prophase chromatid, which itself coils to form metaphase chromosomes. During interphase, mitotic chromosomes decondense through an uncoiling and extension of these chromonema fibers, which can now be more easily visualized as discrete, large-scale chromatin fibers.

Does Chromosome Structure Affect the Accessibility of DNA to Molecular Machines?

Heterochromatin and euchromatin

Correlations have been established between increased chromatin compaction and decreased transcriptional activity. Chromatin at the periphery of the nucleus often appears more dense by light and electron microscopy than chromatin within the nuclear interior. This dense chromatin has been called heterochromatin, and the more diffuse chromatin, euchromatin. In some cases, this cytologically dense heterochromatin corresponds to chromosome regions rich in repetitive DNA and low in gene density. The term heterochromatin has also been applied to chromosome regions rich in repetitive DNA, regardless of chromosome condensation. Transcription appears to be located just outside these dense, heterochromatin regions. (See Chromatin in the Cell Nucleus: Higher-order Organization.)

Variable compaction of entire chromosomes

While mitotic chromosomes are uniformly condensed, the cytological distinction between heterochromatin and euchromatin implies that as cells enter interphase, different chromosome regions assume variable levels of decondensation. In some cases, distinctive levels of decondensation can be observed for entire chromosomes. For example, human chromosomes 18 and 19 contain roughly the same amount of DNA, but chromosome 19 is comparatively gene-rich, while chromosome 18 is comparatively gene-poor. Interestingly, FISH chromosome painting experiments reveal that within interphase nuclei chromosome 18 is much more compact than chromosome 19 (Figure 4a).

Another well-studied set of chromosomes is the XY sex chromosomes. In female mammals most of the genes on one of their two X chromosomes is turned off during development. This process of sex chromosome silencing, also called dosage compensation, equalizes the X-chromosome gene expression in females to that in males, who have only one X chromosome. The inactive X chromosome appears denser than surrounding chromosomes when stained with a DNA-specific dye. In fruitflies, dosage compensation occurs differently. Both female X chromosomes remain active, but the male X chromosome doubles its level of gene expression. Consistent with active chromatin being less compact, the male X

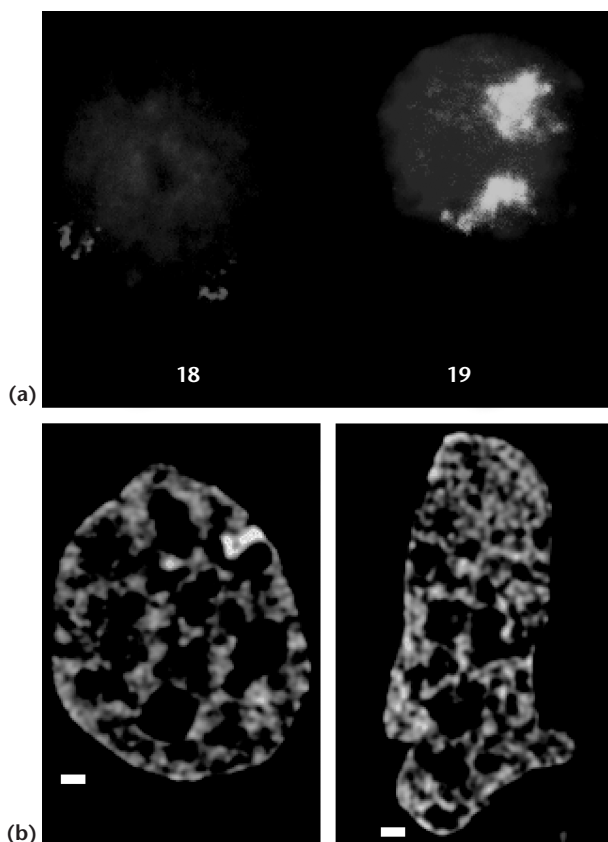


Figure 4 [Figure is also reproduced in color section.] Experiments using fluorescence microscopy yield information about chromatin structure and function. (a) Entire chromosomes are painted by fluorescence *in situ* hybridization (FISH), using chromosome-specific probes, and appear red for chromosomes 18 and green for chromosome 19. DNA in the nucleus is counterstained blue for reference. Each chromosome occupies a fairly distinct region restricted to a small subvolume within the nucleus, known as a chromosome territory. Chromosomes 18 and 19, of similar DNA content, have different gene densities. Chromosome 18, with a lower gene density, appears more condensed and peripherally located relative to the gene-rich chromosome 19. (Original photograph courtesy of Wendy Bickmore.) (b) Many copies of a specific DNA sequence were integrated into mammalian chromosomes. When a fluorescent protein that binds this sequence is added to the cells, the segment is visible as a bright dot (red) within the nucleus (blue-green). On the left, no transcription factor is targeted to the labeled region and the chromatin is condensed. On the right, a strong transcription factor is targeted to the labeled region, and the chromatin unfolds dramatically. The DNA content of the labeled chromosome region is the same for both cells. (Original photographs from the authors' laboratory.)

chromosome in flies has a more diffuse appearance than other chromosomes.

Variable compaction of chromosome bands

Correlations between an entire chromosome's compaction and transcriptional activity are interesting, but

the activity of the 30 000–40 000 genes in the human genome is not likely to be regulated by such a general mechanism. Each chromosome contains hundreds or thousands of genes, which are typically regulated independently. However, this correlation between chromatin decondensation and gene activity holds up when examining smaller chromosome regions surrounding clusters of active genes, or even chromatin domains corresponding to individual gene loci.

For example, mammalian chromosomes display a banded pattern when stained with Giemsa dye – the dark bands are called G bands. G bands are known to have a lower gene density than R bands, which do not stain, and G bands are more compact during interphase. A second example, involving chromatin domains surrounding individual gene loci, is seen in insect polytene chromosomes. Regions of polytene chromosomes containing highly active genes often show dramatic puffs of chromatin. Regions of chromosomes created by insertion of multiple copies of a cloned DNA segment containing an inducible gene have been demonstrated to unfold into an extended large-scale chromatin fiber after the gene was activated. Because chromatin compaction and gene activity seem to be related, scientists predicted that transcription factors, the proteins that bind DNA and activate transcription, also may be able to unfold chromatin. By artificially tethering transcription factors, researchers have indeed observed decondensation of large-scale chromatin structure. By targeting large amounts of transcription factor to a very compact array of DNA sequences in mammalian cells, researchers observed dramatic unfolding of heterochromatic chromosome regions into extended, 80–100 nm diameter large-scale chromatin fibers (Figure 4b).

Unresolved question – what is the physiological significance of chromatin compaction?

In summary, the extent of large-scale chromatin compaction has been observed to correlate with transcriptional activity when examining either entire chromosomes or chromosome bands. Due to technical limitations, however, this correlation has yet to be observed for an individual gene in diploid chromosomes. What still remains unclear is whether there is a causal relationship between large-scale chromatin structure and gene regulation. Specifically, can large-scale chromatin condensation limit accessibility of regulatory proteins, thereby playing a role in gene regulation?

Organization of Chromosomal DNA in the Nucleus

Chromosome territories

Decondensation of chromatin during interphase makes it difficult to distinguish interphase chromosomes. Similarly, the three-dimensional organization of chromosomes within the interphase nucleus is not easily observed. Originally, chromosomes were thought to form distinct structures only during mitosis. However, it is now clear that each interphase chromosome occupies a restricted region or territory of the cell nucleus.

Early evidence for chromosome territories came from labeling local chromosome regions by irradiation with ultraviolet light; repair of damaged DNA was used to incorporate labeled nucleotides. When these labeled regions were followed through subsequent cell cycles, regions of DNA damage were observed to remain in small, distinct nuclear regions, implying that individual chromosome regions occupied small volumes within the nucleus. Distinct chromosome territories can now be directly observed by FISH chromosome painting, using hundreds of unique, labeled DNA sequences distributed over a given chromosome (Figure 4a).

While chromosome territories appear as fairly discrete regions, when examined individually by light microscopy some intermingling of DNA from adjacent chromosome territories is observed. FISH has revealed large protruding loops extending from the chromosome 17 territory into neighboring territories. Some of these loops may correspond to active gene regions. Ribosomal DNA from the short arms of chromosomes 13, 14, 15, 21 and 22 intermingles to form a smaller number of nucleoli. (See Chromatin in the Cell Nucleus: Higher-order Organization.)

Gene position relative to chromosome territories and large-scale chromatin domains

Based on the relatively solid appearance of chromosome territories, as revealed by FISH, an interchromosome domain model was proposed suggesting that chromosome territories might restrict diffusion of macromolecules. This model predicted that active genes would show preferential localization at the surface of chromosome territories. Some initial experiments mapping the location of active and inactive genes relative to the surface of chromosome territories supported this prediction.

However, labeling transcription sites and mapping these relative to chromosome territories has revealed

that newly synthesized ribonucleic acid (RNA) is present throughout chromosome territories. Therefore, the original interchromosome domain model has been modified to become the interchromatin domain model, in which transcription occurs at the edges of condensed, large-scale chromatin domains, even within territories, rather than being restricted to the surface of the territory.

Intranuclear distribution of chromosome regions

We previously noted the tendency for a peripheral distribution of heterochromatin, and a more interior distribution of euchromatin. Centromeres, telomeres and mammalian inactive X chromosomes, all transcriptionally silent, are also preferentially localized to the nuclear periphery. In contrast, gene-rich R bands of chromosomes are found more often in the nuclear interior, while gene-poor G bands are localized toward the nuclear periphery. This behavior of gene-rich versus gene-poor regions of the genome is also seen for entire chromosomes, with the gene-poor human chromosome 18 localizing preferentially to the nuclear periphery and the gene-rich chromosome 19 localizing preferentially toward the nuclear interior (**Figure 4a**). Acetylated histone H4 protein, associated with active genes, is found more toward the interior of the nucleus, implying that this differential localization of active versus inactive chromosomal regions holds for most of the genome.

Manipulating gene localization and gene activity

The experiments described above simply correlate transcriptional activity with intranuclear positioning. They do not establish cause and effect. Using molecular biological techniques it is possible to alter chromatin localization experimentally and to analyze the effect on gene activity. In yeast, a small number of genes important for mating are silenced by creating a repressive chromatin state surrounding the gene locus. Silencing of these genes is related to specific gene regulatory sequences called silencers. These genes normally localize to the nuclear envelope. Mutations in silencer sequences create weakened silencers, which are not as efficient at suppressing gene expression. Artificially tethering a weakened silencer to the nuclear envelope resulted in increased silencing of the gene locus. This and other experiments have led to a model that postulates that higher concentrations of silencing/repressive proteins at the nuclear periphery create a repressive environment for genes. In mammalian cells, targeting large amounts of a strong transcriptional

activator to a chromosome region found preferentially at the nuclear periphery resulted in a repositioning of this chromosome site to the nuclear interior accompanying gene activation.

Gene position relative to centromeres and heterochromatin masses

Centromere chromosomal regions often cluster in interphase nuclei to form heterochromatin masses, making them more easily visible. In some cell types, for instance mouse lymphocytes, inactive genes appear to associate with these centromere clusters. For several genes studied, association follows rather than precedes gene inactivation. These observations have led to a model in which chromosomal location relative to these heterochromatin masses is not required for gene inactivation but may contribute to the stability of the silenced state.

In fruitflies, a large insertion of repetitive DNA next to the *brown* gene locus causes inactivation of the *brown* locus and a looping of the chromosome arm to associate with the centromere cluster. In blood cells, mutations of gene-activating regulatory sequences, called locus control regions, adjacent to the β -globin gene locus result in reduced β -globin or transgene expression. Accompanying this gene inactivation is a change in nuclear localization resulting in association of these DNA sequences with centromeres. Finally, the locus control region of a different gene has been shown to result in the localization of a transgene, inserted experimentally into centromeric heterochromatin, to the surface rather than the interior of the interphase centromeric heterochromatin mass. This localization is dependent on the concentration of transcription factors that bind to this locus control region. These results have led to models where active genes are targeted to euchromatin nuclear compartments, while inactive genes associate with heterochromatin compartments that may reinforce their gene silenced state.

Unresolved questions about chromosomal organization

Many examples now exist in which changes in gene expression are associated with changes in intranuclear localization. The functional significance of these changes in position remains unclear. In addition, the mechanisms by which chromosomes can be repositioned over large distances within the interphase nucleus are completely unknown. Further development of microscope technology and molecular biological tools will be essential in addressing these issues.

See also

Chromatin in the Cell Nucleus: Higher-order Organization
 Chromatin Structure and Domains
 Chromosome
 Chromosomes: Higher-order Organization
 Heterochromatin: Constitutive

Further Reading

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Chromosome Structures: Visualization

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Chromosomes are the functional units of inheritance. Only by visualizing these complex and dynamic macromolecular entities can we begin to understand the implications of their structure and organization for the living cell.

Introduction

Unraveling the complexity of chromosomes is daunting. There are many levels of organization and condensation involved in compacting the 2 m of deoxyribonucleic acid (DNA) double helix of a human cell into the nucleus. Through hierarchical stages of DNA–protein interactions in the chromatin fiber, the DNA polymer is compacted to form the interphase chromosome and, with more extensive condensation, the mitotic chromosome. For purely technical reasons, research in the past has mainly focused on structural organization of mitotic chromosomes. However, improvements in visualization techniques now allow us to observe chromosomes throughout the cell cycle. Application of techniques such as fluorescence *in situ* hybridization (FISH) and immunofluorescence (IF) allows us to discern specific areas of chromatin by fluorescence microscopy. FISH uses specific DNA molecules, directly or indirectly labeled with fluorochromes, to probe whole chromosomes or smaller domains, while IF uses fluorescently tagged antibodies mainly to detect the proteins associated with specific regions of chromatin. Studying the structure and behavior of chromosomes *in vivo* is of increasing importance.

Visualizing Whole Chromosomes

Simple mitotic chromosome preparations allow us to see the gross structure of chromosomes. To increase the yield of mitotic chromosomes, exponentially growing cell cultures are arrested at metaphase by the microtubule-disrupting drug colcemid. Chromosomes are then harvested from these cultures by hypotonic cell swelling, fixed in 3:1 methanol:acetic acid (MAA), and visualized with DNA-binding stains such as orcein, propidium iodide, Giemsa or 4',6-diamidino-2-phenylindole (DAPI). Human chromosomes can be arranged into groups A–G or numbered 1–22 by various criteria. DNA staining techniques produce bands along the length of each chromosome, which allow individual chromosomes to be identified. Characterization of disease-associated chromosomal abnormalities is also possible by these methods. (*See* Banding Techniques; Chromosome Analysis and Identification; Chromosome Preparation.)

Advanced article

Article contents

- Introduction
- Visualizing Whole Chromosomes
- Chromosome Subdomains
- Centromeres and Telomeres
- Scaffold/Matrix
- Visualizing Chromosome Structures in Living Cells – Into the Future