Prokaryotic genome organization

- Each bacterial chromosome is made by a single circular DNA molecule (rarely linear).
- Usually each cell contain one single copy of each chromosome.
- The genetic material can be seen as a fairly compact clump (or series of clumps) that occupies about a third of the volume of the cell named NUCLEOID.
- The DNA of these loops is not found in the extended form of a free duplex, but instead is compacted by association with proteins.

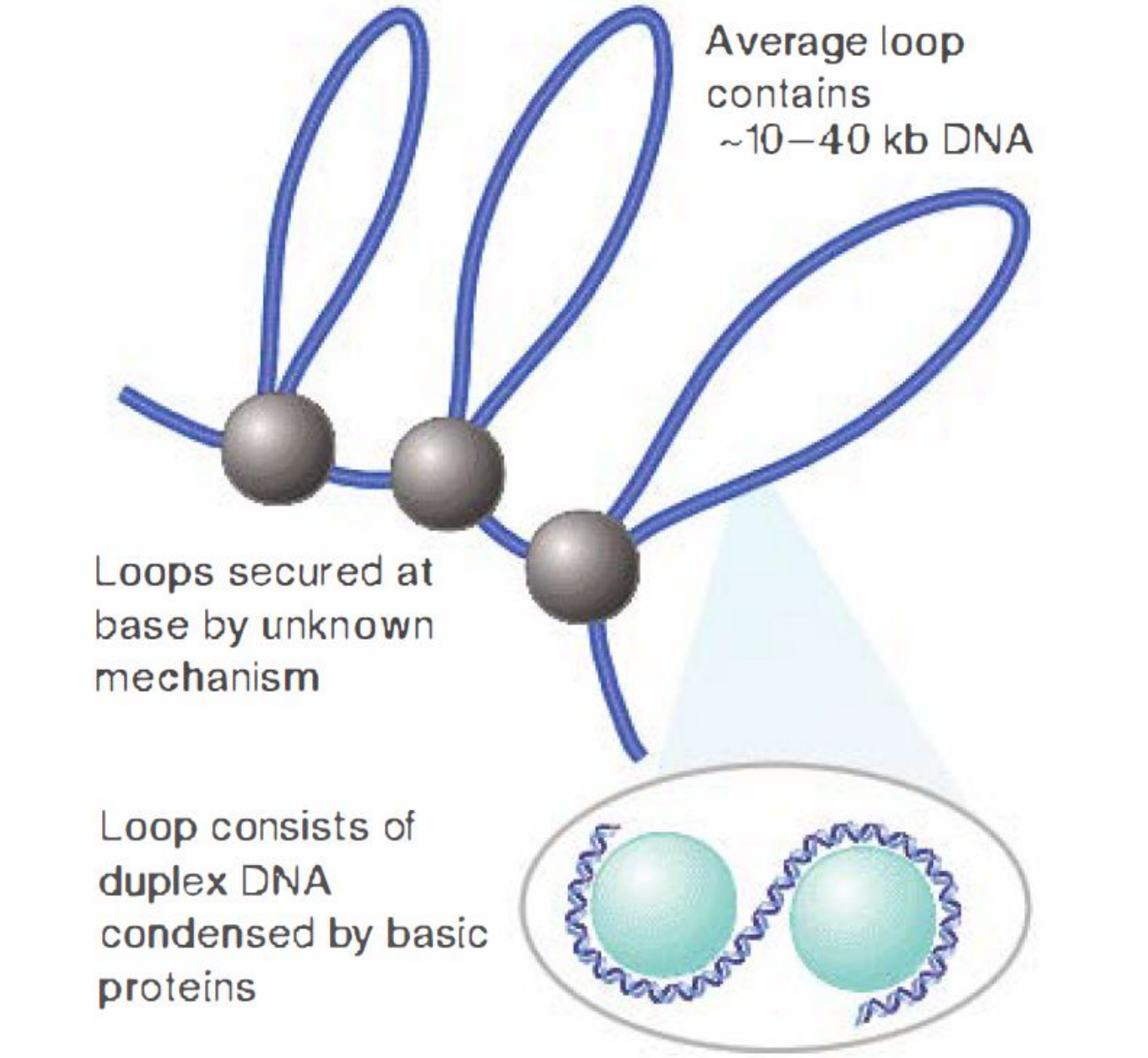


FIGURE 9.7 The bacterial genome consists of a large number of loops of duplex DNA (in the form of a fiber), each of which is secured at the base to form an independent structural domain.

The nucleoid has 400 independent negatively supercoiled domains. The average density of supercoiling is 1 turn/100 bp. Each domain consists of a loop of DNA, the ends of which are secured in some (unknown) way that does not allow rotational events to propagate from one domain to another. (nick caused by Ethidium Bromide)

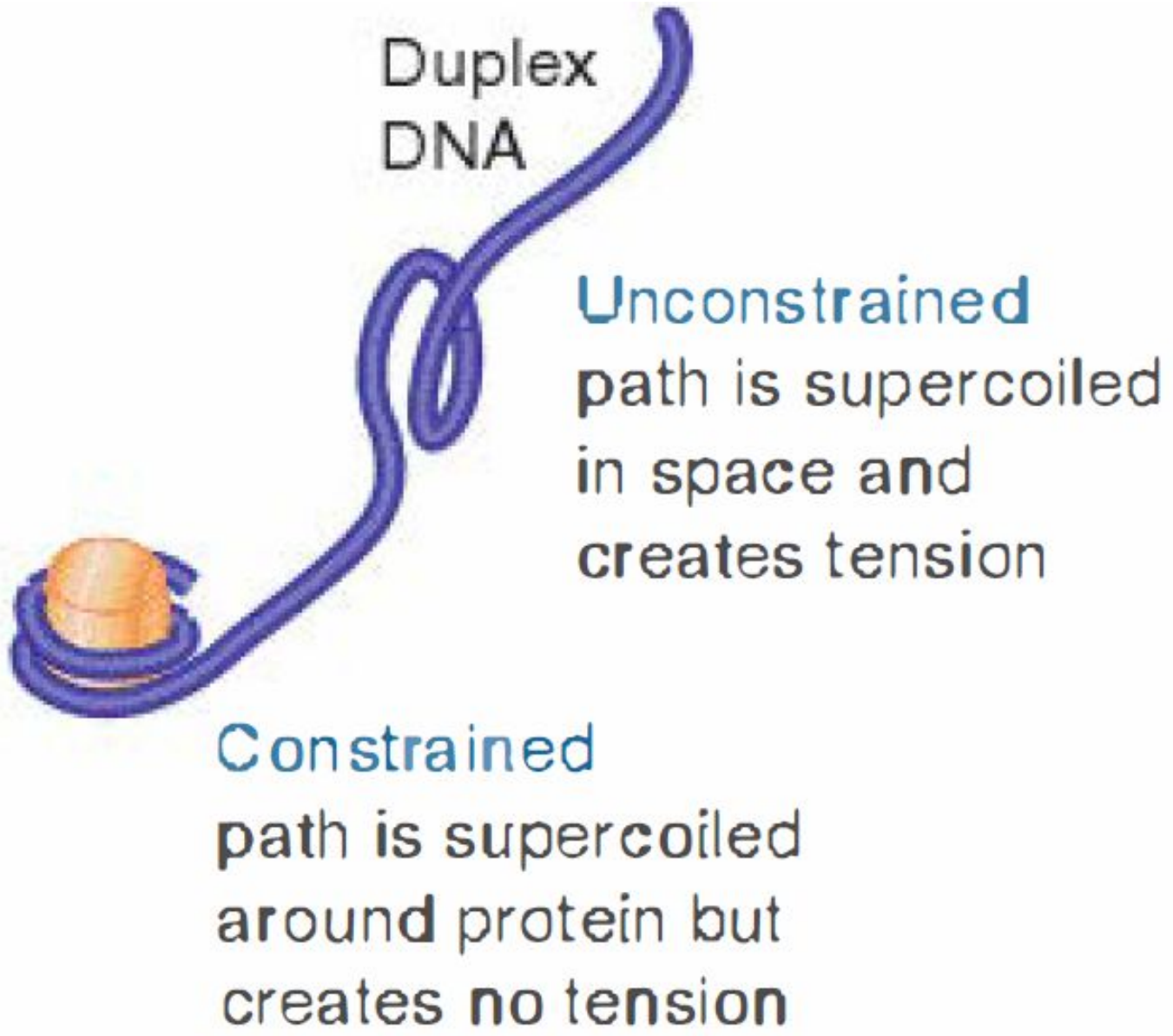


FIGURE 9.8 An unrestrained supercoil in the DNA path creates tension, but no tension is transmitted along DNA when a supercoil is restrained by protein binding.

Eukaryotic genome organization

- Each eukaryotic chromosome is made by a single linear DNA molecule.
- Chromosomes are made of chromatin, some other proteins and are located on the nucleus.
- The cell can have one single copy (haploid), two (diploid) or multiple (polypoid) copies of each chromosome.
- They can be directly seen only during cell mitosis.

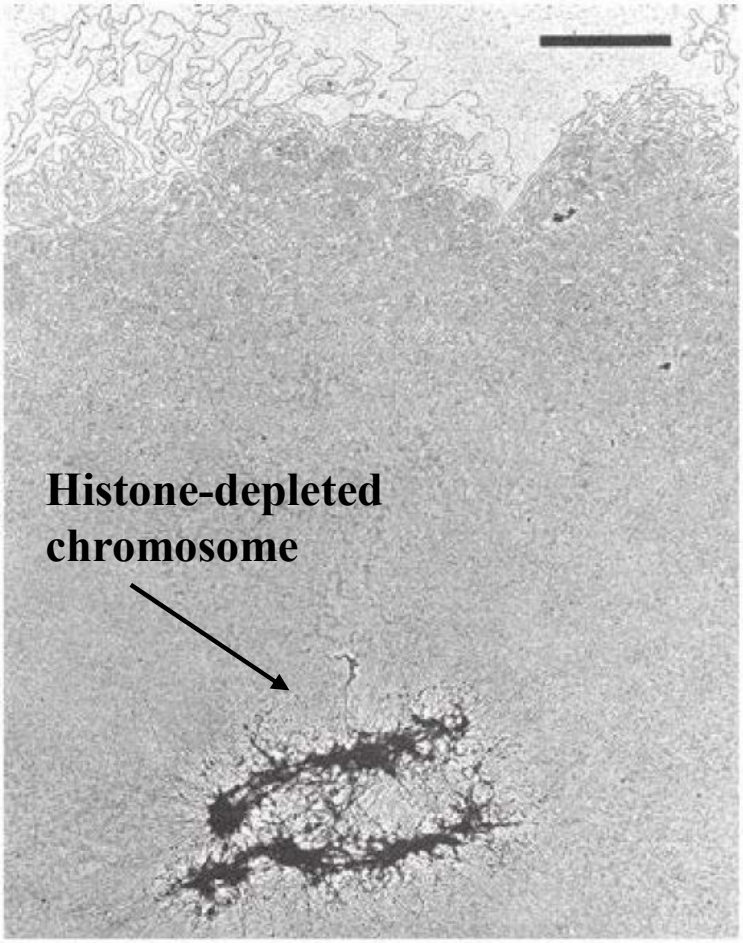


FIGURE 9.9 Histone-depleted chromosomes consist of a protein scaffold to which loops of DNA are anchored. Reprinted from *Cell*, vol. 12, J. R. Paulson and U. K. Laemmli, The structure of histone-depleted metaphase chromosomes, pp. 817–828. Copyright 1977, with permission from Elsevier (http://www.sciencedirect.com/science/article/pii/009286747790280X). Photo courtesy of Ulrich K. Laemmli, University of Geneva, Switzerland.

60Kb loops linked to an interphase matrix

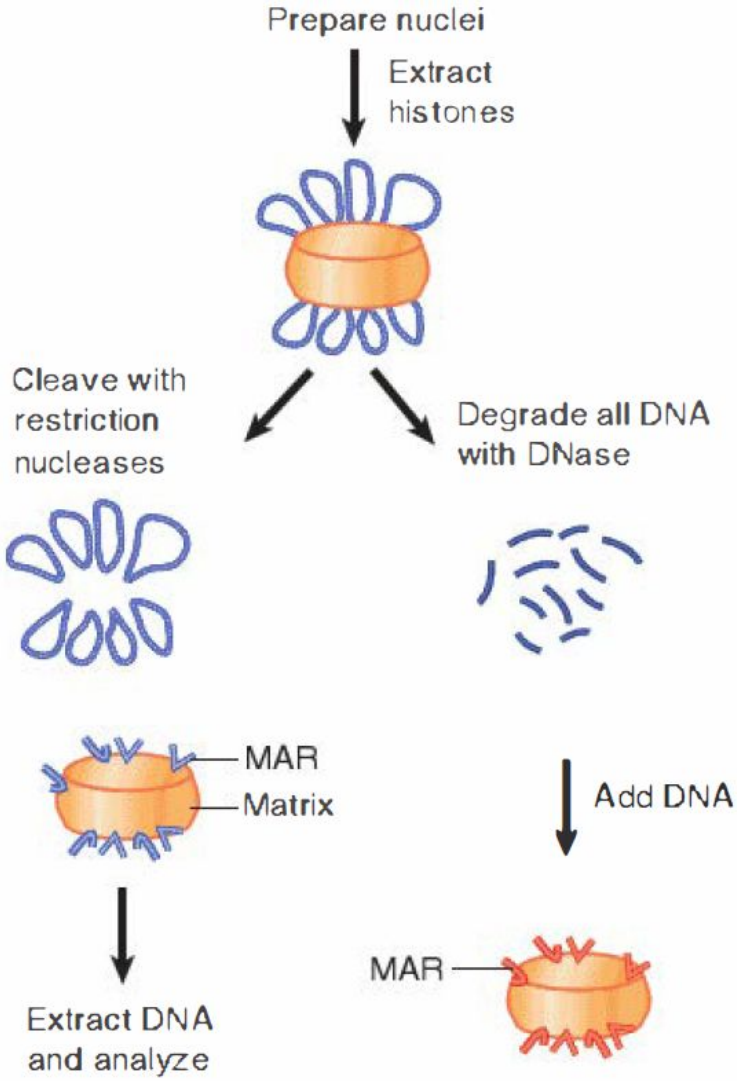


FIGURE 9.10 Matrix-associated regions (MARs) may be identified by characterizing the DNA retained by the matrix isolated *in vivo* (left) or by identifying the fragments that can bind to the matrix from which all DNA has been removed (right).

MAR: matrix attachment region
SAR: scaffold attachment region

Chromatin structure during interphase

- Single chromosomes can be seen only during mitosis.

- In interphase chromatin is present as:

➢ Euchromatin (less dens): expressed genes (not all)
➢ Heterochromatin (more dens): constitutive (E.G. satellite DNA)
facultative

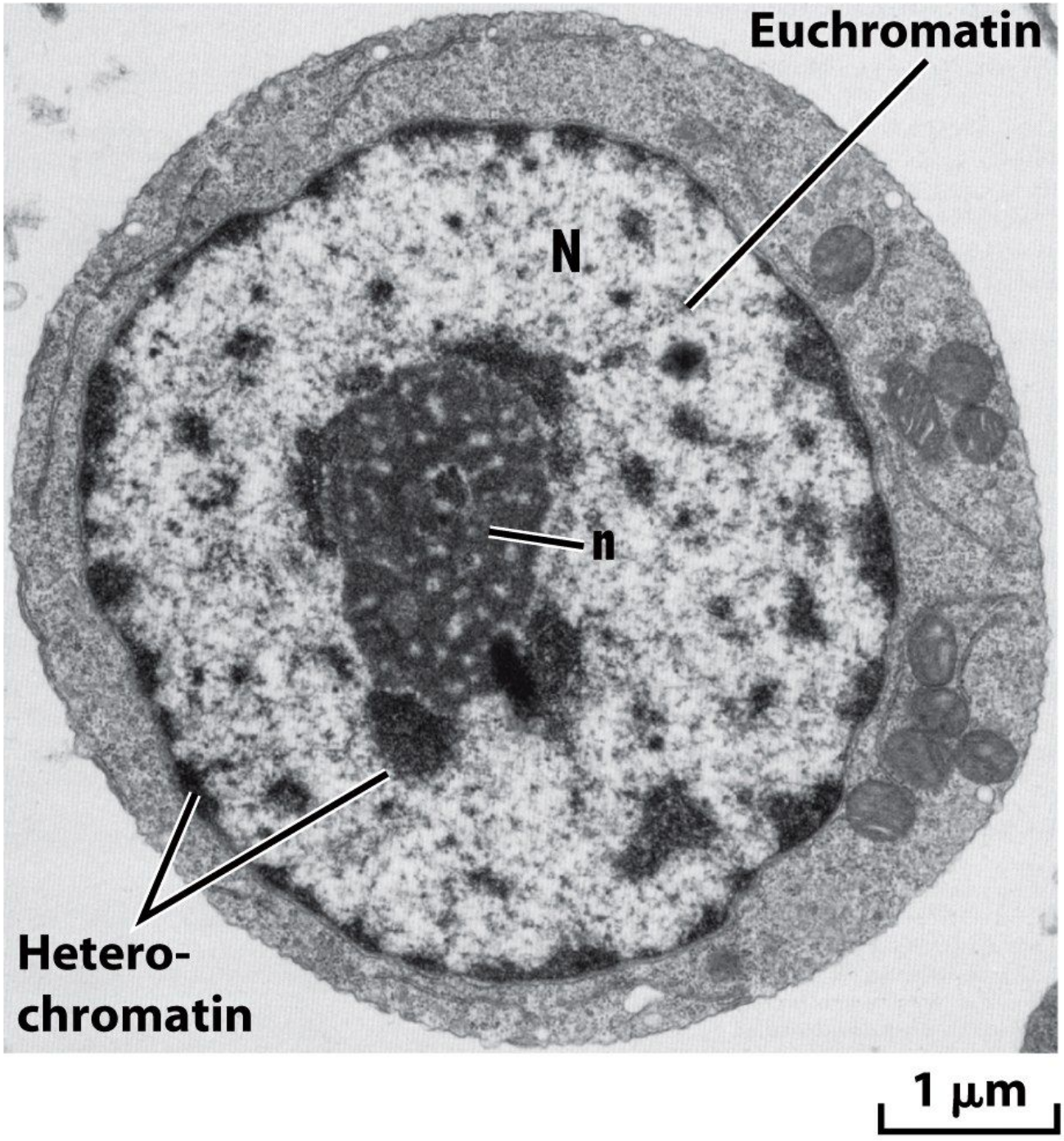


Figure 6-33a
Molecular Cell Biology, Sixth Edition

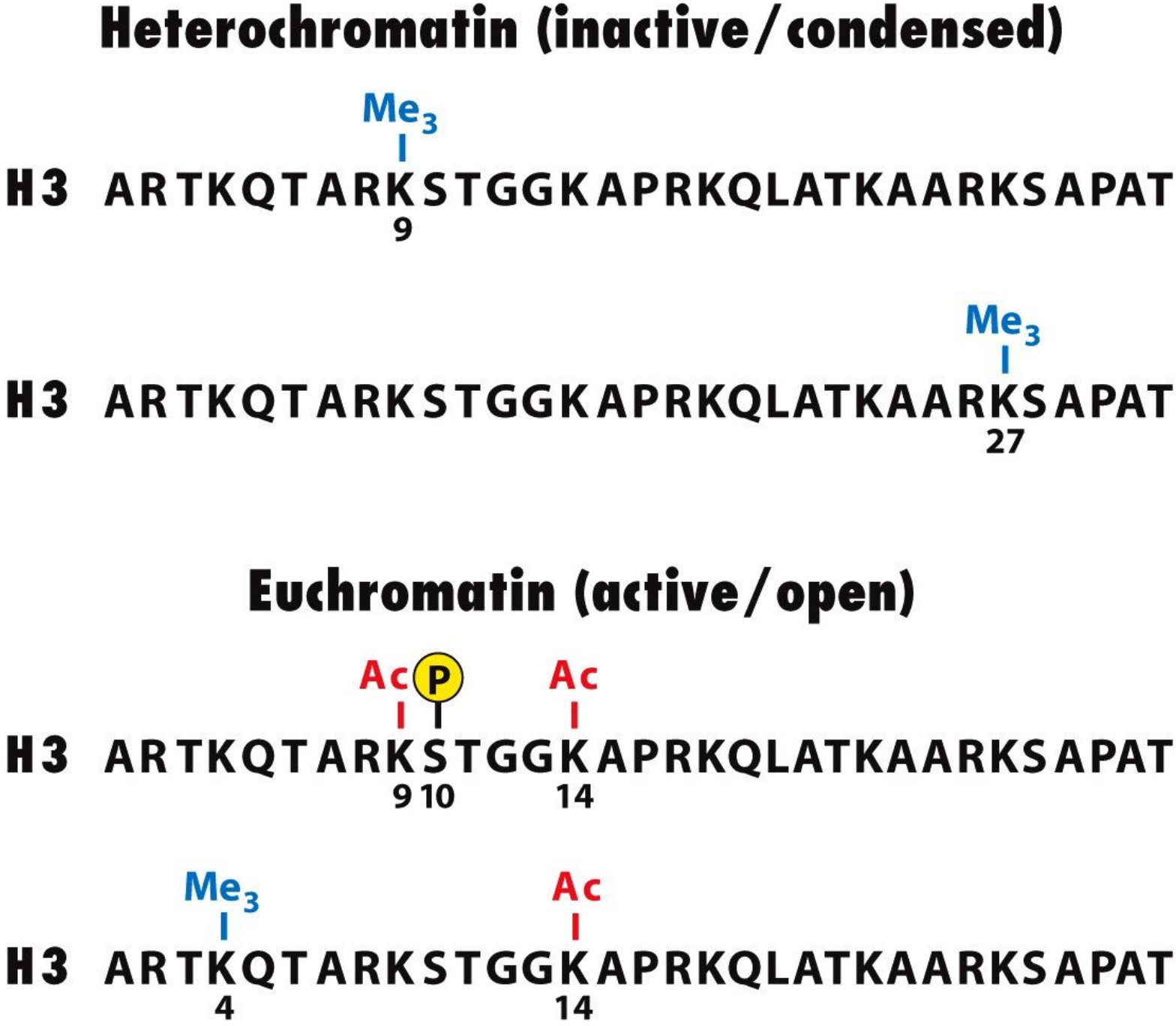


Figure 6-33b
Molecular Cell Biology, Sixth Edition

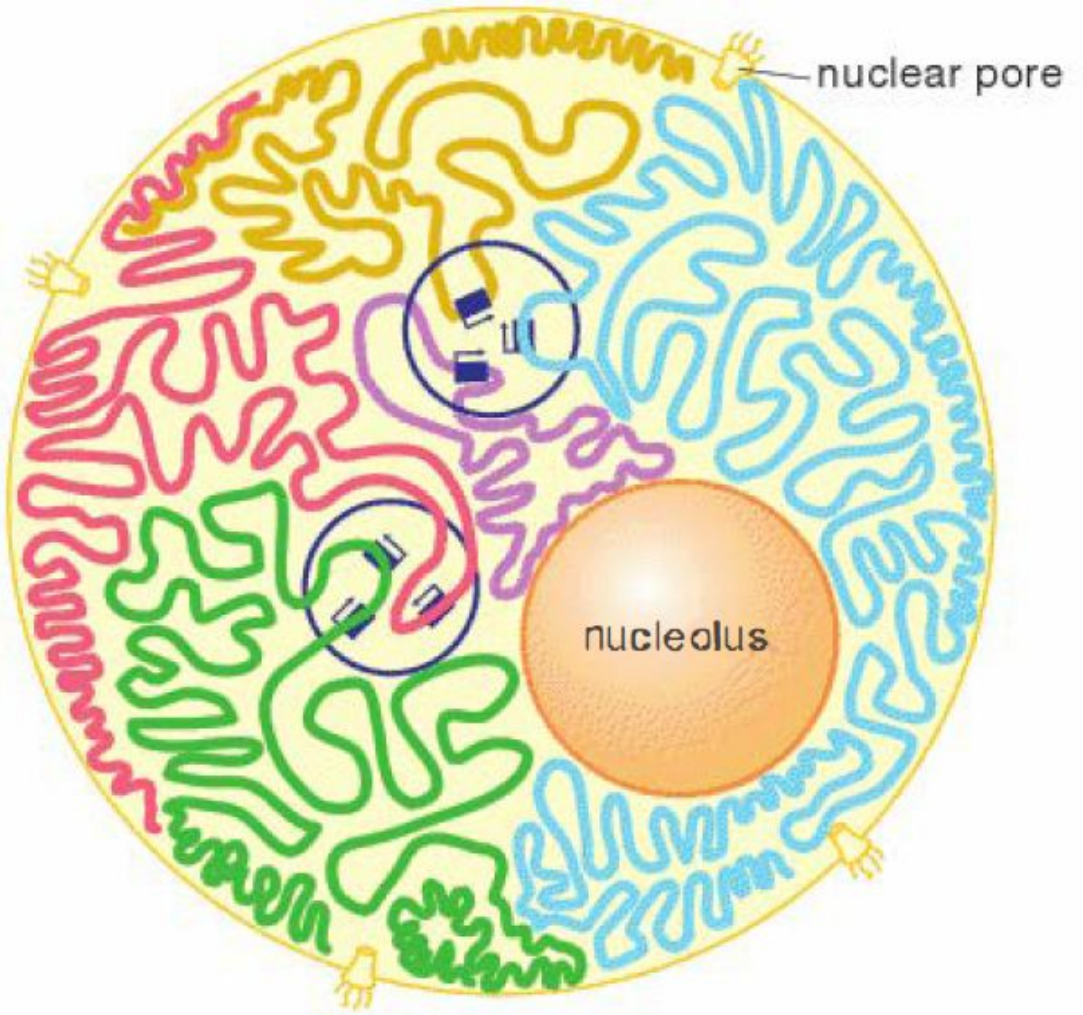
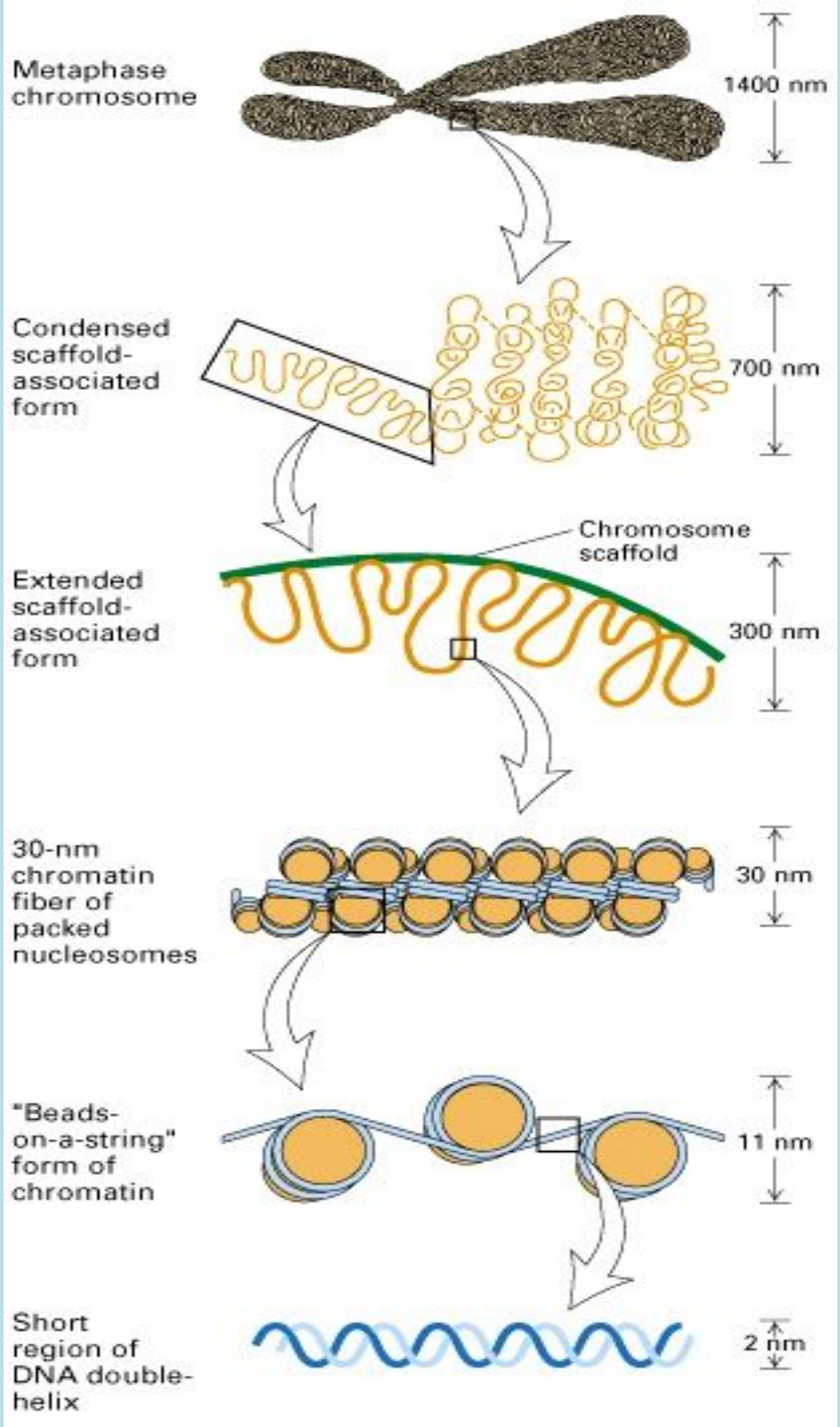


FIGURE 9.13 Chromosomes occupy chromosome territories in the nucleus and are not entangled with each other.. Heterochromatic regions, silenced genes, and gene-sparse regions of chromosomes are typically localized to the nuclear periphery. Active genes are often found at the borders of chromosome territories, and active genes from several chromosomes may cluster in inter-chromosomal territories that are enriched in transcription machinery (circled at the center of the figure).

(during mythosis)



(physiological salt concentration)

(low salt concentration)

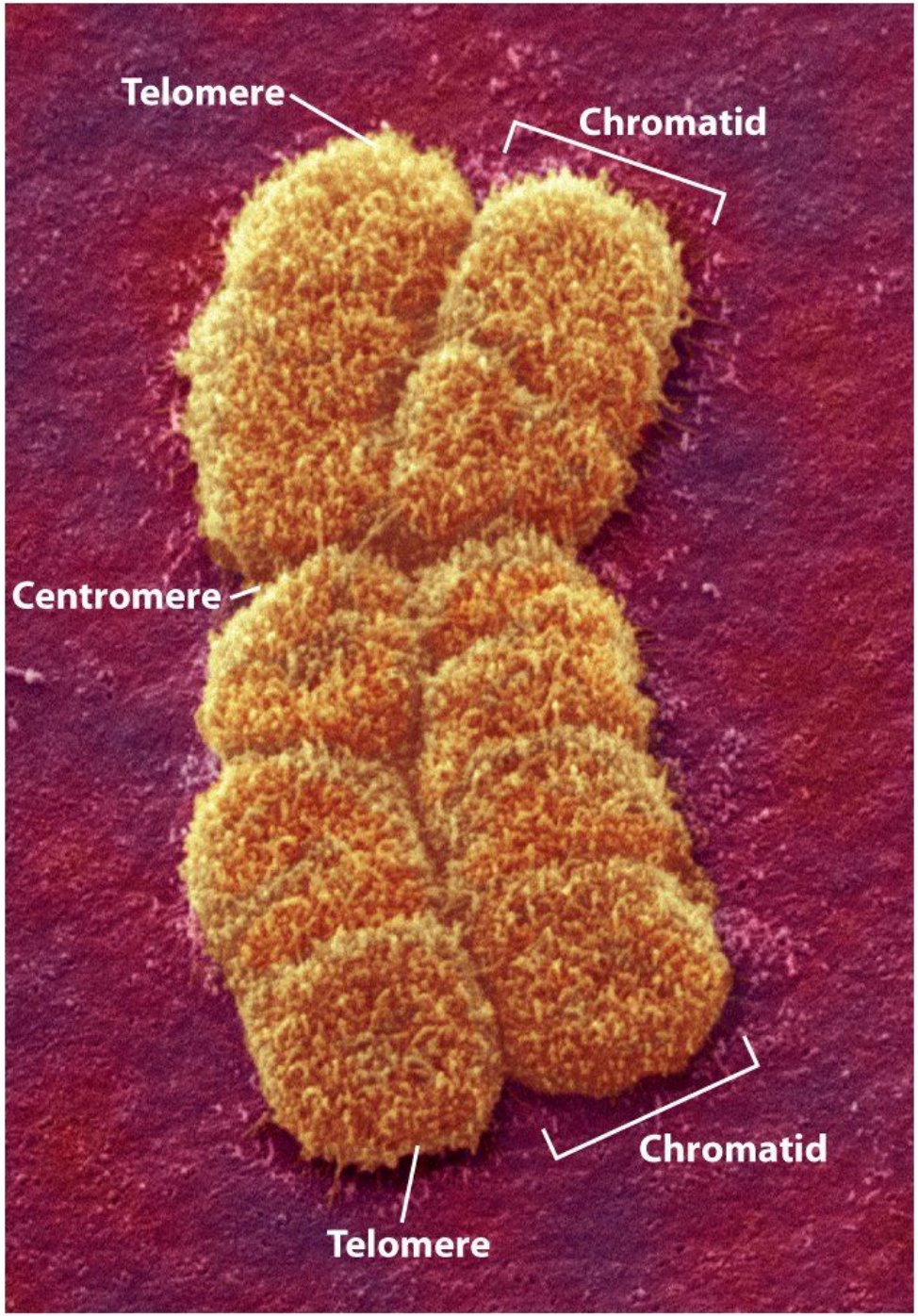


Figure 6-40
Molecular Cell Biology, Sixth Edition

The Giemsa reactive cause the mitosis chromosomes to have the appearance of a series of striations, which are called

G-bands

- The G-bands are lower in G-C content than the interbands.
- Genes are concentrated in the G-C-rich interbands

Giemsa stain, named after Gustav Giemsa, an early malariologist, is used for the histopathological diagnosis of malaria and other parasites. It is a mixture of methylene blue and eosin. The stain is usually prepared from commercially available Giemsa powder. It is specific for the phosphate groups of DNA and attaches itself to regions of DNA where there are high amounts of adenine-thymine bonding. Giemsa stain is used in Giemsa banding, commonly called G-banding, to stain chromosomes and often used to create a karyotype. It can identify chromosomal aberrations such as translocations and interchanges.

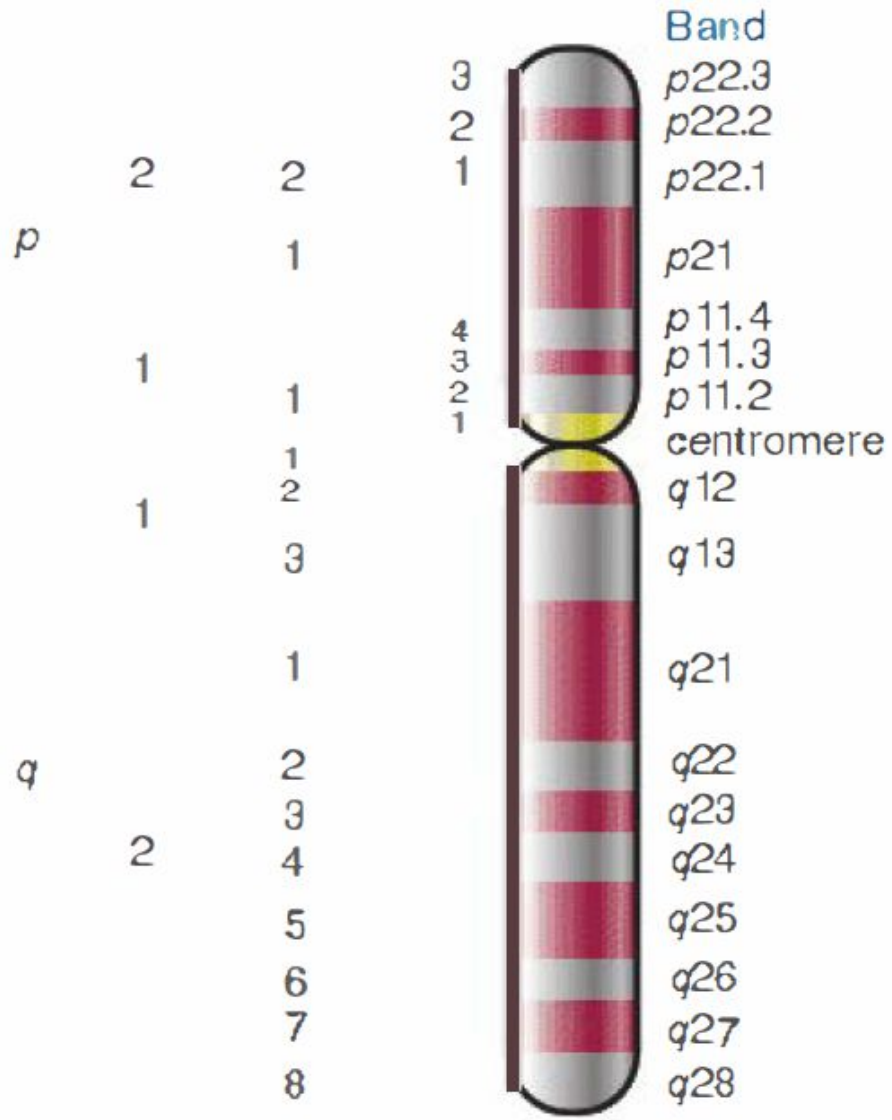


FIGURE 9.15 The human X chromosome can be divided into distinct regions by its banding pattern. The short arm is *p* and the long arm is *q*; each arm is divided into larger regions that are further subdivided. This map shows a low-resolution structure; at higher resolution, some bands are further subdivided into smaller bands and interbands, e.g., *p*21 is divided into *p*21.1, *p*21.2, and *p*21.3.

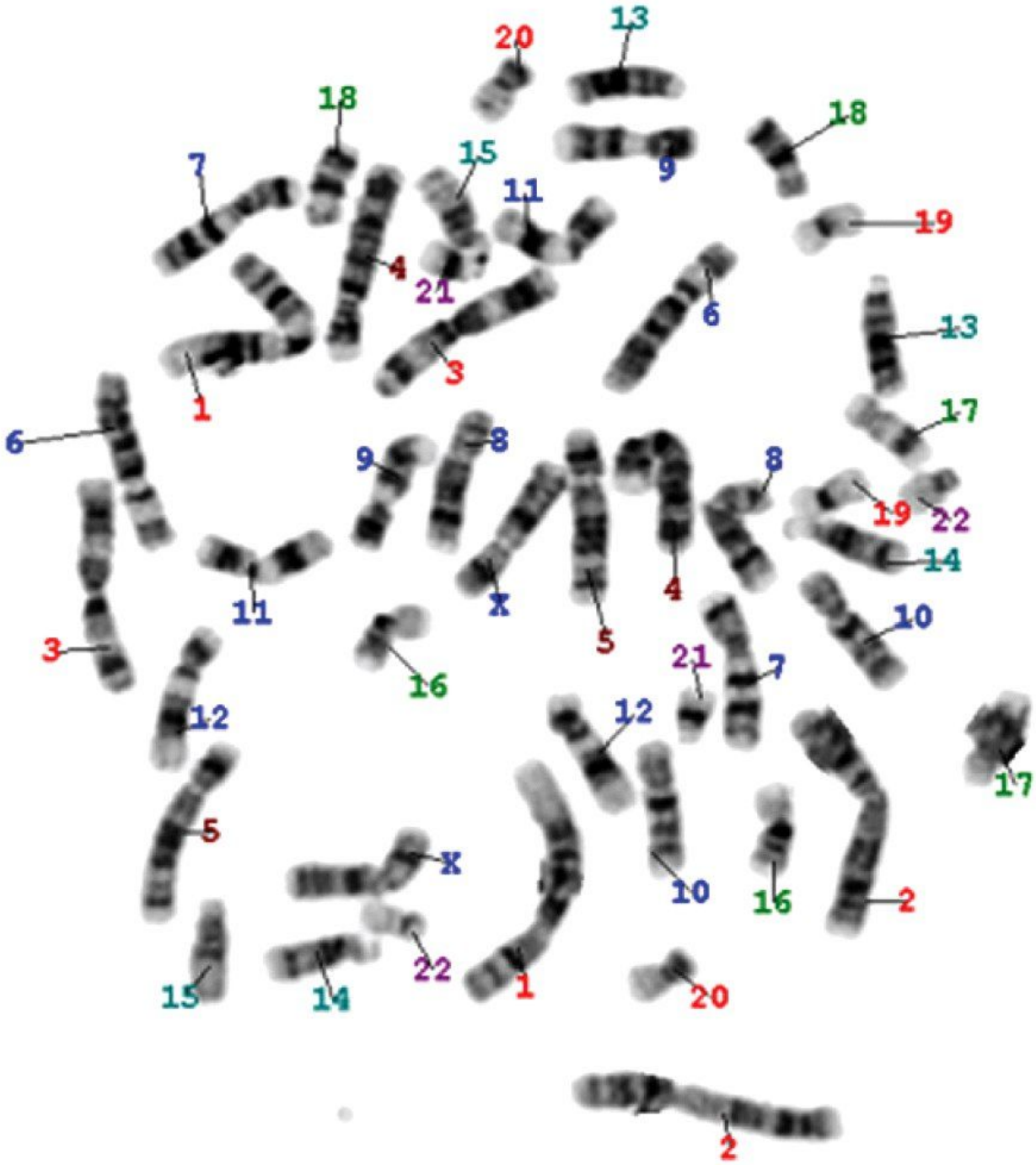


Figure 6-41
Molecular Cell Biology, Sixth Edition

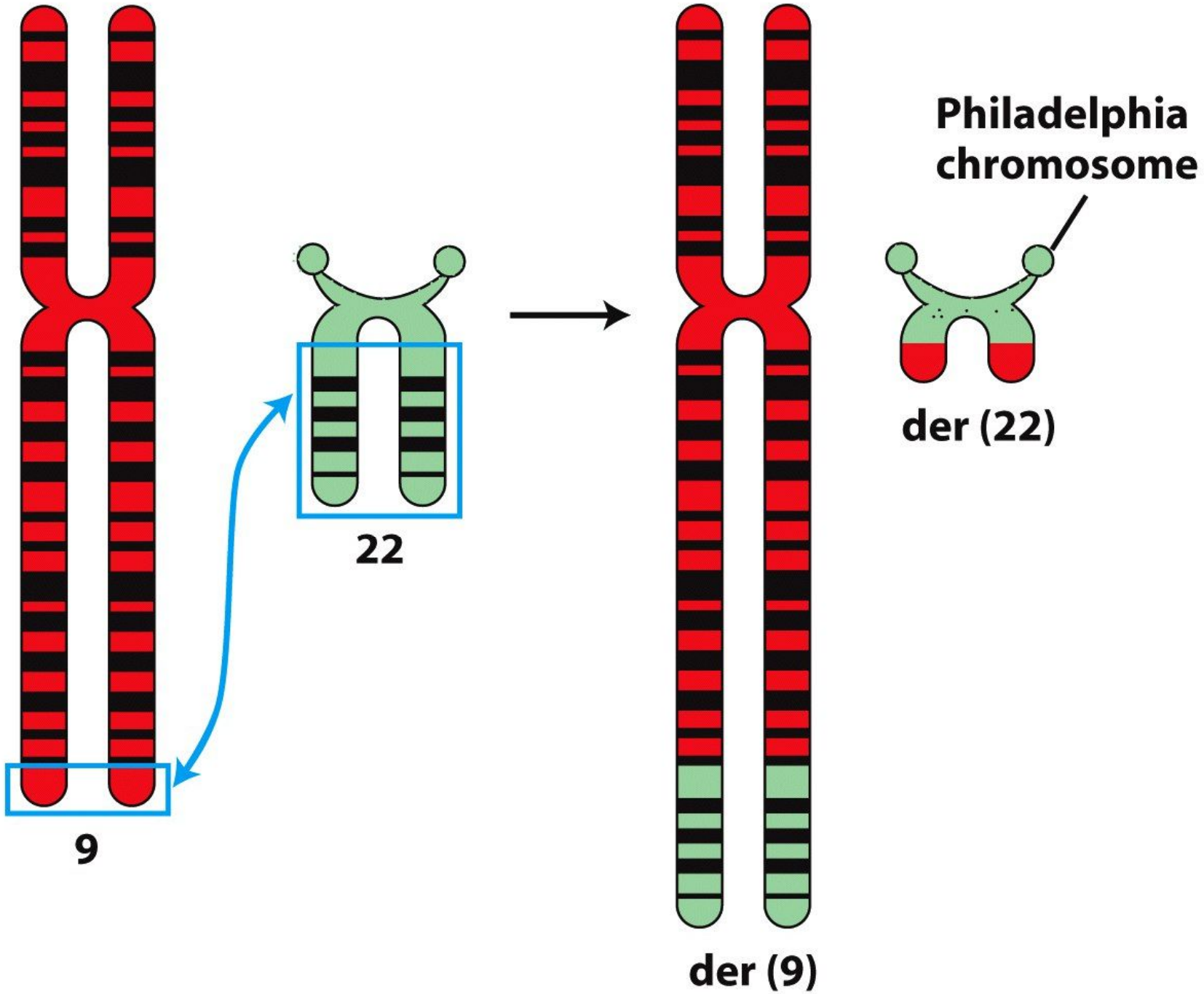


Figure 6-42a
Molecular Cell Biology, Sixth Edition

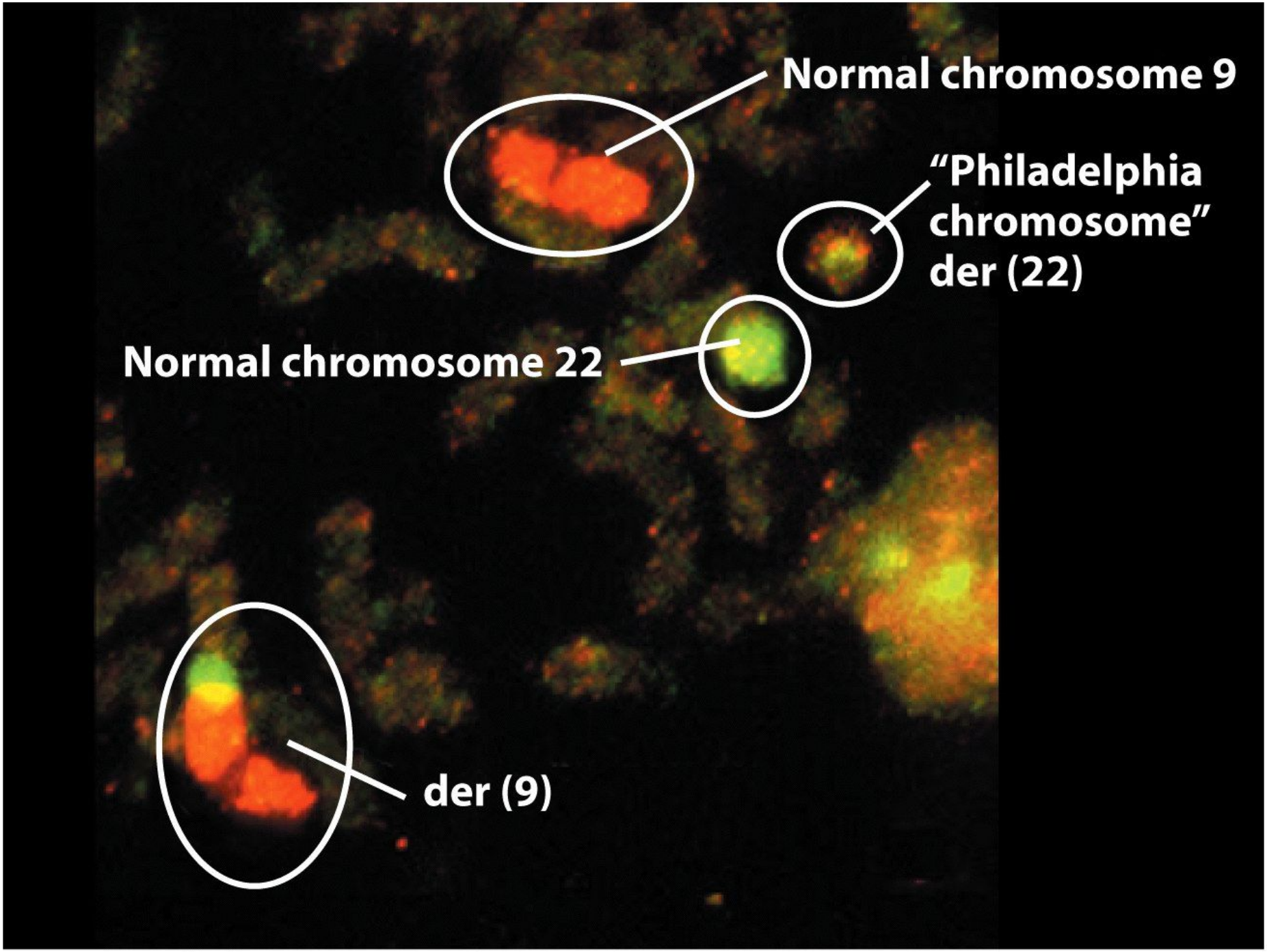


Figure 6-42b
Molecular Cell Biology, Sixth Edition

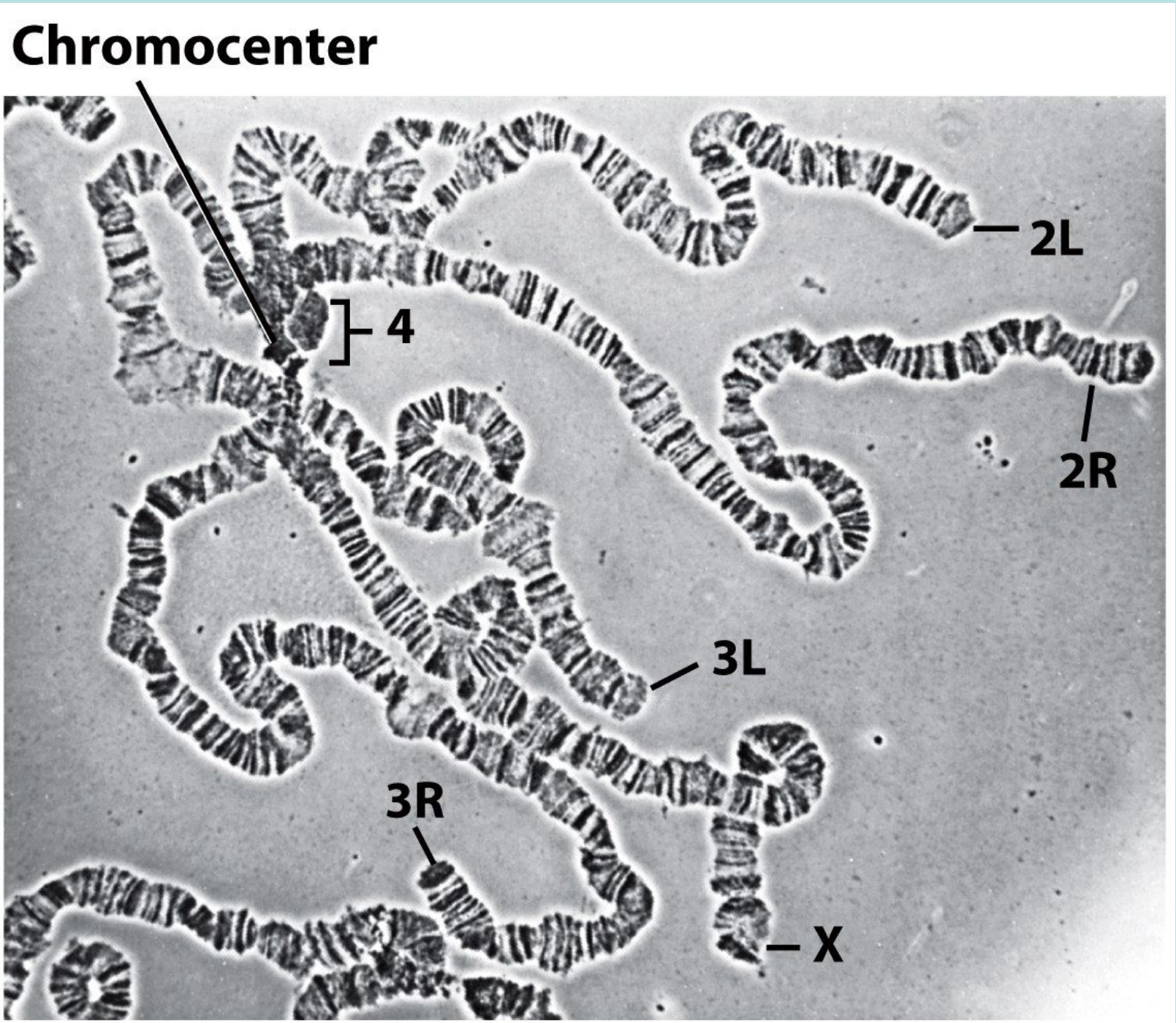


Figure 6-44a
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Cromosoma politenico *D. melanogaster*

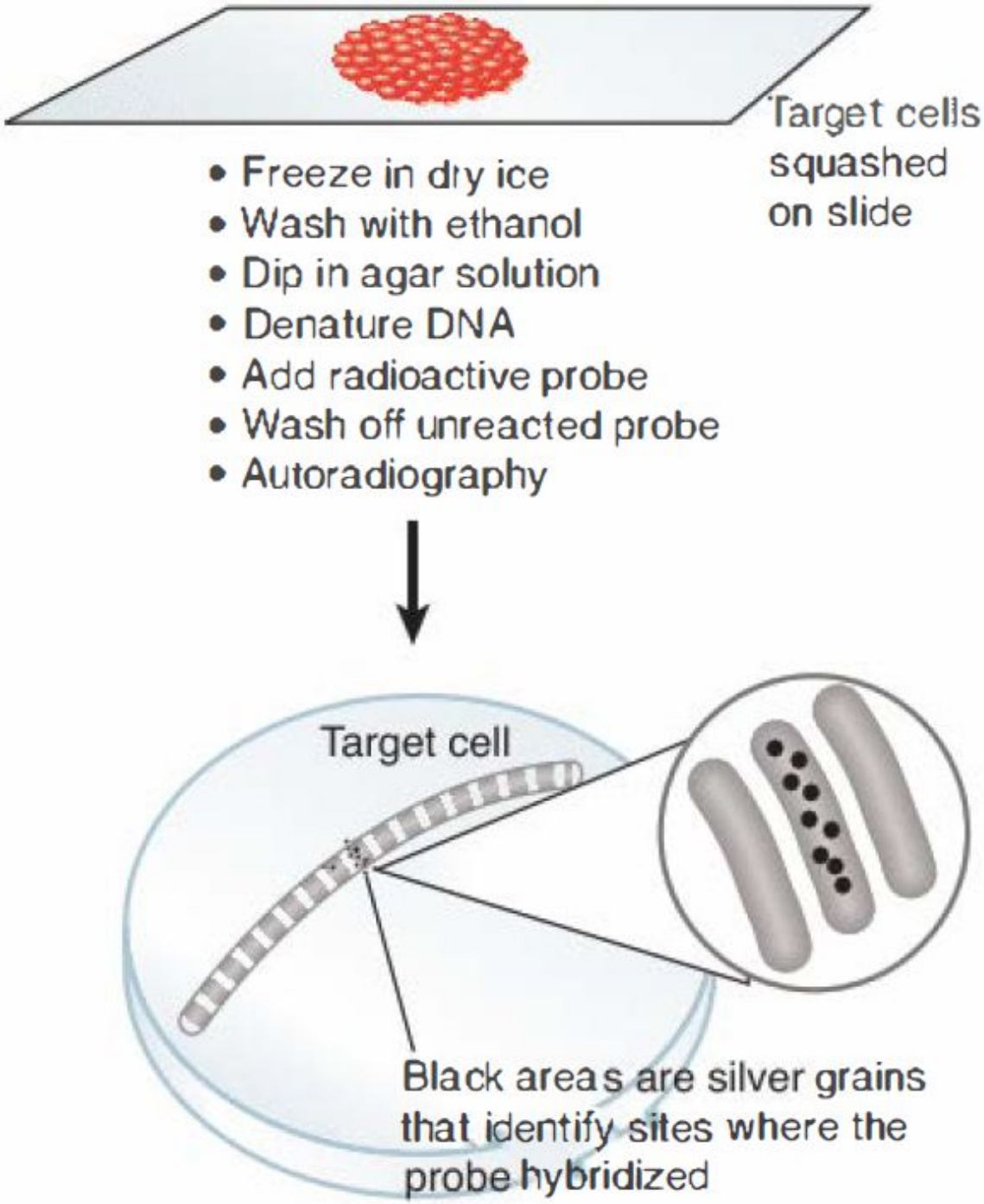

FIGURE 9.19 Individual bands containing particular genes can be identified by *in situ* hybridization.

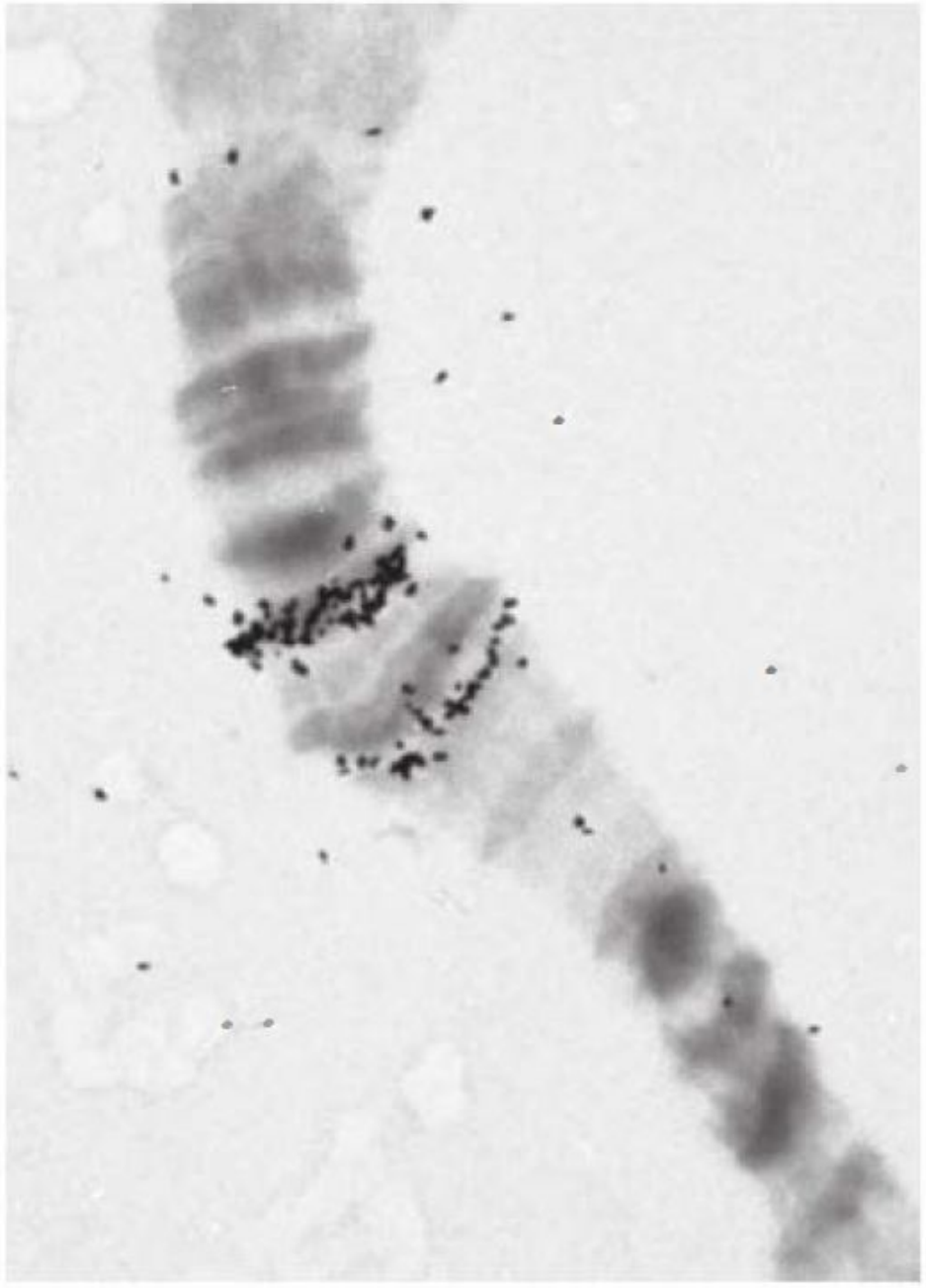

FIGURE 9.20 A magnified view of bands 87A and 87C shows their hybridization *in situ* with labeled RNA extracted from heat-shocked cells. Photo courtesy of José Bonner, Indiana University.

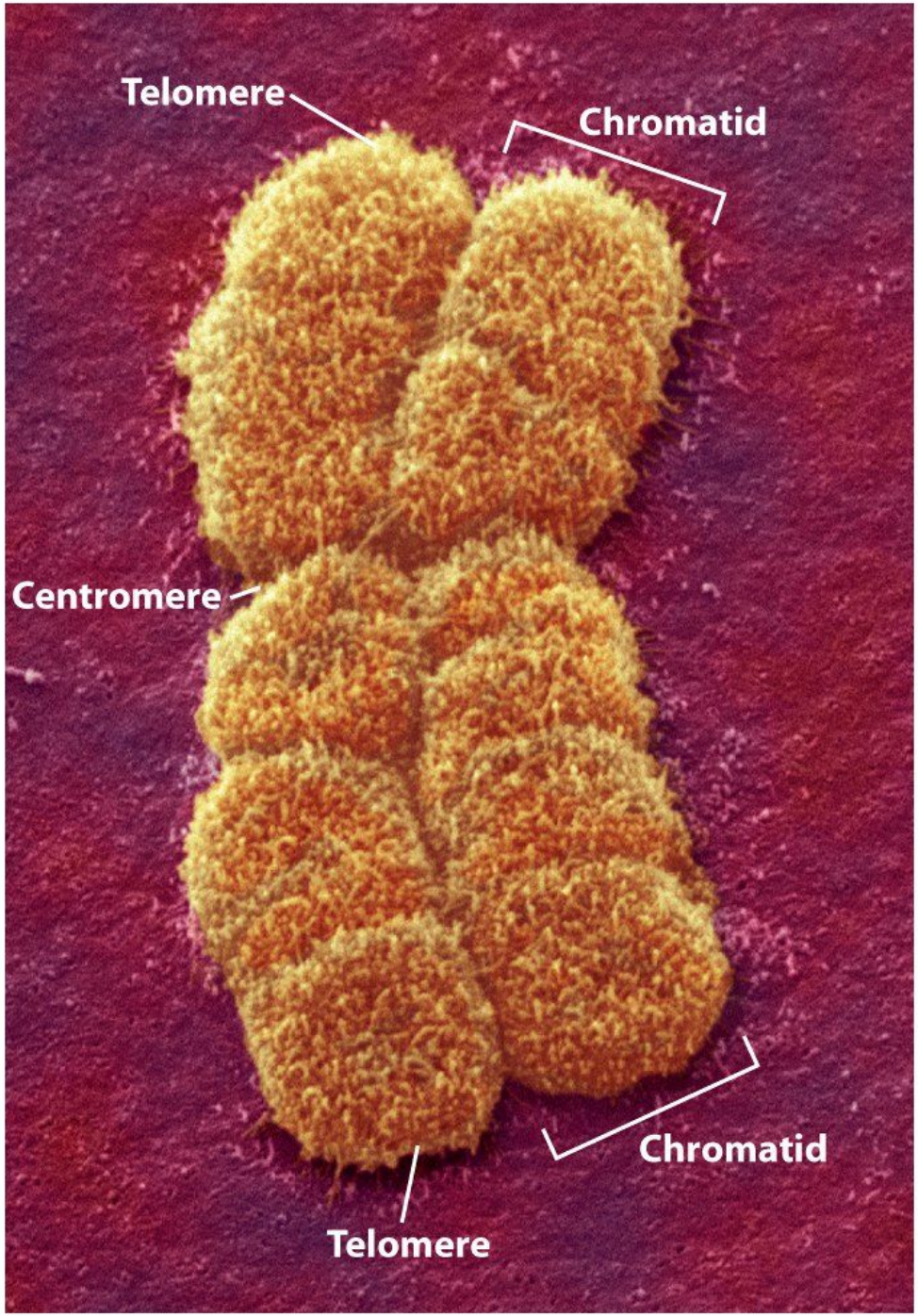

Figure 6-40
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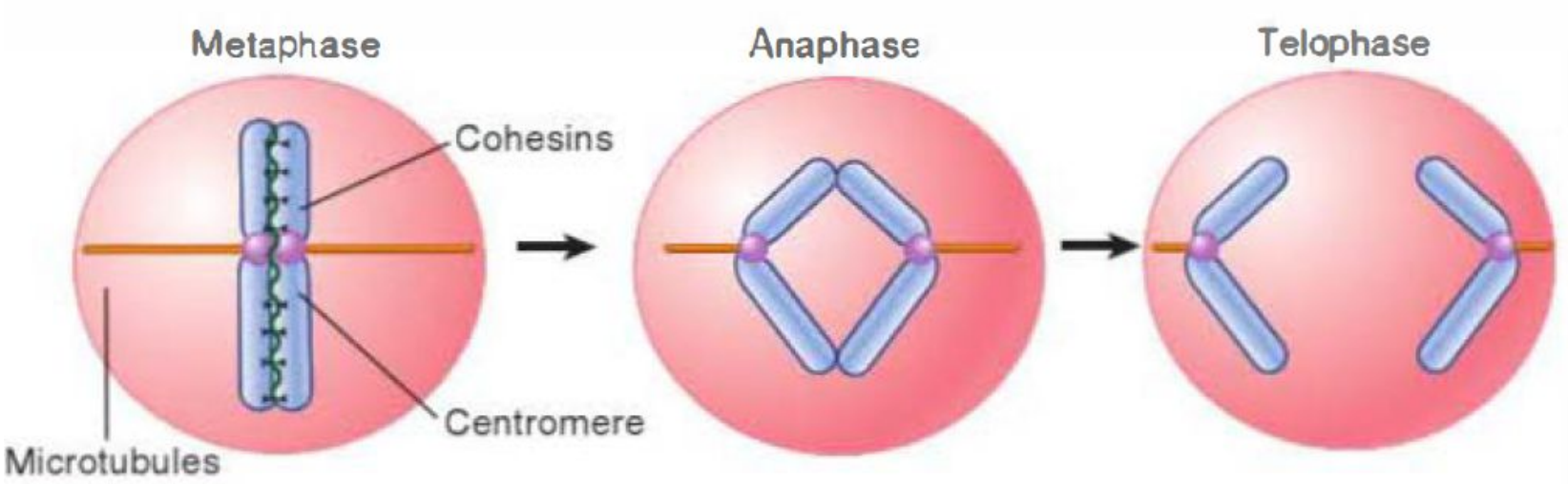
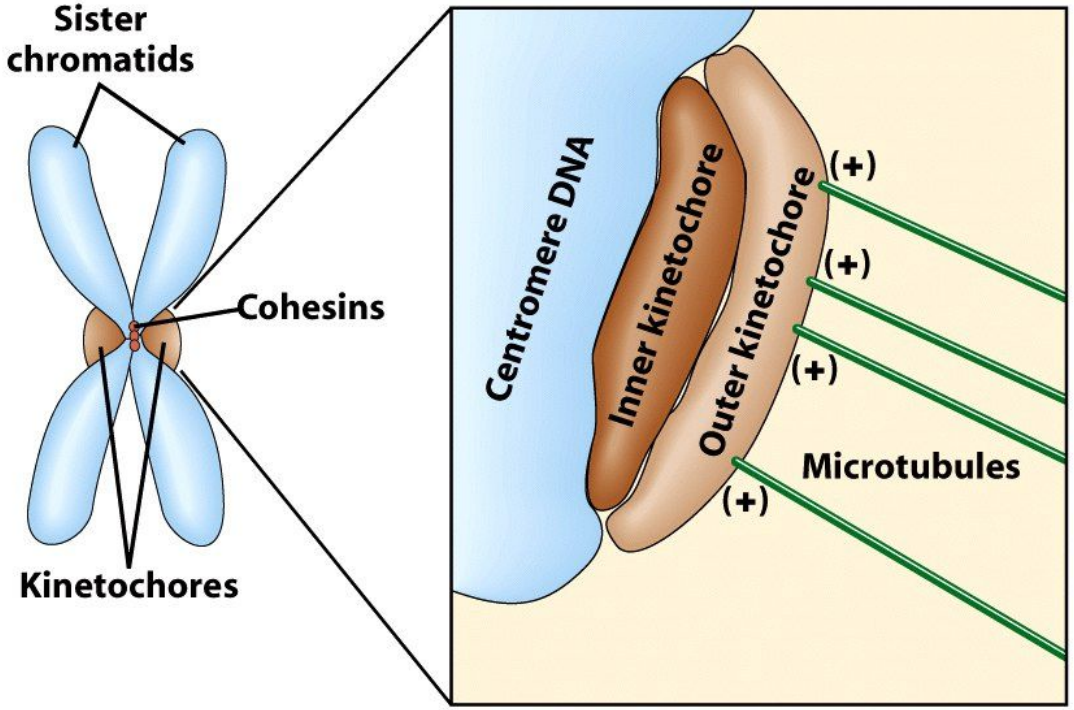


FIGURE 9.23 Chromosomes are pulled to the poles via microtubules that attach at the centromeres. The sister chromatids are held together until anaphase by glue proteins (cohesins). The centromere is shown here in the middle of the chromosome (metacentric), but can be located anywhere along its length, including close to the end (acrocentric) and at the end (telocentric).



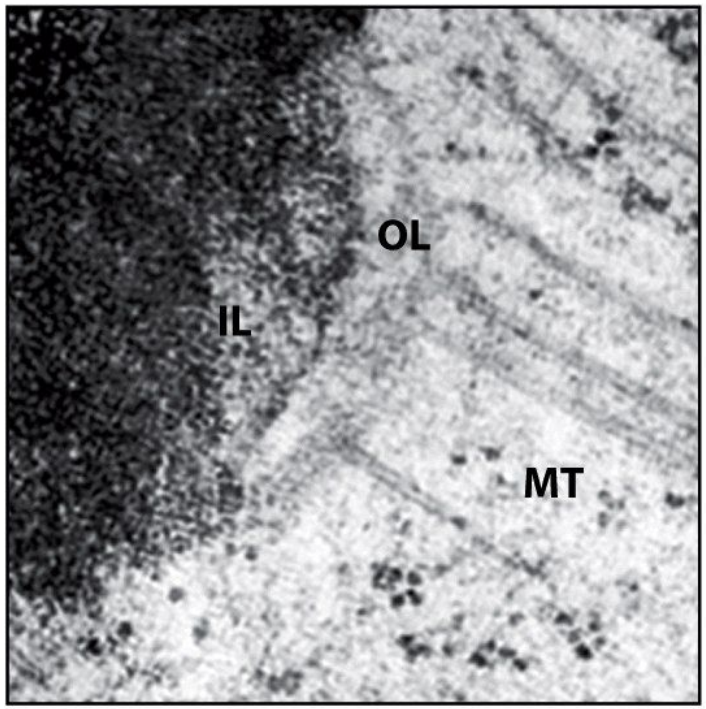


Figure 18-39
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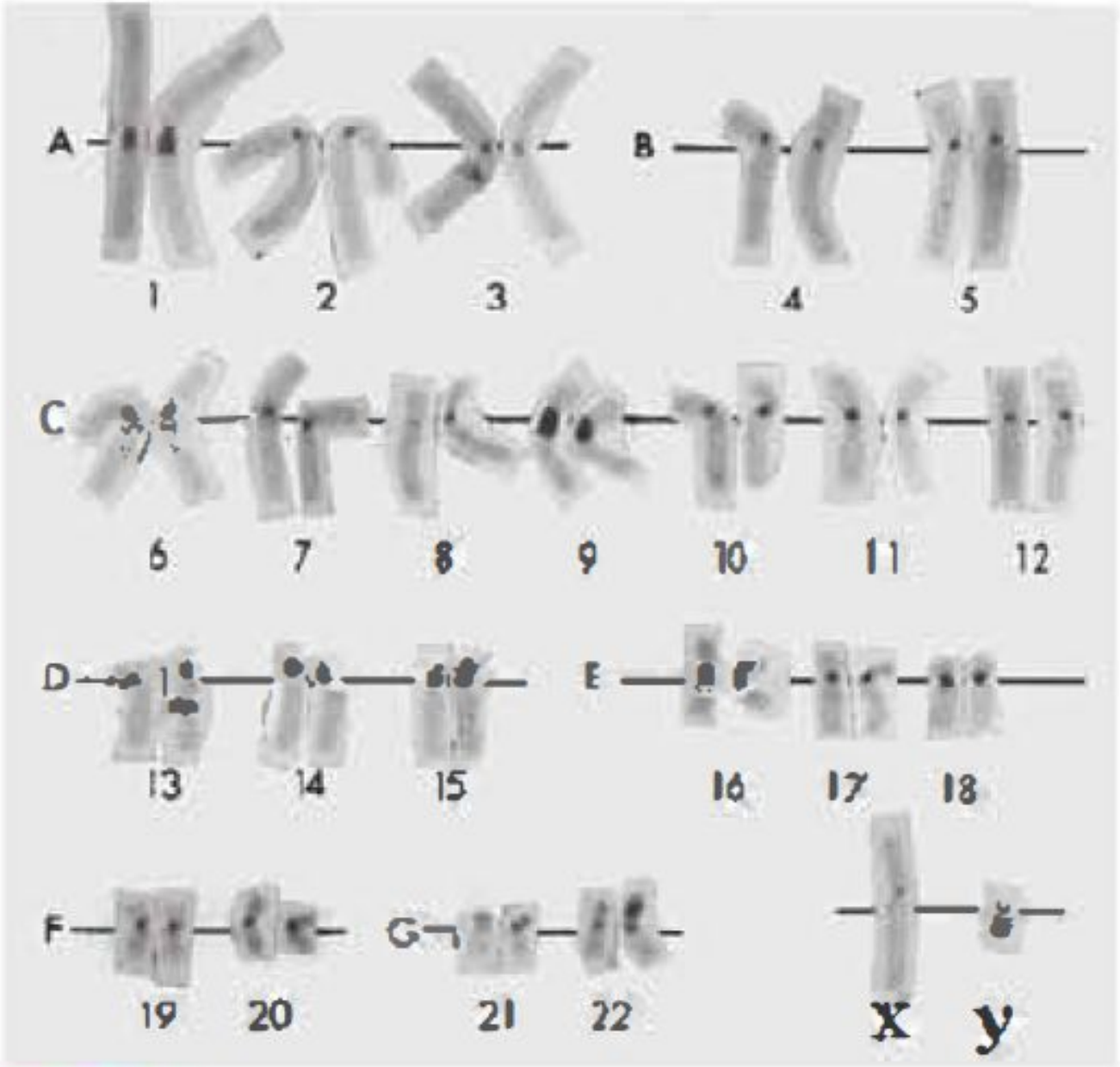

FIGURE 9.24 C-banding generates intense staining at the centromeres of all chromosomes. Photo courtesy of Lisa Shaffer, Washington State University–Spokane.

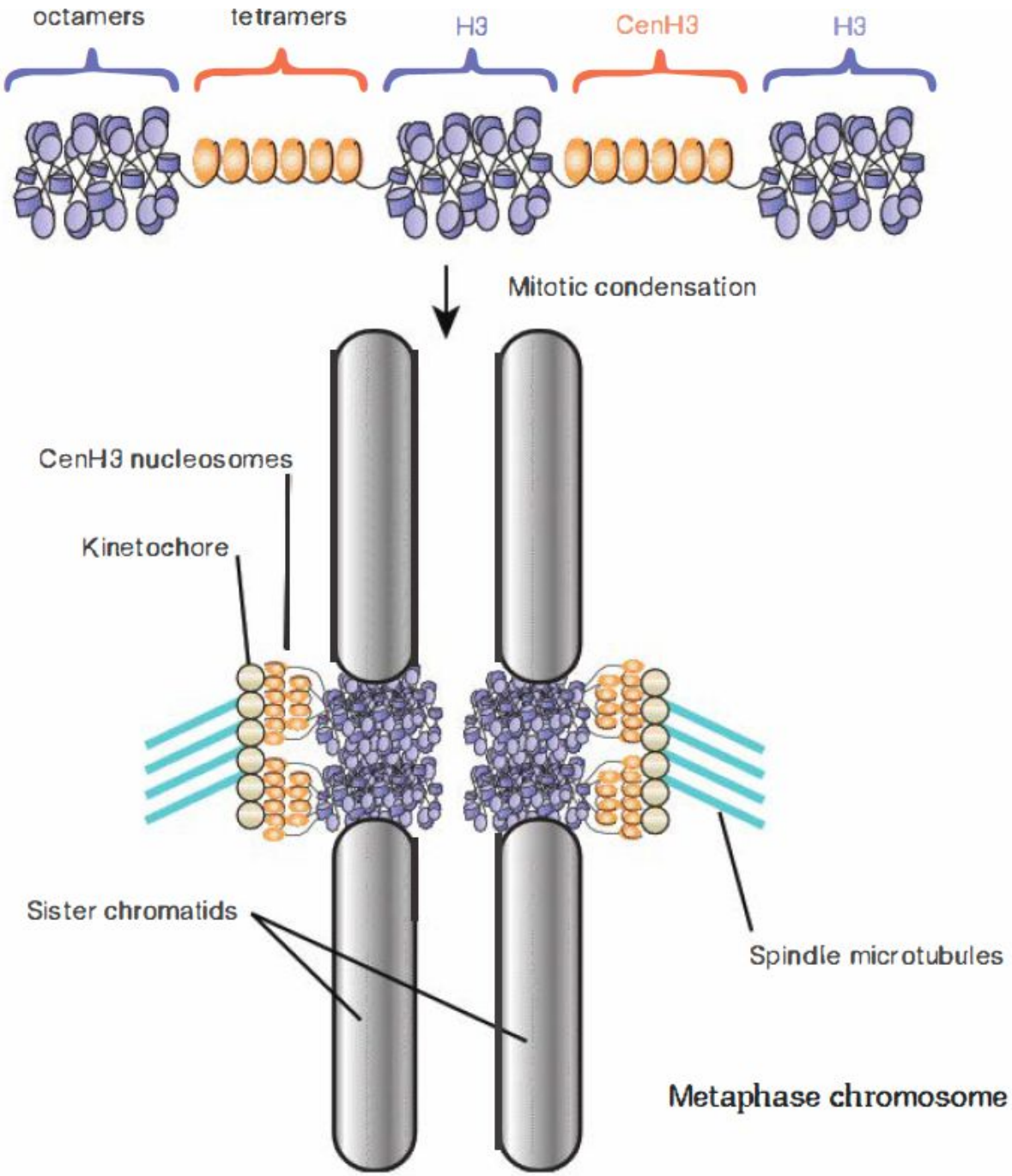

FIGURE 9.25 A model of the overall structure of a regional centromere. The CenH3-containing nucleosomes (orange) occur in clusters that protrude from the chromosome and bind to kinetochore proteins that in turn connect to spindle microtubules. Adapted from Y. Datal, et al., *Proc. Natl. Acad. Sci. USA* 104 (2007): 15974–15981.

Regions in yeast CEN elements

Cell cycle-dependent elements

- CDE-I: 9 bp conserved
- CDE-II: 90% A-T-rich sequence of 80-90 bp
- CDE-III: 11 -bp sequence highly conserved

Mutations in *CCG* inactivate the centromere

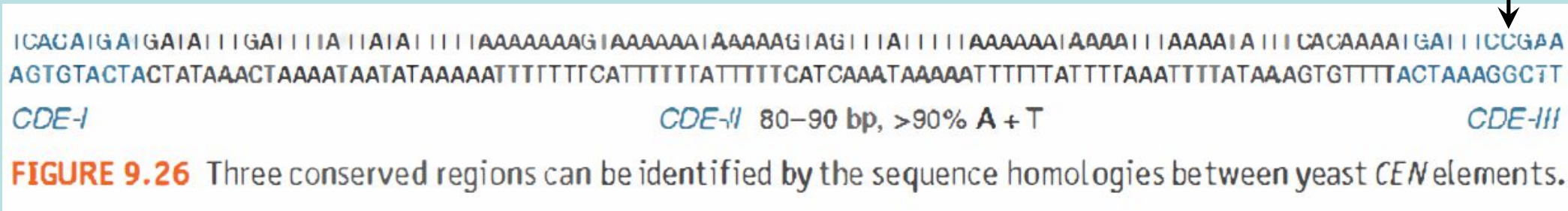

FIGURE 9.26 Three conserved regions can be identified by the sequence homologies between yeast *CEN* elements.

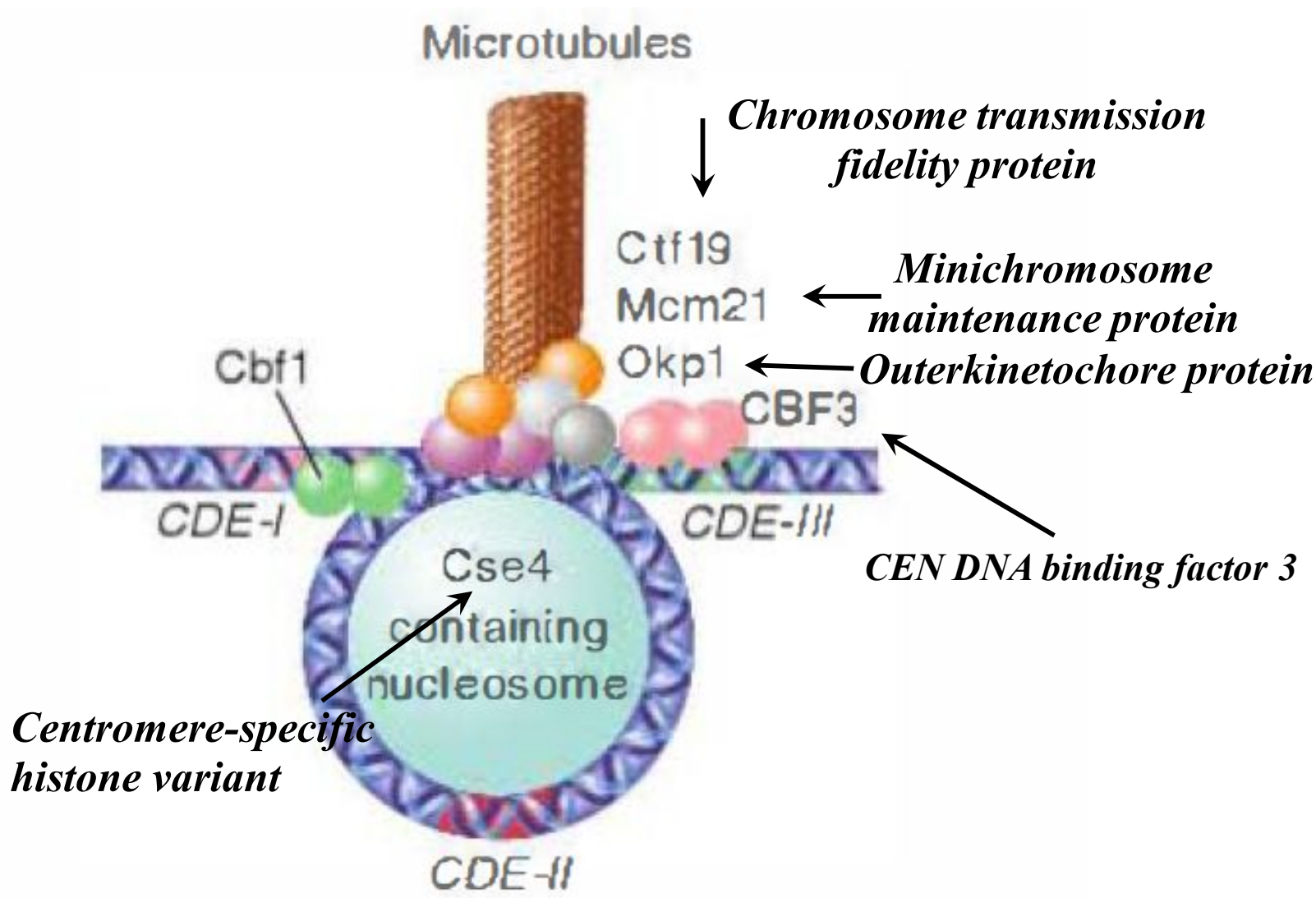

FIGURE 9.27 The DNA at *CDE-II* is wound around an alternative nucleosome containing Cse4, *CDE-III* is bound by the CBF3 complex, and *CDE-I* is bound by a Cbf1 homodimer. These proteins are connected by the group of Ctf19, Mcm21, and Okp1 proteins, and numerous other factors serve to link this complex to a microtubule.

TELOMERES

- TELOMERES consists of a simple repeat where a C+A-rich strand has the sequence $C_{>1}(A/T)_{1-4}$ lying at the end of a chromosome (from 100 to 1000).
- The telomere is required for the stability of the chromosome end.
- The G-tail (14-16 bases) is probably generated because there is a specific limited degradation of the C-A-rich strand.

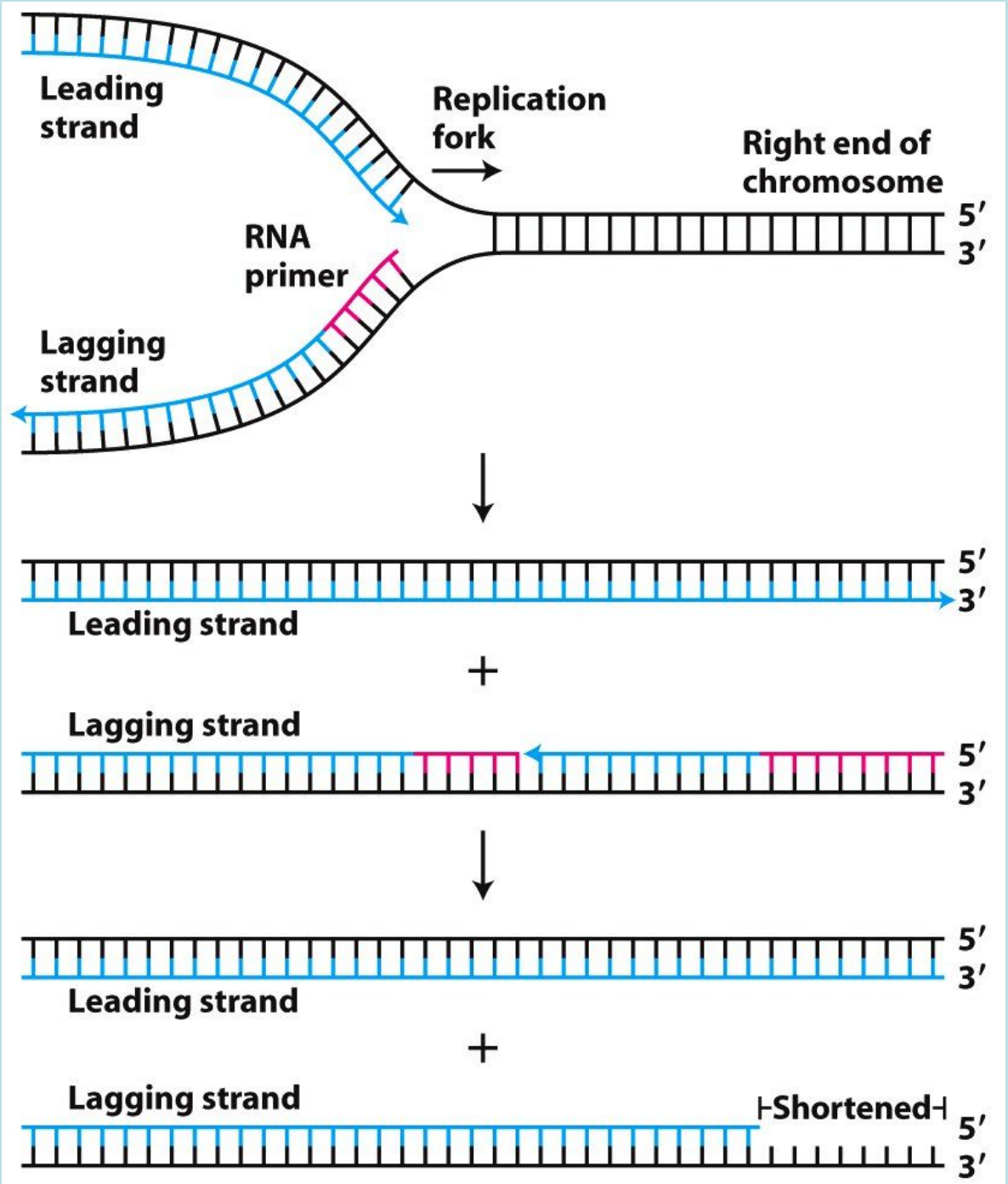

Figure 6-48
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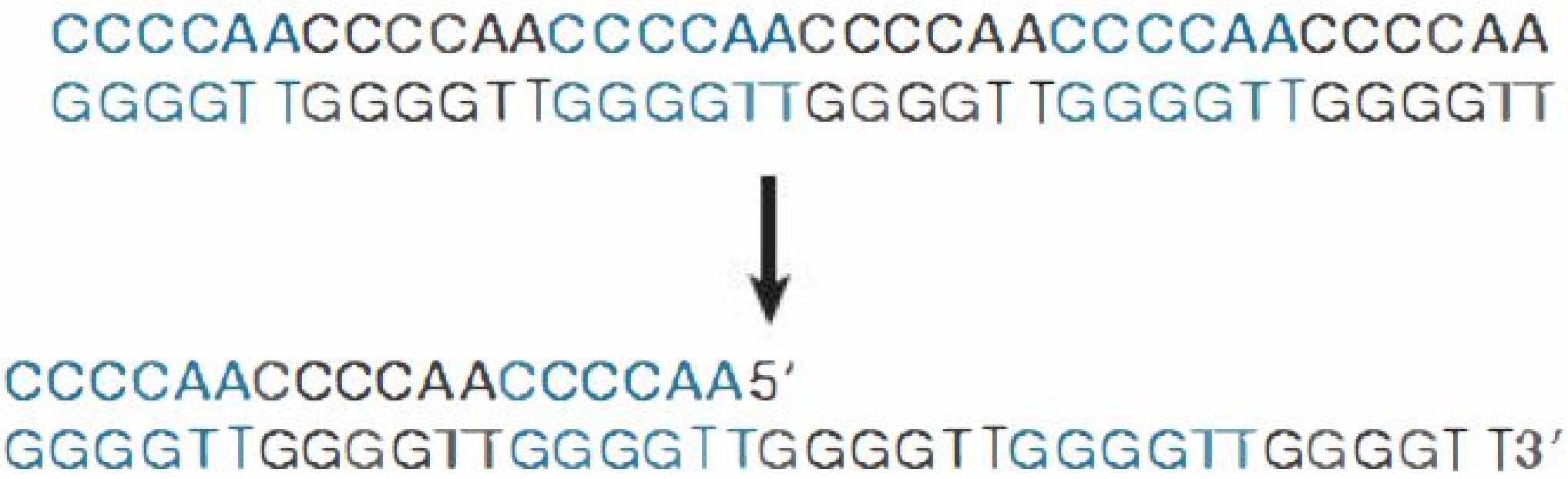

FIGURE 9.28 A typical telomere has a simple repeating structure with a G-T–rich strand that extends beyond the C-A–rich strand. The G-tail is generated by a limited degradation of the C-A–rich strand.

During each round of chromosome replication telomeres shorten their sequences ……

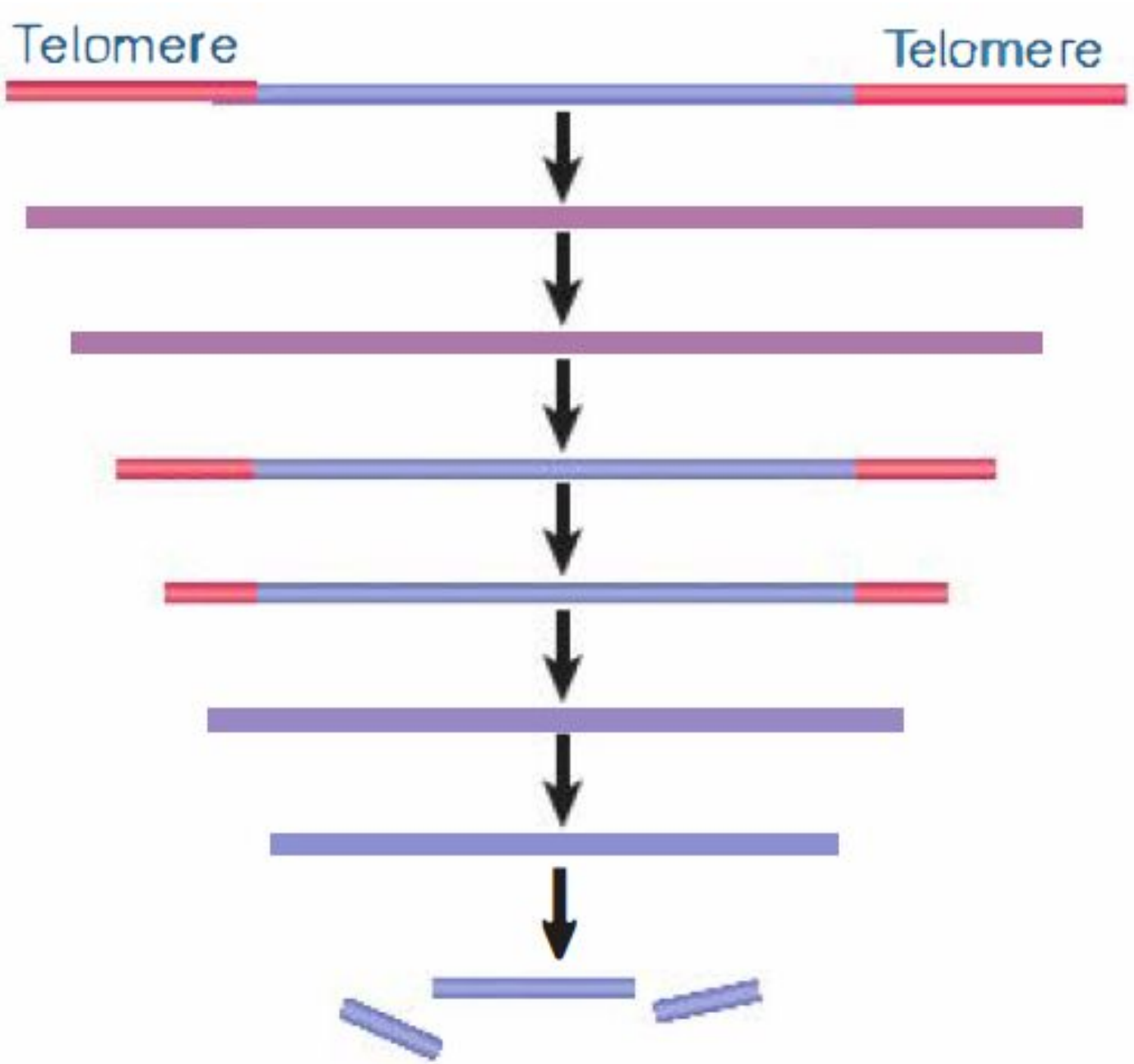

FIGURE 9.34 Mutation in telomerase causes telomeres to shorten in each cell division. Eventual loss of the telomere causes chromosome breaks and rearrangements.

An enzime called TELOMERASE uses the 3'-OH of the G+T telomeric strand as a primer for synthesis of tandem TTGGGG repeats. It is a large ribonucleoprotein that consists of a templating RNA (encoded by TLCJ in yeast, hTERC in humans) and a protein with catalytic activity (encoded by EST2 in yeast, hTERT in humans).