# Molecular Biology
# PG01CBIT01/PG01CMIC01
# Unit 2

Dr. Arif Khan

Assistant Professor, ARIBAS

# Syllabus Unit 2

**Organization of genome and its replication**

Packaging of DNA and organization of chromosome in bacterial cells; Packaging of DNA in eukaryotic nucleosome and chromatin condensation, assembly of nucleosomes upon replication, chromatin modification.

Mechanism of DNA polymerase catalyzed synthesis of DNA, Types of DNA polymerases in bacteria, Initiation of DNA replication and its regulation in prokaryotes, assembly of replisome and progress of replication fork, termination of replication. DNA replication in eukaryotes and archaea. Inhibitors of DNA replication.

# Organization of DNA into chromosomes

# Junk DNA

- "Junk" DNA is defined as the repetitive sequences that make up much of the chromosome in many organisms.
- Junk DNA does not apparently encode proteins or RNA molecules needed for the immediate survival of the cell. Why would “Junk” DNA be so prevalent if it were not important?
- Recently it has been suggested that “Junk DNA" may be involved in preventing excessive genetic recombination between closely related species (Rodman,1991; Kricker et al., 1992).
- One does not wont recombination to tinker with DNA encoding a beautifully designed protein that may have required hundreds of thousands of years to develop.
- The DNA of different species con be distinguished by its repetitive DNA which, since it does not contain information that encodes proteins or RNA, can vary greatly from one species to the next.
- In the presence of enzymes that carry out genetic recombination very efficiently, divergent repetitive DNA would prevent genetic recombination between closely related species by stimulating the destruction of recombination events containing mismatches from recombining "junk" DNA.
- This is done by the mismatch repair system which is designed to detect and then correct regions of DNA containing mismatches.

# Packaging of DNA and organization of chromosome in bacterial cells

**The Bacterial HU Protein**

- Griffith (1976) published pictures of DNA spilling out of an *E. coli* cell very quickly after the cell was broken open, in which the DNA complexed with proteins looked like "beads on a string.“
- The protein responsible for the bacterial nucleosomes observed by Griffith may be the abundant HU protein.
- The bacterial HU protein is a small basic protein of 18,000 daltons.
- It exists as a heterodimer of two nearly identical subunits (HU-1 and HU-2). HU binds to DNA and changes the shape and supercoiling of the DNA.
- There are also a number of other small abundant DNA binding proteins including H-NS (a histone-like protein previously called H1) in *E. coli* that may be involved in chromosome organization.
- These may act alone or they may interact with the HU protein to organize the chromosome *in vivo*.

- Rouviere-Yaniv et al. (1979) and Broyles and Pettijohn (1986) showed that the bacterial HU protein can act in a fashion consistent with the requirements for a DNA packaging protein.
- A packaging protein must compact the DNA or shorten its end-to-end distance. This can be accomplished by folding DNA back and forth or by coiling.
- The HU protein utilize coiling as a mechanism of DNA compaction. The DNA is coiled around the body of the protein, thereby greatly reducing its overall length.
- In addition, coiling DNA defines a path or writhe of DNA in space. As discussed in Chapter 3, the negative superhelical energy of supercoiled DNA is manifest as changes in the twist of the double helix (T) and the writhe of DNA in space (W).
- Therefore, wrapping DNA around HU proteins in prokaryotes or around histones in eukaryotes influences the level of DNA supercoiling and the amount of free energy potentially available for biological reactions.

## DNA Supercoiling and DNA Packaging

- Chromosomes in prokaryotes are frequently circular, which reduces the end-to-end length by a factor of at least 2.
- In addition, most circular DNAs isolated from cells are negatively supercoiled. The formation of interwound supercoils compacts DNA.
- The energy from negative DNA supercoiling can also be used to drive the wrapping of DNA into a toroidal coil around a protein.
- The writhe associated with wrapping around a protein will affect the superhelical topology of the rest of the DNA molecule.
- One 360° wrap around a protein will introduce a writhe of +1 or -1 depending on whether the wrap is clockwise (+1) or counterclockwise (-1).
- One toroidal 360° wrap is equivalent to one interwound supercoil provided there is no change in the twist (T) of the helix.

- **Restrained and Unrestrained Supercoils**
- A negative supercoil can exist in two forms, ***restrained*** or ***unrestrained****.*
- A circular DNA molecule with one negative supercoil that exists as an interwound negative supercoil or as a toroidal coil that is not stably wrapped around a protein is considered *unrestrained.*
- The deficiency in linking number ($L - L_0$) is distributed in part as the negative supercoil W = -1 and in part as a deviation from the preferred twist (where Tpreferred = $L_0$).
- In the unrestrained supercoil, torsional strain or torsional stress is felt in the winding of the DNA double helix over the entire DNA molecule.
- If a nick were introduced anywhere in the circular molecule, the linking number would increase (to $L = L_0$) as negative supercoils were relaxed by the rotation of one strand about the other.
- Resealing the nick by the action of DNA ligase would result in the formation of a relaxed DNA molecule (Figure 9.1).

- A negative supercoil is said to be restrained when the linking number deficit ($L - L_0$) is restrained by the stable coiling or writhing (W) of the DNA around a protein or by the stable interaction with a denaturing protein or a protein that changes twist.
- In the example shown in Figure 9.1, the single supercoil is restrained by wrapping around the protein. The part of the DNA molecule that is not physically associated with the protein is effectively relaxed since there is no linking number deficit distributed over this part of the DNA molecule. If this molecule is nicked, no unwinding occurs.
- When this molecule is resealed by DNA ligase, it retains its original linking number deficit ($\Delta L = -1$).

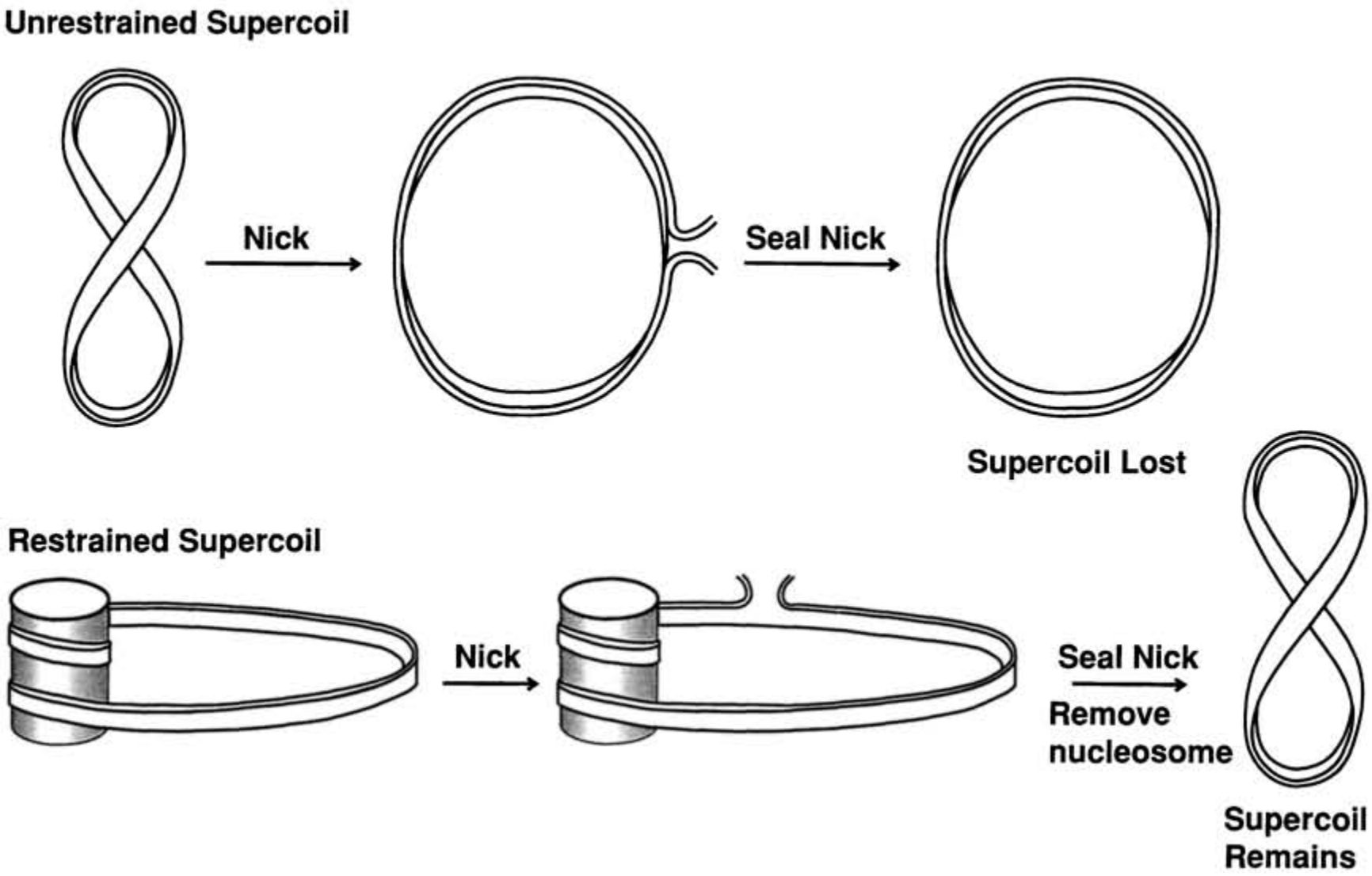


**Fig. 9. 1 Restrained and unrestrained supercoils.**

**Are Supercoils Restrained in Escherichia coli?**

- Pettijohn and Pfenninger (1980) showed that supercoils were restrained in living *E. coli* cells.
- To demonstrate this, they introduced nicks into DNA in vivo by irradiating cells with X-rays.
- Following incubation in media, the breaks were repaired in cells by the action of DNA repair enzymes and DNA ligase.
- Before being sealed, however, the breaks provided a swivel through which supercoils could be lost by the rotation of one strand around the other.
- In vitro, following the introduction of nicks into purified protein-free DNA, all supercoils are rapidly lost.
- To determine if all supercoils would be lost from a large circular DNA (an F plasmid) packaged in E. coli, it was necessary to prevent the introduction of new supercoils into the DNA.
- Thus, during repair, cells were incubated in media containing coumermycin, an inhibitor of DNA gyrase (the enzyme responsible for supercoiling DNA).
- Consequently, if all supercoils were lost by the introduction of a nick in the DNA, no new supercoils could be reintroduced into DNA by the action of DNA gyrase.
- When DNA was purified from cells in which repair occurred in the presence of coumermycin, the F plasmid DNA contained about half the number of supercoils as DNA purified before nicking in vivo. This result demonstrated that about half the supercoils present in DNA in living E. coli cells are restrained, presumably by association with specific proteins in cells.

## The HU Protein Can Restrain Supercoils in *Vitro*

- Rouviere-Yaniv et at. (1979) and Broyles and Pettijohn (1986) demonstrated that, when bound to DNA in vitro, HU compacts DNA and restrains DNA supercoils.
- Topoisomerases can relax a protein-free supercoiled DNA molecule. If supercoils are restrained by the interaction of DNA with a protein, they will not be lost through the action of a topoisomerase.
- Moreover, if relaxed DNA wraps around a protein to restrain a negative supercoil, a positive supercoil must be introduced into another part of the molecule (to satisfy the relationship L = T + W).
- If topoisomerase activity is present to relax the positive supercoil introduced by the negative superhelical wrapping, then there will be a net introduction of one negative supercoil into DNA.
- By incubating DNA in the presence of HU protein and topoisomerase, HU can be shown to restrain supercoils in negatively supercoiled DNA and introduce restrained supercoils in relaxed DNA.

- The number of supercoils that could be restrained by HU was linearly dependent on the HU concentration. At a 1 : 1 weight ratio of HU protein to DNA, a maximum number of supercoils was restrained by HU protein.
- The addition of more HU did not result in the restraint of additional supercoils.
- The maximum number of supercoils HU restrained *in vitro was (J "" -0.03 or* about half that found in DNA purified from cells.
- Perhaps not surprisingly, this was the same level of restraint that Pettijohn and Pfenninger (1980) found *in vivo after nicking DNA with X-rays. (See the box entitled "The* Topology of the DNA-Hu Interaction.")

## HU Binds Transiently to DNA

- Griffith found that, following lysis of cells, the DNA-protein complexes rapidly dissociated, indicating that the DNA packaging that existed in *E. coli* was not very stable.
- The in vitro results of Broyles and Pettijohn supported the idea that HU binds weakly to DNA.
- In physiological levels of salt, the half-time ($t_{1|2}$) for the dissociation of DNA was too rapid to measure.
- At a lower salt concentration (0.05 M), $t_{1|2}$ of HU binding was longer and kinetic measurements could be made.
- This rapidly dissociable binding may be advantageous for *E. coli* in which most of the genome must be accessible to regulatory proteins and RNA polymerase.

## HU Bends DNA

- From the topology of DNA supercoiling on interaction of HU with DNA, it is expected that HU will bend DNA sharply into a tight circle.
- Hodges-Garcia et al. (1989) tested this possibility by measuring the circularization rate of small DNA fragments (< 200 bp).
- Typically, it is very difficult to ligate DNA fragments shorter than the persistence length of DNA into a circle.
- For example, 80·bp fragments could not easily bend or be ligated into a circle in the absence of the HU protein. However, in the presence of HU, 80-bp fragments of DNA were efficiently ligated into a circle.
- HU has been implicated in a variety of reactions involving DNA.
- In some cases the increased bendability or flexibility of DNA in the presence of HU is important. For example, the HU protein may facilitate wrapping the *E. coli* chromosome replication origin, *oriC*, into a complex with the initiation specific DnaA protein.

# The Packaging of DNA in the Eukaryotic Nucleasome

- DNA in eukaryotic cells is organized into nucleosomes, in which the DNA is wrapped into two left-handed coils around a histone octamer.
- A core nucleosome consists of two subunits each of small basic histone proteins H2A, H2B, H3, and H4.
- Histories bind tightly to DNA, forming very stable complexes.

**The Structure of the Nucleosome**

- In 1984, Richmond *et al*. reported the structure of the nucleosome determined by X-ray crystallography.
- The nucleosome is a disk 57 Å thick and 110 Å across around which 145 bp of DNA wraps in a left-handed coil. The two H3 and H4 sub units form a symmetrical unit at the center of the disk.
- Two pairs of H2A-H2B dimers then bind to the outside of the H3-H4 complex completing the octameric histone core. The H3-H4 central protein complex is believed to contact the central 70- 80 bp of the DNA.
- The outer regions of the DNA are associated with the H2A-H2B subunits as well as with the H3 subunit.
- The DNA completes 1.6- 1.8 turn s around the nucleosome within 145 bp.

- Arents and Moudrianakis (1993) have described a detailed view of the organization of DNA around a nucleosome.
- A single fifth histone called H1 binds to the outside of the nucleosome and is believed to contact the DNA where it enters and exits the nucleosome.
- With H1 present, about 167 bp of DNA are associated with the nucleosome.
- The 167 bp of DNA form two complete helical turns. The nucleosome with an H1 histone bound is called a chromatasome.
- DNA in a chromatasome is organized in a more stable and more compact form than in the nucleosome.
- Regions of DNA that are transcriptionally inactive are believed to be organized as chromatasomes.

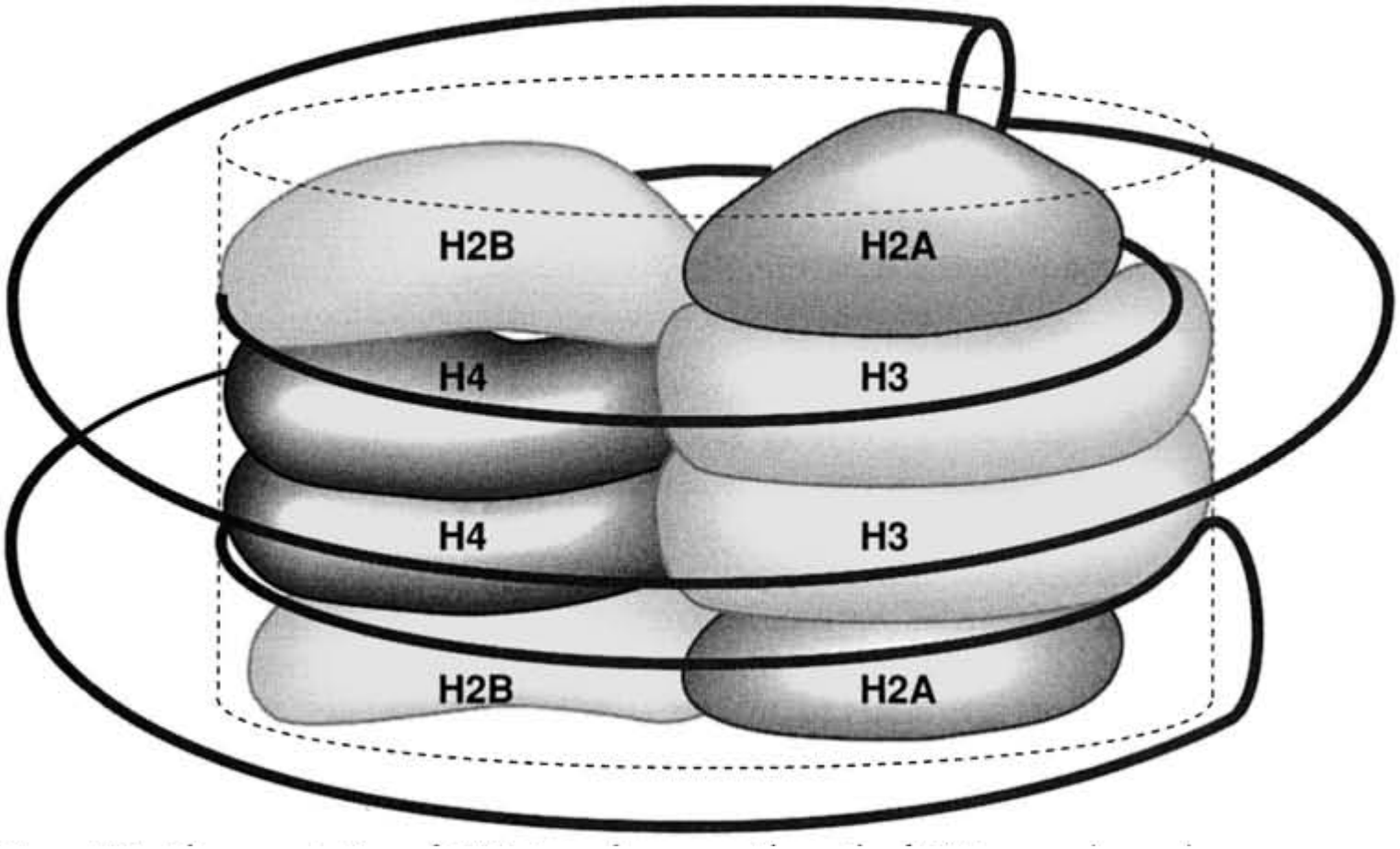


**Fig. The organization of DNA in a nucleosome**

- Other proteins called HMG (high mobility group) proteins are often found with nucleosomes in regions of chromosomes that are transcriptionally active.
- These proteins have recently been shown to bind to cruciform structures. Biologically, they may bind two helices of DNA together as they cross in space, stabilizing an X structure of DNA.
- Histone subunits are also subject to covalent modifications such as phosphorylation and acetylation.
- These modifications can influence the protein-protein-DNA interactions and may affect the biology of the histone-DNA interaction.
- For example, the acetylation of histones H3 and H4 on the amino group of lysines can destabilize the nucleosome . The level of restraint of negative superhelical tension may be altered when histones are acetylated .
- Acetylation is associated with regions of chromatin that are active in transcription. The destabilization of the nucleosome should facilitate the passage of RNA polymerase through the nucleosome during transcription.
- In addition, histone acetylation can lead to binding of transcription factor TFIIIA to a *Xenopus borealis* 5S RNA gene organized in a nucleosome. Without acetylation, the transcription factor cannot bind its recognition sequence.

## Nucleosomes and the Restraint of Supercoiling

- The path of DNA on the nucleosome constitutes about 1.75 left-handed turns. However, a nucleosome appears to restrain only one negative supercoil, a phenomenon known as *the linking number paradox.*

## Nucleosome Positioning

- The organization of DNA into nucleosomes can have dramatic consequences for gene expression and DNA repair.
- Knowledge of the factors that govern the placement and level of association of nucleosomes on DNA is critical to understanding the regulation of gene expression.

## Models for Positioning

- Nucleosomes can **be randomly or precisely** positioned on a DNA molecule.
- A random organization would exist if all DNA molecules had an equal ability to wrap around histones.
- With the immense variability of helical structure and deformability inherent in different sequence organizations, a totally random organization is unlikely.
- In fact, sequences of DNA that resist bending are not usually associated with nucleosomes, whereas certain bent regions strongly associate with nucleosomes.
- The term "**nucleosome phasing**" has been used to describe the relationship between nucleosomes on DNA.
- Phased nucleosomes have a uniform spacing, resulting in a uniform length of the linker DNA. Phased nucleosomes can be random or positioned.

**Translational and Rotational Positioning**

**Bent DNA Positions Nucleosomes**.

- A number of studies have extended the work of Drew and Travers to show that bent DNA strongly positions nucleosomes (Shrader and Crothers, 1990).
- DNA sequences from the bacterial plasmids can position nucleosomes.
- There is a strong bend near the terminus of SV40 DNA replication that binds nucleosomes very tightly (Hsieh and Griffith, 1988).
- Typically, binding will occur first at bent regions of DNA as nucleosomes are reconstituted onto DNA.
- There must be a lower energy barrier for tightly wrapping curved DNA around histones than for wrapping a relatively straight or randomly coiled molecule.

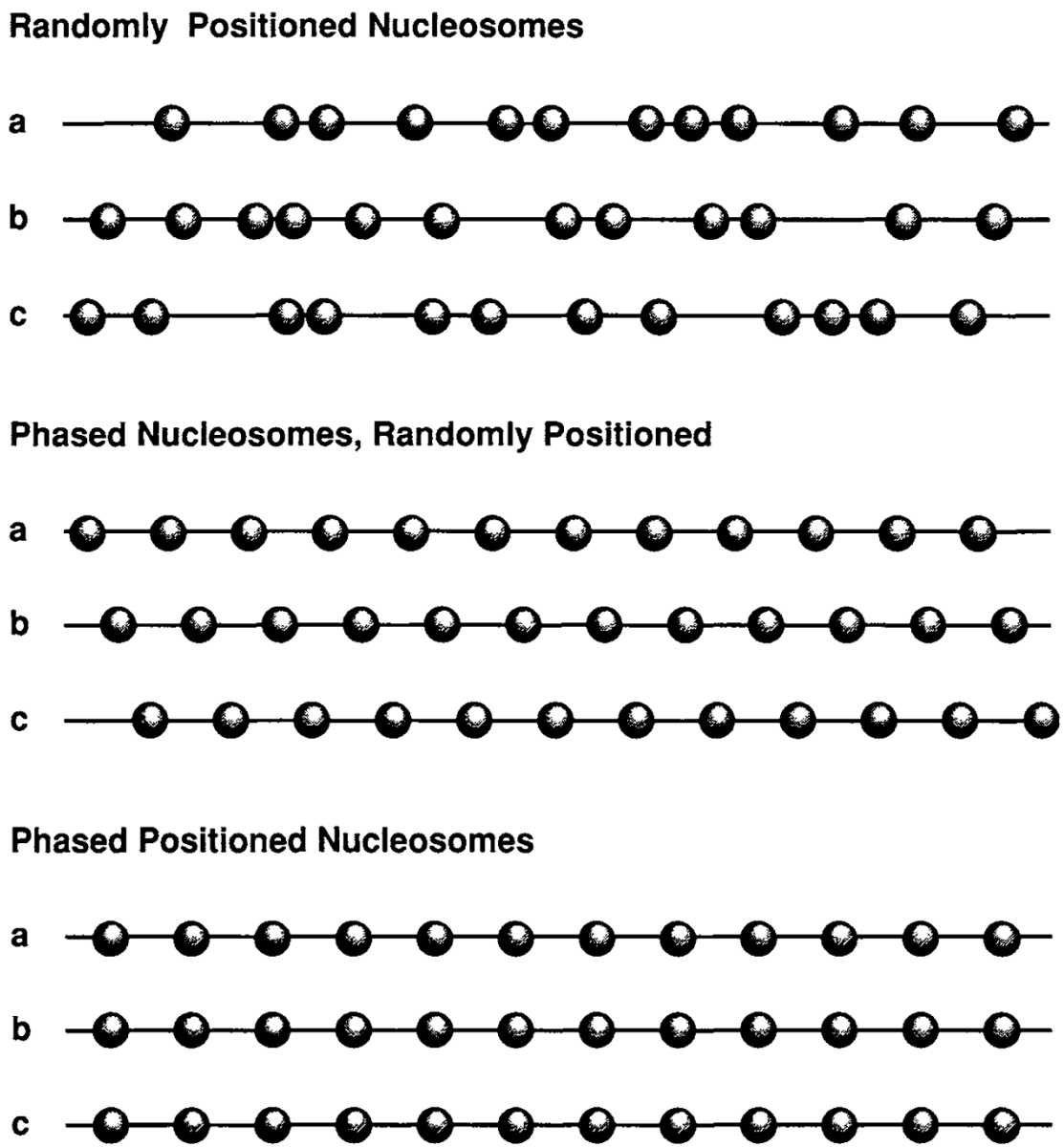


Fig. Nucleosome Phasing

**Sequences That Resist Organization into Nucleosomes**.

- Certain sequences of DNA do not readily associate with nucleosomes.
- Sequences that are less flexible than "garden variety DNA" (or average sequence DNA) such as long A tracts or G·C runs may resist wrapping around histones.
- Long A tracts are rarely found near the center of nucleosomes but tend to be organized at the ends of DNA associated with nucleosomes.
- *At the* region where DNA enters and exits the nucleosome, the DNA may be more flexible and may be less tightly wrapped than DNA at the center of the nucleosome.

***Nucleosomes and Gene Expression****.*

- The organization of DNA into nucleosomes generally represses gene expression.
- The level of nucleosomal organization in terms of higher order packing affects the level or degree of repression.
- Nucleosomal DNA has been divided into two classes: ***heterochromatin*** *and* ***euchromatin****.*
- Heterochromatin has historically been defined as densely staining regions of chromosomes that condense early in prophase and that replicate late in S phase. These regions include centromeres and telomeres, and represent regions of the genome that may be inert transcriptionally.
- Females contain two X chromosomes, one of which is inactive. The inactive X chromosome is maintained as a heterochromatic structure called a Barr body.
- If a recombinant DNA construct containing a gene that can be expressed in the eukaryotic genome is introduced into a heterochromatic region it will become transcriptionally inactive, a situation known as a *position effect.*
- Euchromatin refers to the rest of the genome that does not stain as intensely. Euchromatin contains regions of DNA that are transcriptionally active or that may need to be transcriptionally active at some point during the growth and development of the cell.

- Gene expression in euchromatin is not controlled by the level of DNA accessibility alone, but involves the presence and action of transcription factors.
- The deposition of nucleosomes on promoters can lead to an inability of RNA polymerase II to initiate transcription of the gene.
- Knezetic and Luse (1986) showed an inverse correlation between the level of deposition of nucleosomes on DNA and transcriptional initiation activity.
- As more nucleosomes were reconstituted onto DNA, transcription initiation was inhibited.
- RNA polymerase is apparently unable to bind to a promoter if it is wrapped around a nucleosome. If nucleosomes are reconstituted onto a DNA template that has one or more transcription factors bound to the promoter, the transcription factor-bound promoter will not become organized into a repressive nucleosome.
- Even after the heavy deposition of nucleosomes, transcription initiation can occur from these templates when relevant transcription factors and RNA polymerase are supplied.

## What Happens When RNA Polymerase Encounters a Nucleosome?

- When RNA polymerase encounters a nucleosome, it can either stop or transcribe through the nucleosome.
- Nucleosomes may be inhibitory and prevent movement of RNA polymerase. Under certain experimental conditions nucleosomes can prevent transcription elongation.
- Alternatively, RNA polymerase may transcribe through nucleosomes.
- For a gene to be transcribed, the DNA must presumably unwrap from the core histones, although the nucleosome may not entirely dissociate from the DNA.

- There are several possible mechanisms by which transcription could occur through a nucleosome.
- First, DNA could unwrap one turn from the nucleosome, allowing transcription of 70 bp of DNA.
- Once transcribed, the DNA might rewrap around the histone core while the second 70-bp turn of DNA begins to unwrap and be transcribed.
- Second, the nucleosome may dissociate and unfold into two tetrameric subunits (half nucleosomes) each consisting of one subunit of H2A, H2B, H3, and H4.
- *There is chemical and biophysical evidence that the nucleosomes* on transcriptionally active chromatin are somewhat unfolded or less tightly associated into a core octamer. Each of these half nucleosomes may be loosely bound to the DNA.
- Third, positive supercoiling generated ahead of transcription by RNA polymerase may weaken the nucleosome organization, leading to dissociation from the DNA.
- Nucleosomes may rapidly reform behind RNA polymerase in a localized region of negative supercoiling.

- Biochemically, what distinguishes a nucleosome on inactive DNA from a nucleosome on DNA that is transcriptionally active? The answer to this question is not yet known with certainty, but chemical modifications of histones may be associated with transcriptionally active nucleosomes.
- There are  a number of sites on histones that can be either phosphorylated or acetylated.
- Most of these sites are within the $NH_2$-terminal ends of the core histones.
- The amino group of lysine, which has a positive charge that may be involved in binding the histone to the negatively charged DNA phosphate backbone, is the site of acetylation.
- The addition of the acetyl group (-$COCH_3$ ) to the amino group removes the positive charge on the lysine. If the tenacity of histone binding to DNA involves electrostatic interaction, acetylation will weaken histone-DNA binding. Thus, acetylation may "loosen up" the nucleosome and facilitate movement of RNA polymerase through DNA.
- In fact, when histones are purified from transcriptionally active chromatin, histones H3 and H4 are hyperacetylated. Moreover, histone acetylation can result in a change in the amount of supercoils restrained by nucleosomes suggesting that some supercoiling might be introduced by histone modification.

# Transcriptionally Active Genes Can Contain Precisely Positioned Nucleosomes in Vivo

# The Organization of Chromosomes in Eukaryotic Cells

**Histones**

- The protein components of chromatin, which comprises somewhat more than half its mass, consist mostly of **histones.**
- Discovered in 1884 by Albrecht Kossel.
- Five major classes of histones **H1,H2A,H2B, H3, and H4.**
- All have a large proportion of positively charged residues (Arg &Lys).
- These proteins therefore ionically bind DNA's negatively charged phosphate groups.
- Histones are almost entirely synthesized during the relatively short S phase of the cell cycle (when DNA is replicated), when they are needed in massive amounts for chromatin replication.

**Table: Calf Thymus Histones**

| Histone | Number of residues | Mass (kD) | % Arg | % Lys | UEP ($x10^{-6}$ year) |
|---|---|---|---|---|---|
| H1 | 215 | 23.0 | 1 | 29 | 8 |
| H2A | 129 | 14.0 | 9 | 11 | 60 |
| H2B | 125 | 13.8 | 6 | 16 | 60 |
| H3 | 135 | 15.3 | 13 | 10 | 330 |
| H4 | 102 | 11.3 | 14 | 11 | 600 |

## Histones Are Evolutionarily Conserved

- The amino acid sequences of histones H2A, H2B, H3, and H4 have remarkably high evolutionary stability.
- For example, histones H4 from cows and peas, species that diverged 1.2 billion years ago, differ by only two conservative residue changes, which makes histone H4, the most invariant histone, among the most evolutionarily conserved proteins known.
- *Such rigid evolutionary stability implies that the above four histones have critical functions to which their structures are so well tuned that they are all but intolerant to change*.
- The fifth histone, histone H1, is more variable than the other histones;

## Histones May Be Modified and Have Variant Forms

- Histones are subject to post-translational modifications that include acetylations, methylations, phosphorylations, ubiquitination, sumoylation and ADP-ribosylation of specific Arg, Glu, His, Lys, Ser, Thr, and Tyr side chains.
- Most of these modifications, many of which are reversible, decrease the histones' positive charges, thereby significantly altering histone–DNA interactions.
- Yet, despite the histones' great evolutionary stability, their degree of modification varies enormously with the species, tissue, and the stage of the cell cycle.

- Most, if not all, eukaryotes have numerous genetically distinct subtypes of the canonical histones.
- These variant histones have important roles in such essential cellular processes as transcriptional initiation and termination, DNA repair, homologous recombination, and telomere and centromere function.
- For example, **H2A.Z** (64% identical to H2A) is associated with the promoters of actively transcribed genes, **H2A.X** (95% identical to H2A) binds to DNA at double-strand breaks, thereby marking them for repair, and the H3-like **CenH3** (also called CENP-A; 50% identical to H3) functions in organizing chromatin structure at the centromere.
- In fact, the erythroid cells of chick embryos contain a histone H1 variant that differs so greatly from H1 that it is named **histone H5** (38% identical to chicken H1; avian erythrocytes, unlike those of mammals, have nuclei).
- Variant histones are synthesized independent of DNA replication and many are only synthesized during specific stages of embryonic development and in the differentiation of certain cell types. How they carry out their various specialized functions is, for the most part, unknown.

# Nucleosomes: The First Level of Chromatin Organization

- The first level of chromatin organization was pointed out by Roger Kornberg in 1974 through the synthesis of several lines of evidence:

  1. Chromatin contains roughly equal numbers of molecules of histones H2A, H2B, H3, and H4, and no more than half that number of histone H1 molecules.

  2. X-ray diffraction studies indicate that chromatin fibers have a regular structure that repeats about every10 nm along the fiber direction. This same X-ray pattern is observed when purified DNA is mixed with equimolar amounts of all the histones except histone H1.

  3. Electron micrographs of chromatin reveal that it consists of 10-nm-diameter particles connected by thin strands of apparently naked DNA, rather like beads on a string.These particles are presumably responsible for the foregoing X-ray pattern.

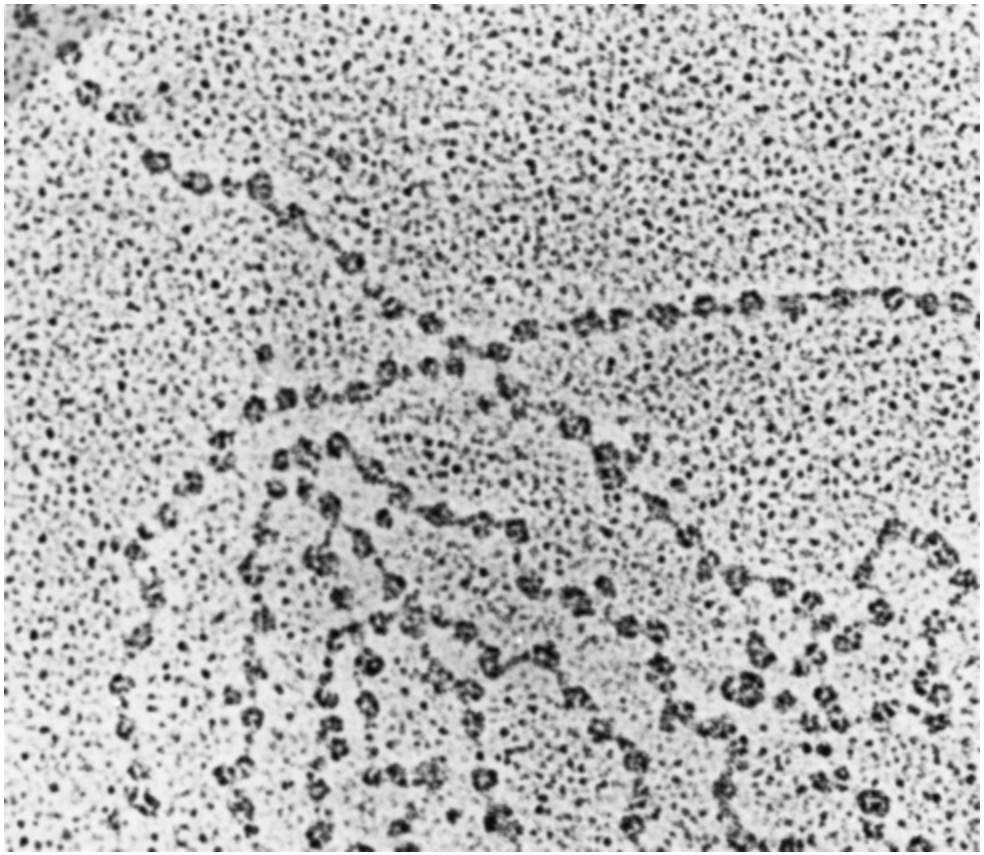

**Fig. Electron micrograph of *D. melanogaster* chromatin showing that its 10-nm fibers are strings of closely spaced nucleosomes.**

4. Brief digestion of chromatin by micrococcal nuclease (which cleaves double-stranded DNA) cleaves the DNA between some of the above particles*; apparently* the particles protect the DNA closely associated with them from nuclease digestion. Gel electrophoresis indicates that each particle *n-mer* contains 200n bp of DNA.
5. Chemical cross-linking experiments, indicate that histones H3 and H4 associate to form the tetramer (H3)2(H4)2. These observations led Kornberg to propose that *the chromatin particles, which are called* ***nucleosomes****, consist of the octamer (H2A)2(H2B)2(H3)2(H4)2 in association with 200 bp of DNA. The fifth histone, H1,* was postulated to be associated in some manner with the outside of the nucleosome.

## DNA Coils around a Histone Octamer to Form the Nucleosome Core Particle

- Micrococcal nuclease, initially degrades chromatin to particles known as **chromatosomes** that each consist of 167 bp of DNA in complex with a histone octamer and one molecule of histone H1.
- On further digestion, some of the chromatosome's DNA is trimmed away in a process that releases histone H1.
- This yields the 205-kD **nucleosome core particle,** which consists of a 147**-** bp strand of dsDNA in association with the above histone octamer.
- The DNA cumulatively removed by this digestion, which had previously joined neighboring nucleosome core particles, is known as **linker DNA.**
- Its average length, which varies from 10 to 50 bp from organism to organism and tissue to tissue, is 18 bp in yeast, 28 bp in *Drosophila*, and 38 bp in humans.

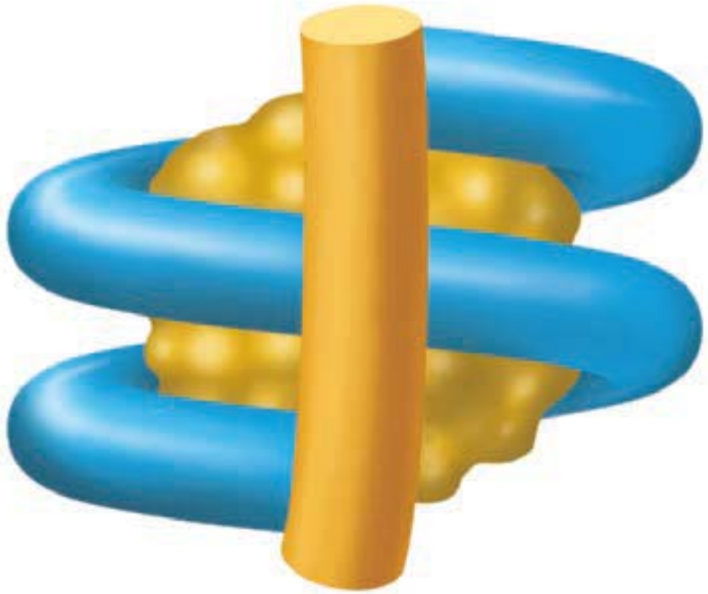

**Fig. Model of the interaction of histone H1 with the DNA of the 166-bp chromatosome**

# *30-nm Fibers: The Second Level of Chromatin Organization*

- The 167-bp nucleosomal DNA has a packing ratio of 7 (its 560-Å contour length is wound into an 80-Å-high supercoil).
- Clearly, the filament of nucleosomes, which only occurs at low ionic strengths and hence is unlikely to havean independent existence *in vivo*, represents only the first level of chromosomal DNA compaction. Only at physiological ionic strengths does the next level of chromosomal organization become apparent.
- As the salt concentration is raised, the H1-containing nucleosome filament initially folds to a zigzag conformation, whose appearance suggests that nucleosomes interact through contacts between their H1 molecules. Then, as the salt concentration approaches the physiological range, chromatin forms an 30-nm-diameter fiber in which the nucleosomes are visible.Two models for this **30-nm fiber** are given the greatest credence.

- The first model proposed by Richmond, *is* based on the X-ray structure that he determined of a tetranucleosome in which the DNA has a strong nucleosome positioning sequence that repeats every 167 bp.
- In this model, the nucleosomes follow a zigzag path in which all the odd-numbered nucleosomes and all the even-numbered nucleosomes stack next to each other.
- This 25-nm-diameter structure, in which the two stacks wind round each other in two left-handed helices (a so-called **two-start helix;** the DNA forms a right-handed supersuperhelix), has 18.9 nucleosomes per turn, a pitch of 31.6 nm, and hence a rise of 0.6 nucleosome/nm.
- The short 20-bp linker DNAs cross back and forth through the center of the fiber between successive nucleosomes, which is only possible because these linker DNAs are unconstrained by linker histones.
- Longer linker DNAs would allow the binding of linker histones and thus increase the fiber diameter, but would require the rigid dsDNA to bend and/or disrupt the contacts between nucleosomes.

- An alternate model of the 30-nm fiber, formulated by Daniela Rhodes, in which the DNA and the nucleosomes follow a single left-handed helix (a **one-start helix;** the DNA forms a left-handed super-superhelix) that has a diameter of 34 nm, 5.4 nucleosomes per turn, a pitch of 5.4 nm, and hence a rise of 1 nucleosome/nm.
- The observation that the dimensions of the nucleosome assemblies are constant over a wide range of linker DNA lengths suggests that the linker DNA, which is not resolved in the electon micrographs, is bent and occupies the interior of the fiber.
- Kensal van Holde has argued that the 30-nm fiber lacks a regular structure; because of the varying lengths of the presumably straight and stiff linker DNAs (every base pair added to a linker DNA rotates the adjacent nucleosomes by 36° with respect to each other), it has an irregular helix-like structure that simulations indicate forms a fiber with an average diameter of 30 nm.
- This would account for the difficulty in definitively determining the structure of the 30-nm fiber despite numerous attempts to do so over more than three decades.

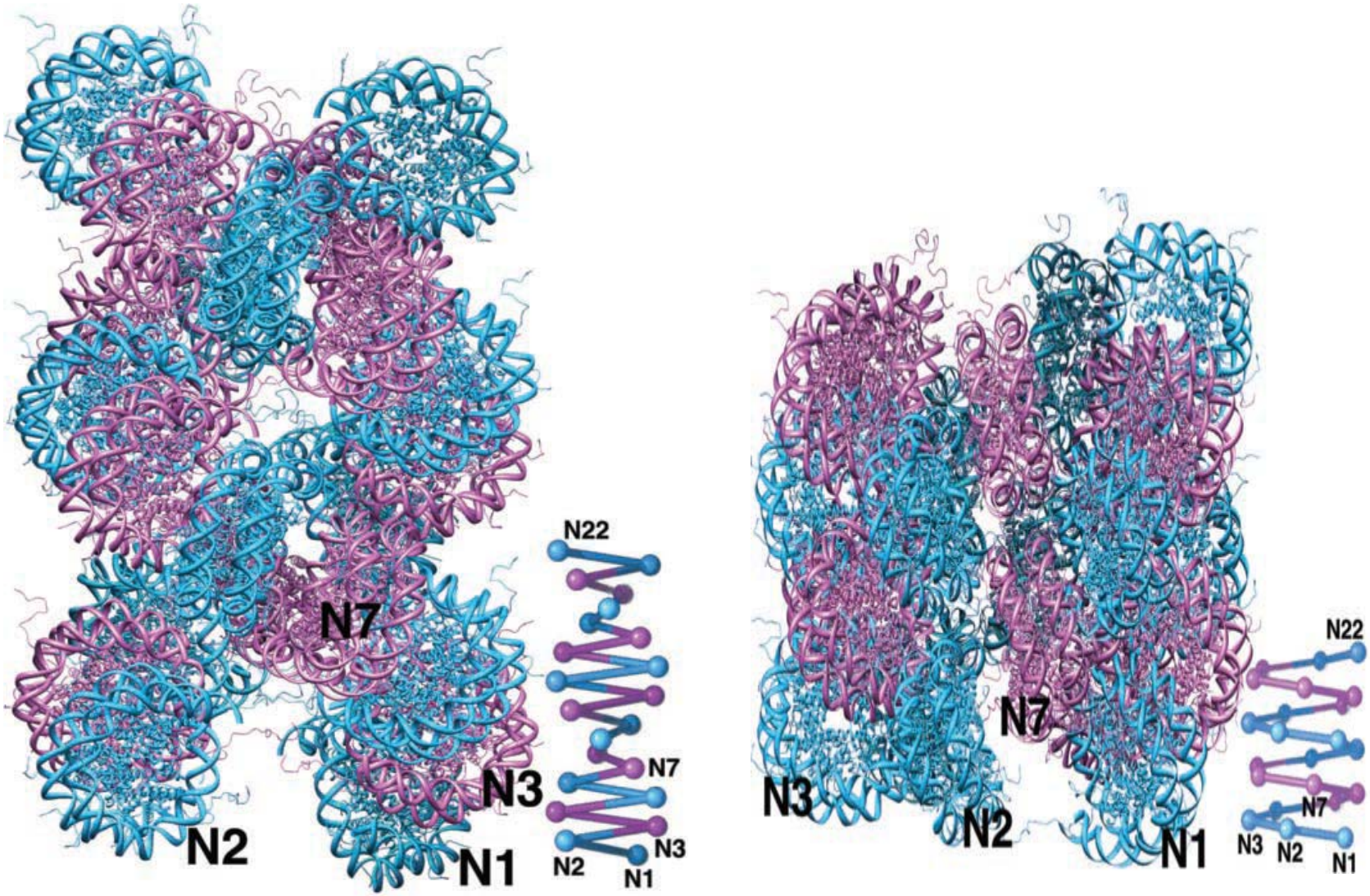


**Fig. Models of the 30-nm chromatin fiber consisting of 22 nucleosomes in which the fiber axis is vertical. (a) Richmond Model (b) Rhodes Model**

# Radial Loops: The Third Level of Chromatin Organization

- Histone-depleted metaphase chromosomes exhibit a central fibrous protein matrix or scaffold surrounded by an extensive halo of DNA .
- The strands of DNA that can be followed are observed to form loops that enter and exit the scaffold at nearly the same point.
- Most of these loops have lengths in the range 15 to 30 µm (which corresponds to 45–90 kb), so that when condensed as 30-nm fibers they would be 0.6 µm long.
- Electron micrographs of chromosomes in cross section, strongly suggest that the chromatin fibers of metaphase chromosomes are radially arranged. If the observed loops correspond to these radial fibers, they would each contribute 0.3 µm to the diameter of the chromosome (a fiber must double back on itself to form a loop). Taking into account the 0.4-µm width of the scaffold, this model predicts the diameter of the metaphase chromosome to be 1.0 µm, in agreement with observation.
- A typical human chromosome, which contains 140 million bp, would therefore have 2000 of these 70-kb radial loops (Fig. 34-16c). The 0.4-µm-diameter scaffold of such a chromosome has sufficient surface area along its 6- µm length to bind this number of radial loops.

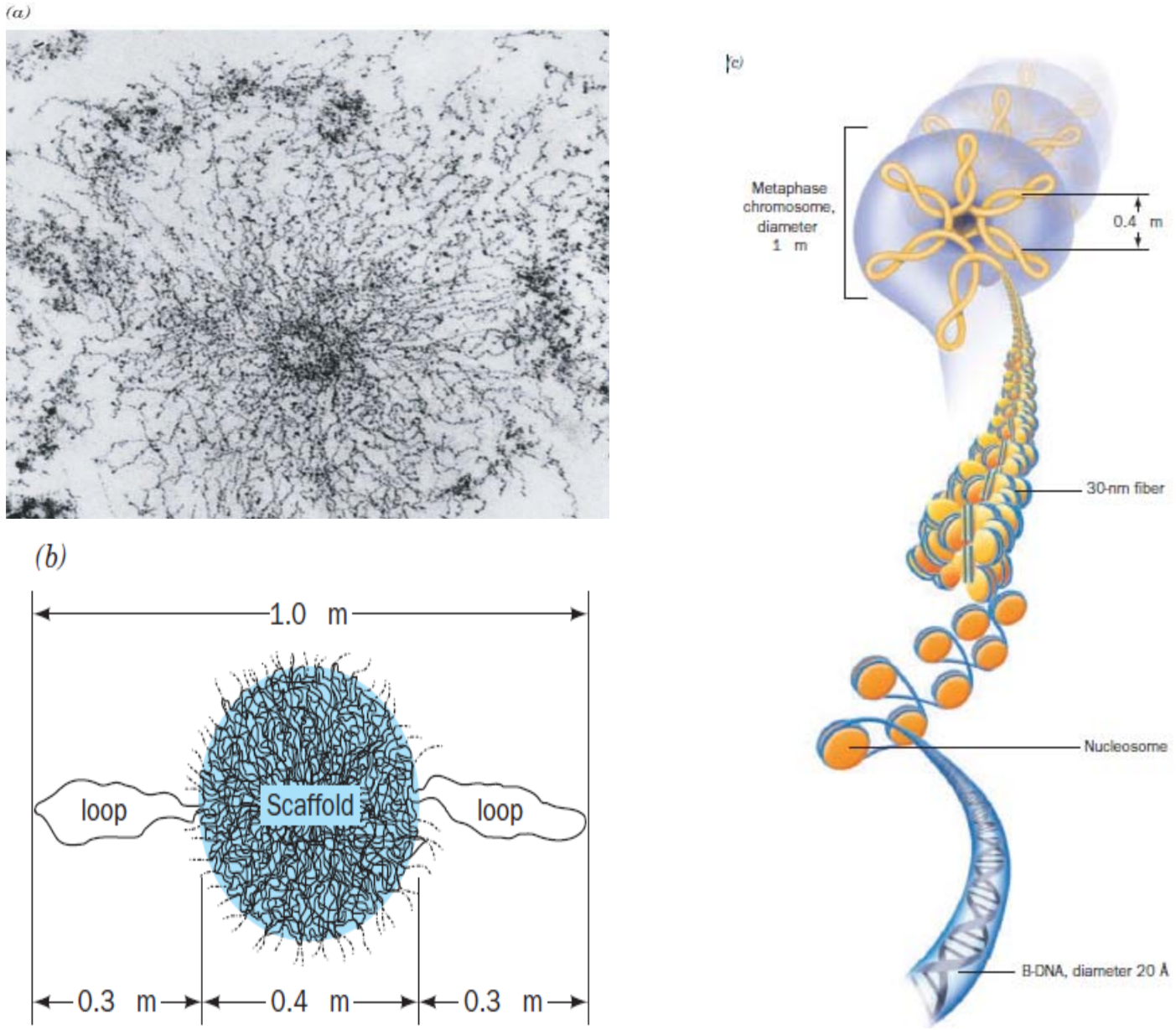


**Fig. Organization of DNA in a metaphase chromosome.**

# Metaphase Chromosomes Are Organized by Cohesin and Condensin

- The radial DNA loops are attached to the matrix via AT-rich **matrix-associated regions [MARs;** alternatively, **scaffold attachment regions (SARs)].**
- They are organized by **nonhistone proteins,** whose thousands of varieties constitute 10% of chromosomal proteins. Among the most prominent nonhistone proteins are the **structural maintenance of chromosomes (SMC) proteins**, which are members of a protein family that is expressed by prokaryotes as well as eukaryotes.
- SMC proteins are large polypeptides (1000–1300 residues) that have a unique domain organization.
- Their N- and C-terminal domains, which collectively form an ATPase domain resembling that of ABC transporters, are linked by two 50-nm-long helical segments that come together to form an antiparallel coiled coil and which are joined by a nonhelical, so-called hinge domain.
- Two different family members join at their hinge regions to form a V-shaped heterodimer

- Eukaryotes have two major species of SMC proteins, **cohesin**, whose SMC subunits are **Smc1** and **Smc3**, and **condensin**, whose SMC subunits are **Smc2** and **Smc4.**
- A third protein, a member of the ***kleisin*** (Greek: *kleisimo, closure*) superfamily*, binds to the* ATPase domains of both members of the SMC heterodimer to form a closed loop whose diameter is large enough to encompass two 10-nm fibers.
- During the G1 phase of the cell cycle cohesin loads onto the dispersed chromatin fibers in an ATP-dependent manner.
- When the DNA is subsequently replicated during S phase the replisome passes through the cohesin rings, thus keeping the resulting sister chromatids together.
- As the cell enters M phase (mitosis), condensin organizes the radial loops of the metaphase chromosomes.
- Concurrently, most of the cohesin rings open up to allow the parallel sister chromatids to separate everywhere but at their centromeres, a process that also requires the action of topoisomerase II to decatenate (untangle) the two dsDNA strands.

- Then, as the sister chromatids complete their separation during the anaphase portion of mitosis, the remaining cohesin rings open up.
- Finally, as the two daughter cells enter interphase and their chromosomes disperse, the condensin rings open up to release the chromatin fibers.
- The release of cohesin during the onset of M phase is induced by the phosphorylation, via a poorly understood
- process, of control proteins that bind to cohesin's kleisin subunit.
- A small population of cohesin bound near the centromere escapes this release; during anaphase, however, their kleisin subunits are cleaved by a protease named **separase,** thereby permitting the sister chromatids to fully separate.

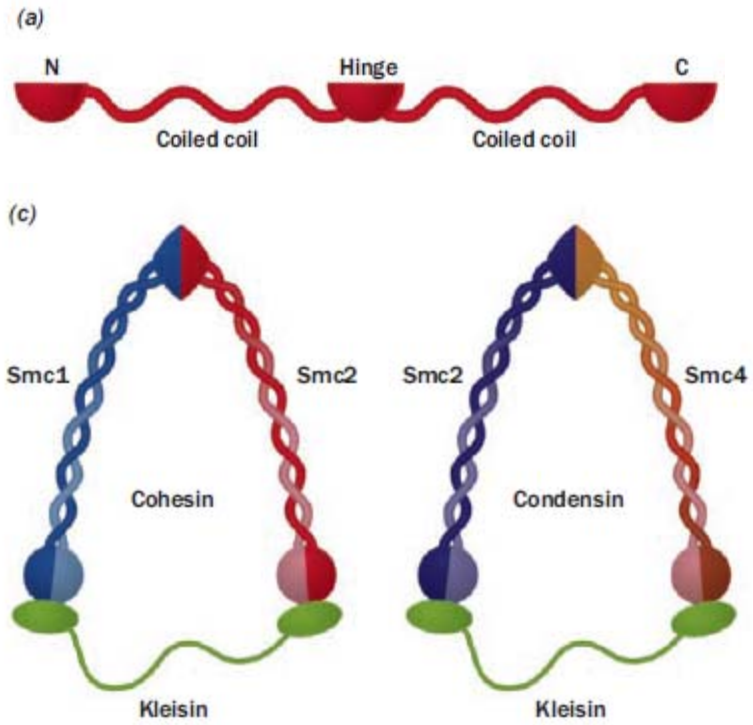


Fig. **SMC proteins.** (a) The domain organization of SMC proteins. The N- and C-terminal domains associate to form an ATPase, whereas the long helical segments joining these terminal domains to the hinge domain form an antiparallel coiled coil. (c) Schematic diagram of cohesin (left) and condensin (right). A kleisin (green) closes the loops of these large assemblies.

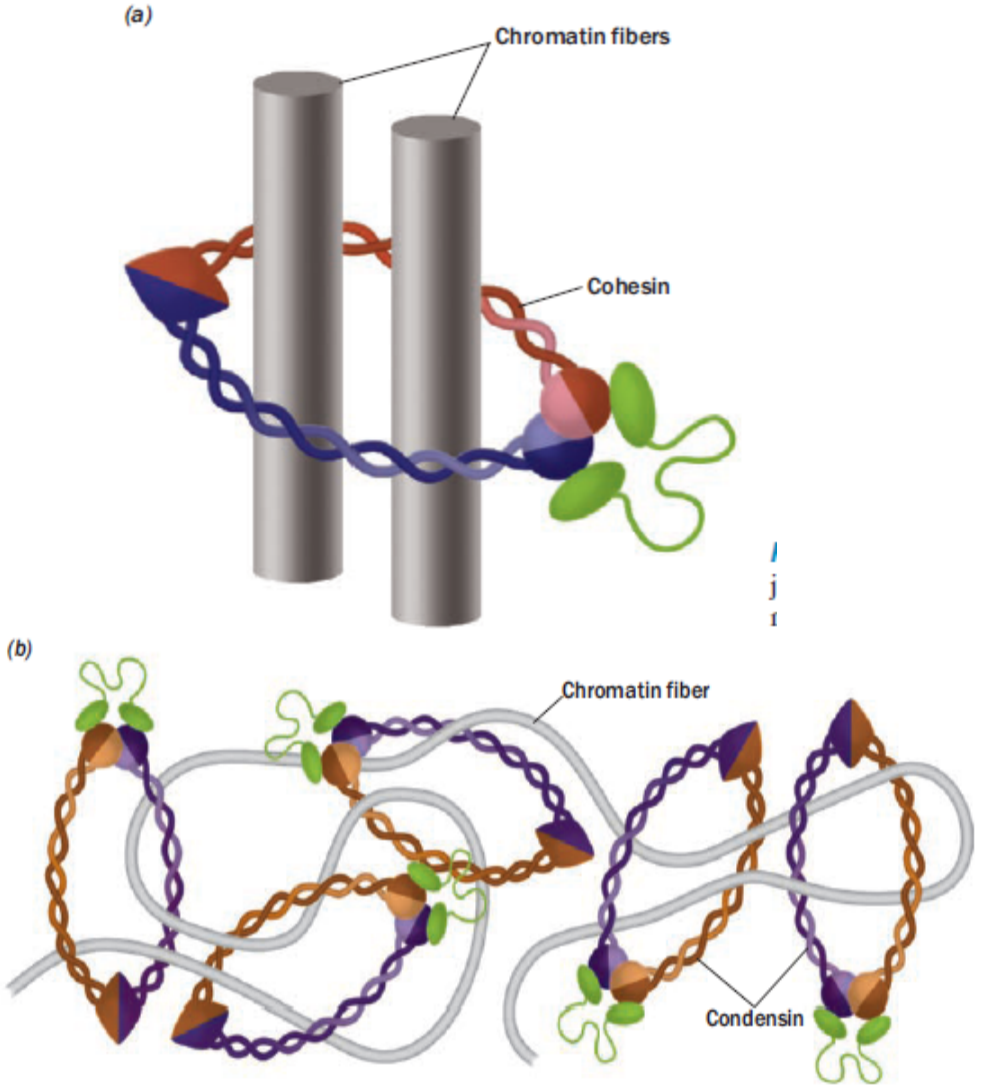


Fig. **Functions of cohesin and condensin.**
(a) Cohesin topologically joins the fibers of sister chromatids.
(b) Condensin topologically organizes the metaphase chromosome's radial loops.

# Interphase Chromosomes

**a. Polytene Chromosomes Contain Numerous Parallel DNA Strands**

- Until the 1980s, the diffuse structure of most interphase chromosomes made it all but impossible to characterize them at the level of individual genes.
- However, this difficulty was greatly ameliorated by the existence of “giant” banded chromosomes in certain terminally differentiated (nondividing) secretory cells of dipteran (two-winged) flies.
- These chromosomes, of which those from the salivary glands of *D. melanogaster* larvae were the most extensively studied, are produced by multiple replications of a synapsed (joined in parallel) diploid pair in which the replicas remain attached to one another and in register.
- Each diploid pair may replicate in this manner as many as nine times so that the final **polytene** (**Greek: *tainia,*** band or ribbon) chromosome contains up to $2*2^9$ =1024 DNA strands.
- The function(s) of polytene chromosomes is unclear although probably they permit a greatly increased rate of transcription of certain genes.

- The four giant chromosomes of *D. melanogaster* have an aggregate length of ~2 mm so that its haploid genome of
- $1.80 * 10^8$ bp has an average packing ratio in these chromosomes of 30.
- About 95% of this DNA is concentrated in chromosomal bands.
- These bands (more properly, **chromomeres),** as microscopically visualized through staining, form a pattern that is characteristic of each *D. melanogaster* strain.
- Indeed, chromosomal rearrangements such as duplications, deletions, and inversions result in a corresponding change in the banding pattern.
- A polytene chromosome's banding pattern therefore forms a cytological map that parallels its genetic map.

- The characteristic banding pattern of each polytene chromosome suggests that its component DNA molecules are precisely aligned.
- This hypothesis was corroborated by the application of ***in situ*** (on site) **hybridization**.
- In this technique, developed by Mary Lou Pardue and Joseph Gall, an immobilized chromosome preparation is treated with NaOH to denature its DNA; it is then hybridized with a purified species of radioactively labeled mRNA (or its corresponding cDNA), and the chromosomal binding site of the radioactive probe is determined by autoradiography.
- A given mRNA hybridizes with one, or no more than a few, chromosomal bands.

- *D. melanogaster's* four polytene chromosomes exhibit an aggregate of ~5000 bands.
- It originally appeared that the number of *D. melanogaster* genes was roughly equal to this number of bands and hence it was thought that each band corresponds to a single gene.
- However, the genome sequence of *D. melanogaster* contains 14,000 genes, nearly three times its number of bands.
- In fact, genes have been shown to be located in both band and interband regions, with some bands containing several genes and others containing none.
- Thus, it appears that the banding pattern of polytene chromosomes is a consequence of different levels of gene expression due to variations in chromatin structure, with the genes in the relatively open interband regions presumably more highly expressed than those in the more condensed and hence less accessible bands.

**b. Interphase Chromosomes Occupy Discrete Nuclear Territories**

- The experimental difficulties and lack of sensitivity of using radioactive probes stimulated the development of an improved method of *in situ hybridization known as* ***fluorescence in situ hybridization (FISH).***
- ***In this technique, the*** probe mRNA or DNA is fluorescently labeled and the chromosomal site to which it hybridized is identified by fluorescence microscopy.
- How are the chromosomes in an interphase nucleus distributed? This question was answered using a method known as **three-dimensional FISH** in which the chromosomes in an interphase nucleus are each "painted" with a mixture of probes specific for sites that are scattered throughout the chromosome and in which the probes are labeled by a chromosome-specific combination of **fluorophores** (fluorescent groups).
- Then, using a sophisticated form of fluorescence microscopy that acquires a series of images at different levels in the nucleus and spectrally differentiates the various combinations of fluorophores, the three-dimensional locations of the various chromosomes in a nucleus are determined.
- The resulting images reveal that each chromosome occupies a specific space or **territory in** the nucleus that does not greatly overlap the territories of other chromosomes.
- The territory occupied by a particular chromosome does not greatly vary during the lifetime of a cell, but it changes after cell division.
- However, the observation that **translocations,** in which a segment from one chromosome becomes attached to another, are more frequent between certain pairs of chromosomes suggests that their territories are often in proximity.

- Images such as Fig. 34-22 might naively suggest that the nucleus is tightly packed with chromatin. However, a rough calculation indicates that chromatin as 10-nm fibers occupies only 17% of the nuclear volume.
- Moreover, electron micrographs of interphase nuclei reveal that their chromatin is dispersed mainly as 10- and 30-nm fibers, and FISH experiments have shown that RNA transcripts are distributed throughout chromosomal territories.
- Evidently, there is sufficient space within chromosomal territories to allow free access of the transcriptional machinery and presumably the replicational machinery to its target DNA.