Alberts • Johnson • Lewis • Raff • Roberts • Walter

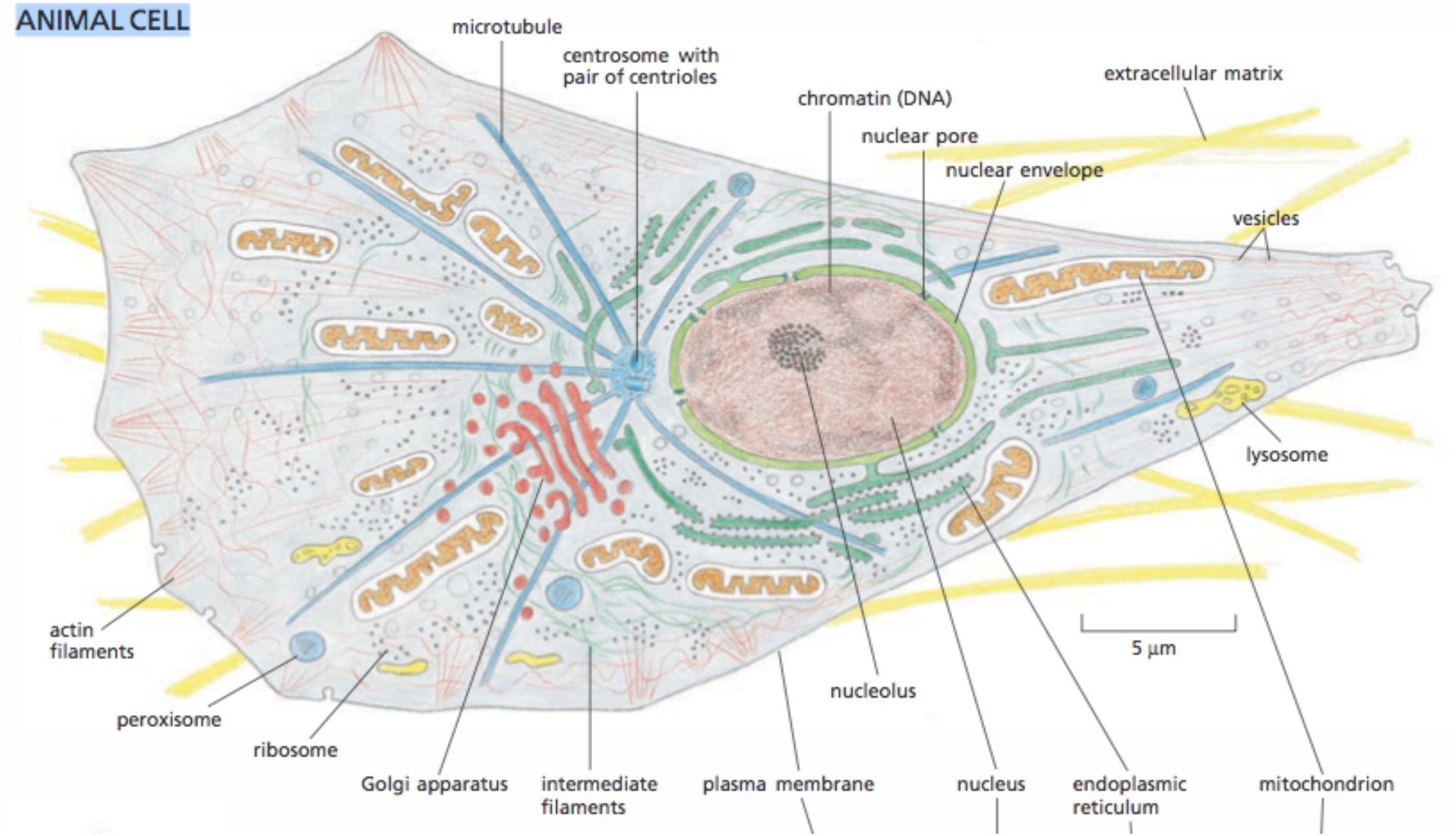
Molecular Biology of the Cell

Fifth Edition

Chapter 4

DNA, Chromosomes, and Genomes

PANEL 1–2 Cell architecture



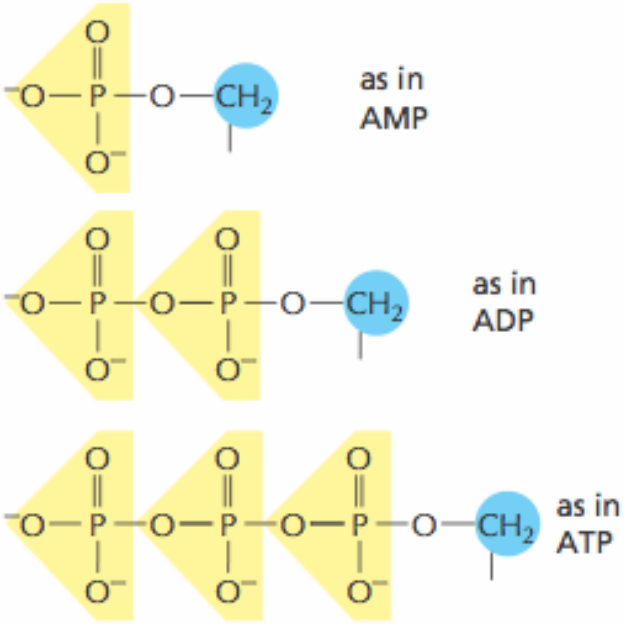
뉴클레오타이드/핵산

염색체

뉴클레오타이드는 핵산의 기본단위

PHOSPHATES

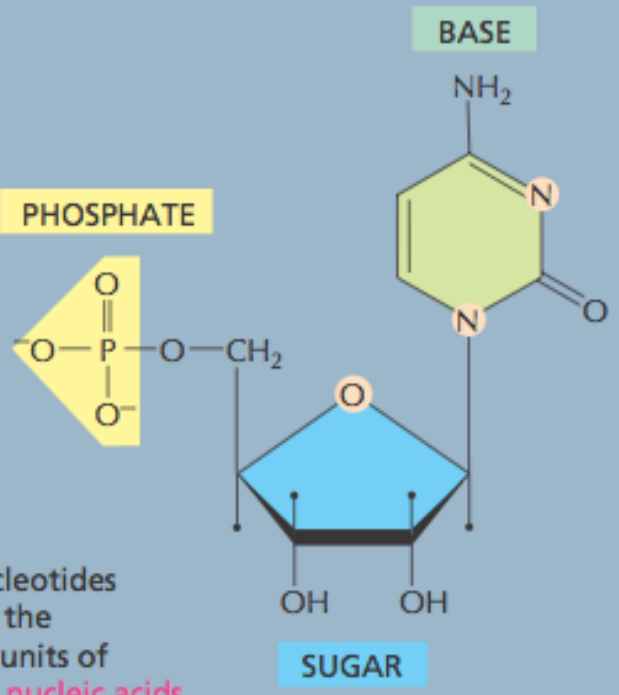
The phosphates are normally joined to the C5 hydroxyl of the ribose or deoxyribose sugar (designated 5'). Mono-, di-, and triphosphates are common.



The phosphate makes a nucleotide negatively charged.

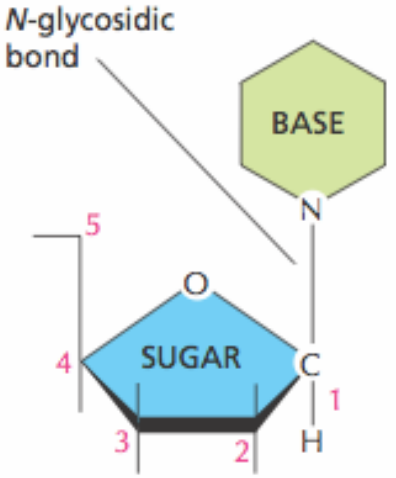
NUCLEOTIDES

A nucleotide consists of a nitrogen-containing base, a five-carbon sugar, and one or more phosphate groups.



Nucleotides are the subunits of the nucleic acids.

BASIC SUGAR LINKAGE

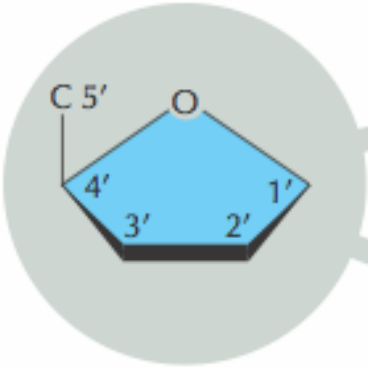


The base is linked to the same carbon (C1) used in sugar–sugar bonds.

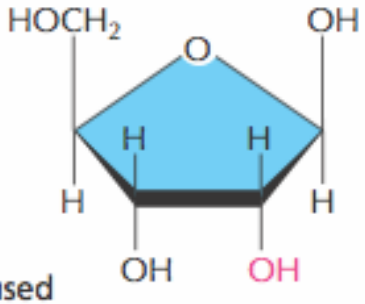
SUGARS

PENTOSE

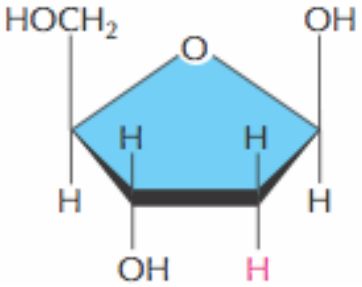
a five-carbon sugar



two kinds are used

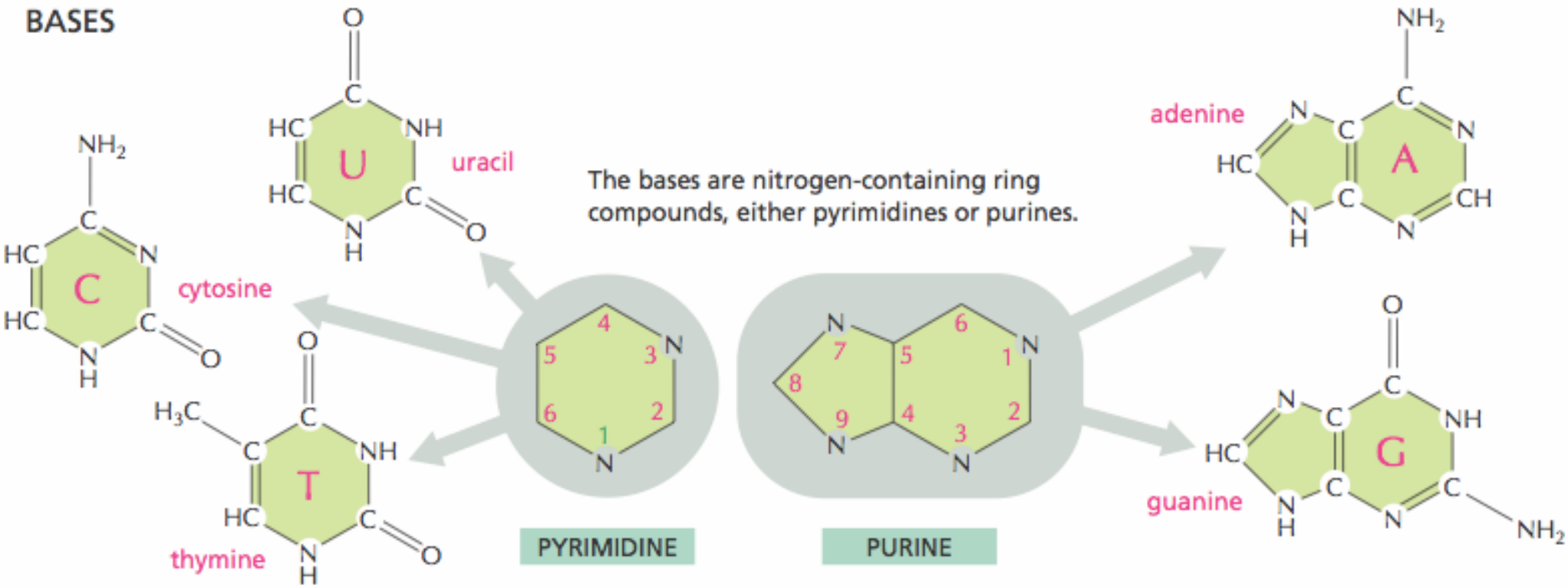


β-D-ribose
used in ribonucleic acid



β-D-2-deoxyribose
used in deoxyribonucleic acid

Each numbered carbon on the sugar of a nucleotide is followed by a prime mark; therefore, one speaks of the "5-prime carbon," etc.


BASES
uracil
cytosine
thymine
adenine
guanine
The bases are nitrogen-containing ring compounds, either pyrimidines or purines.
PYRIMIDINE
PURINE

Depurination & Deamination

The *most frequent* chemical reactions.

Create serious *DNA damage* in cells.

- *Depurination*

  Release guanine as well as *adenine* from DNA (does not break the phosphodiester backbone).

  Spontaneous reaction: 10^{12} purines will be lost.

- *Deamination*

  Converts *cytosine to Uracil.*

  Spontaneous reaction.

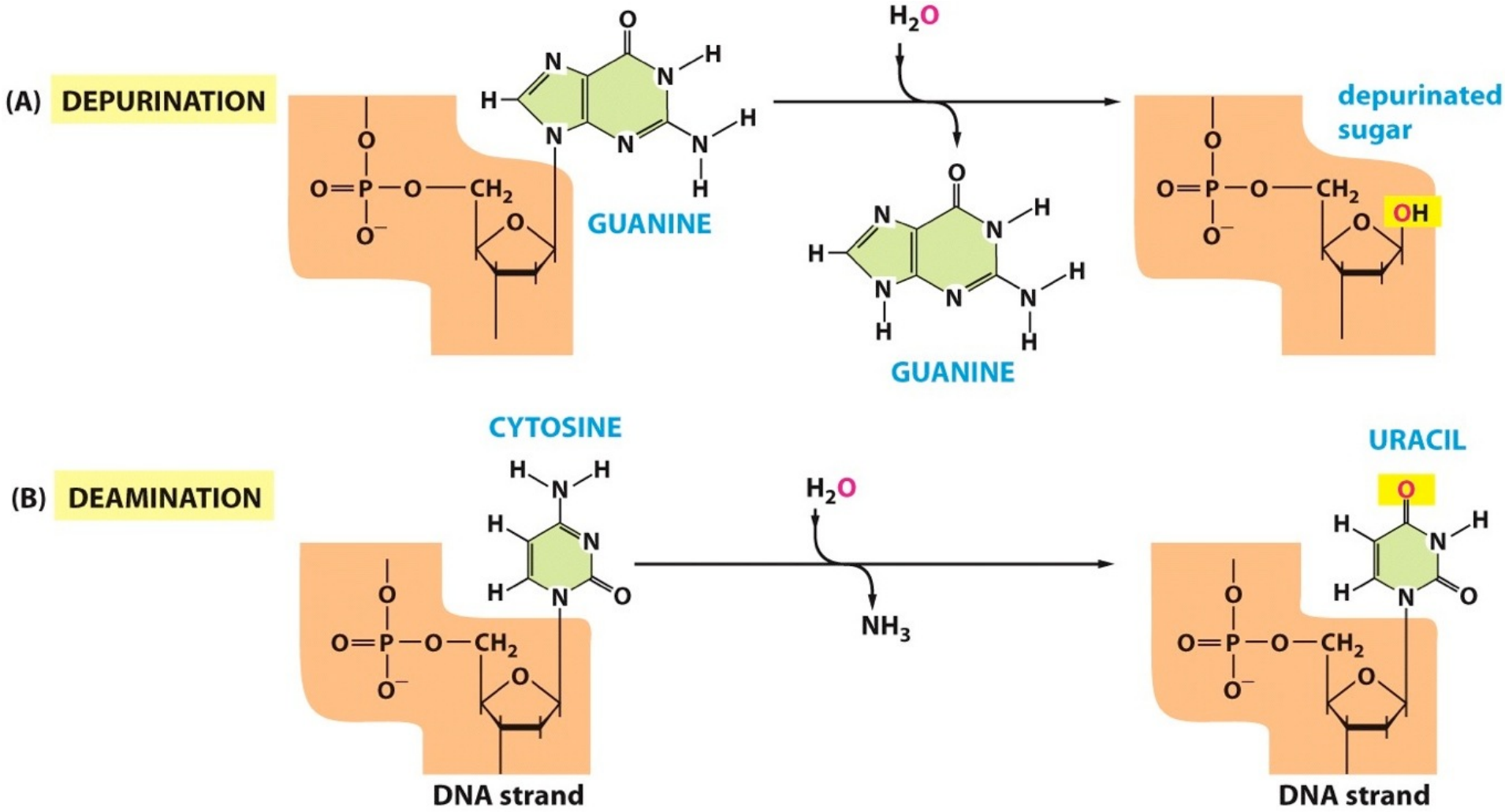


Figure 6-23 Essential Cell Biology 3/e (© Garland Science 2010)

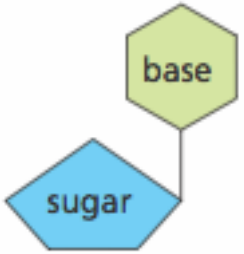
NOMENCLATURE

The names can be confusing, but the abbreviations are clear.

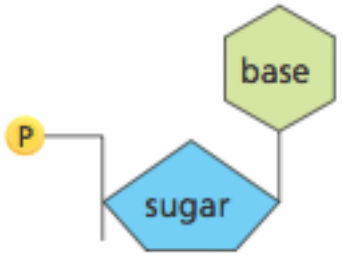
BASE	NUCLEOSIDE	ABBR.
adenine	adenosine	A
guanine	guanosine	G
cytosine	cytidine	C
uracil	uridine	U
thymine	thymidine	T

Nucleotides are abbreviated by three capital letters. Some examples follow:

AMP = adenosine monophosphate
dAMP = deoxyadenosine monophosphate
UDP = uridine diphosphate
ATP = adenosine triphosphate



BASE + SUGAR = NUCLEOSIDE



BASE + SUGAR + PHOSPHATE = NUCLEOTIDE

뉴클레오타이드는 핵산의 기본단위

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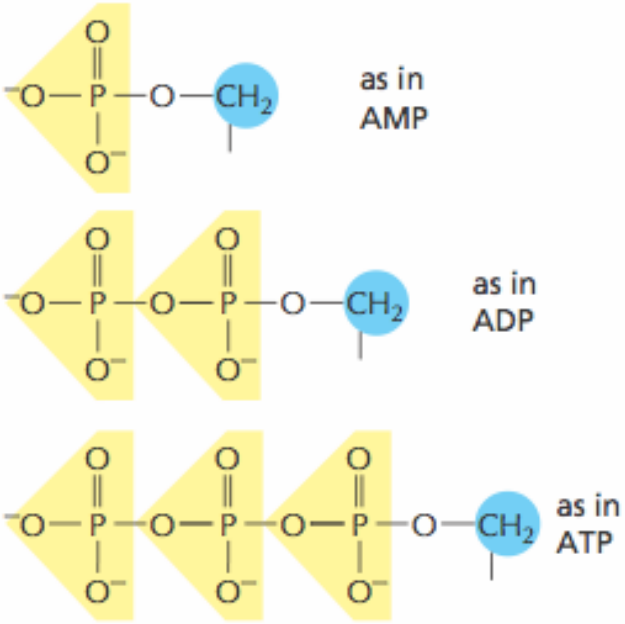

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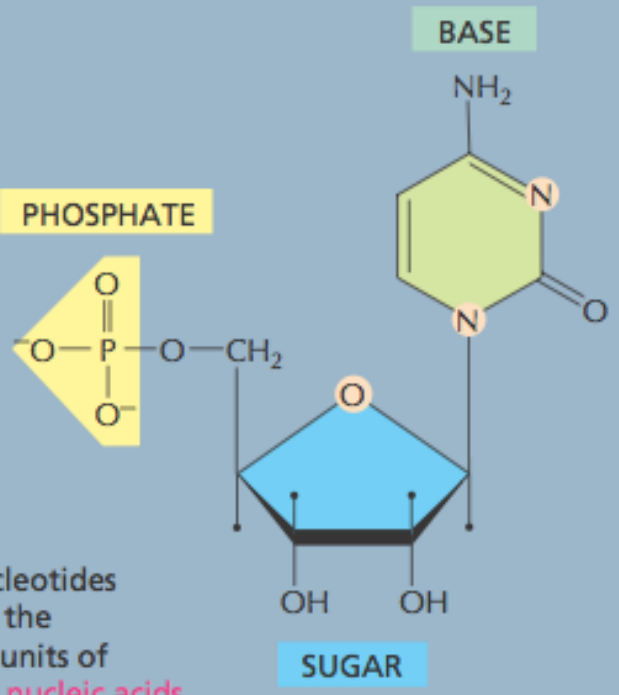
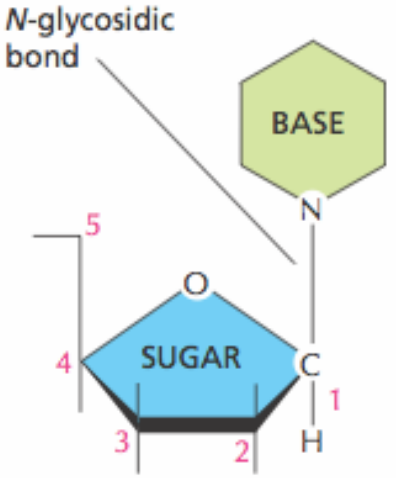


Nucleotides are the subunits of the nucleic acids.

BASIC SUGAR LINKAGE



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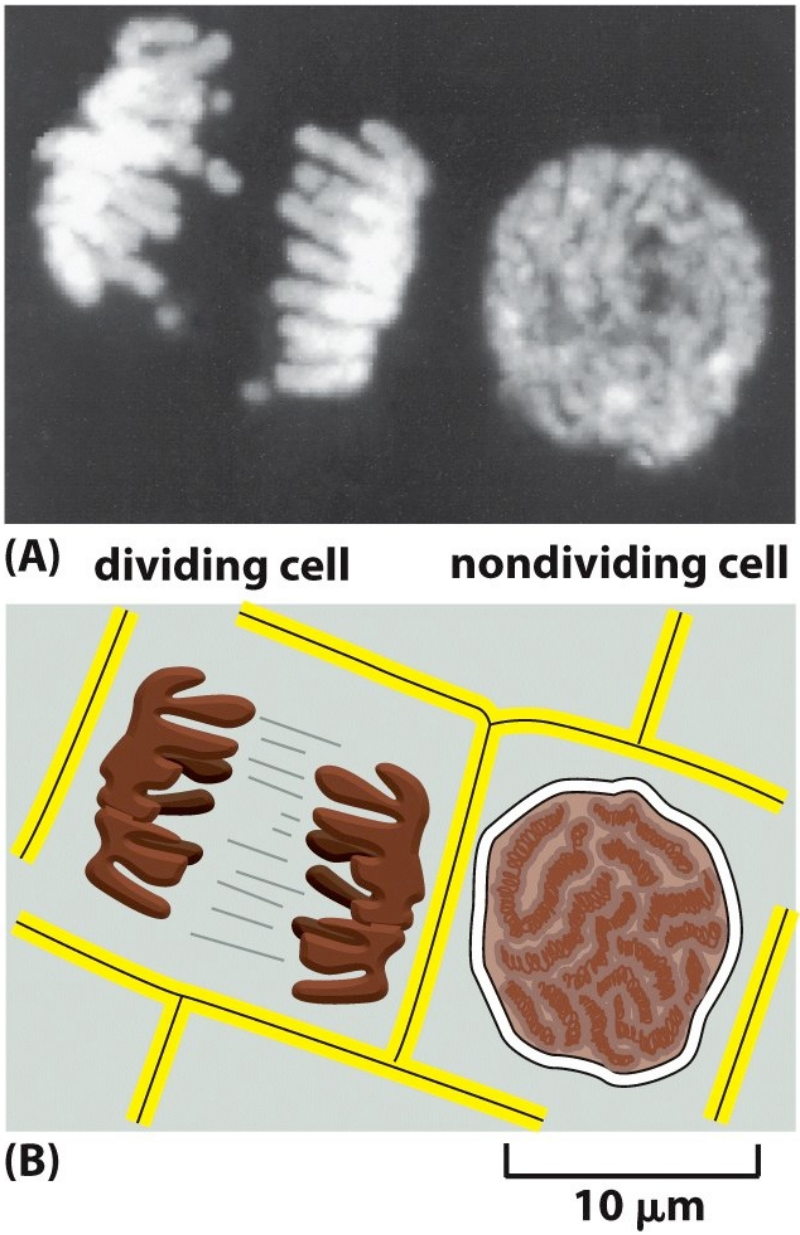
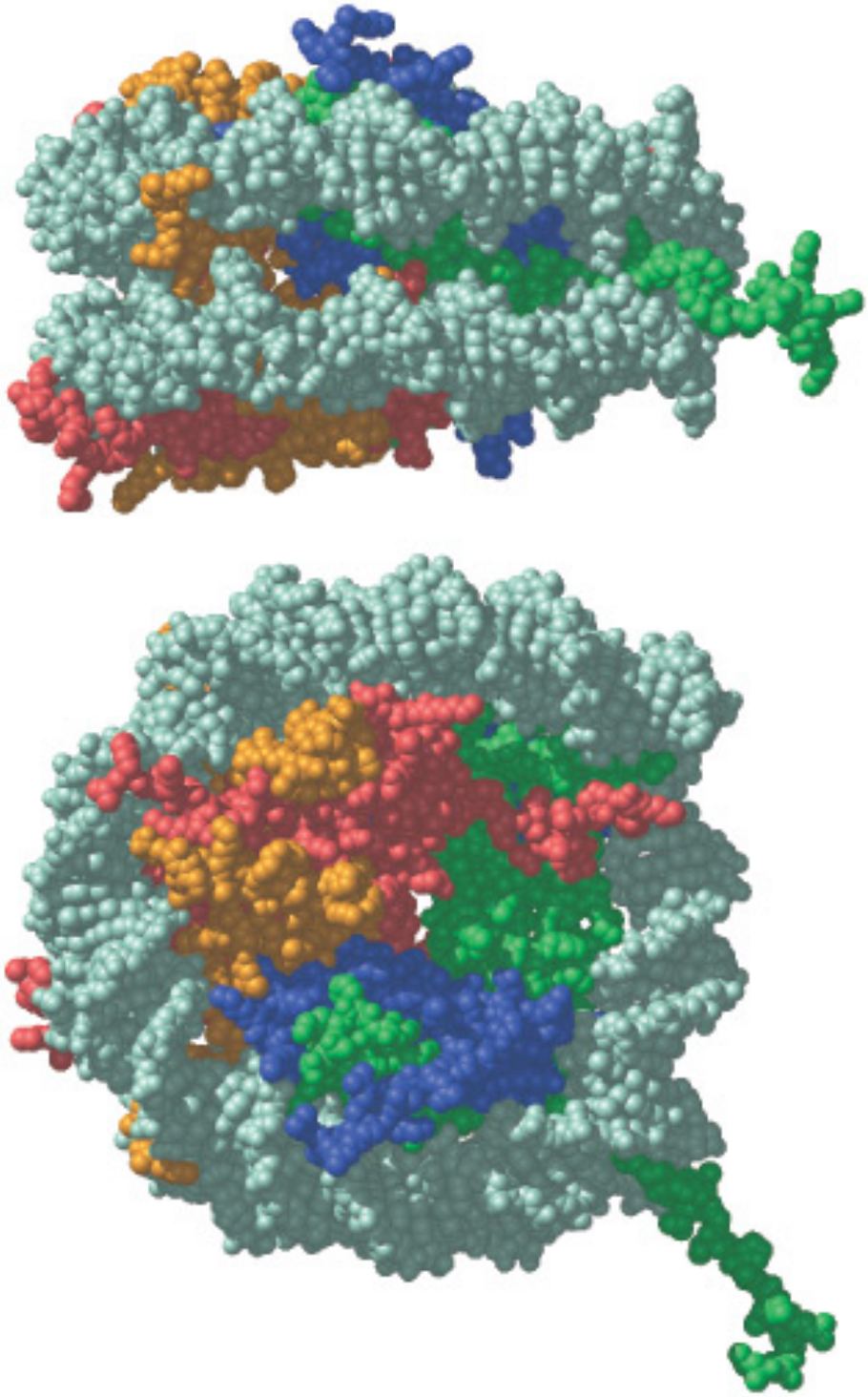


Figure 5-1 Essential Cell Biology 3/e (© Garland Science 2010)

Chromosomes become visible as cells prepare to divide.
(A) Two adjacent plant cells. The DNA has been stained with a fluorescent dye (DAPI) that binds to it. The DNA is present in chromosomes, which become visible as distinct structures, as shown on the *left*. The cell on the *right*, which is not dividing, contains the identical chromosomes; they cannot be distinguished as individual chromosomes.



The nucleosome. The basic structural unit of all eucaryotic chromosoms is the nucleosome. The DNA double helix (*gray*) is wrapped around a core particle of hisotne proteins (colored) to create the nucleosome. Nucleosomes are spaced roughly 200 nucleotide pairs apart along the chromosomal DNA.

(Nature 389:251-260, 1997)

- The Structure and Function of DNA
- The Structure of Eucaryotic Chromosomes
- The Regulation of Chromosome Structure

The Structure and Function of DNA

A DNA molecule consists of *two complementary chains of nucleotides*

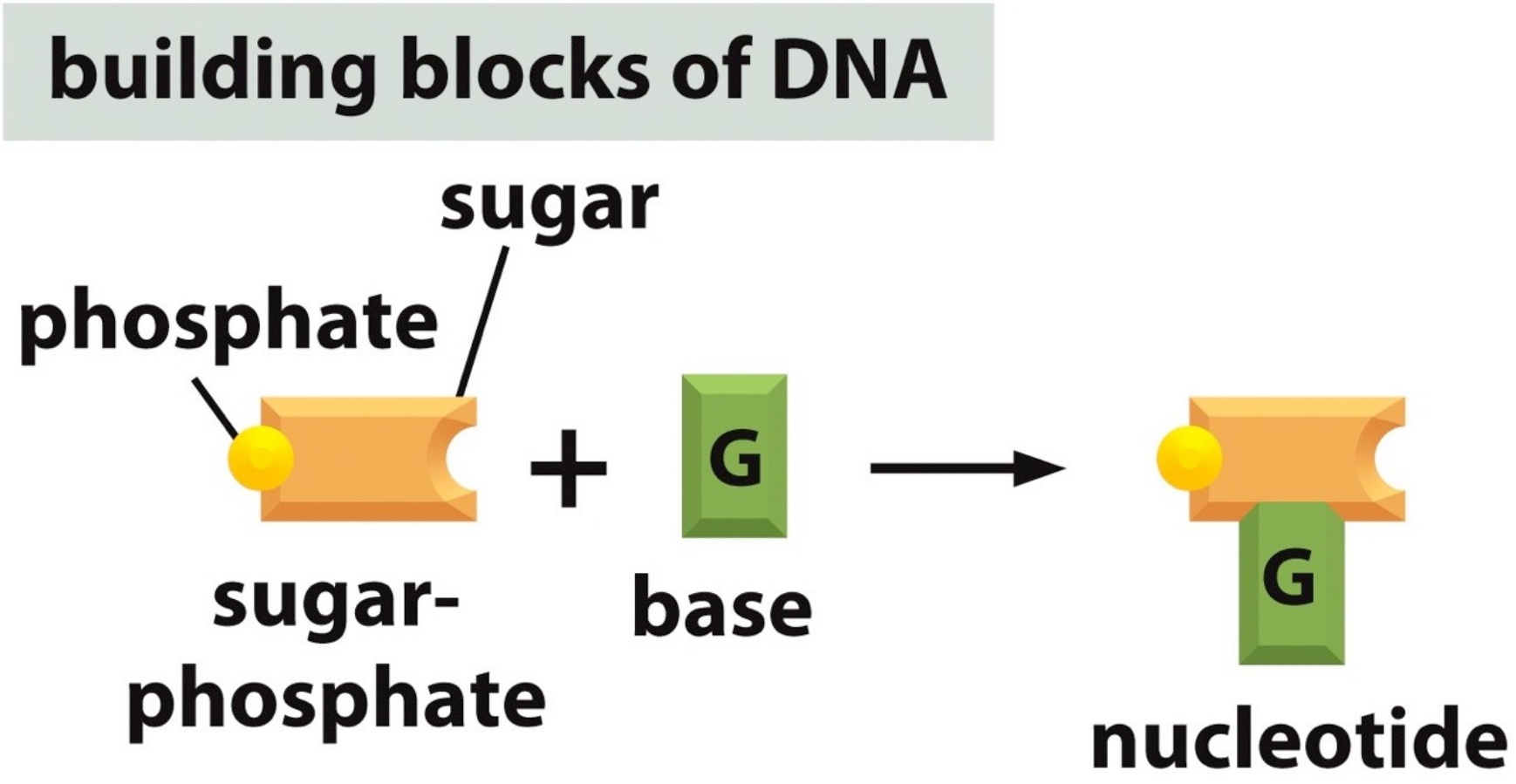


Figure 5-2a Essential Cell Biology 3/e (© Garland Science 2010)

- *Chemical polarity*

DNA is made of *four nucleotide building blocks*

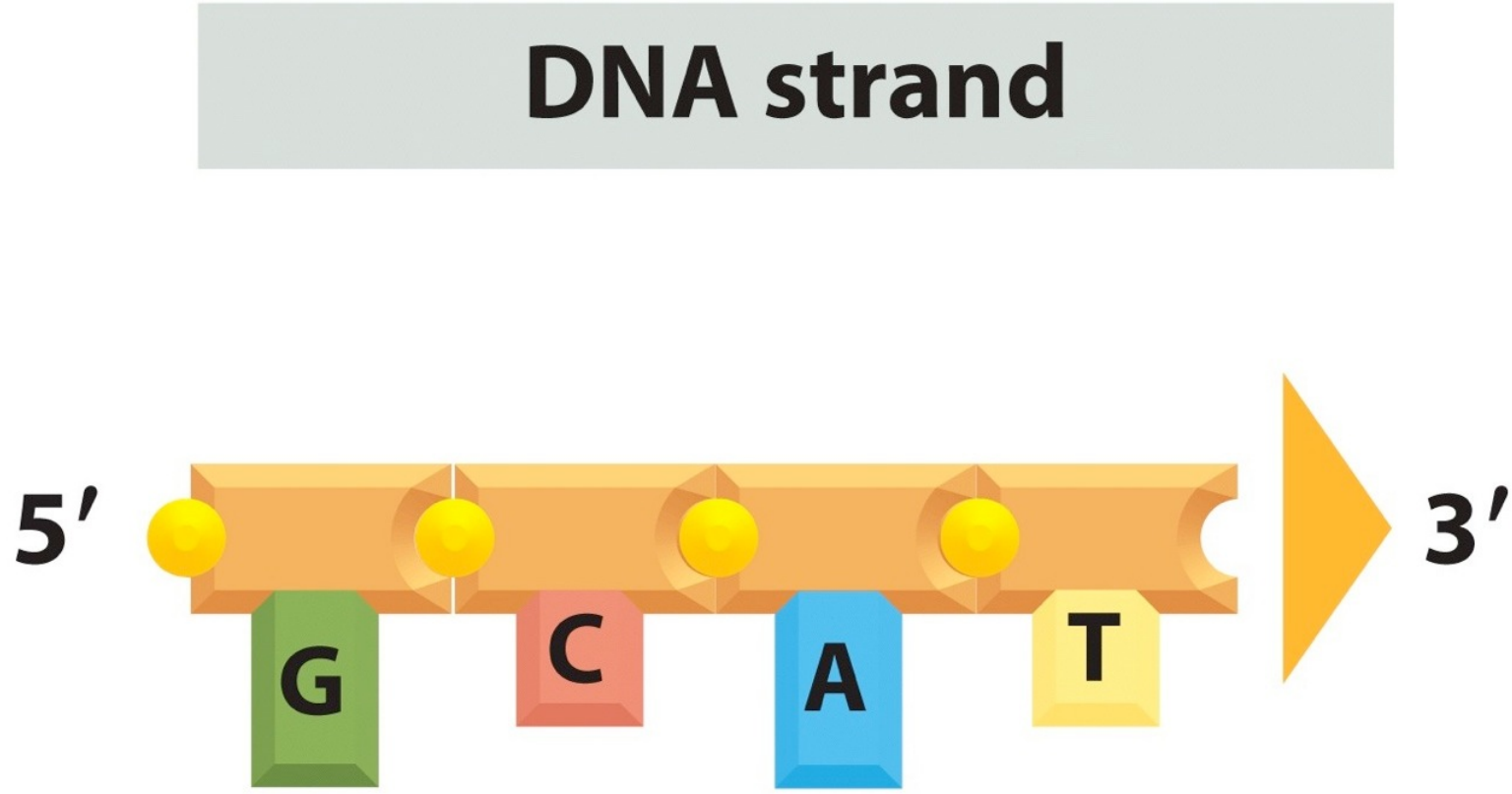


Figure 5-2b Essential Cell Biology 3/e (© Garland Science 2010)

by 'DNA polymerase'

- *3.2 x 10⁹ (3.2 billion) nt (2 m)* over *24 chromosomes*

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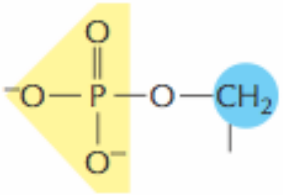
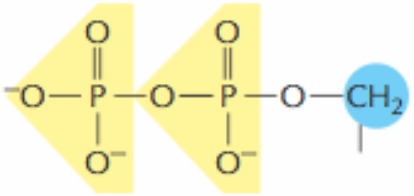
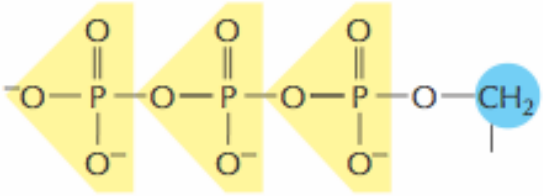


as in AMP



as in ADP



as in ATP

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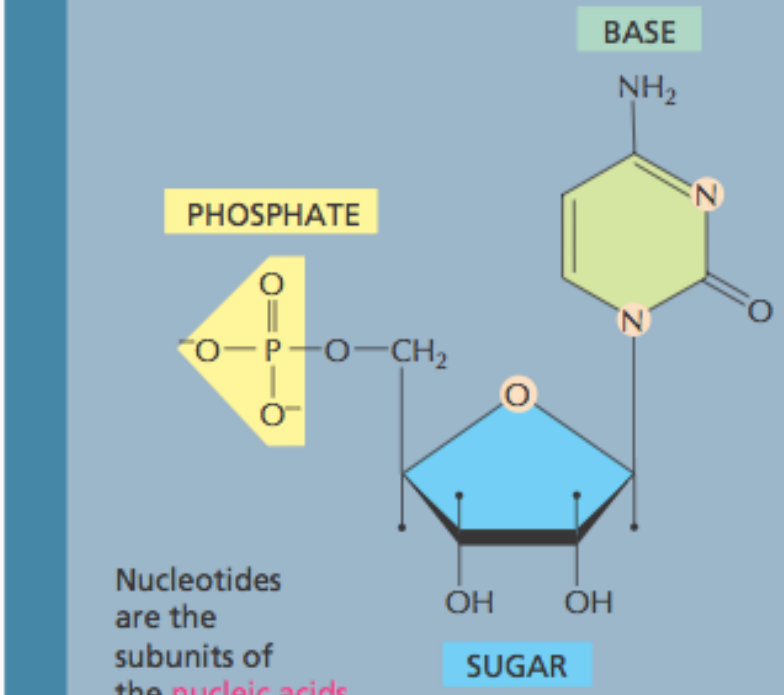

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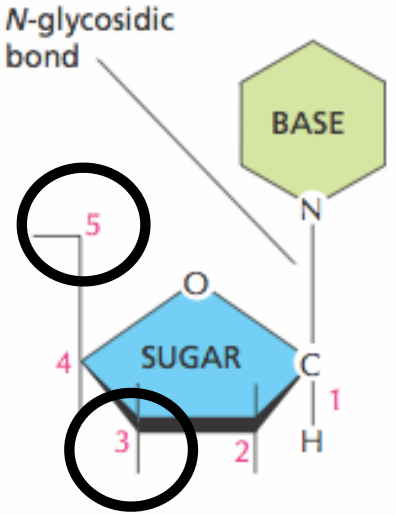
BASIC SUGAR LINKAGE



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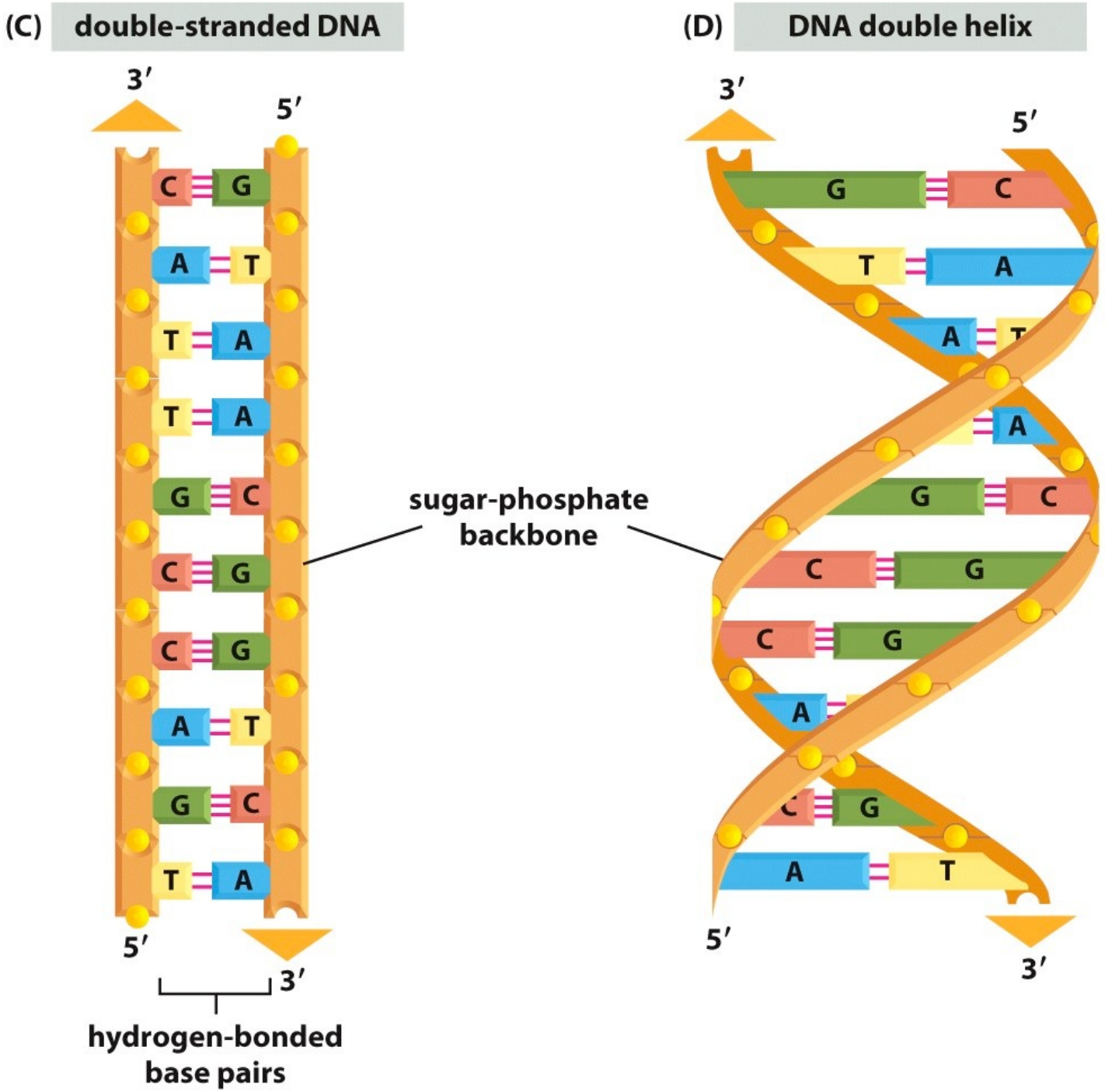
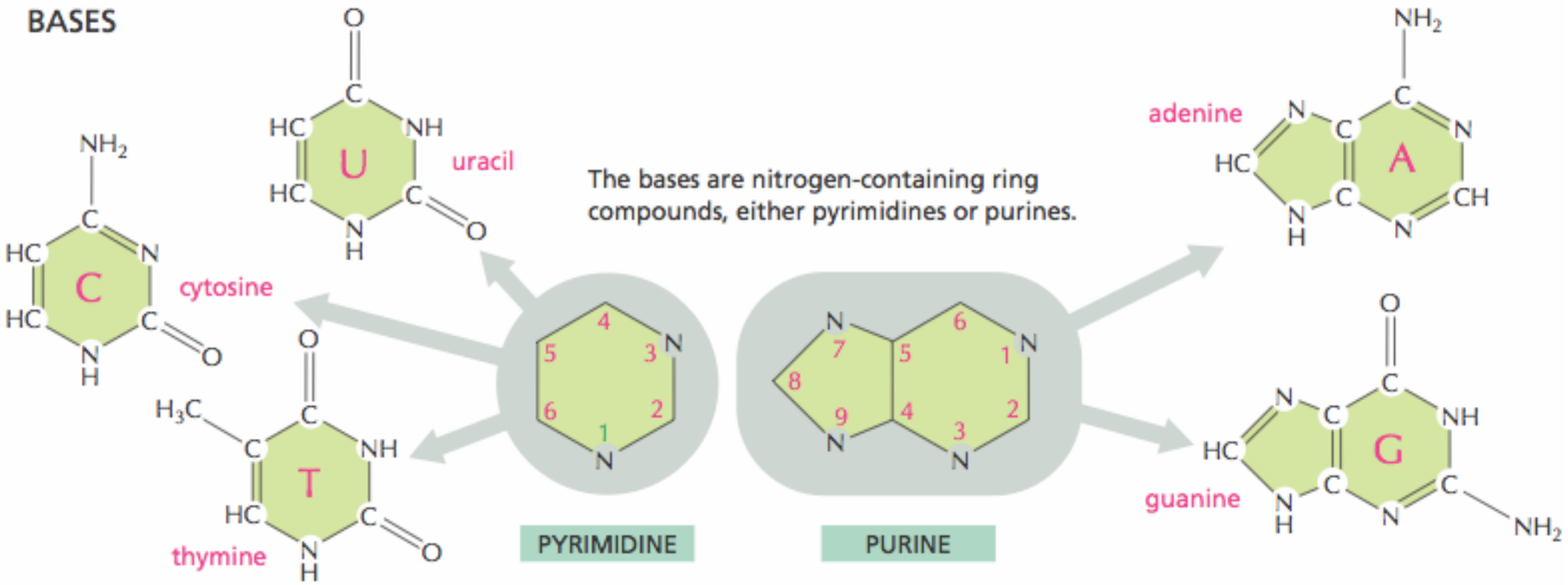


Figure 5-2cd Essential Cell Biology 3/e (© Garland Science 2010)


BASES
uracil
cytosine
thymine
adenine
guanine
The bases are nitrogen-containing ring compounds, either pyrimidines or purines.
PYRIMIDINE
PURINE

The DNA double helix has a major and minor groove

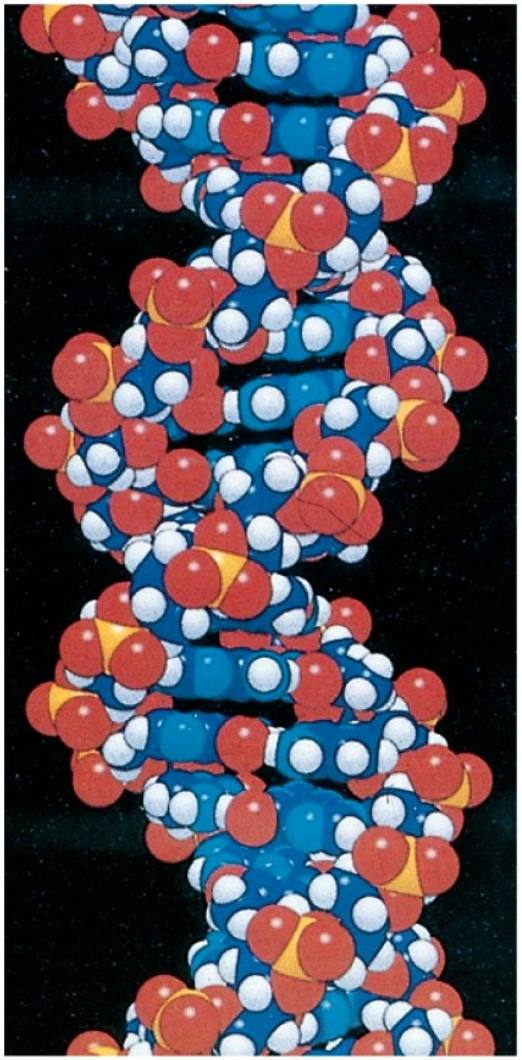


Figure 5-7 Essential Cell Biology 3/e (© Garland Science 2010)

The strand *twist around each other* to form a double helix containing 10 base pairs per helical turn.

A DNA molecule

DNA strand (or DNA chain)

The base: A (adenine), C (cytosine), G (guanine), T (thymine).

Hydrogen bonds (AT and GC).

The way in which the nucleotide subunits are linked together gives a DNA strand *a chemical polarity*.

- In the *same orientation*.
- Easily *distinguishable*.
- *3' end, 5' end* (3' hydroxyl and 5' phosphate)

TABLE I

Fidelity Comparison of Thermostable DNA Polymerases Using a *lacI*OZα-Based Fidelity Assay[a]

Thermostable DNA polymerase	Error rate[b]	Percentage (%) of mutated PCR products[c]
Pfu DNA polymerase	1.3×10^{-6}	2.6
Taq DNA polymerase	8.0×10^{-6}	16.0
Vent$_R$® DNA polymerase	2.8×10^{-6}	5.6
Deep Vent$_R$® DNA polymerase	2.7×10^{-6}	5.4
Tfl DNA polymerase	8.3×10^{-6}	16.6
Tbr DNA polymerase	9.5×10^{-6}	19.0
UlTma™ DNA polymerase	55.3×10^{-6}	110.6[d]

[a] Fidelity is measured using a PCR-based forward mutation assay based on the *lacI* target gene.[5]

[b] The error rate equals mutation frequency per base pair per duplication.

[c] The percentage of mutated PCR products after amplification of a 1-kb target sequence for 20 effective cycles.

[d] Some PCR products will exhibit more than one error.

Complementary base pairs are formed in the DNA double helix

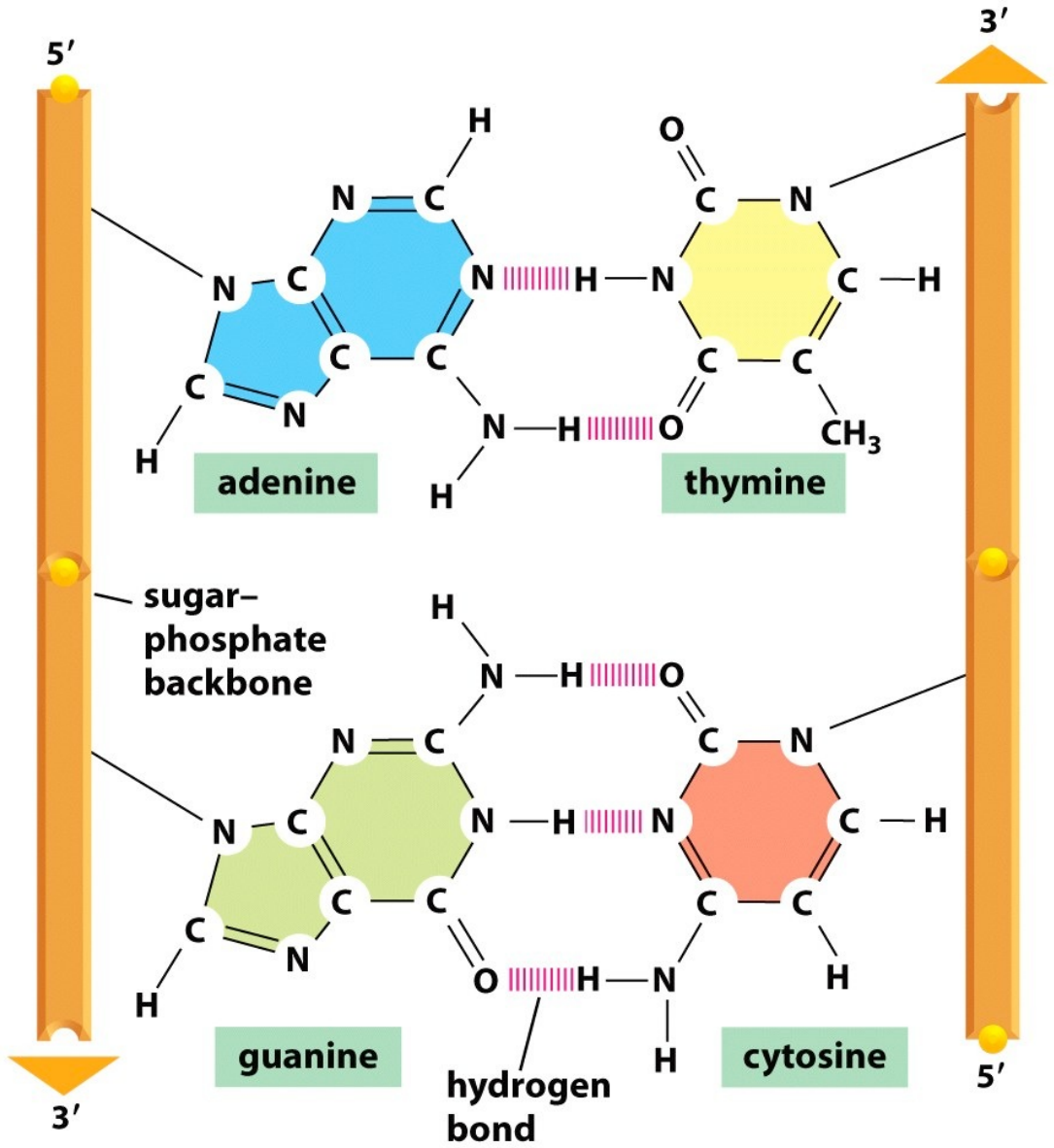


Figure 5-6a Essential Cell Biology 3/e (© Garland Science 2010)

DNA double helix

1. Complementary base-pairing

   Hydrogen bonds to form *AT and GC* (purine + pyrimidine).

   Each base pair is of *similar width, an equal distance* apart along the DNA.

   Important for both *copying and repairing the DNA*.

   Antiparallel (oriented in opposite polarities)

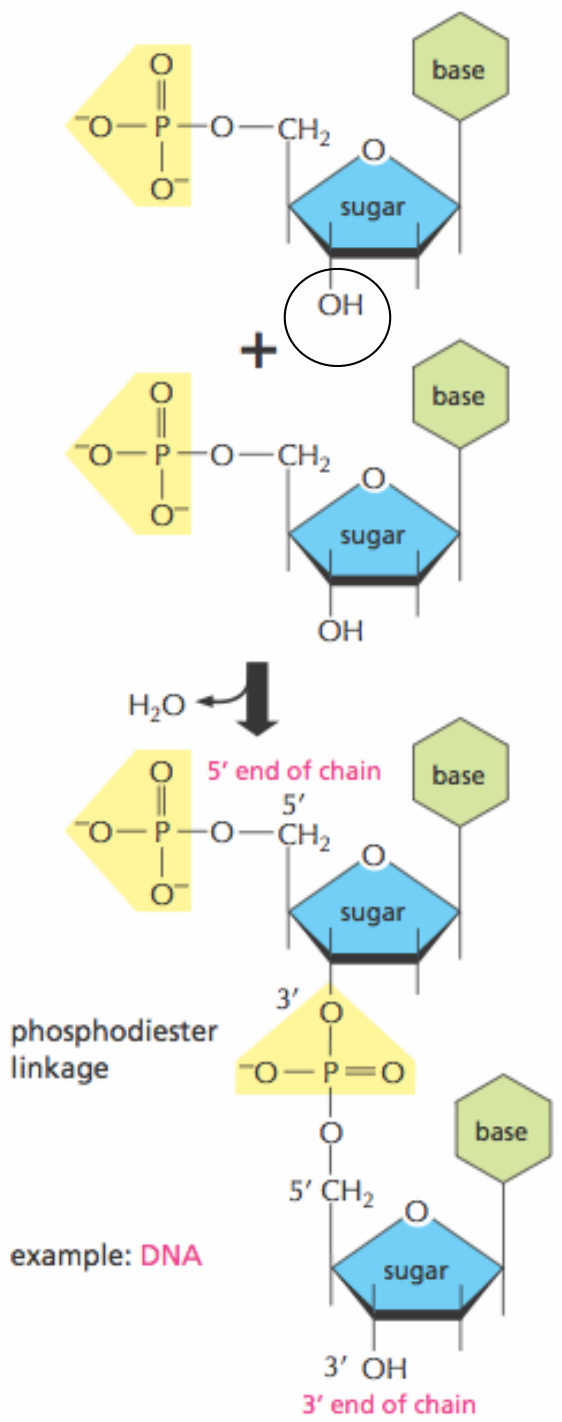
Primer Melting Temperature:

Primer Melting Temperature (Tm) by definition is the temperature at which one half of the DNA duplex will dissociate to become single stranded and indicates the duplex stability. Primers with melting temperatures in the range of 52-58 oC generally produce the best results.

$$Tm = 4(G+C) + 2(A+T)$$

GC 함량이 많아지면 Tm 값이 높아짐 (Primer는 보통 Tm = 50-60 정도.
Tm 값이 높아지면 낮은 온도에서도 annealing이 쉽게 됨.

The nucleotides are linked together covalently by *phosphodiester bonds*



The 3’ end carries – *OH group*; the 5’ end carries *a free phosphate group*

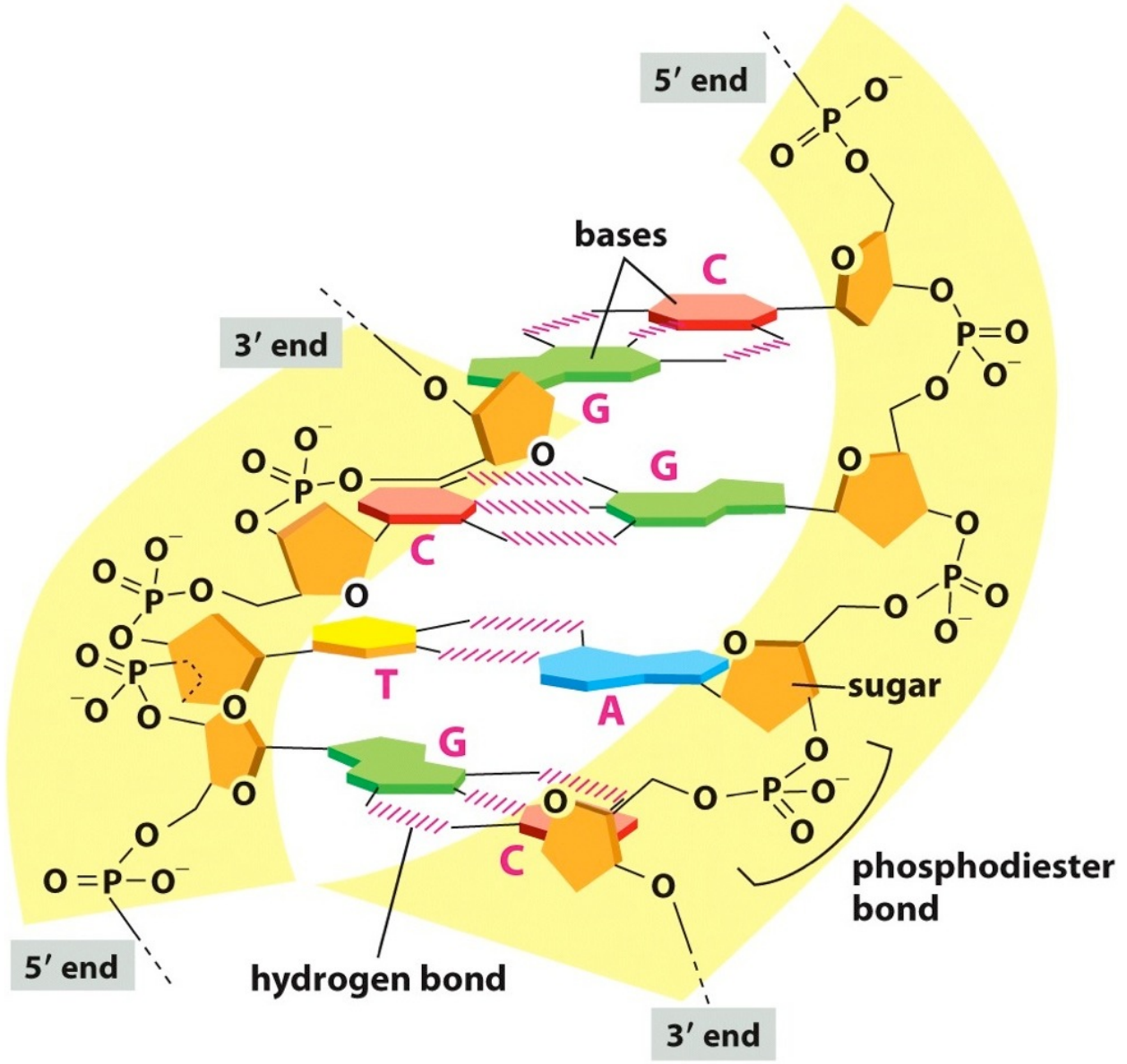


Figure 5-6b Essential Cell Biology 3/e (© Garland Science 2010)

2. Held together by base pairing

The nucleotides are linked together covalently by *phosphodiester bonds*

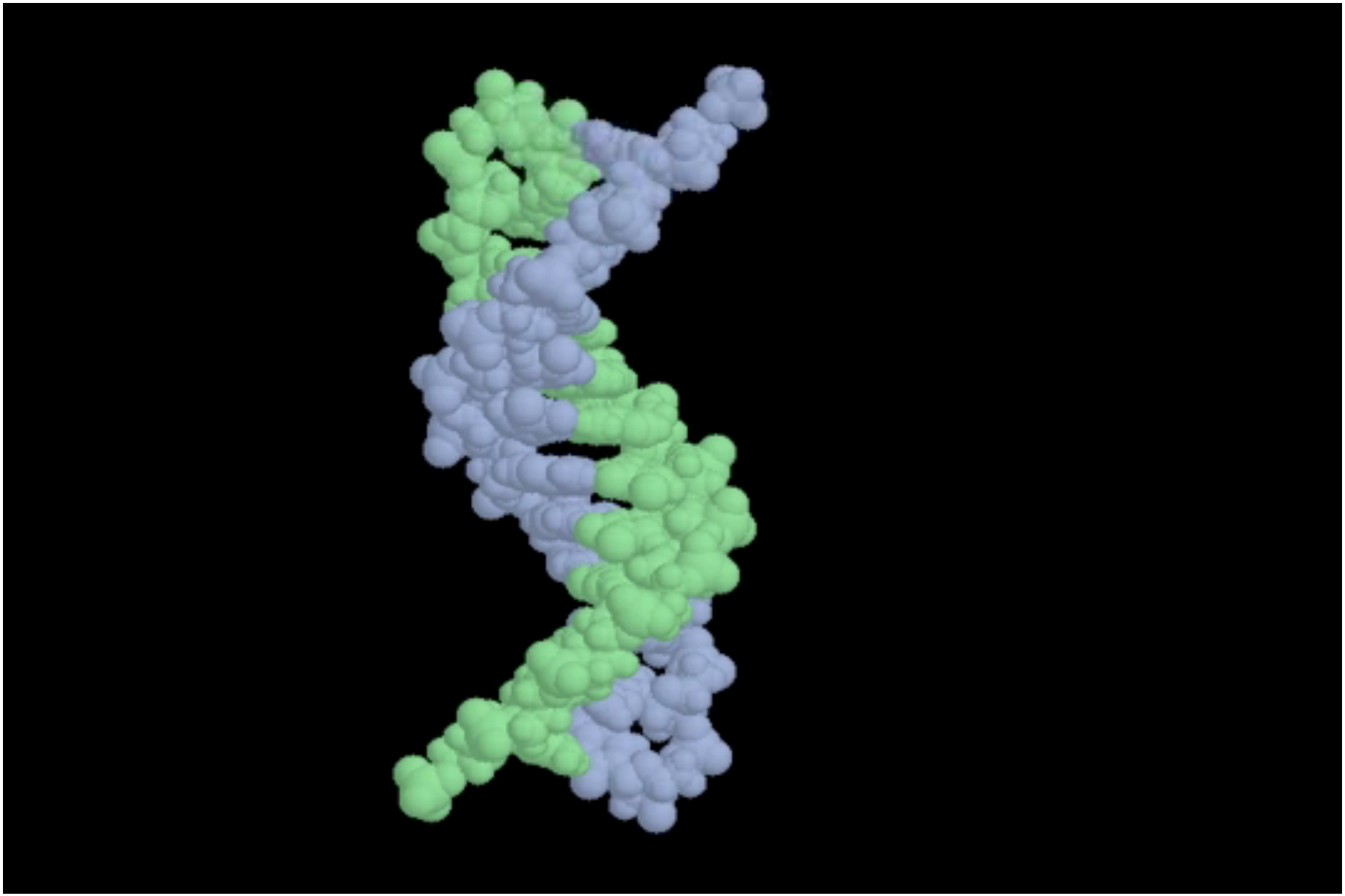
The 3' end carries – *OH group*; the 5' end carries *a free phosphate group*

Summary

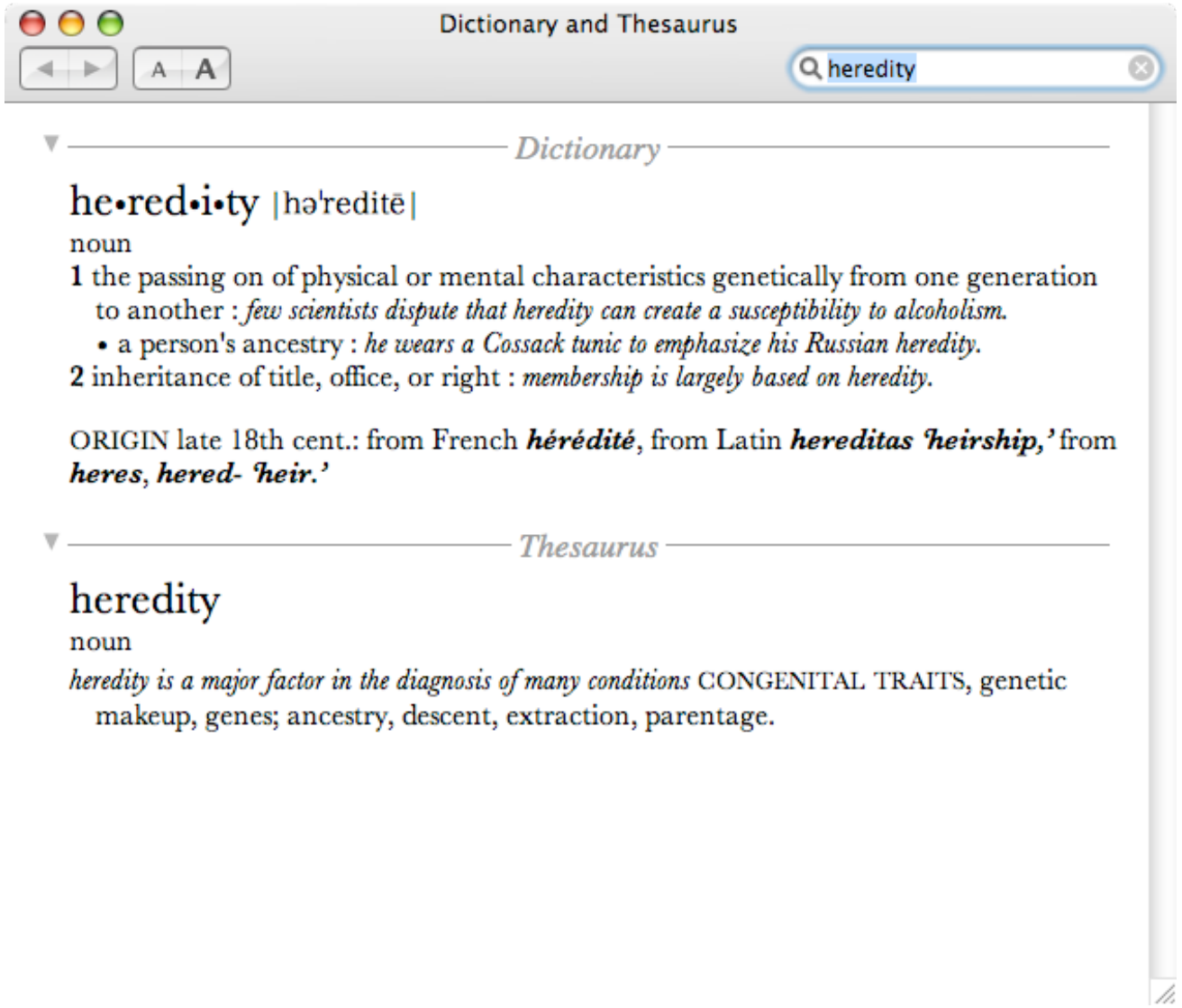
1. Complementary base-pairing
   - Hydrogen bonds to form *AT and GC* (purine + pyrimidine).
   - Each base pair is of *similar width, an equal distance* apart along the DNA.
   - Important for both *copying and repairing the DNA*.
   - *Antiparallel* (oriented in opposite polarities)
2. Held together by base pairing
   - The nucleotides are linked together covalently by *phosphodiester bonds*
   - The 3' end carries – *OH group*; the 5' end carries *a free phosphate group*.
3. Major and minor groove

   The strand *twist around each other* to form a double helix containing 10 base pairs per helical turn.

DNA structure



The structure of DNA provide a mechanism for *heredity*



heredity (유전형질)

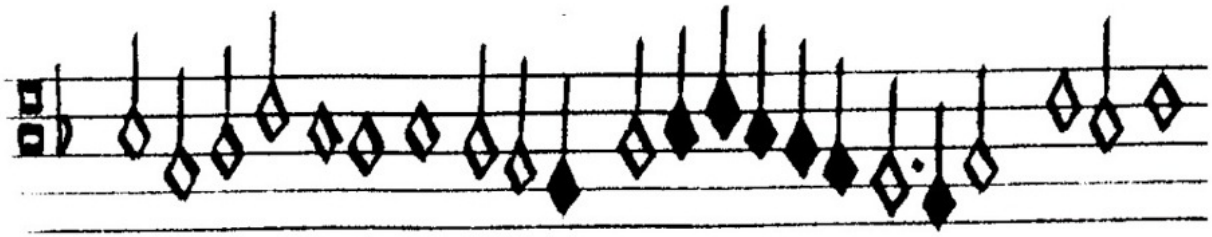
How can *the information for specifying an organism* be carried *in chemical form*?

How is it *accurately copied?* (next chapter)

Linear messages come in many forms

(A) molecular biology is...

(B)

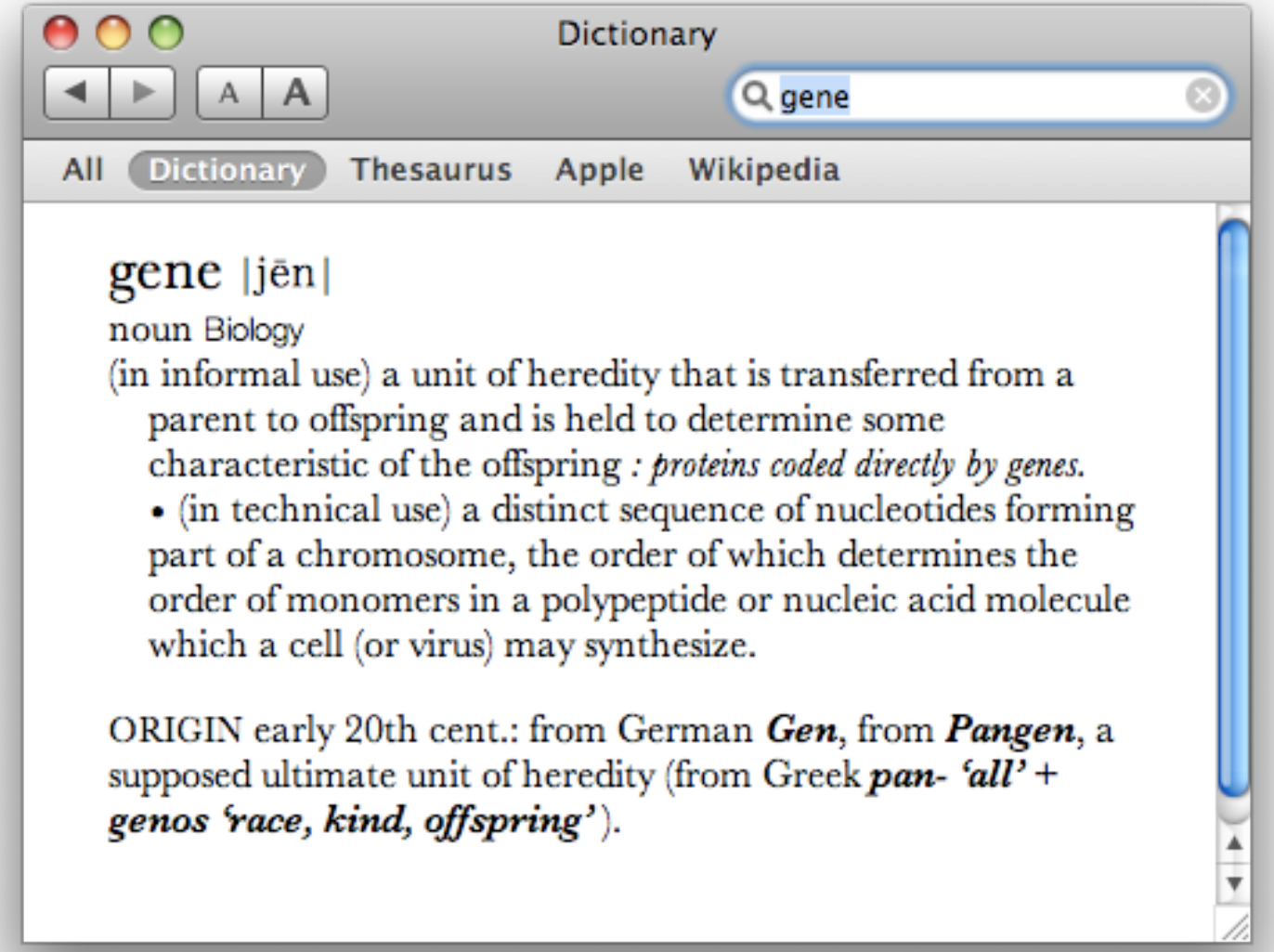


(C) •— •—•• •• —•—• •

(D) 细胞生物学乐趣无穷

(E) TTCGAGCGACCTAACCTATAG

Figure 5-8 Essential Cell Biology 3/e (© Garland Science 2010)


Dictionary
gene
All
Dictionary
Thesaurus
Apple
Wikipedia
gene |jēn|
noun Biology
(in informal use) a unit of heredity that is transferred from a parent to offspring and is held to determine some characteristic of the offspring : *proteins coded directly by genes.*
• (in technical use) a distinct sequence of nucleotides forming part of a chromosome, the order of which determines the order of monomers in a polypeptide or nucleic acid molecule which a cell (or virus) may synthesize.
ORIGIN early 20th cent.: from German ***Gen***, from ***Pangen***, a supposed ultimate unit of heredity (from Greek ***pan- 'all'*** + ***genos 'race, kind, offspring'***).

Genes contain information to make *proteins*

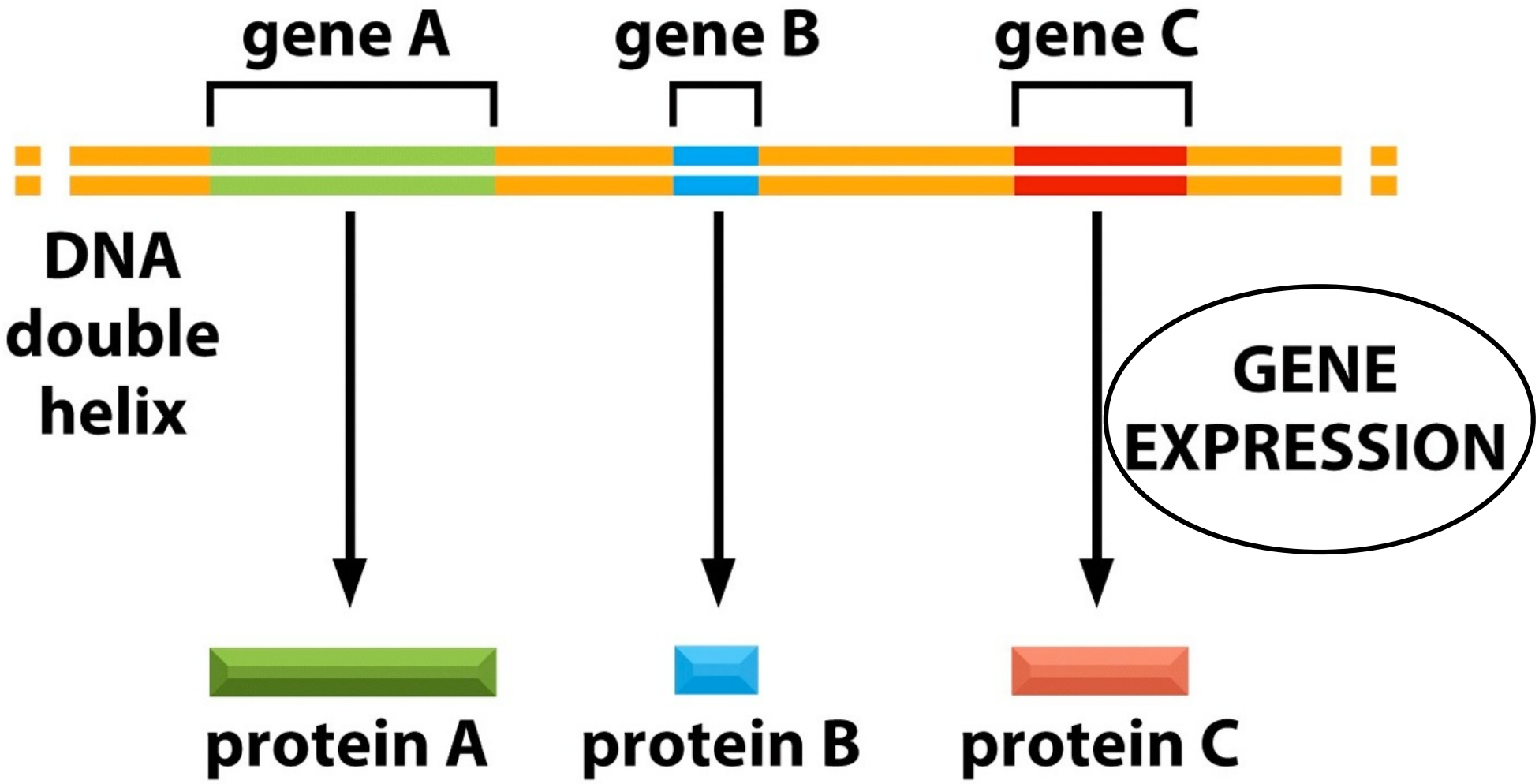


Figure 5-9 Essential Cell Biology 3/e (© Garland Science 2010)

Gene sequences can be written and read like any text

- human β-globin gene.
- the sequence (seq) should be read from left to right in successive lines
- the DNA seq highlighted in yellow color show the three regions of the gene that specify the amino seq of the β-globin protein

Figure 4–7. Molecular Biology of the Cell, 4th Edition.

Genetic Code

'The exact correspondence between the 4-letter nucleotide of DNA and the 20-letter amino acid'

GCA	AGA						GGA			UUA					AGC				
GCC	AGG						GGC		AUA	UUG				CCA	AGU	ACA			GUA	
GCG	CGA								AUC	CUA				CCC	UCA	ACC			GUC	UAA
GCU	CGC						GGG		AUU	CUC				CCG	UCC	ACG		UAC	GUG	UAG
	CGG	GAC	AAC	UGC	GAA	CAA	GGU	CAC		CUG	AAA		UUC	CCU	UCG	ACU	UGG	UAU	GUU	UGA
	CGU	GAU	AAU	UGU	GAG	CAG		CAU		CUU	AAG	AUG	UUU		UCU					
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
A	R	D	N	C	E	Q	G	H	I	L	K	M	F	P	S	T	W	Y	V	

Figure 7-24 Essential Cell Biology 3/e (© Garland Science 2010)

Genome

'The complete set of information in an organism's DNA'

TGA:
Stop codon

Gal10 gene (2,100 bp)

- Promoter
- ORF (open reading frame)
- 3'-UTR

Length (a.a.)	699
Molecular Weight (Da)	78,195
Isoelectric Point (pI)	5.84

```
  1 MTAQLQSEST SKIVLVTGGA GYIGSHTVVE LIENGYDCVV ADNLSNSTYD
 51 SVARLEVLTK HHIPFYEVDL CDRKGLEKVF KEYKIDSVIH FAGLKAVGES
101 TQIPLRYYHN NILGTVVLLE LMQQYNVSKF VFSSSATVYG DATRFPNMIP
151 IPEECPLGPT NPYGHTKYAI ENILNDLYNS DKKSWKFAIL RYFNPIGAHP
201 SGLIGEDPLG IPNNLLPYMA QVAVGRREKL YIFGDDYDSR DGTPIRDYIH
251 VVDLAKGHIA ALQYLEAYNE NEGLCREWNL GSGKGSTVFE VYHAFCKASG
301 IDLPYKVTGR RAGDVLNLTA KPDRAKRELK WQTELQVEDS CKDLWKWTTE
351 NPFGYQLRGV EARFSAEDMR YDARFVTIGA GTRFQATFAN LGASIVDLKV
401 NGQSVVLGYE NEEGYLNPDS AYIGATIGRY ANRISKGKFS LCNKDYQLTV
451 NNGVNANHSS IGSFHRKRFL GPIIQNPSKD VFTAEYMLID NEKDTEFPGD
501 LLVTIQYTVN VAQKSLEMVY KGKLTAGEAT PINLTNHSYF NLNKPYGDTI
551 EGTEIMVRSK KSVDVDKNMI PTGNIVDREI ATFNSTKPTV LGPKNPQFDC
601 CFVVDENAKP SQINTLNNEL TLIVKAFHPD SNITLEVLST EPTYQFYTGD
651 FLSAGYEARQ GFAIEPGRYI DAINQENWKD CVTLKNGETY GSKIVYRFS*
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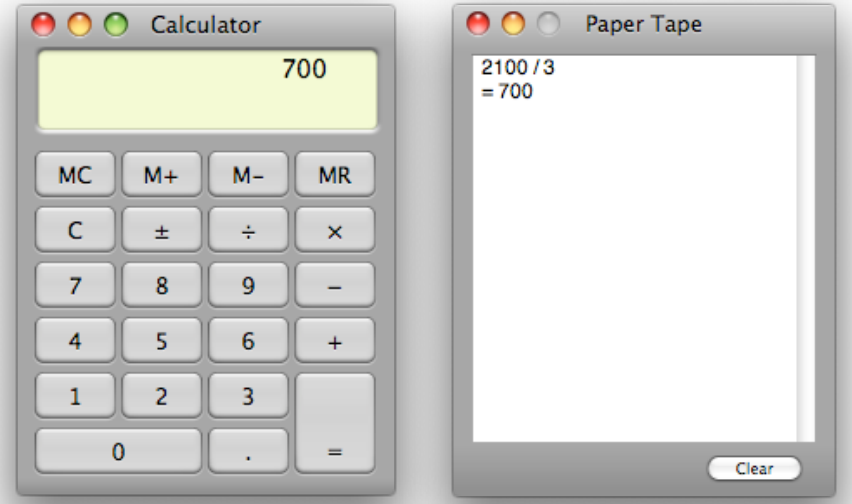


The Structure of Eucaryotic Chromosomes

Human genome : 3.2 x 10^9 nt over 24 chromosomes.
Human 32억개의 Nucleotide 쌍으로 구성 (3.2 x 10^9 nt)
3.2 x 10^9 mm = 3.2 x 10^8 cm = 3.2 x 10^6 m = 3.2 x 10^3 km
(만일 mm단위로 그린다면 Africa를 횡단할 수 있는 길이)

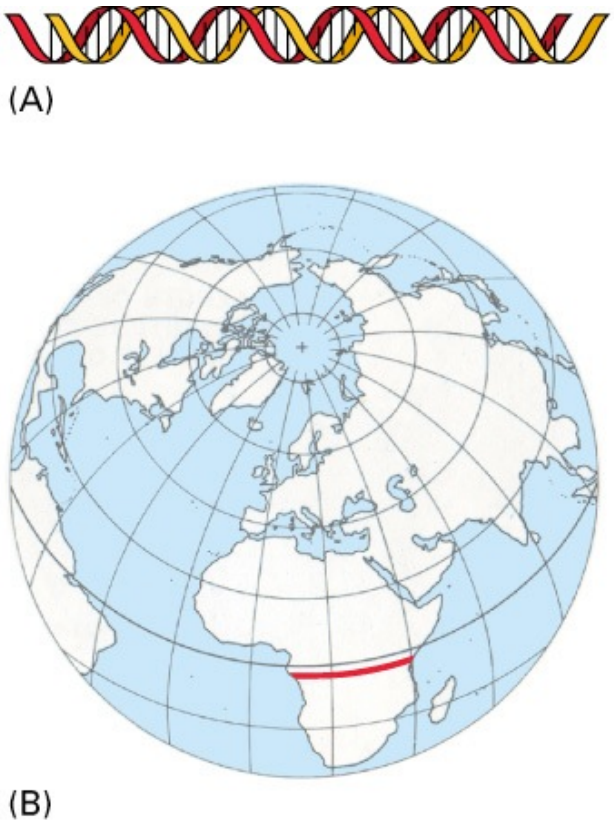
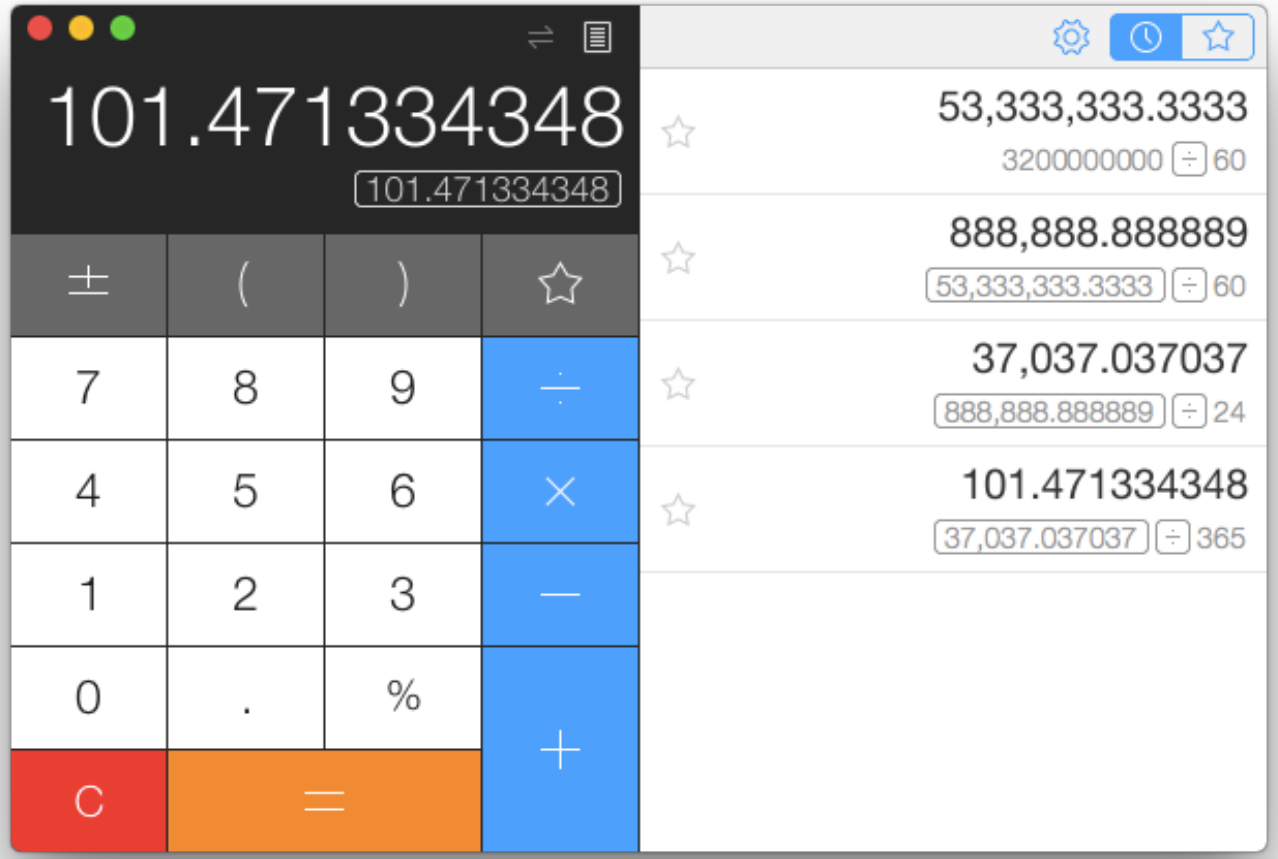


Figure 4–16. Molecular Biology of the Cell, 4th Edition.

Fig. Scale of the human genome
If each nucleotide pair is drawn as 1 mm as in (A), then the human genome would extend 3,200 km, far enough to stretch across the center of Africa (B).

101년



5천3백 분

8십8만 시간

3만7천 일

101년

Backgrounds

Each human cell contains about *2 m of DNA*; yet the cell *nucleus is only 5 to 8 μm* in diameter (the equivalent of trying to fold 40 km of extremely fine thread into a tennis ball).

In eukaryotic cells, enormously long double-stranded DNA molecules are *packaged into chromosomes*.

The complex task of packing DNA is accomplished by *specialized proteins* that bind to and fold the DNA.

Amazingly, the DNA is compacted in a way that allows it to *remain accessible to* all of the enzymes and other proteins that *replicate it, repair it, and direct the expression of its gens*.

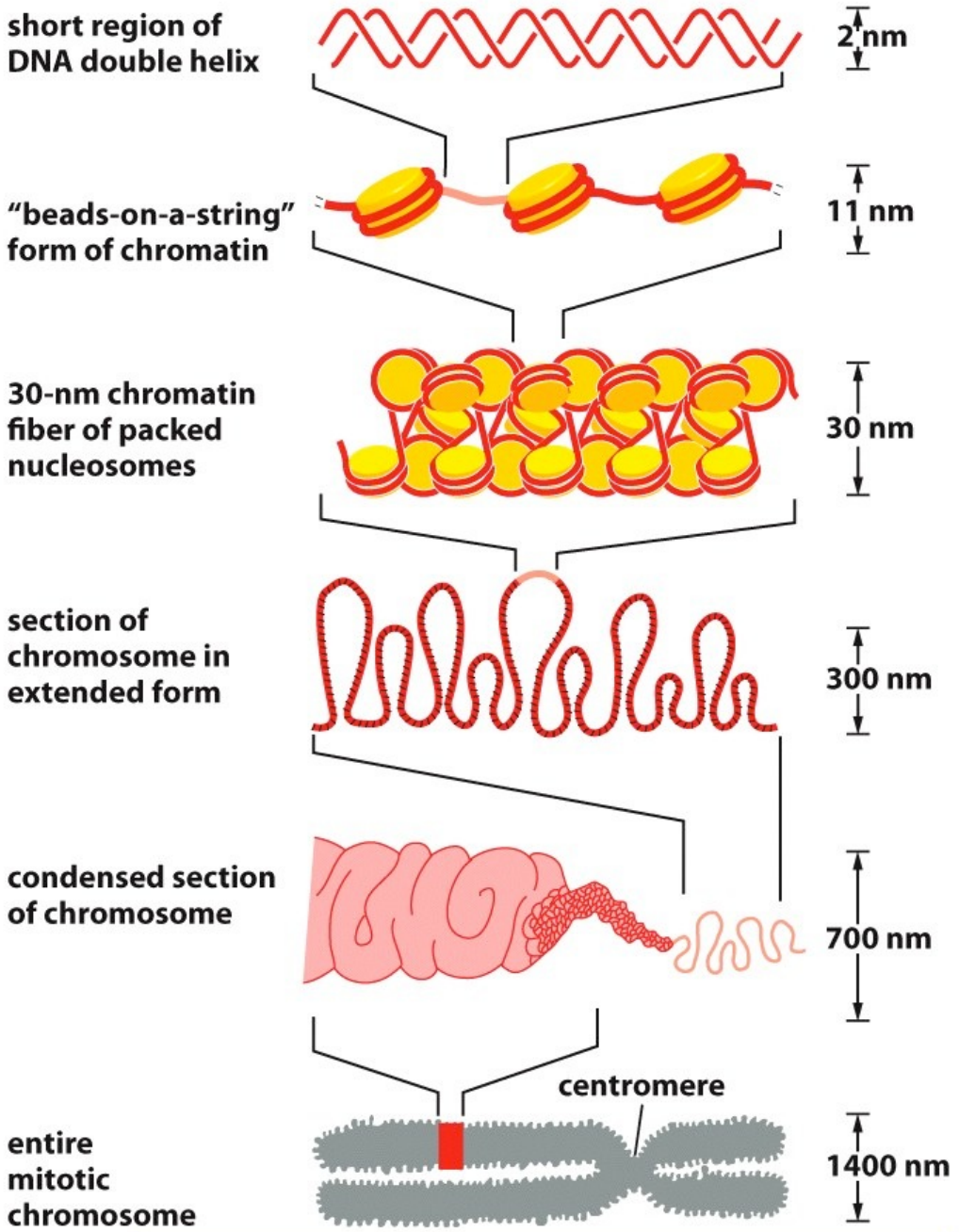
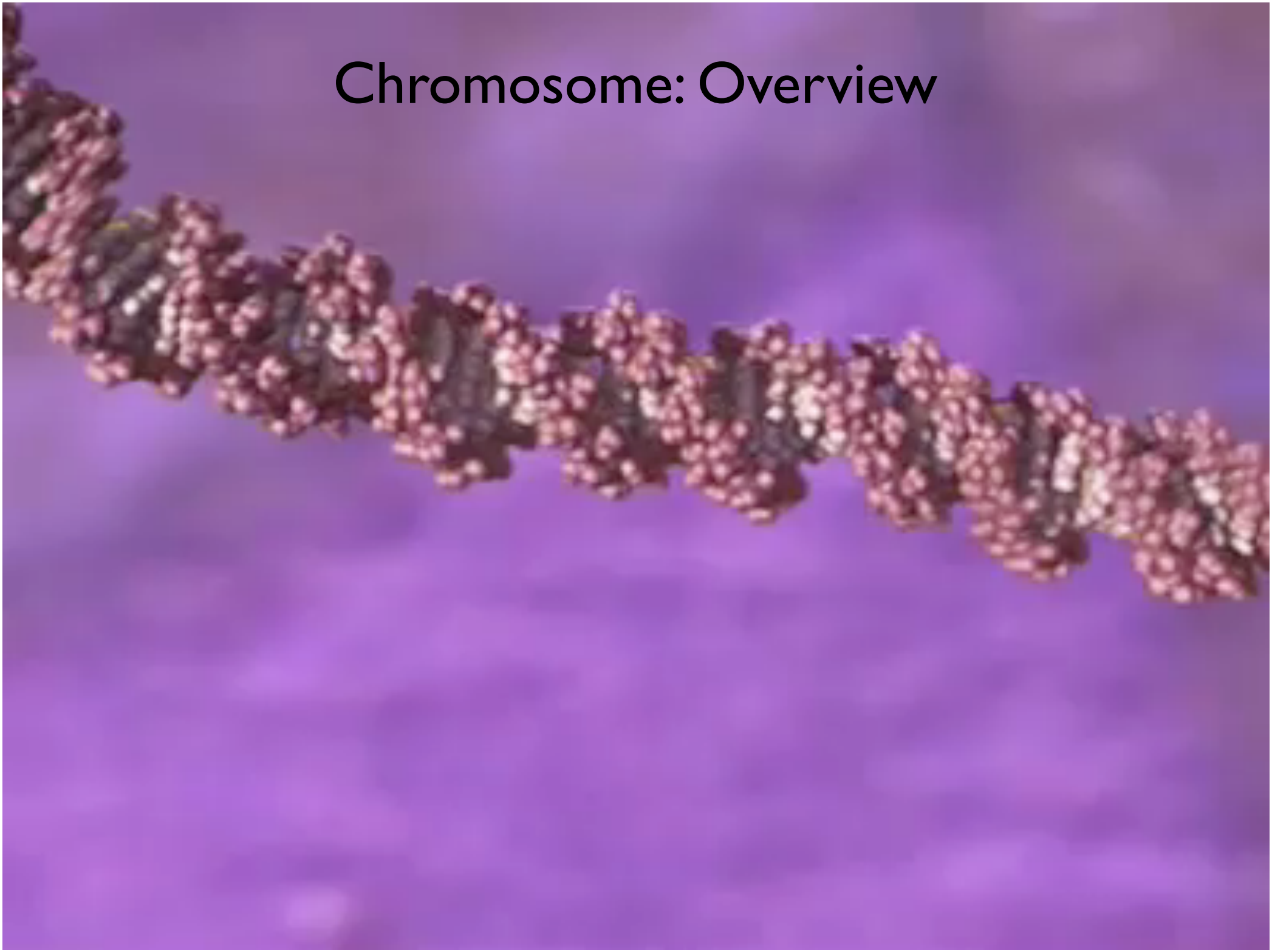


Figure 5-25 Essential Cell Biology 3/e (© Garland Science 2010)

Chromosome: Overview



Eucaryotic DNA is *packed into multiple chromosomes*

Chromosomes: The human genome contains about *3.2 x 10^9 (3.2 billion) nucleotides* distributed over *24 chromosomes*.

Chromatin: Each chromatin consists of *a single, enormously long linear DNA* molecules associated with *proteins* that fold and pack the fine thread of DNA into a more *compact* structure. Also associated with proteins in gene expression, DNA replication, and DNA repair.

Homologous chromosomes (상동염색체, 22쌍) *Two copies of each chromosome*, one inherited from the mother and one from the father.

Sex chromosomes (성염색체, 1쌍): *X and Y* chromosome.

- *DNA hybridization*: the technique uses a set of DNA molecules coupled to fluorescent molecules to "paint" each chromosome a different color.

- *Human karyotype*: a display of the full set of 46 human chromosomes.

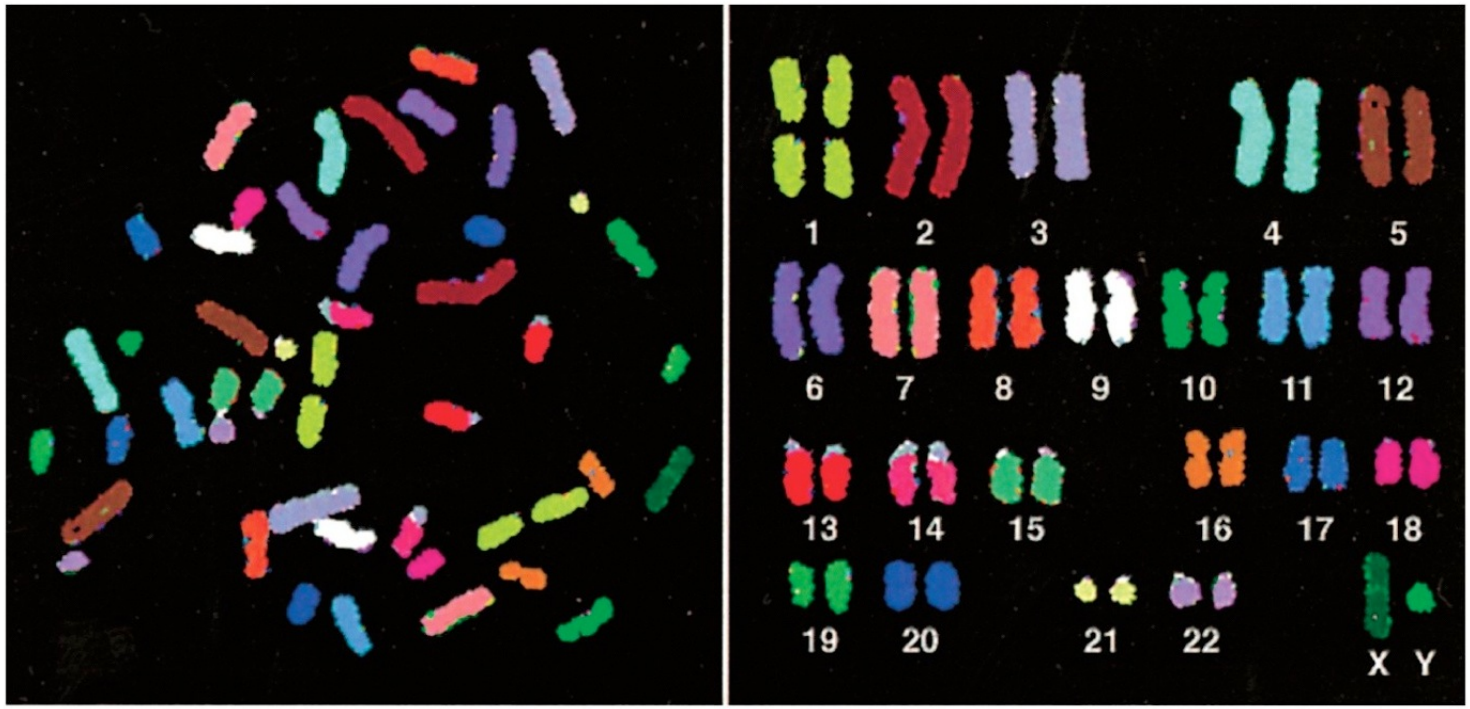
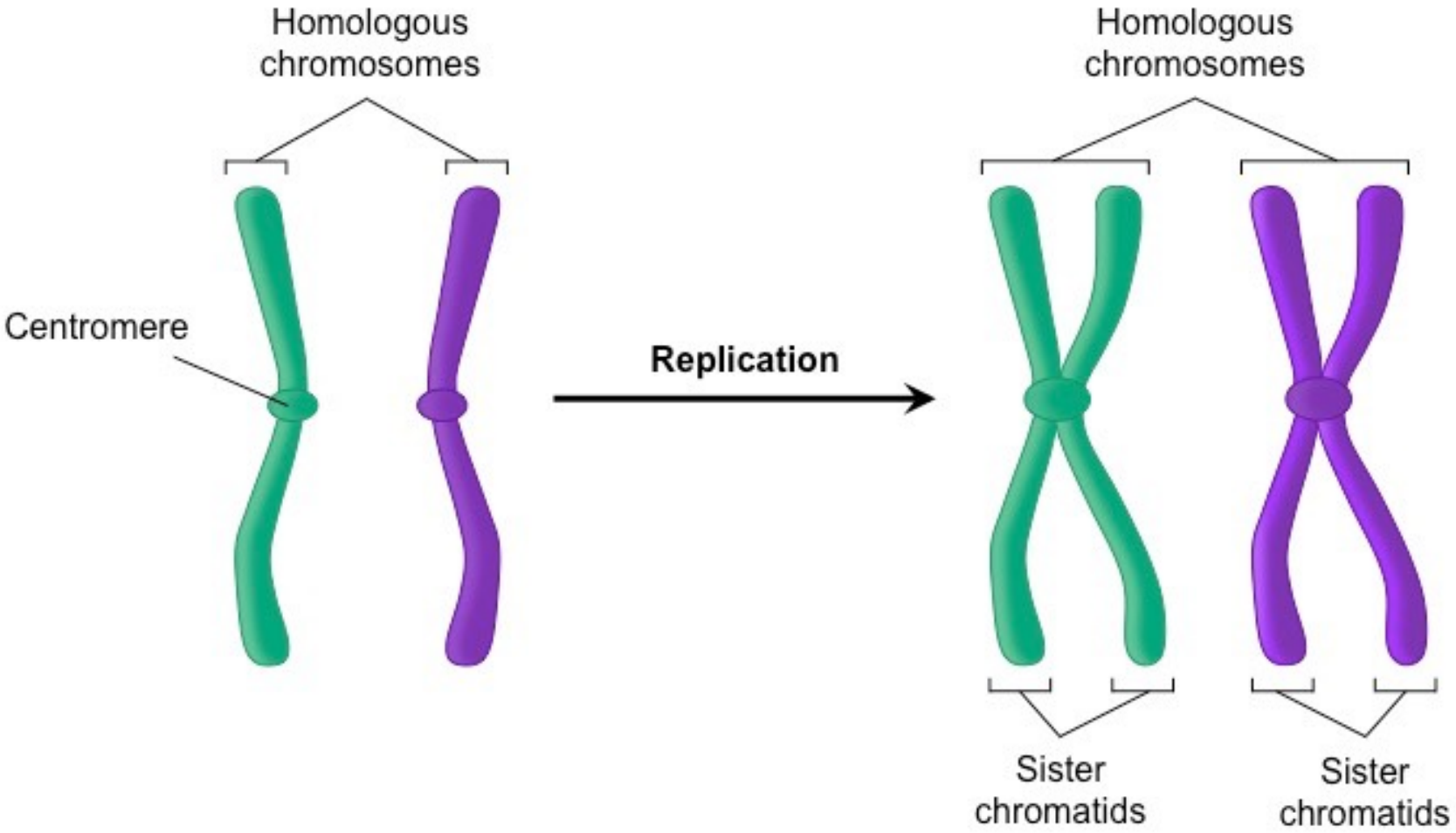
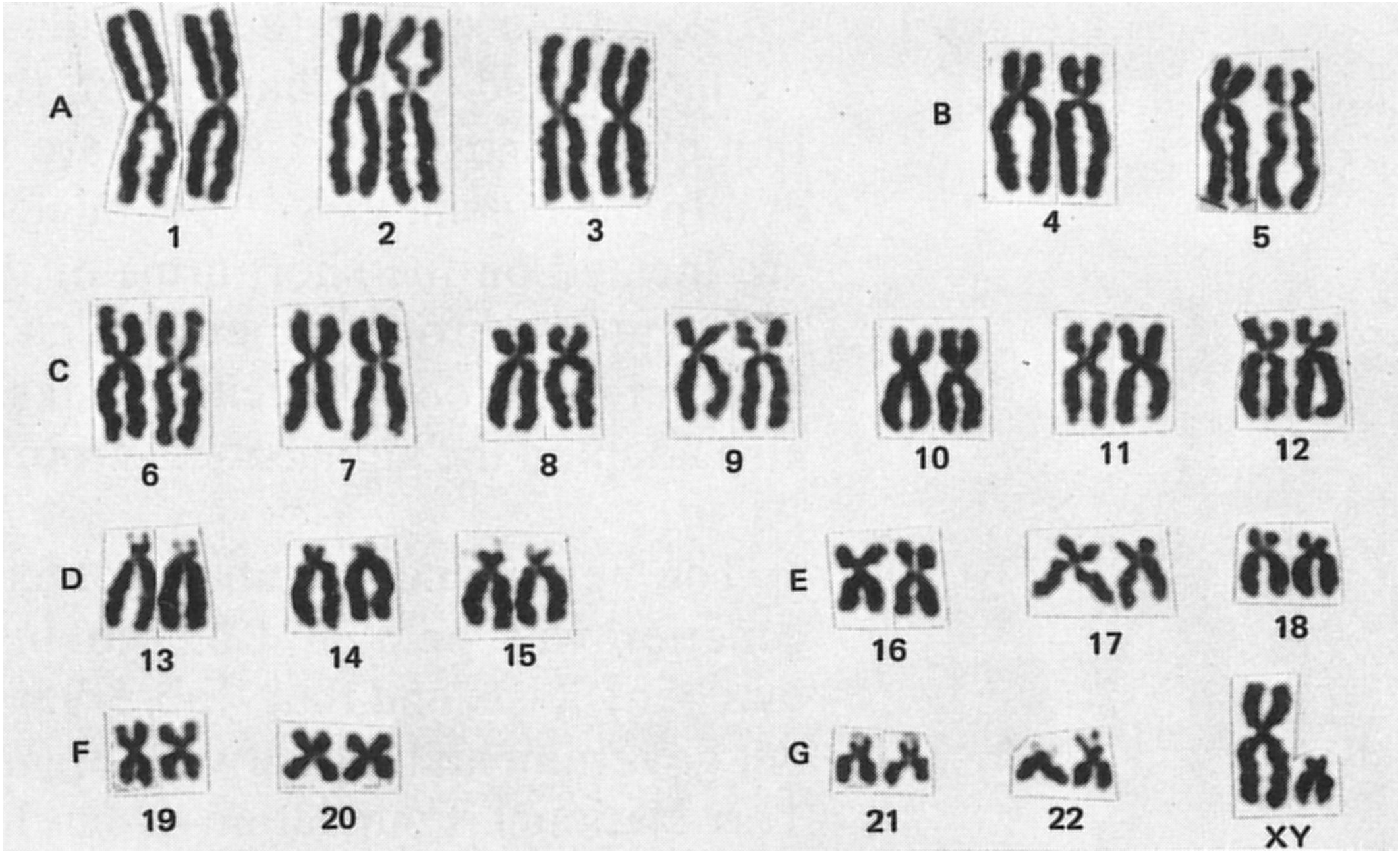


Figure 5-10 Essential Cell Biology 3/e (© Garland Science 2010)


Homologous chromosomes
Centromere
Replication
Homologous chromosomes
Sister chromatids
Sister chromatids


A
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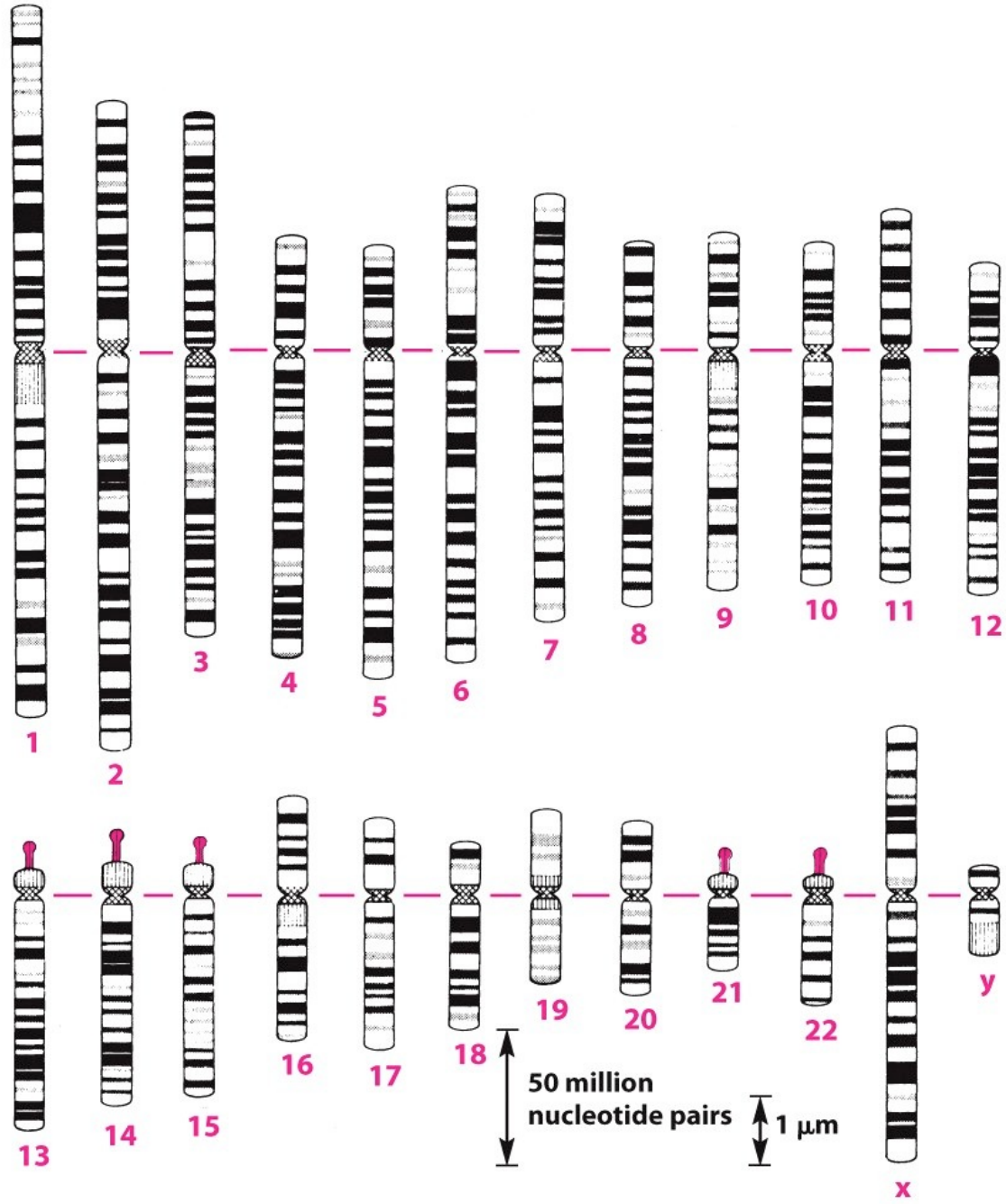


Figure 5-11 Essential Cell Biology 3/e (© Garland Science 2010)

Unique banding patterns allow the identification of each human chromosome

- To stain the chromosomes with dyes that bind to certain types of DNA sequences.
- These dyes mainly distinguish between DNA that is rich in A-T nucleotide pairs and DNA that is G-C rich, and they produce a striking and reliable pattern of bands along each chromosome.
- *Giemsa stain*: produce dark bands in AT rich region.
- Giemsa stain is named after German chemist and bacteriologist Gustav Giemsa.
- Giemsa's solution is a mixture of methylene blue, eosin, and Azure B.

Abnormal chromosomes are associated with some inherited genetic defects

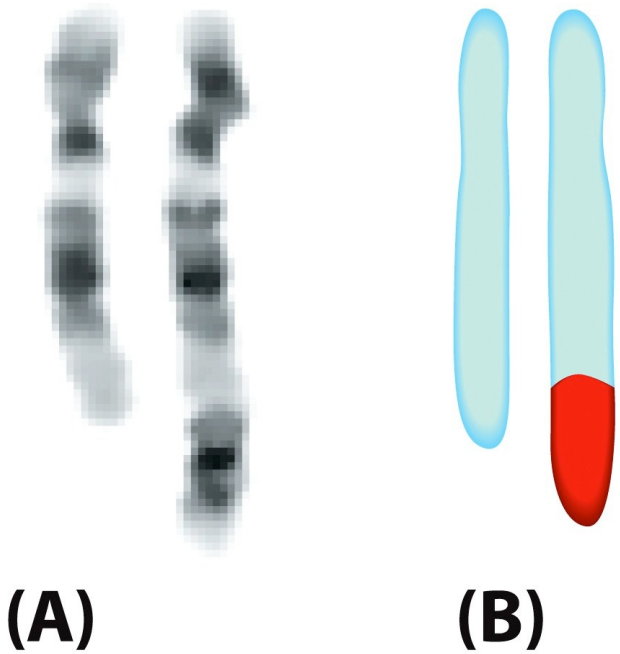


Figure 5-12 Essential Cell Biology 3/e (© Garland Science 2010)

(A) Inherited ataxia (운동실조). The patient has one normal Chromosome 12 (left) and one aberrant chromosome 12 (B) Chromosome 4 DNA "painted" red and the parts corresponding to Chromosome 12 DNA painted blue.

Chromosomes contain *long stings of genes*

The most important function of chromosomes is *to carry genes (the functional units of heredity)*

Gene: a segment of DNA that contains the instructions for *making a particular protein.*

(cf, Some genes produce an *RNA* molecules)

Genes are arranged along the chromosomes

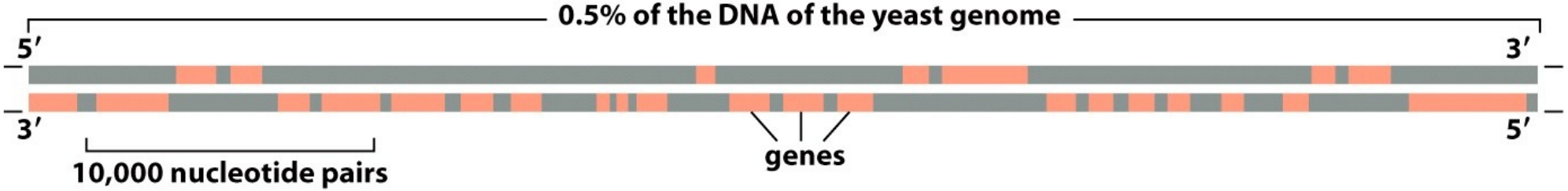


Figure 5-13 Essential Cell Biology 3/e (© Garland Science 2010)

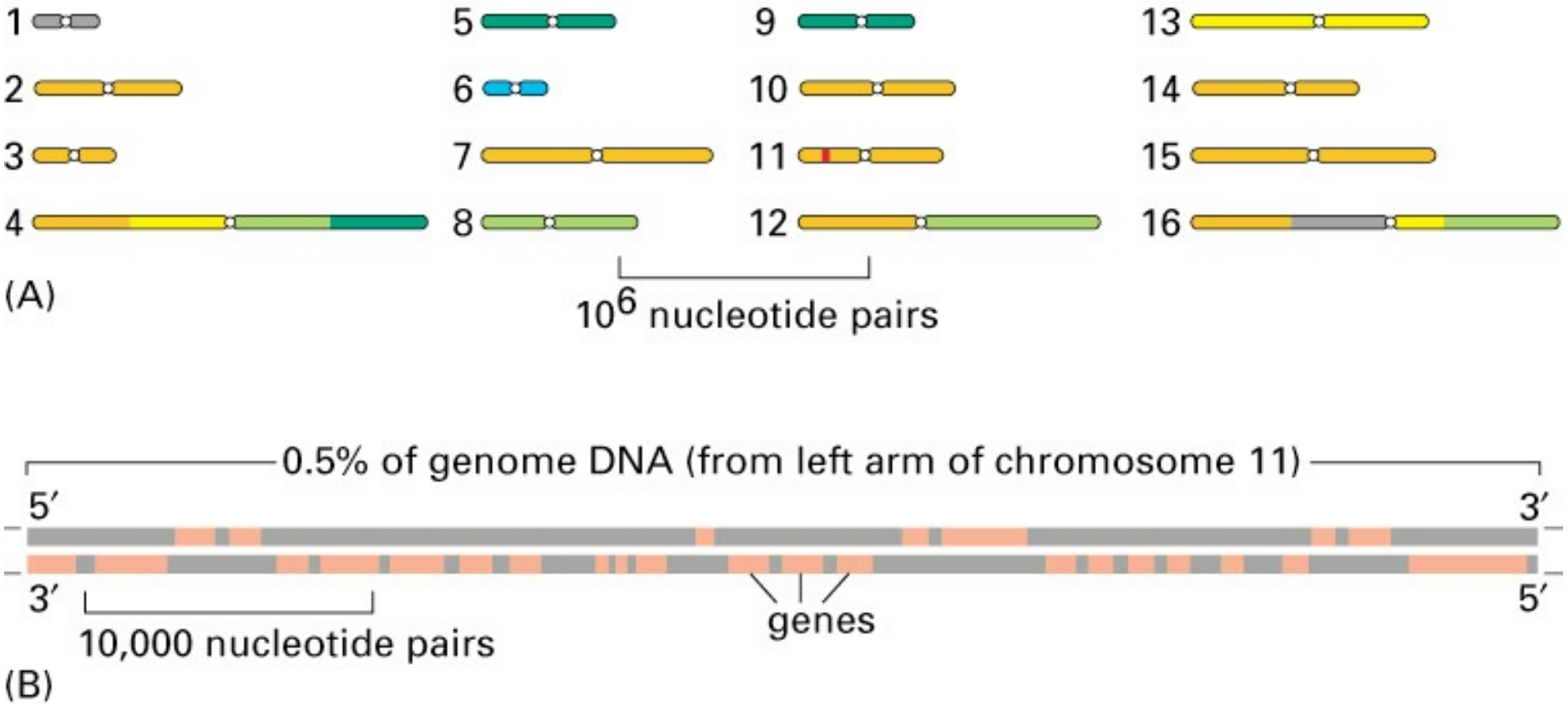


Figure 4–13. Molecular Biology of the Cell, 4th Edition.

Fig. The genome of *S. cerevisiae* (budding yeast)
(A) The genome : 16 chromosomes
(B) A small region of chromosome 11

The nucleotide sequence of the human genome shows how our genes are arranged

The *conserved regions* include both functionally important exons and regulatory DNA sequences.

The *nonconserved regions* represent DNA whose sequence unlikely to be critical for function.

Roughly *5%* of the human genome consists of "multi-species conserved sequences".

Only about one- third of these sequences code for proteins.

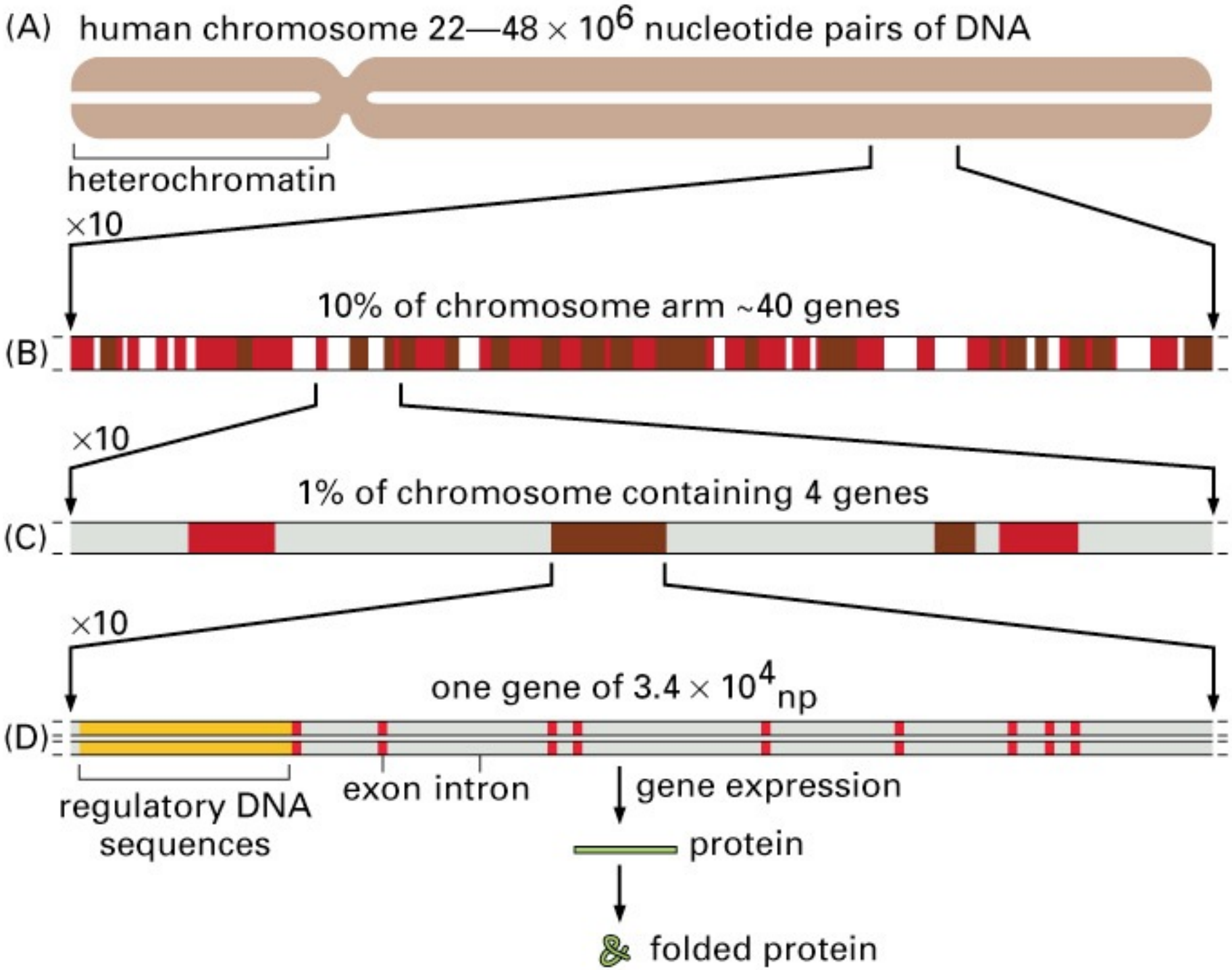


Figure 4–15. Molecular Biology of the Cell, 4th Edition.

Table 4–1 Some Vital Statistics for the Human Genome

	HUMAN GENOME
DNA length	3.2×10^9 nucleotide pairs*
Number of genes	approximately 25,000
Largest gene	2.4×10^6 nucleotide pairs
Mean gene size	27,000 nucleotide pairs
Smallest number of exons per gene	1
Largest number of exons per gene	178
Mean number of exons per gene	10.4
Largest exon size	17,106 nucleotide pairs
Mean exon size	145 nucleotide pairs
Number of pseudogenes**	more than 20,000
Percentage of DNA sequence in exons (protein coding sequences)	1.5%
Percentage of DNA in other highly conserved sequences***	3.5%
Percentage of DNA in high-copy repetitive elements	approximately 50%

* The sequence of 2.85 billion nucleotides is known precisely (error rate of only about one in 100,000 nucleotides). The remaining DNA primarily consists of short highly repeated sequences that are tandemly repeated, with repeat numbers differing from one individual to the next.

** A pseudogene is a nucleotide sequence of DNA closely resembling that of a functional gene, but containing numerous mutations that prevent its proper expression. Most pseudogenes arise from the duplication of a functional gene followed by the accumulation of damaging mutations in one copy.

*** Preserved functional regions; these include DNA encoding 5′ and 3′ UTRs (untranslated regions), structural and functional RNAs, and conserved protein-binding sites on the DNA.

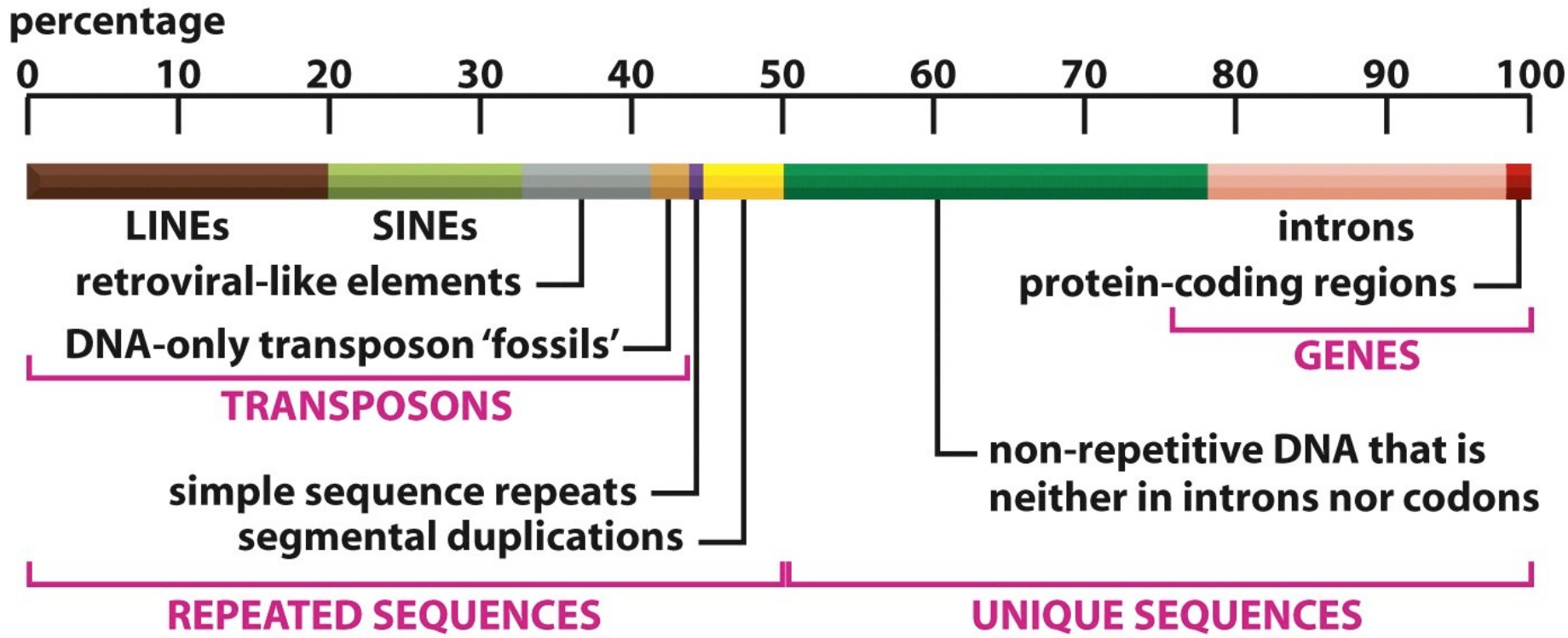


Figure 4-17 Molecular Biology of the Cell 5/e (© Garland Science 2008)

This unexpected discovery has led scientists to conclude that *we understand much less about the cell biology of vertebrates* than we had previously imagined.

Certainly, there are *enormous opportunities for new discoveries*, and we should expect *many surprises ahead*.

'Junk DNA': a large excess of interspersed DNA, the majority of which does not seem to carry critical information.

may be crucial for the *long-term evolution*.

this extra DNA is highly *conserved among related species*.

In general, the *more complex* an organism is, *the larger* its genome.

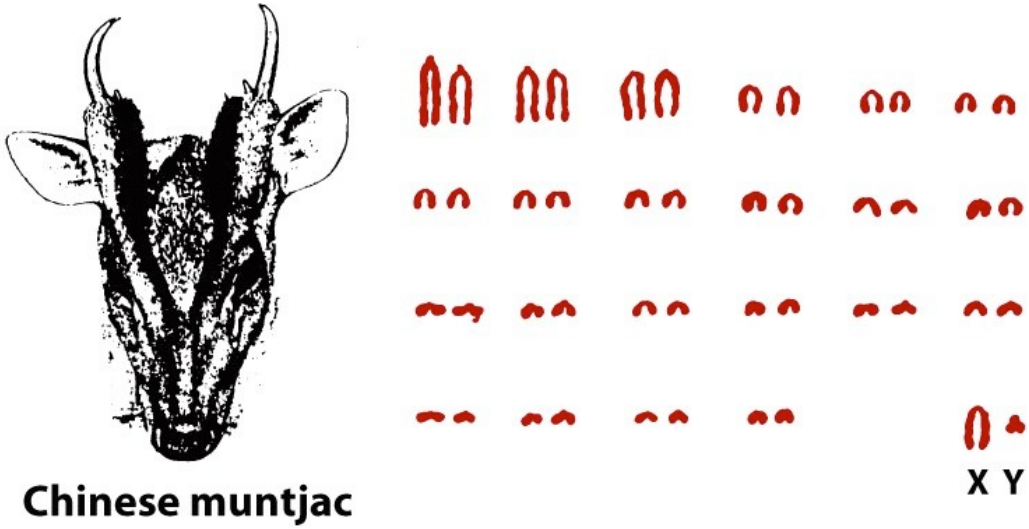
But, not always true.

e.g., closely related species (see fig. 5-14).

No simple relationship between gene number, chromosome number, and the total size.

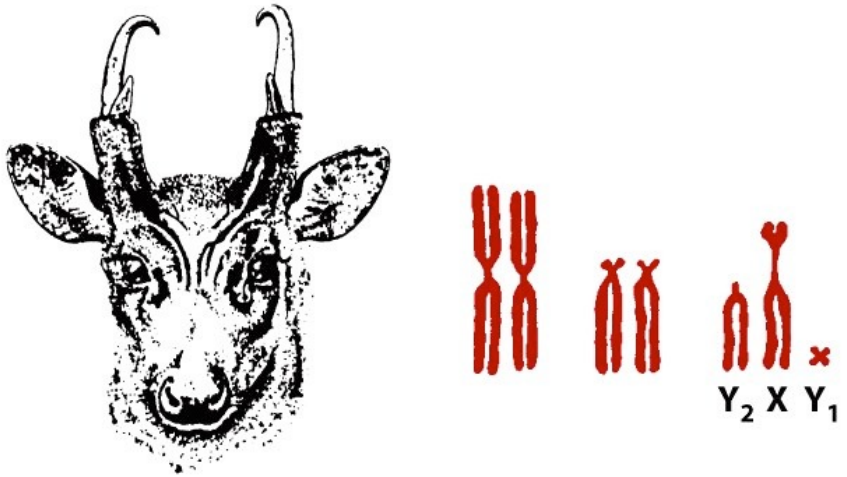
e.g., Human vs (*S. cerevisiae* / plant / amoeba).

22 pairs + XY



Chinese muntjac

2 pairs + XYY



Indian muntjac

Figure 5-14 Essential Cell Biology 3/e (© Garland Science 2010)

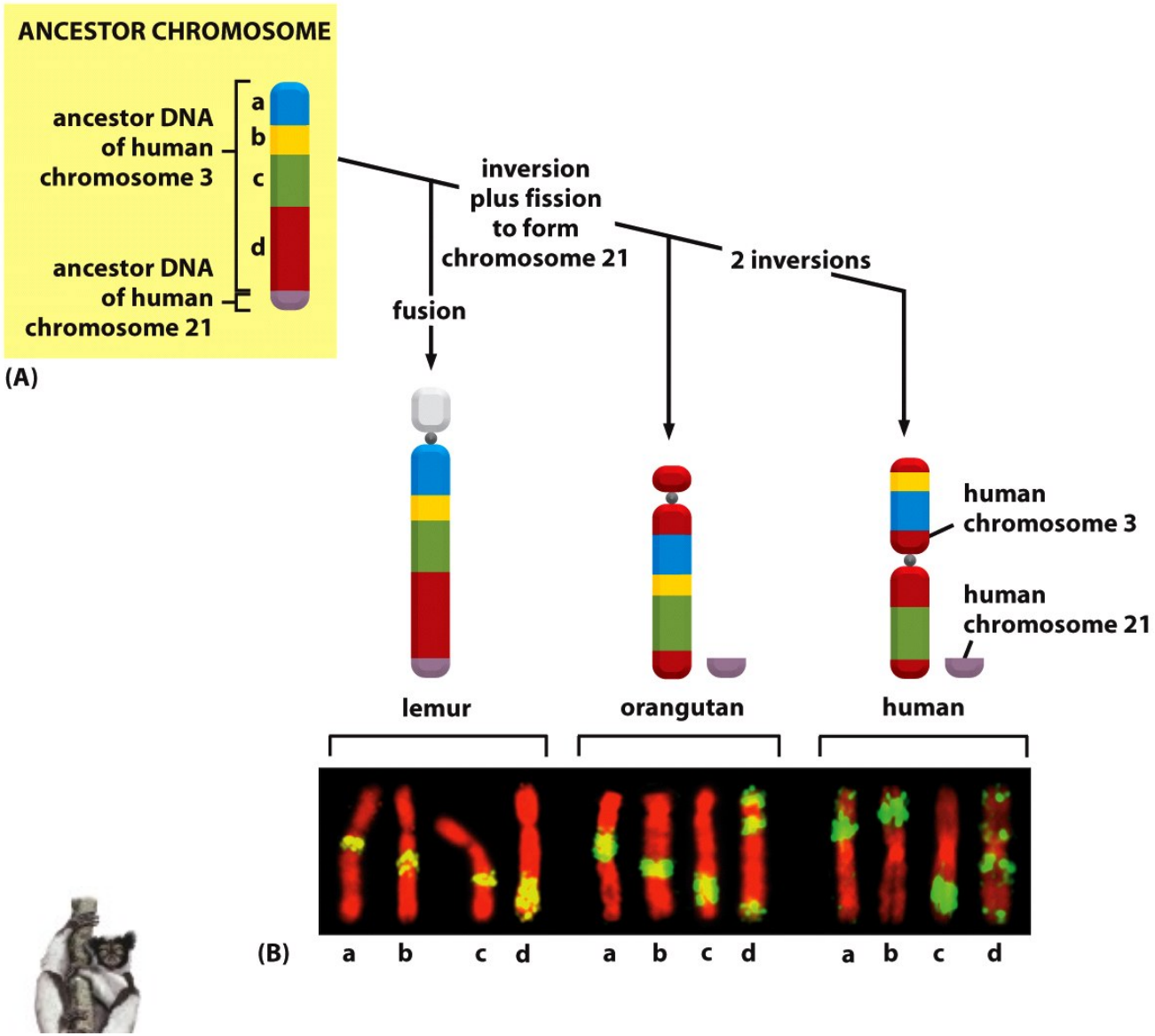
중국 문자크(동남아시아 원산의 작은 사슴)

인도 문자크(동남아시아 원산의 작은 사슴)

Genome comparisons reveal evolutionarily conserved DNA sequences

Major obstacle in interpreting the nucleotide sequences of human chromosome

1. Much of the sequence is probably *unimportant*.
2. Coding regions in *short* segments (ave about 145 nt pairs).
3. Where a gene begins and ends?
4. Exactly *how many exons* a gene spans?



lemur 미국·영국 [ˈliːmə(r)]
[명사] (마다가스카르산) 여우원숭이

Figure 4–18 A proposed evolutionary history of human chromosome 3 and its relatives in other mammals. (A) The order of chromosome 3 segments hypothesized to be present on a chromosome of a mammalian ancestor is shown (*yellow* box). The minimum changes in this ancestral chromosome necessary to account for the appearance of each of the three modern chromosomes are indicated. (The present-day chromosomes of humans and African apes are identical at this resolution.) The *small circles* depicted in the modern chromosomes represent the positions of centromeres. A fission and inversion that leads to a change in chromosome organization is thought to occur once every 5–10 × 10^6 years in mammals. (B) Some of the chromosome painting experiments that led to the diagram in (A). Each image shows the chromosome most closely related to human chromosome 3, painted *green* by hybridization with different segments of DNA, lettered a, b, c, and d along the *bottom* of the figure. These letters correspond to the colored segments of the diagrams in (A), as indicated on the ancestral chromosome. (From S. Müller et al., *Proc. Natl Acad. Sci. U.S.A.* 97:206–211, 2000. With permission from National Academy of Sciences.)

Comparative studies have revealed not only that humans and other mammals *share most of the same genes*, but also that large blocks of our genomes contain these genes in the *same order*, a feature called *conserved synteny*.

As a result, large blocks of our chromosomes can be recognized in other species. This allows the chromosome painting technique to be used to reconstruct the *recent evolutionary history of human chromosomes*.

synteny 미국·영국 síntəni
명사 (유전) 신터니 ((복수의 유전자가 동일 염색체 위에 있는 일))

Chromosomes exist *in different states* throughout the life of a cell

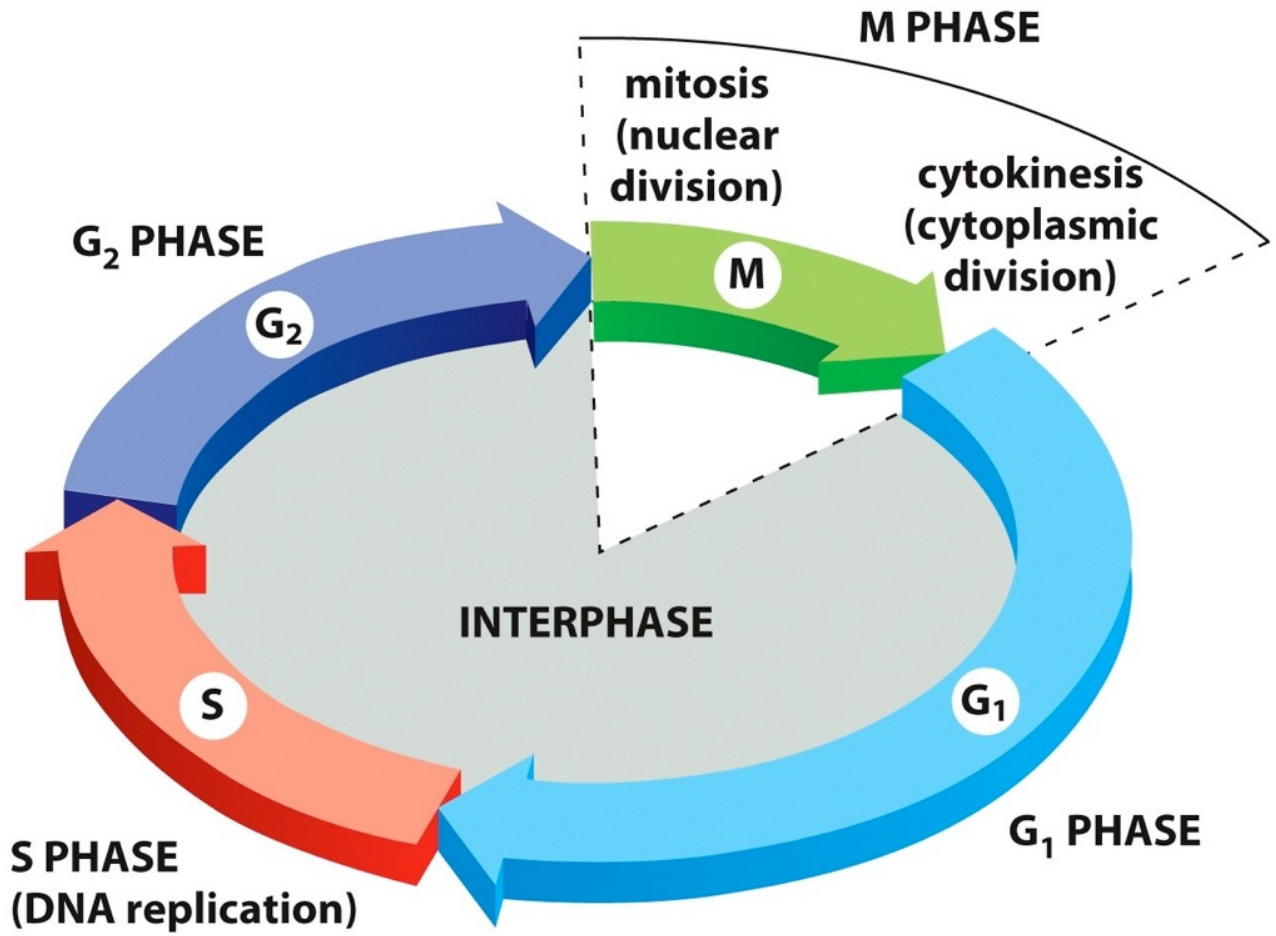


Figure 18-2 Essential Cell Biology 3/e (© Garland Science 2010)

Cell cycle: *Cell growth* and *division* (when the *replication* and *segregation* of chromosomes occurs).

Interphase:

Interphase Chromosomes

Extended as *long, thin, tangled thread of DNA.*

Not be easily distinguished.

Mitosis: Chromosomes are distributed to the two daughter nuclei.

Mitotic chromosomes

More and more compact structure (highly condensed).

- *Most easily visualized.*

Mitotic chromosome is highly compact

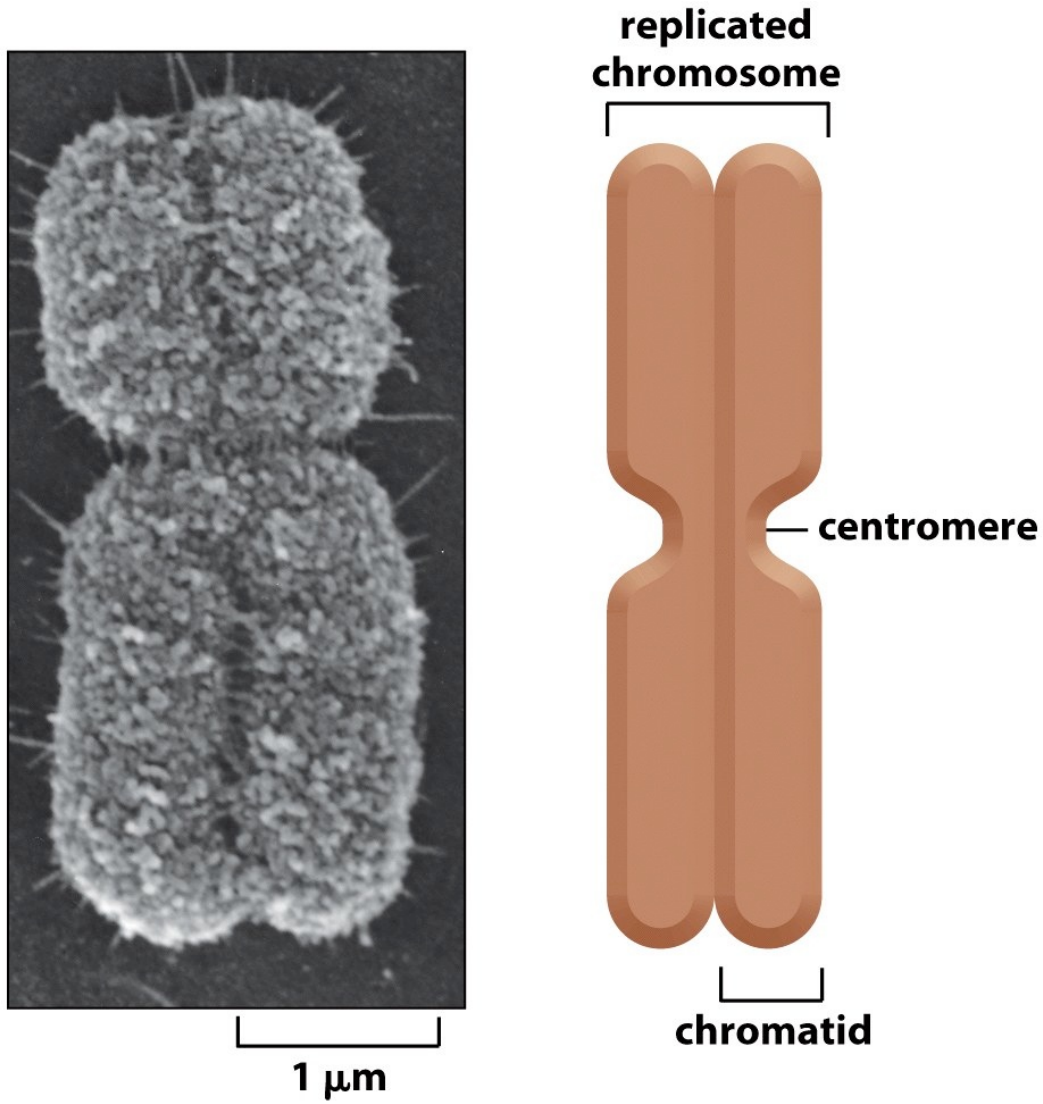


Figure 5-17 Essential Cell Biology 3/e (© Garland Science 2010)

Replication and segregation of chromosomes occur through the cell cycle

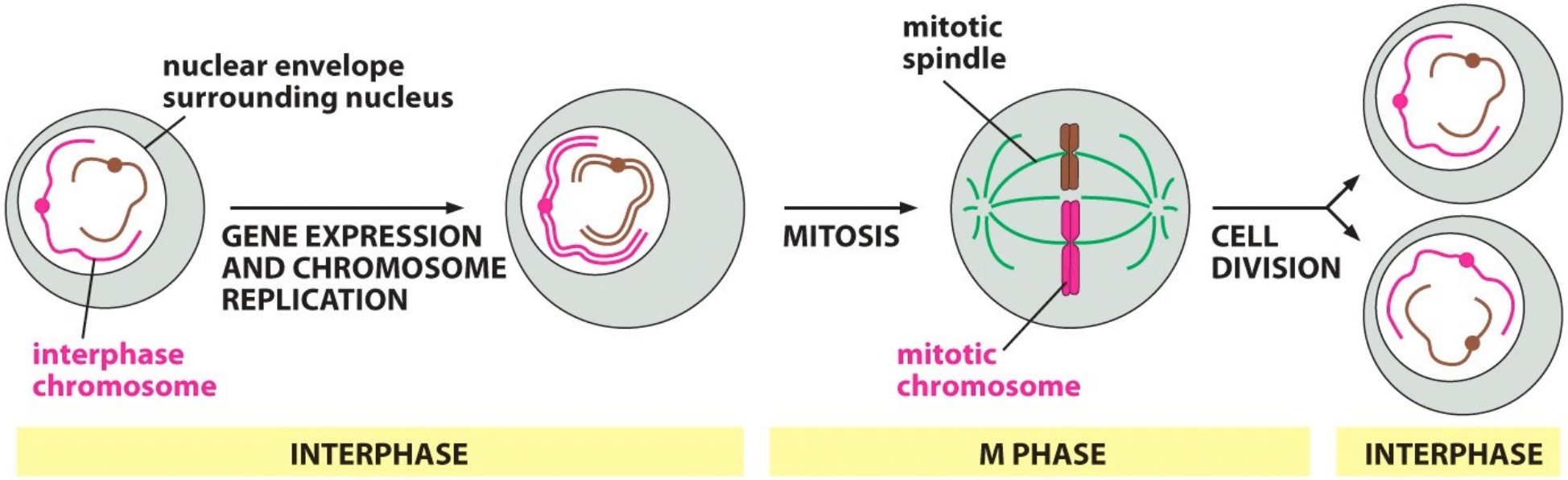


Figure 5-15 Essential Cell Biology 3/e (© Garland Science 2010)

Interphase, when chromosomes are duplicated
Mitosis, when chromosomes are distributed to the two daughter nuclei

The duration of the cell cycle varies greatly from one cell type to another

TABLE 18–1 SOME EUCARYOTIC CELL-CYCLE TIMES

CELL TYPE	CELL-CYCLE TIMES
Early frog embryo cells	30 minutes
Yeast cells	1.5–3 hours
Mammalian intestinal epithelial cells	~12 hours
Mammalian fibroblasts in culture	~20 hours
Human liver cells	~1 year

Table 18-1 Essential Cell Biology 3/e (© Garland Science 2010)

Three DNA sequence elements are needed to produce a eucaryotic chromosome

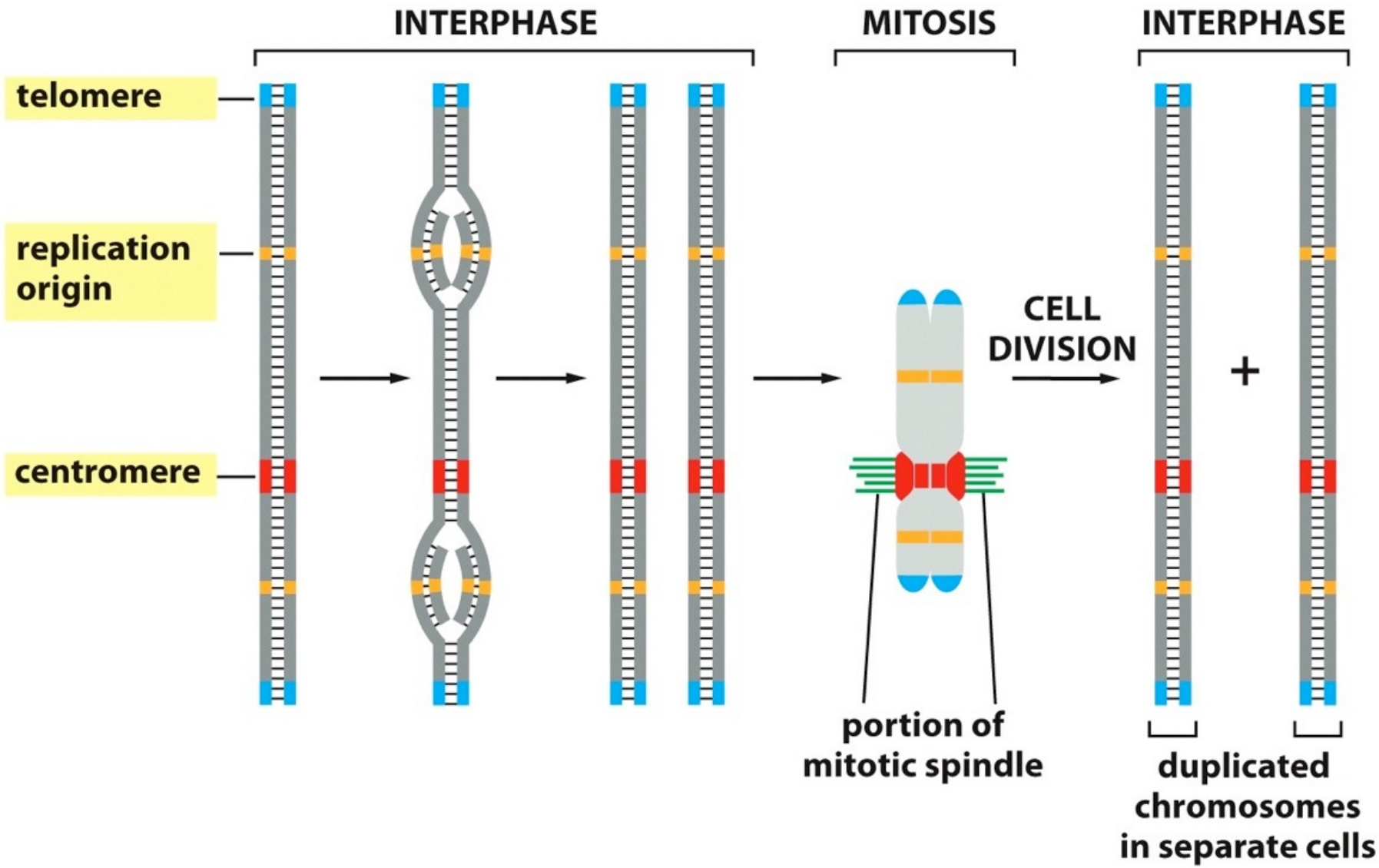


Figure 5-16 Essential Cell Biology 3/e (© Garland Science 2010)

Replication origin

Duplication of the DNA begins.

Many replication origins to be replicated *rapidly*.

Telomeres

Found at each of the *two ends* of a chromosome.

Repeated nucleotide sequences (*GGGGTTA* repeats in human) that enable the ends of chromosomes to be replicated.

Protect the end of the chromosome from being mistaken by the cell as a broken DNA molecule in need of repair.

Centromere

Allows one copy of each duplicated chromosome to be *apportioned* to each daughter cell.

Interphase chromosomes are organized within the nucleus

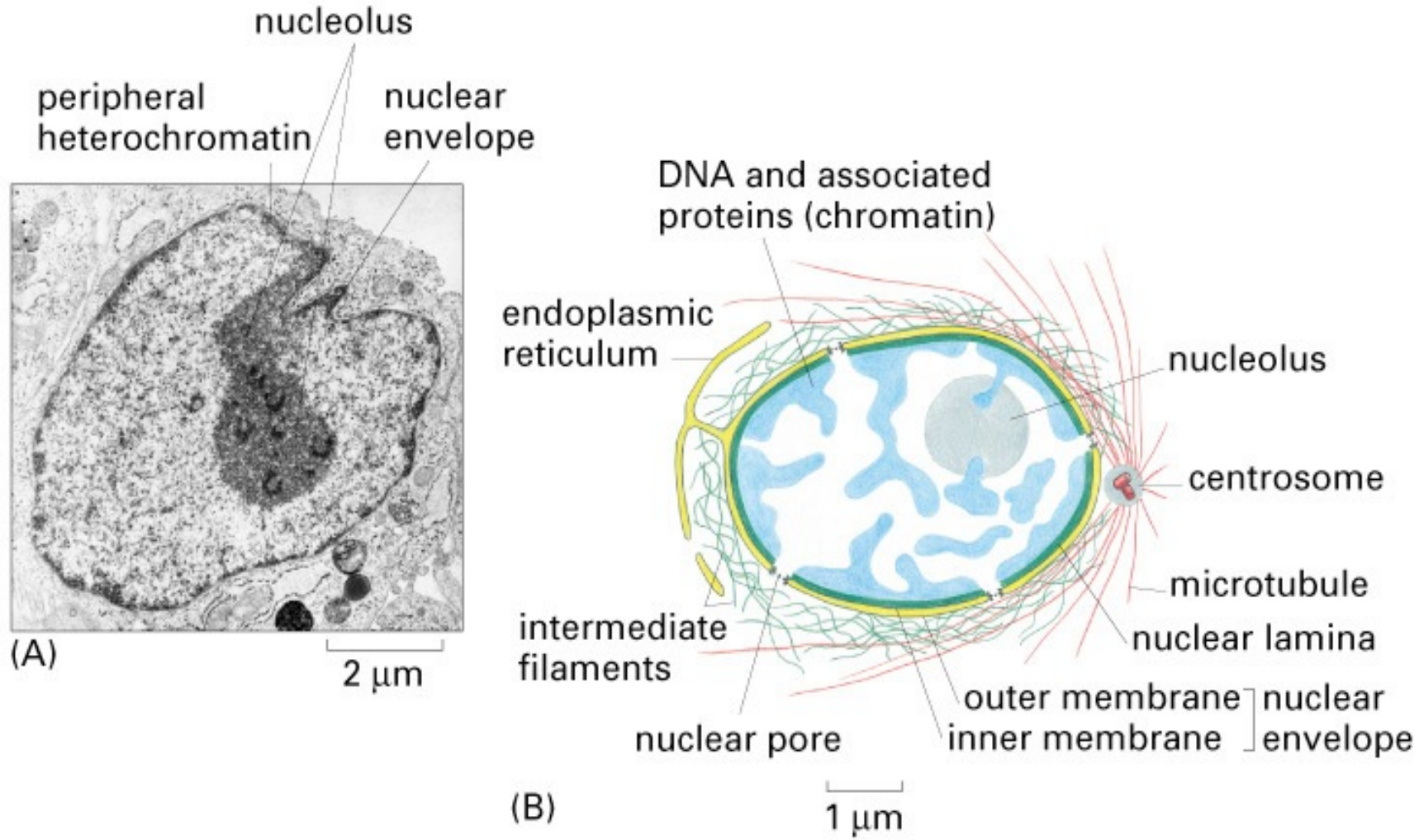


Figure 5-19 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Nuclear envelop: formed by *two concentric membranes; punctuated* at intervals by nuclear pores.

Nuclear pores: *actively transport* selected molecules to and from the cytosol.

Nuclear lamina: *a network of protein filaments* that forms a thin layer underlying and the inner membrane.

Nucleolus

The most obvious example of chromosome organization in the interphase nucleus.

Parts of the different chromosomes carrying genes for *ribosomal RNA* cluster together.

"...Not being mixed with each other like spaghetti in a bowl..."

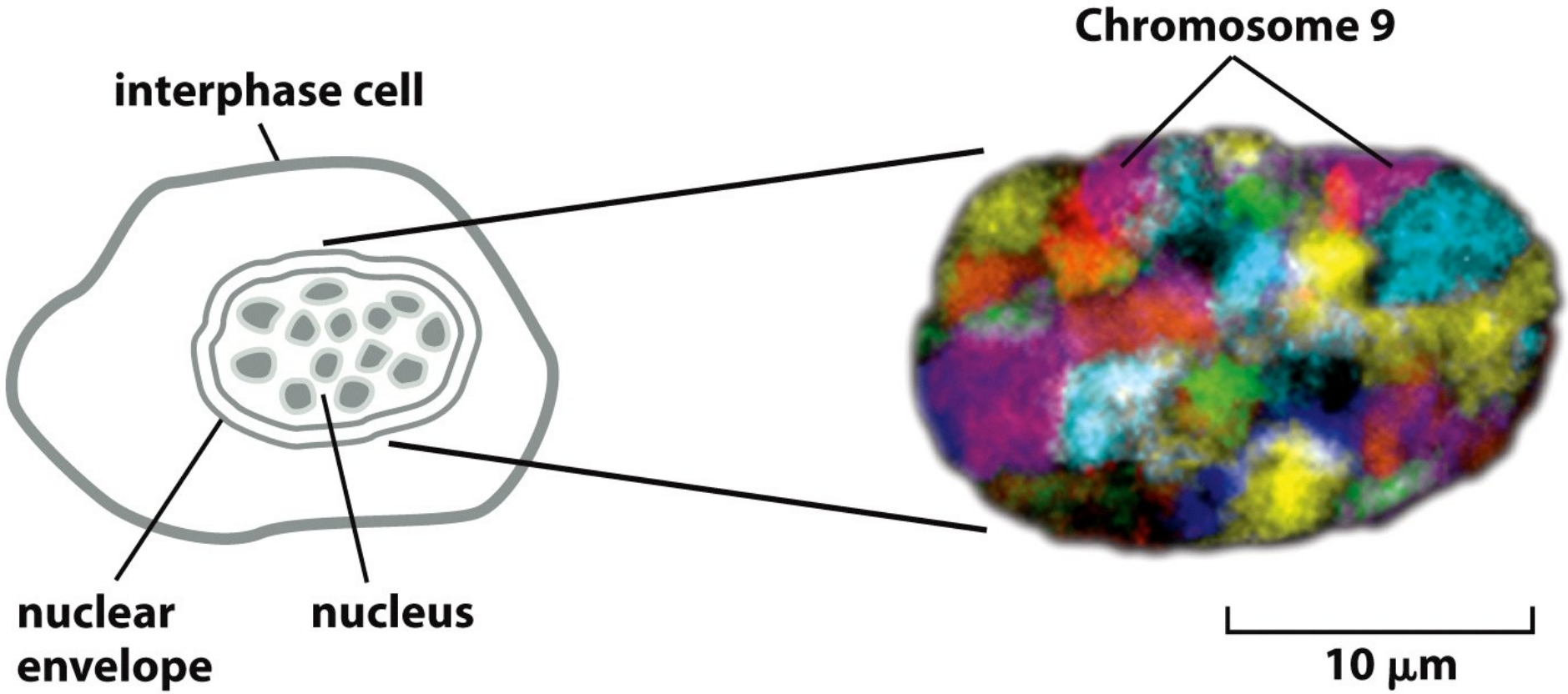


Figure 5-18 Essential Cell Biology 3/e (© Garland Science 2010)

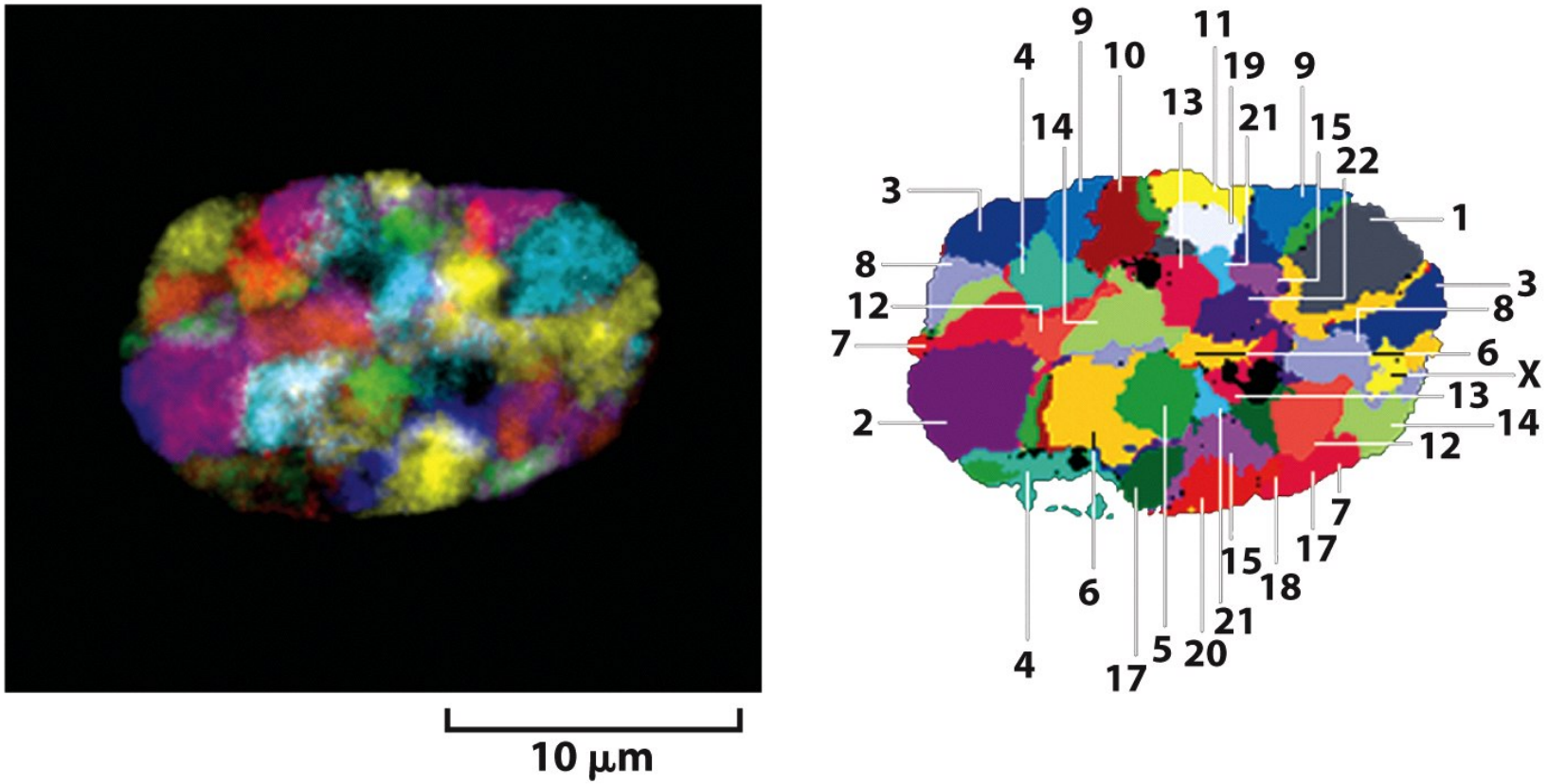


Figure 4–63 Simultaneous visualization of the chromosome territories for all of the human chromosomes in a single interphase nucleus. A FISH analysis using a different mixture of fluorochromes for marking the DNA of each chromosome, detected with seven color channels in a fluoresence microscope, allows each chromosome to be distinguished in three-dimensional reconstructions. Below the micrograph, each chromosome is identified in a schematic of the actual image. Note that the two homologous chromosomes (e.g., the two copies of chromosome 9), are not in general co-located. (From M.R. Speicher and N.P. Carter, *Nat. Rev. Genet.* 6:782–792, 2005. With permission from Macmillan Publishers Ltd.)

Nuclear envelop: formed by *two concentric membranes; punctuated* at intervals by nuclear pores.

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Nuclear lamina: *a network of protein filaments* that forms a thin layer underlying and the inner membrane.

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The most obvious example of chromosome organization in the interphase nucleus.

Parts of the different chromosomes carrying genes for *ribosomal RNA* cluster together.

Chromatin Can Move to Specific Sites Within the Nucleus to Alter Gene Expression

The heterochromatic regions of a chromosome are often closely associated with *the nuclear lamina*, regardless of the chromosome examined.

Different average positions for *active and inactive genes*.

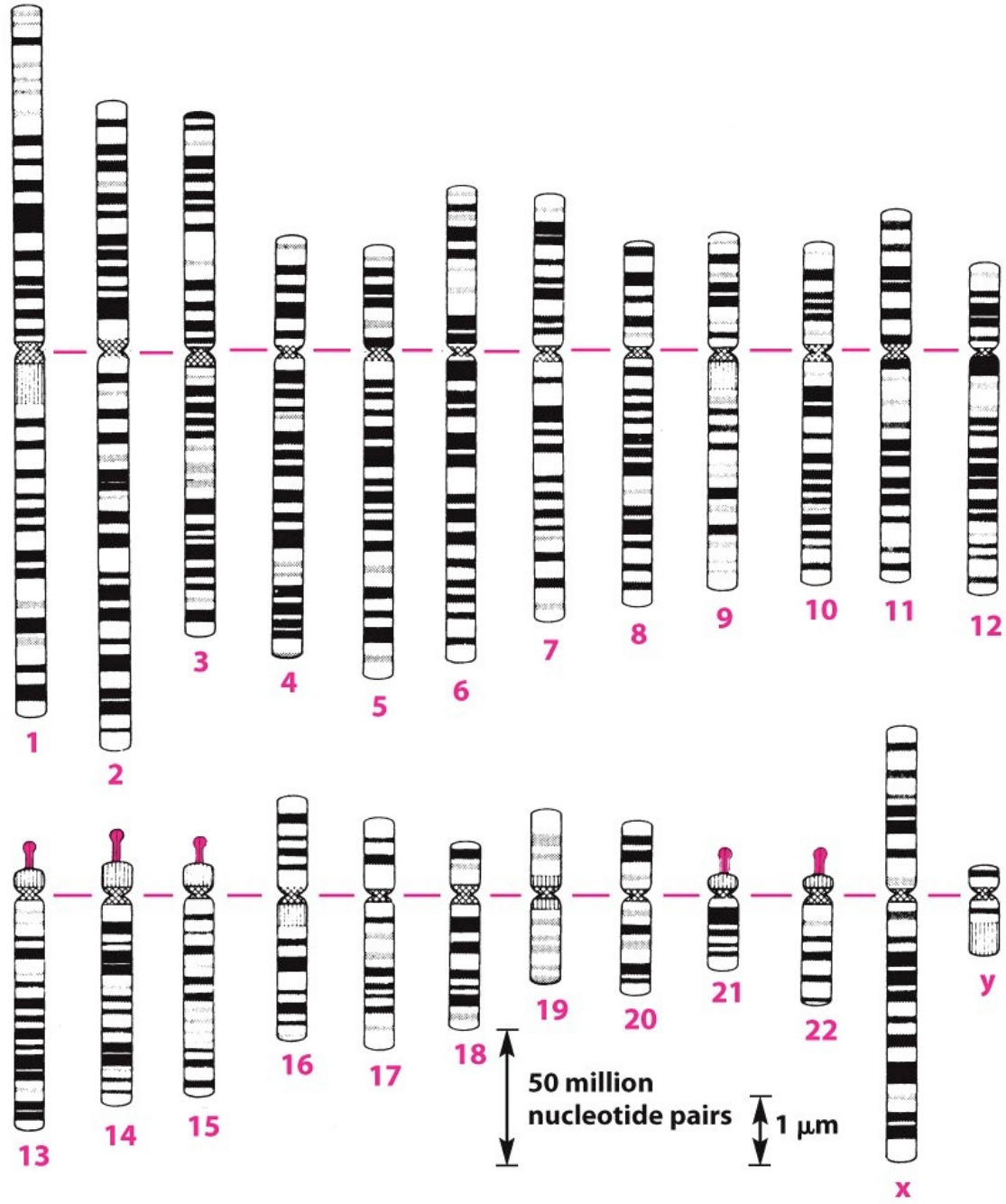

Figure 5-11 Essential Cell Biology 3/e (© Garland Science 2010)

Unique banding patterns allow the identification of each human chromosome

- To stain the chromosomes with dyes that bind to certain types of DNA sequences.
- These dyes mainly distinguish between DNA that is rich in A-T nucleotide pairs and DNA that is G-C rich, and they produce a striking and reliable pattern of bands along each chromosome.
- *Giemsa stain*: produce dark bands in AT rich region.
- Giemsa stain is named after German chemist and bacteriologist Gustav Giemsa.
- Giemsa's solution is a mixture of methylene blue, eosin, and Azure B.

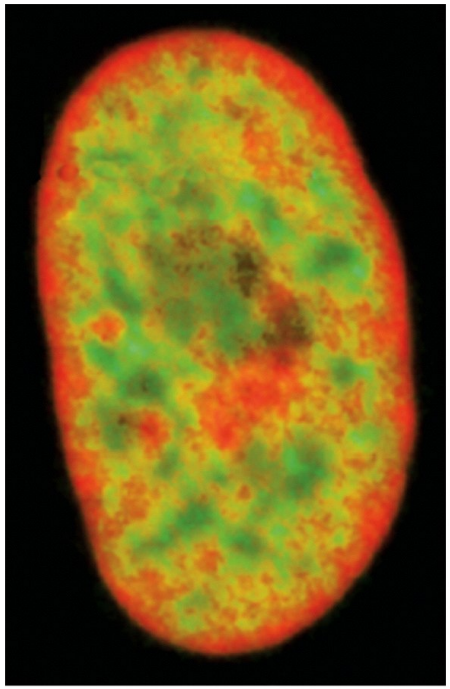

Figure 4–64 The distribution of gene-rich regions of the human genome in an interphase nucleus. Gene-rich regions have been visualized with a fluorescent probe that hybridizes to the Alu interspersed repeat, which is present in more than a million copies in the human genome (see Figure 5–75). For unknown reasons, these sequences cluster in chromosomal regions rich in genes. In this representation, regions enriched for the Alu sequence are *green,* regions depleted for these sequences are *red,* while the average regions are *yellow.* The gene-rich regions are seen to be depleted in the DNA near the nuclear envelope. (From A. Bolzer et al., *PLoS Biol.* 3:826–842, 2005. With permission from Public Library of Science.)

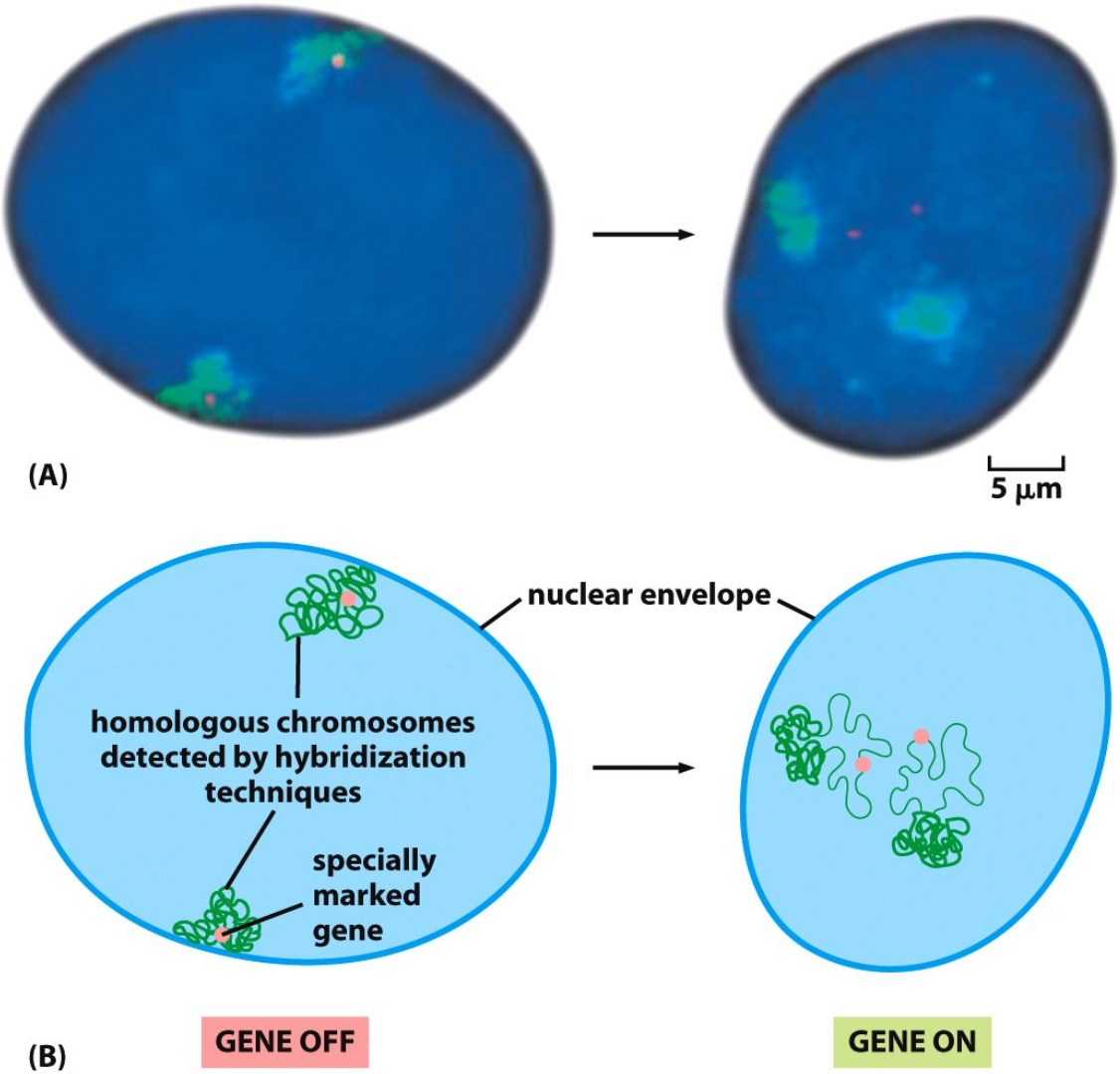

Figure 4–65 An effect of high levels of gene expression on the intranuclear location of chromatin. (A) Fluorescence micrographs of human nuclei showing how the position of a gene changes when it becomes highly transcribed. The region of the chromosome adjacent to the gene *(red)* is seen to leave its chromosomal territory *(green)* only when it is highly active. (B) Schematic representation of a large loop of chromatin that expands when the gene is on, and contracts when the gene is off. Other genes that are less actively expressed can be shown by the same methods to remain inside their chromosomal territory when transcribed. (From J.R. Chubb and W.A. Bickmore, *Cell* 112:403–406, 2003. With permission from Elsevier.)

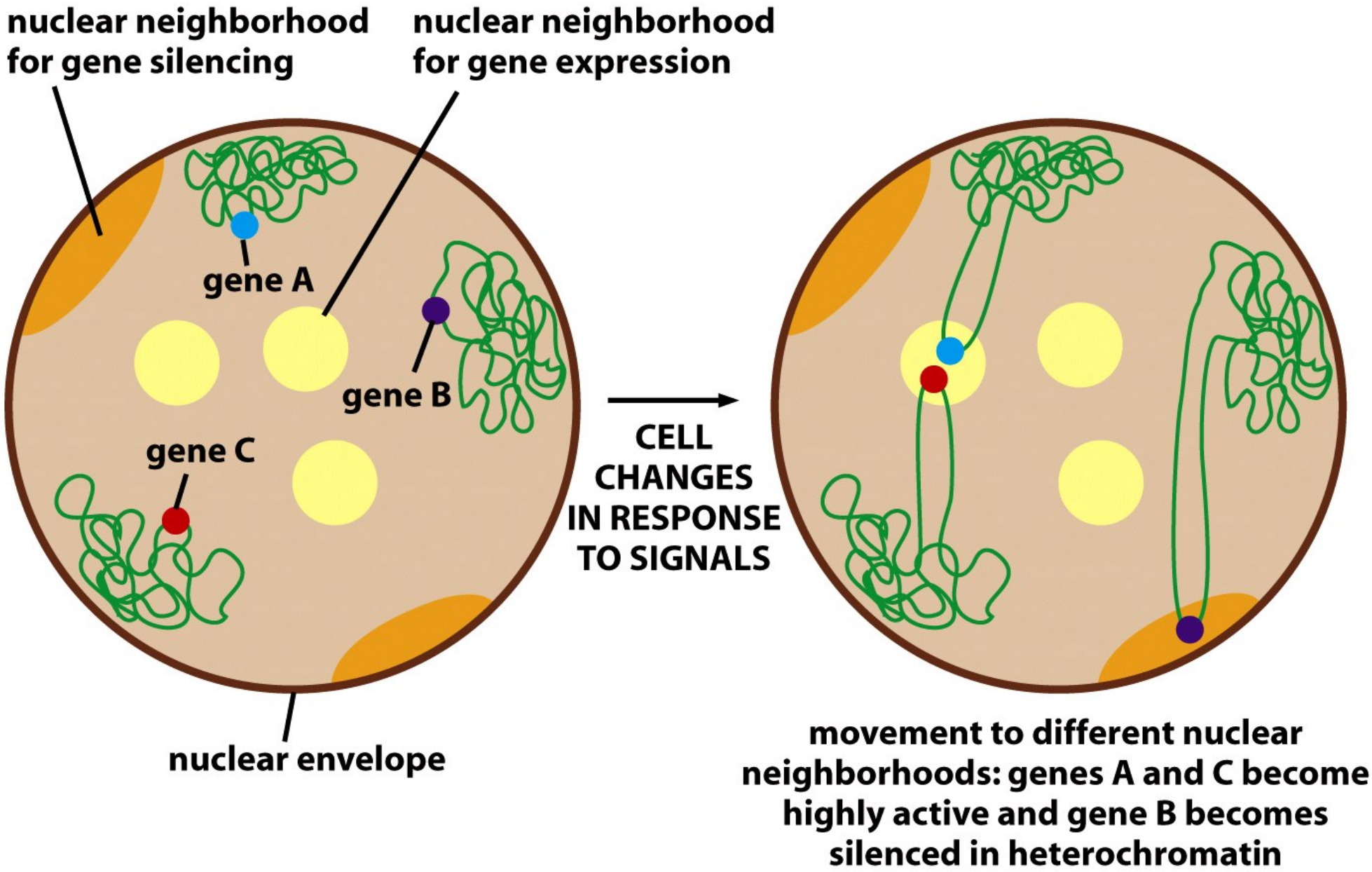

Figure 4-66 *Molecular Biology of the Cell* (© Garland Science 2008)

The position of a gene in the interior of the nucleus *changes* when it becomes highly expressed.

Thus, *a region* that becomes very *actively transcribed* is often found to extend out of its chromosome territory, as if *in an extended loop*.

The initiation of transcription, the first step in gene expression, requires the assembly of *over 100 proteins*, and it makes sense that this would *occur most rapidly in regions* of the nucleus particularly rich in these proteins.

The DNA in chromosomes is *highly condensed*

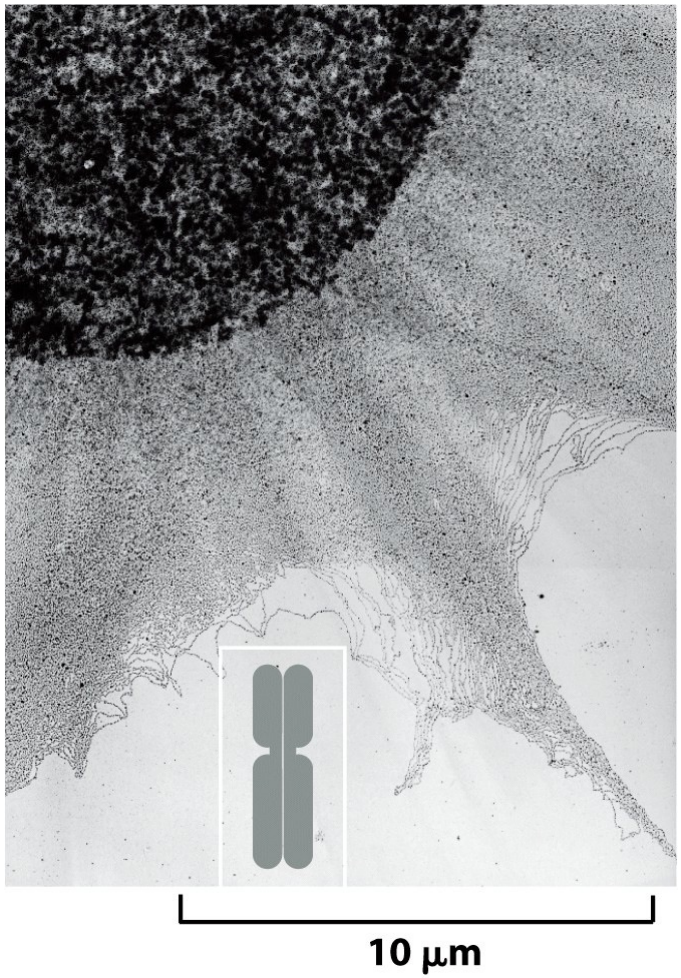

Figure 5-20 Essential Cell Biology 3/e (© Garland Science 2010)

- *The interphase chromosomes*

  Longer and *finer* than mitotic chromosomes

  Nonetheless *organized* in various ways.

    tends to *occupy a particular region.*

    do *not* become extensively *entangled.*

    specific regions of chromosomes are *attached to sites* *on* the nuclear envelop or the nuclear lamina.

All eucaryotic cells package *DNA tightly into chromosomes.*

Chromosome structure is *dynamic*.

Not only do chromosomes *condense and relax* in concert with the cell cycle,

but different *regions* of the interphase chromosome must *unpack* to allow cells to access specific DNA sequence for replication, repair, or gene expression.

Chromosomes packing occurs on *multiple levels*

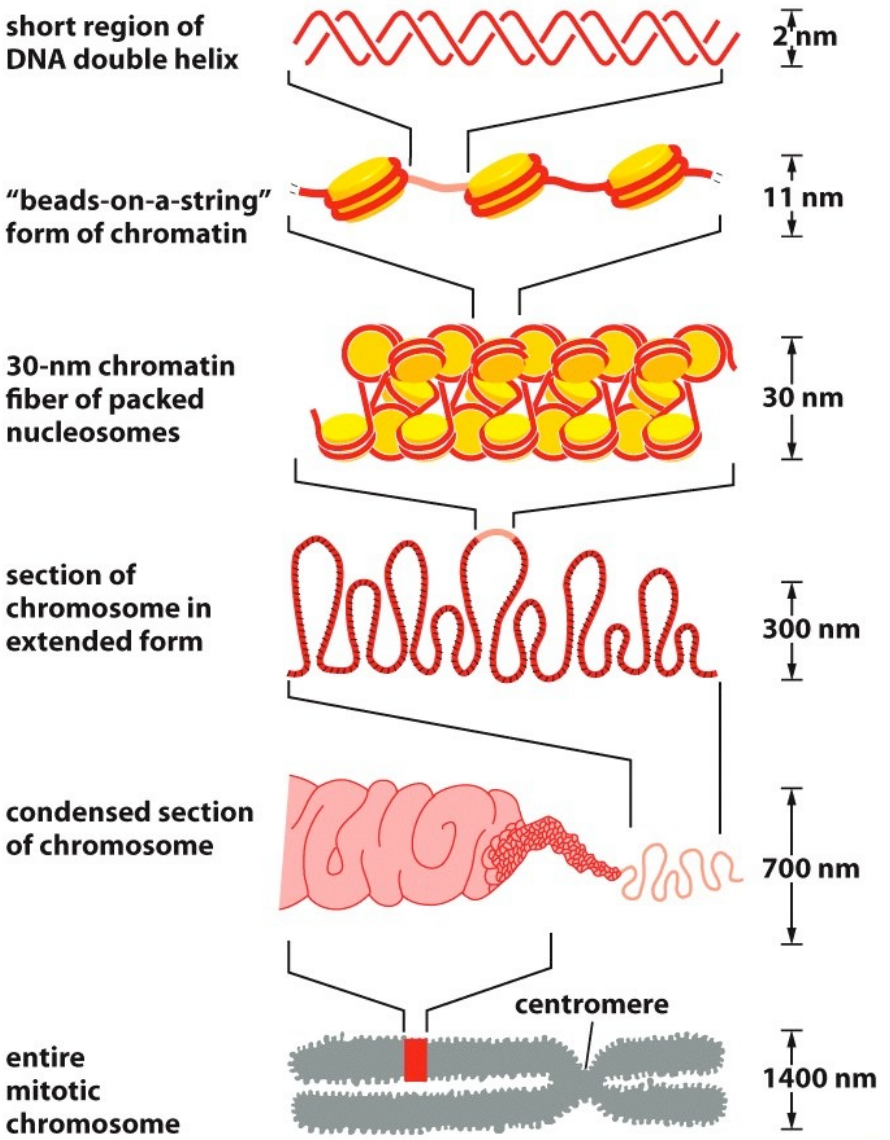

Figure 5-25 Essential Cell Biology 3/e (© Garland Science 2010)

Controlling the double helix

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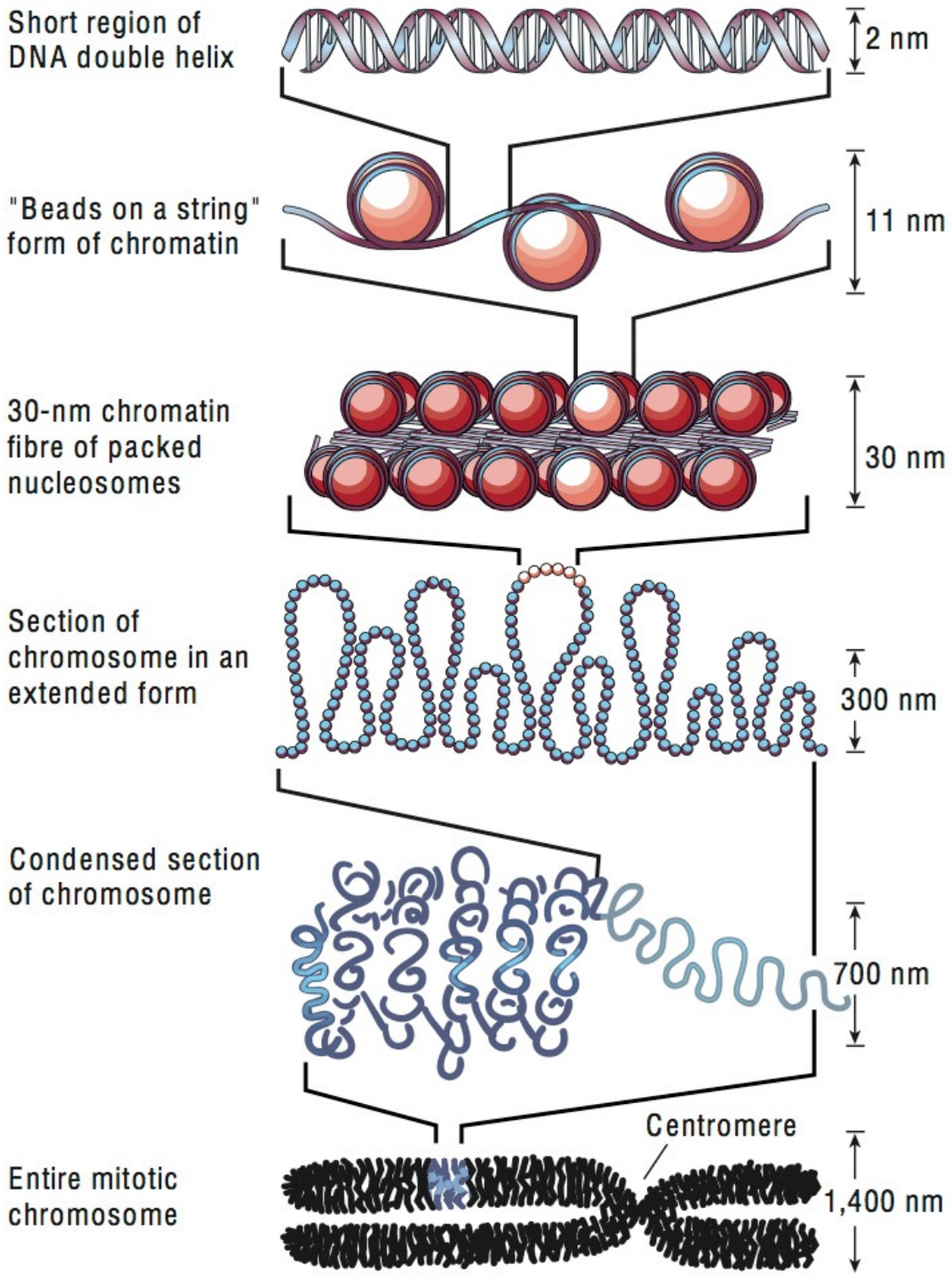


DNA double helix

→ "*beads-on-a-string*" form of chromatin
→ *30-nm chromatin fiber*
→ Chromosome *in extended form*
→ *Condensed* section of chromosome
→ Entire mitotic chromosome

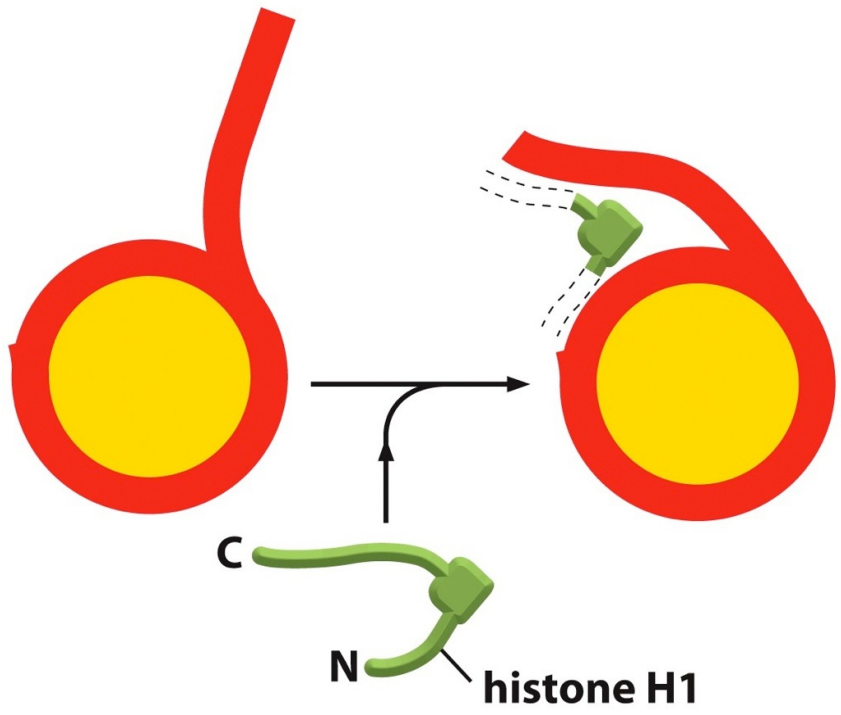


Figure 5-24 Essential Cell Biology 3/e (© Garland Science 2010)

Histone H1

Histone H1 is one of the five **main histone protein families** which are components of chromatin in eukaryotic cells. Though **highly conserved**, it is nevertheless **the most variable histone** in sequence across species.

H1 is involved with **the packing** of the "beads on a string" sub-structures **into a high order structure**, whose details have not yet been solved

https://en.wikipedia.org/wiki/Histone_H1

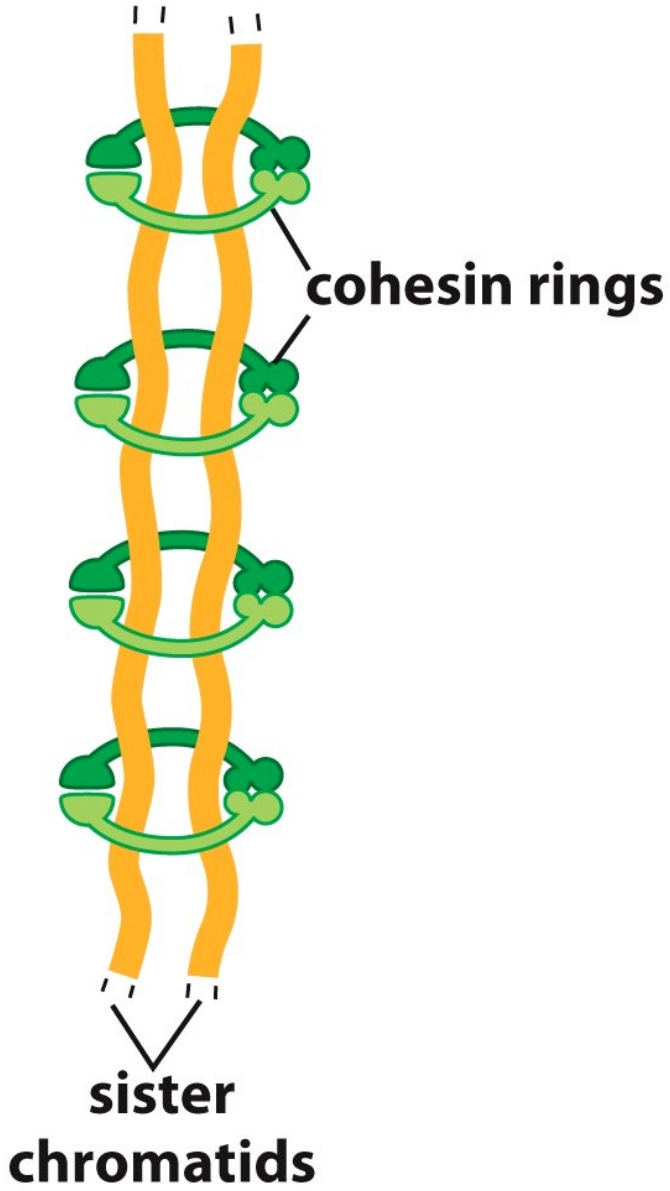


Figure 18-15 Essential Cell Biology 3/e (© Garland Science 2010)

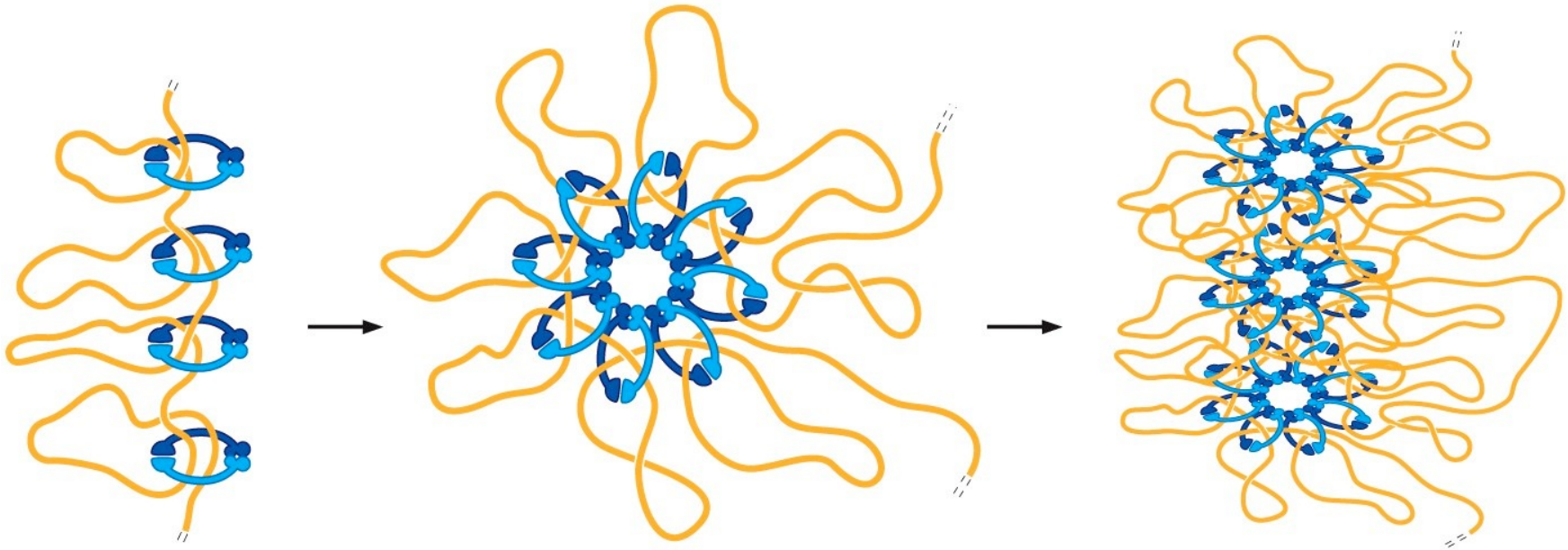


Figure 18-19a Essential Cell Biology 3/e (© Garland Science 2010)

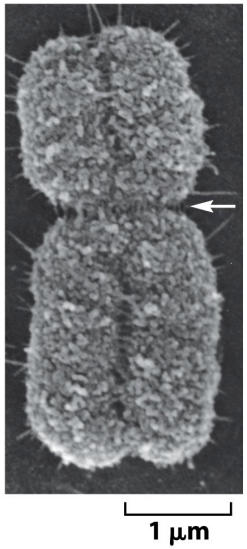
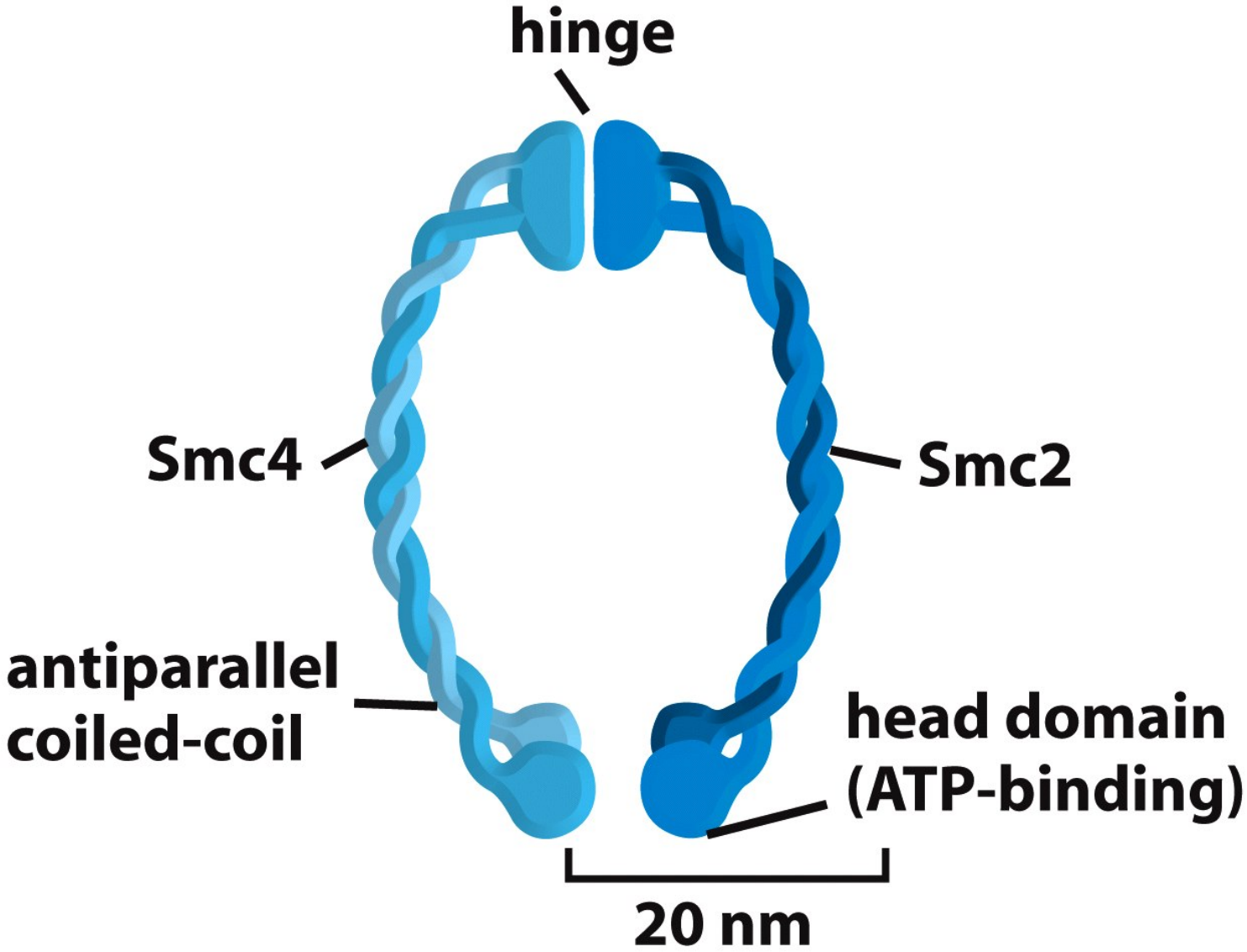


Figure 18-19b Essential Cell Biology 3/e (© Garland Science 2010

‘Condensins help to **coil** the mitotic chromatids into, smaller, **more compact** structures that can be more easily segregated during mitosis.’



The structure of a SMC dimer. The long central region of this protein is an antiparallel coiled-coil with a flexible hinge in its middle.

Figure 4-73b *Molecular Biology of the Cell* (© Garland Science 2008)

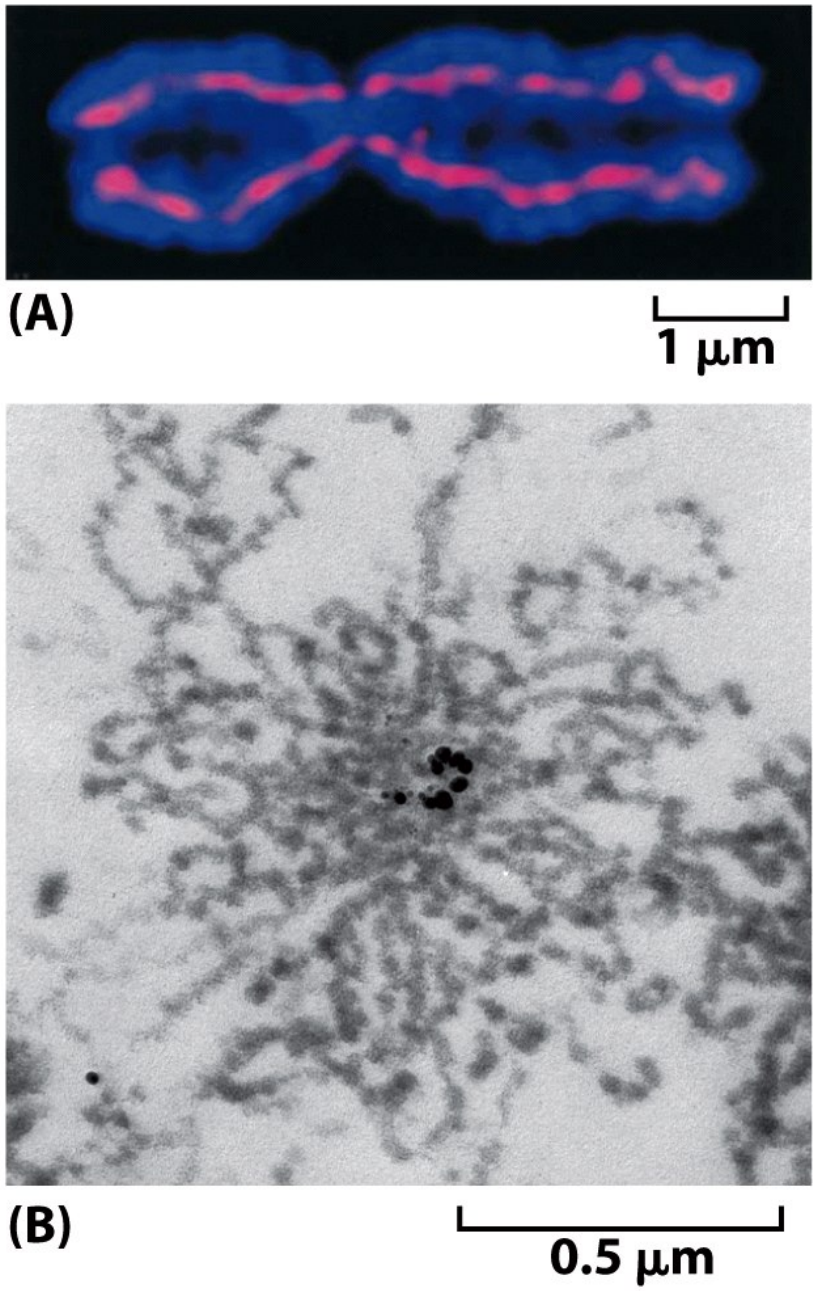


Figure 4–74 The location of condensin in condensed mitotic chromosomes. (A) Fluorescence micrograph of a human chromosome at mitosis, stained with an antibody that localizes condensin. In chromosomes that are this highly condensed, the condensin is seen to be concentrated in punctate structures along the chromosome axis. Similar experiments show a similar location for DNA topoisomerase II, an enzyme that makes reversible double-strand breaks in DNA that allow one DNA double helix to pass through another (see Figure 5–23). (B) Immunogold electron microscopy reveals localization of condensin (*black* dots). Here a chromatid is seen in cross section, with the chromosome axis perpendicular to the plane of the paper. (A, from K. Maeshima and U.K. Laemmli, *Dev. Cell* 4:467–480, 2003. With permission from Elsevier. B, courtesy of U.K. Laemmli, from K. Maeshima, M. Eltsov and U.K. Laemmli, *Chromosoma* 114:365–375, 2005. With permission from Springer.)

Chromatin in the living cell *rarely adopts* the extended beads-on-a-sting form. Instead, the nucleosomes are *further packed* to generate a more compact structure, the *30-nm fiber*.

1) Histone H1

- *5th histone; Linker histone*.

  Pull the nucleosomes together into a regular repeating array into 30-nm fiber.

Mitotic chromosome

30-nm fiber is *folded* into a series of *loops*.

Further condensed to produce the *interphase chromosome*.

Undergo *at least one more level of packing* for the *mitotic chromosome*.

2) Condensin

Nucleosomes are the basic units of chromatin structure

(A) Chromatin from an interphase nucleus as a thread 30 nm thick
(B) Chromatin experimentally *unpacked* to show the nucleosomes.

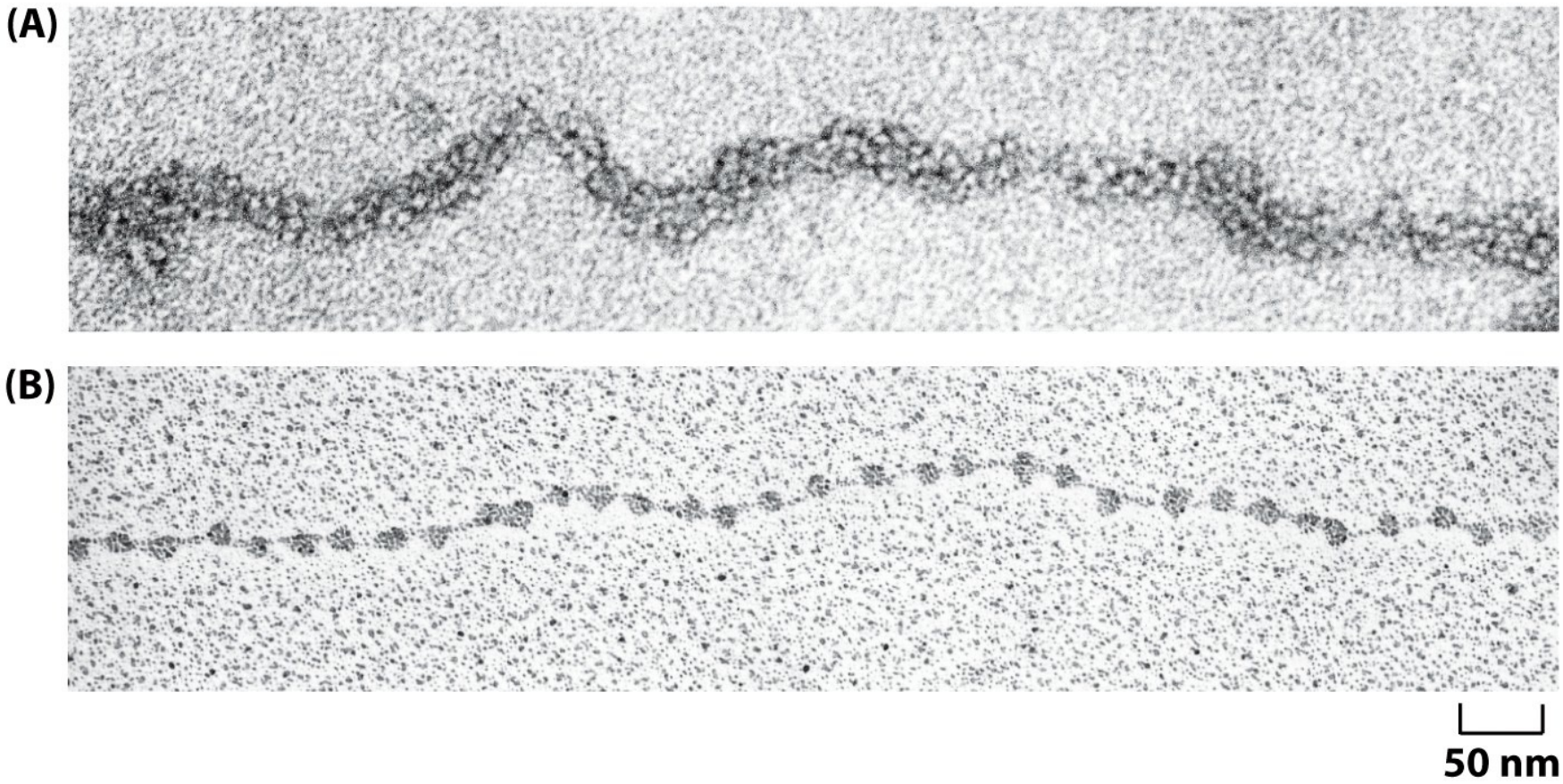


Figure 5-21 Essential Cell Biology 3/e (© Garland Science 2010)

The *proteins* that bind to the DNA

Histones

Nonhistone chromosomal proteins

- *Histones*

  Enormous quantities: 60 million molecules / cell.

  Equal mass to that of DNA itself.

Chromatin

The complex of both classes of *proteins + nuclear DNA.*

Exist in the form of *fiber* with a 30 nm diameter.

Nucleosome

Consists of *DNA wound around a core histones.*

"*beads on a string*"

Nucleosomes contain DNA + Histones

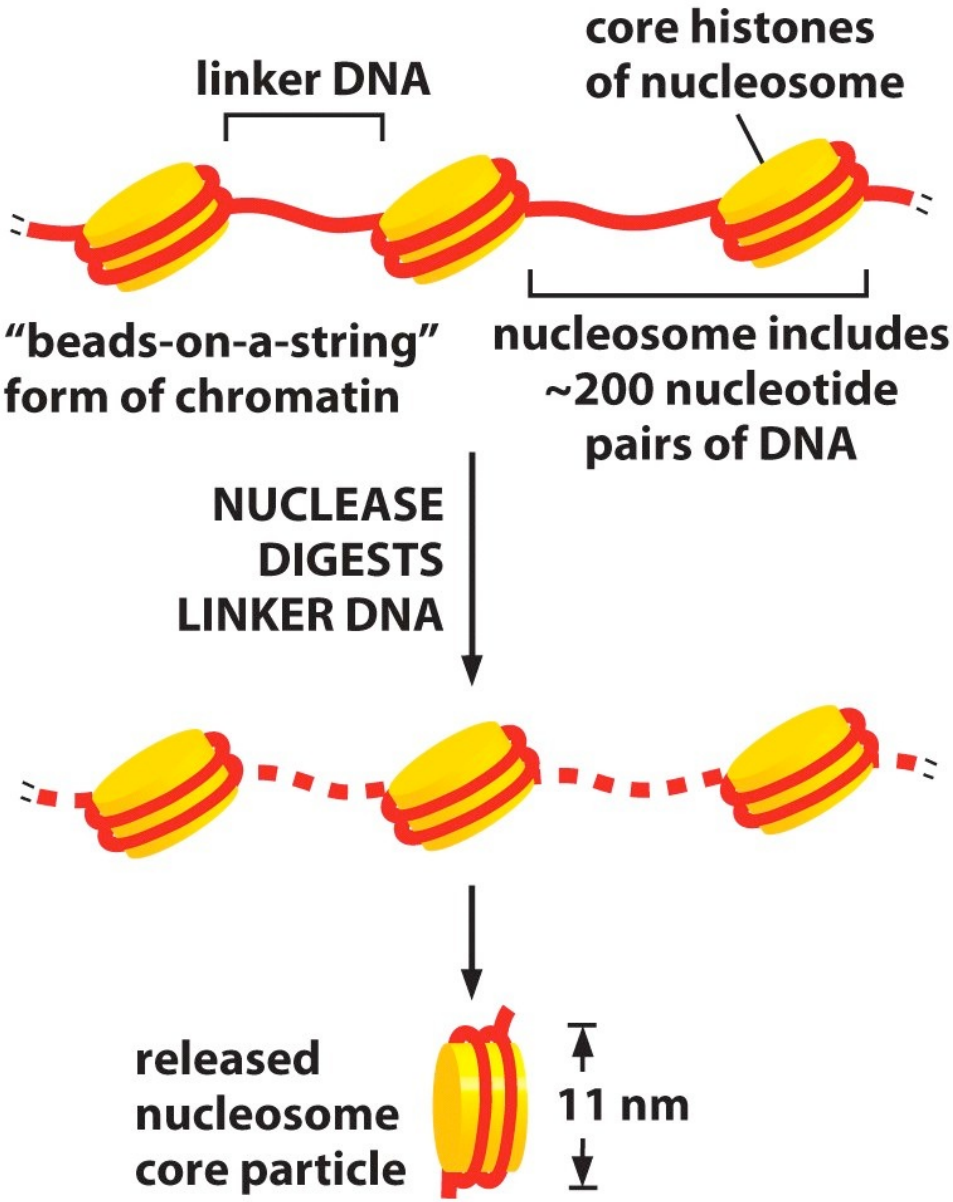


Figure 5-22 part 1 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

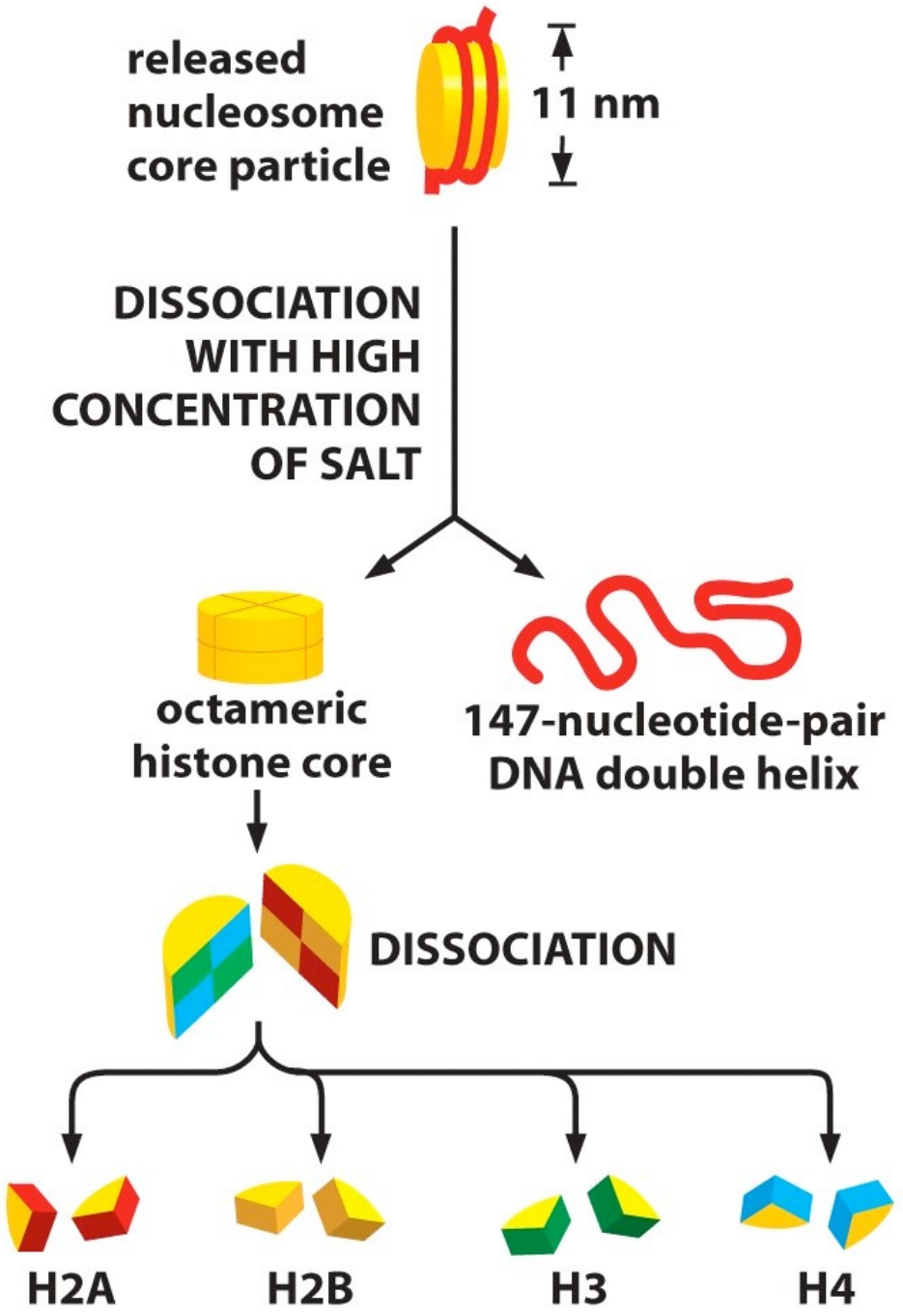


Figure 5-22 part 2 of 2 Essential Cell Biology 3/e (© Garland Science 2010)

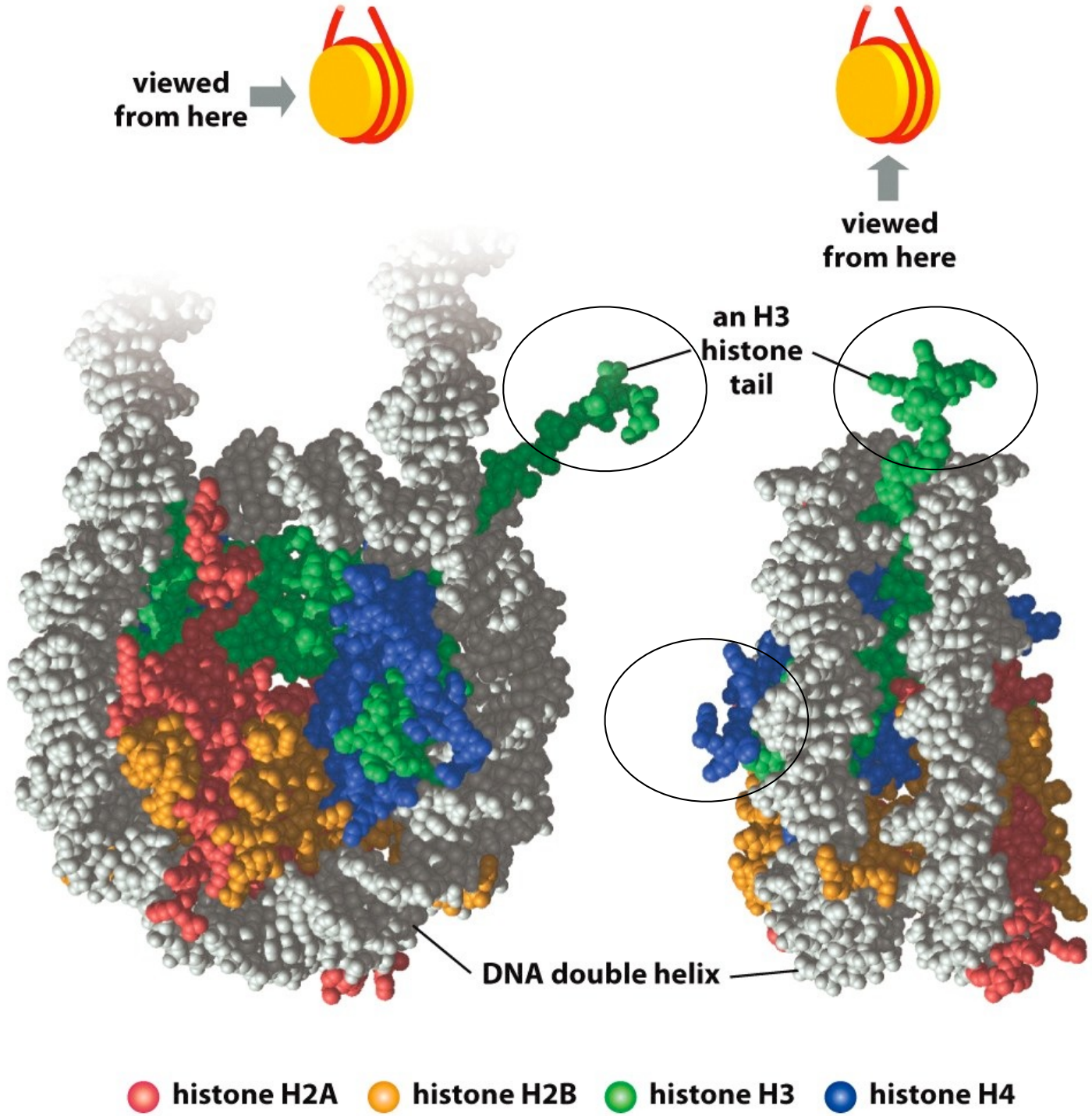


Figure 5-23 Essential Cell Biology 3/e (© Garland Science 2010)

The structure of *nucleosome*

Isolation of nucleosome : '*nuclease*'.

An individual 'nucleosome core particle'

Eight histone proteins (H2Ax2, H2Bx2, H3x2, and H4x2) and *double-stranded DNA*.

DNA is 147 nt pairs long, *winds* around the histone octamer.

High resolution structure of the nucleosome

Solved in *1997* in atomic detail.

Disc-shaped histone complex around which the DNA is *tightly wrapped,* making *1.7 turns in a left-handed coil.*

Linker DNA

DNA between nucleosomes : ~ *80 nt.*

Meaning...

Converts a DNA molecule into a chromatin thread ~ 1/3 of its initial length.

First level of DNA packing.

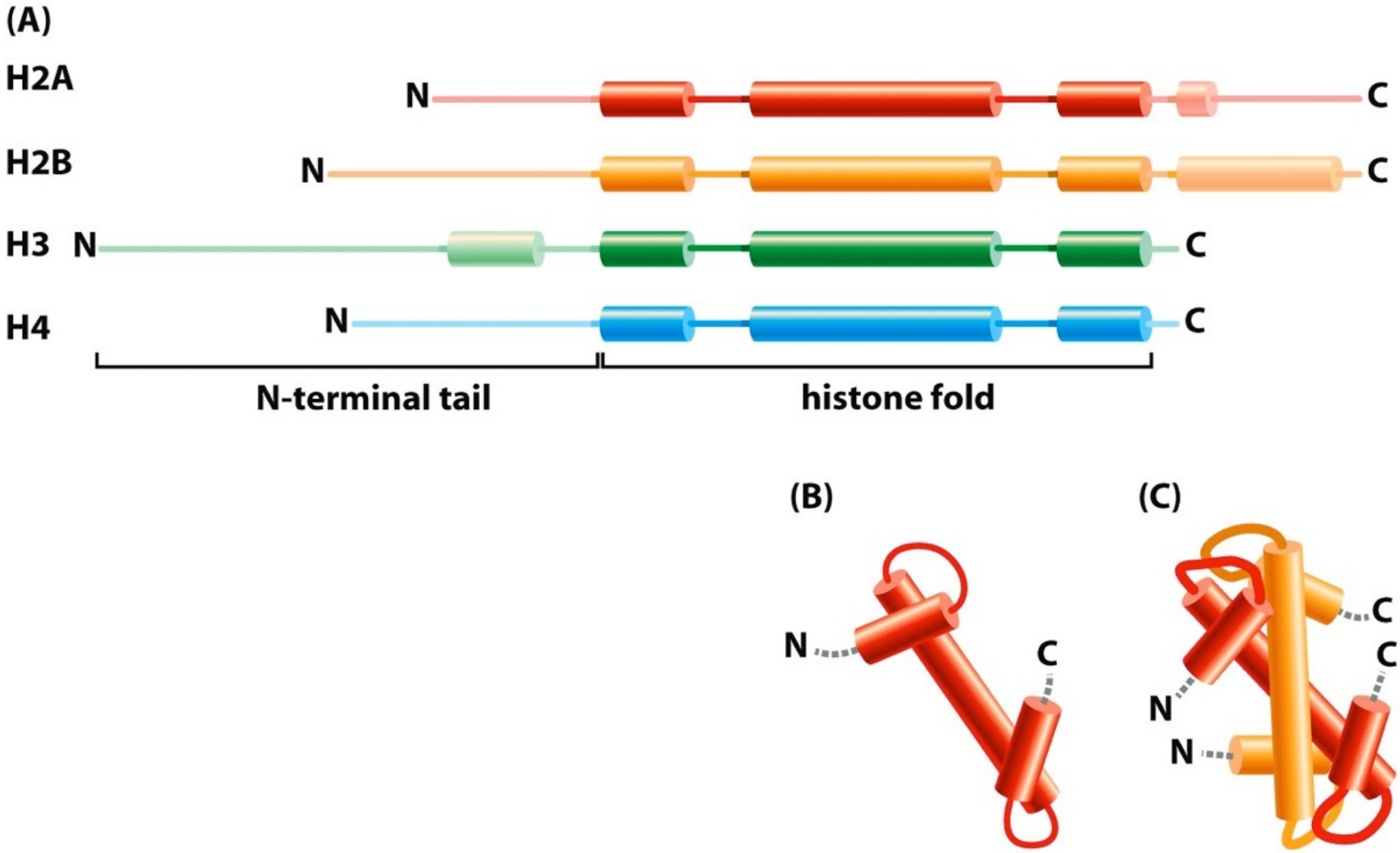


Figure 4-25 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Histones

Small proteins (H2A, H2B, H3, H4 and H1)

Contain *positively charged* amino acids (K or R).

Bind tightly to the negatively charged DNA.

Histone N-terminal tail

- Several types of *covalent modification (epigenetically inherited)*
- *Control* many aspects of *chromatin structure* and *gene expression* (acetylation; methylation; phosphorylation; ubiquitination).

Highly conserved of all known eukaryotic proteins.

- *Extreme evolutionary conservation* (e.g. peas and cows).
- Vital role in *controlling eucaryotic chromosome structure.*
- Also found in *archaea.*

The Regulation of Chromosome Structure

Changes in nucleosome structure allow access to DNA

Two ways to adjust the *local structure* of their chromatin *rapidly*

1. *Chromatin Remodeling Complexes*
2. *Chemical Modifications of the Histones*

Dynamic Nucleosome

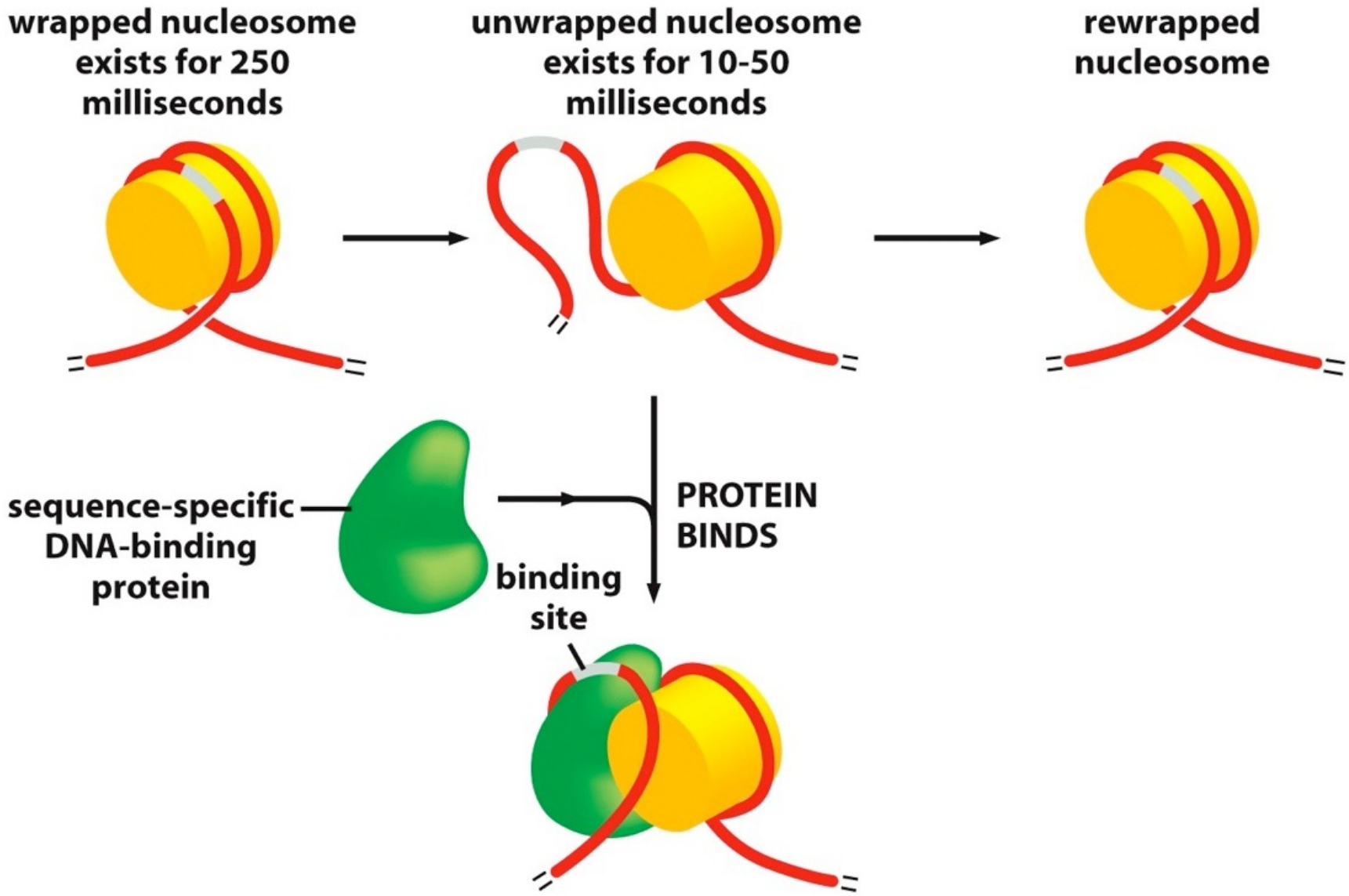


Figure 4-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

1. Chromatin Remodeling Complexes

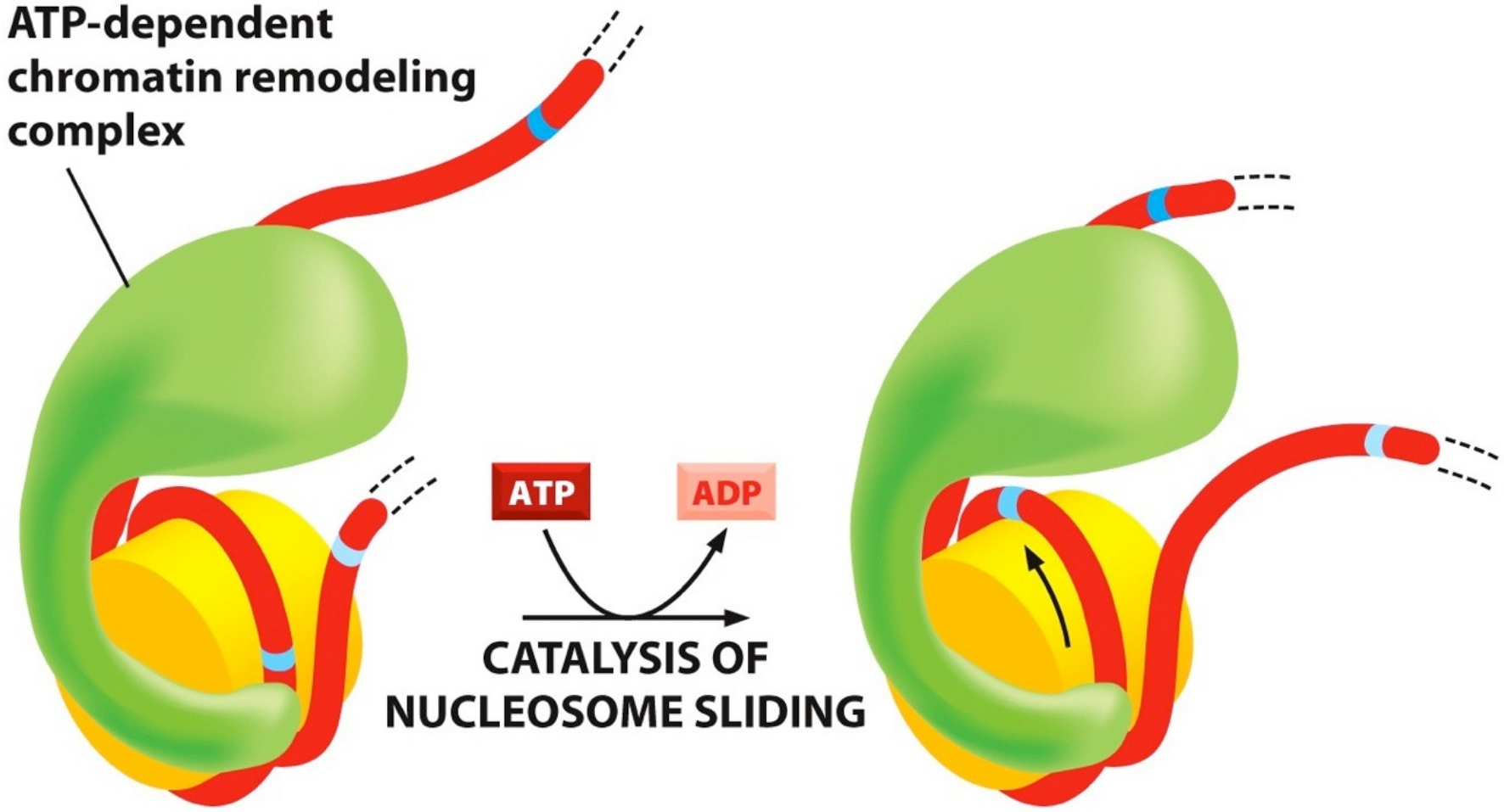


Figure 5-27a Essential Cell Biology 3/e (© Garland Science 2010)

adjustment; sliding; movement; altering

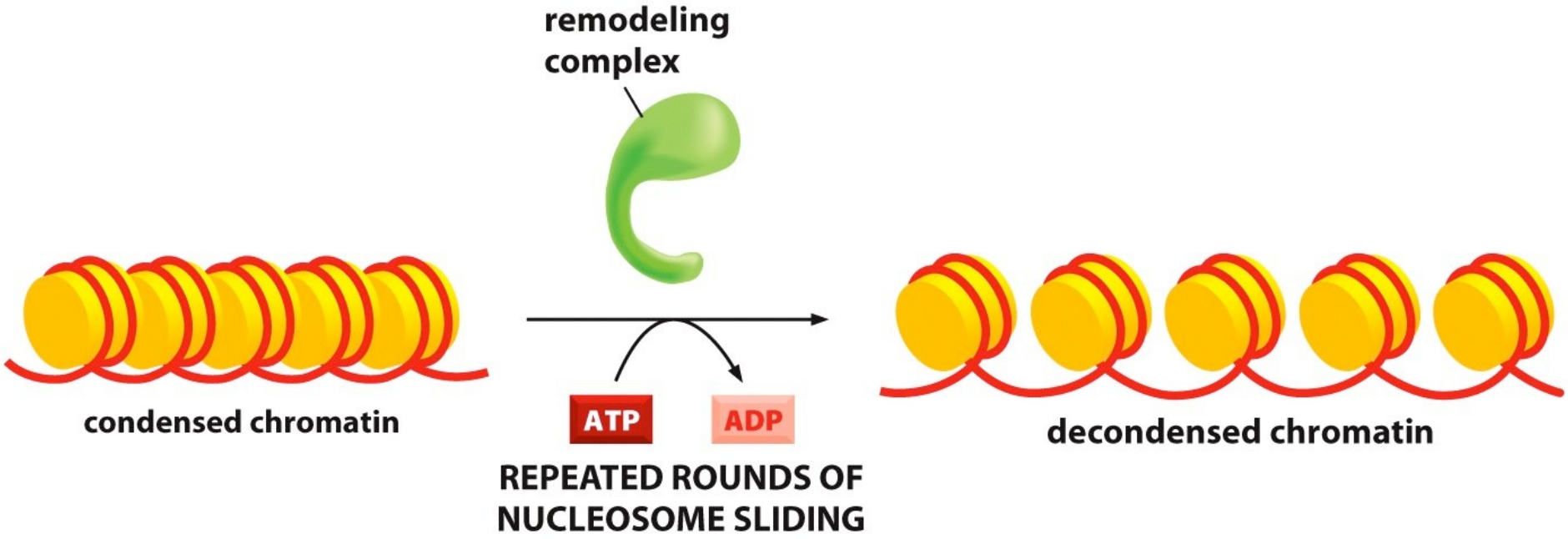


Figure 5-27b Essential Cell Biology 3/e (© Garland Science 2010)

Nucleosome removal and histone exchange

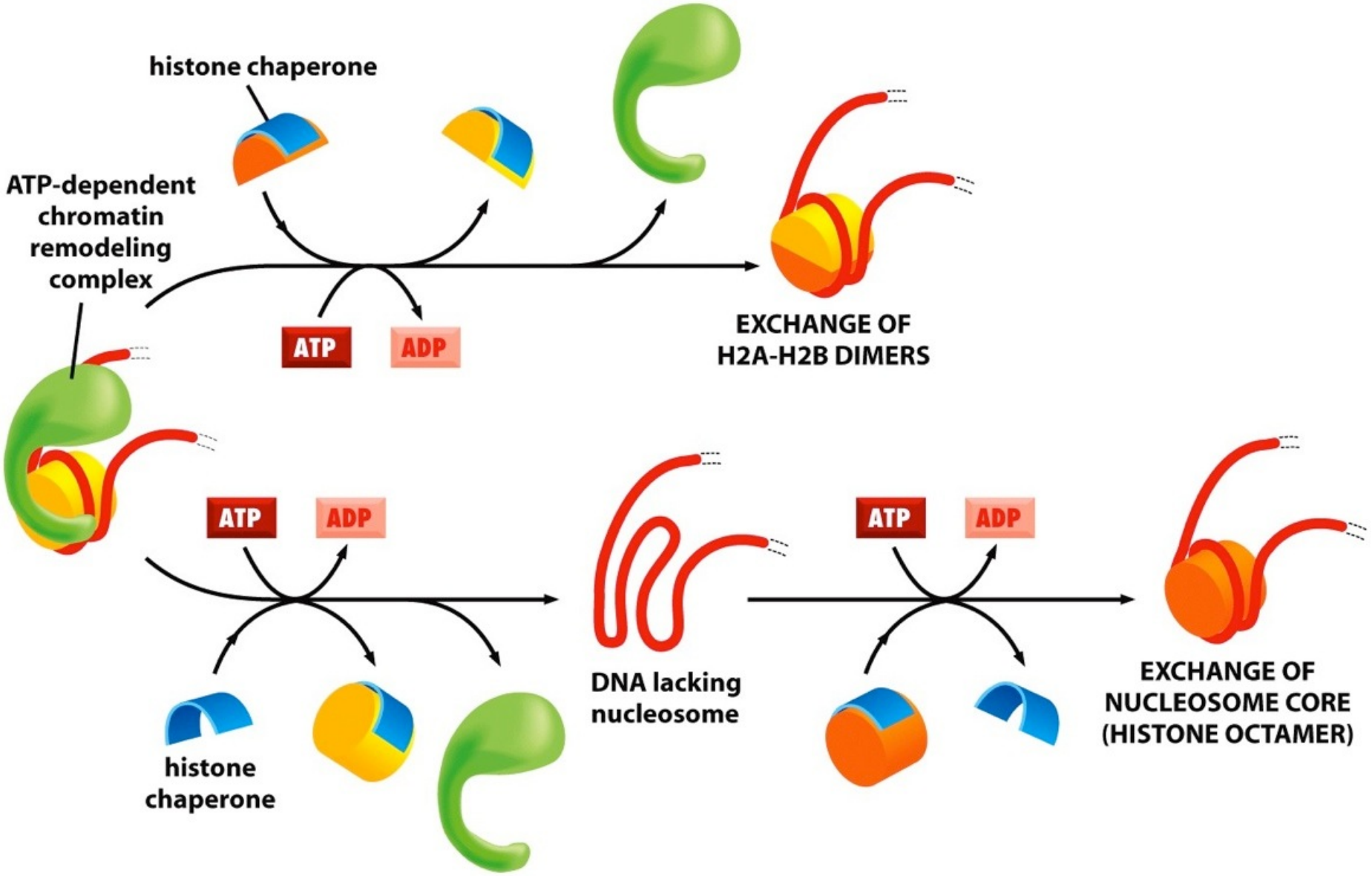


Figure 4-30 Molecular Biology of the Cell 5/e (© Garland Science 2008)

1. Chromatin Remodeling Complexes

Protein complex

Use the *ATP hydrolysis* to change the position of the DNA *(adjustment; sliding; movement; altering)*

Loosen the nucleosomal DNA by *pushing* it along the histone core.

Make *DNA* *more accessible* to other proteins.

Inactivated during mitosis.

Other types of *sliding* *can also condense* the chromatin in a particular region.

In addition, they catalyze *nucleosome removal* and *histone exchange*.

Alberts • Johnson • Lewis • Raff • Roberts • Walter

Molecular Biology of the Cell

Fifth Edition

Chapter 4

DNA, Chromosomes, and Genomes

2. Chemical Modifications of the Histones

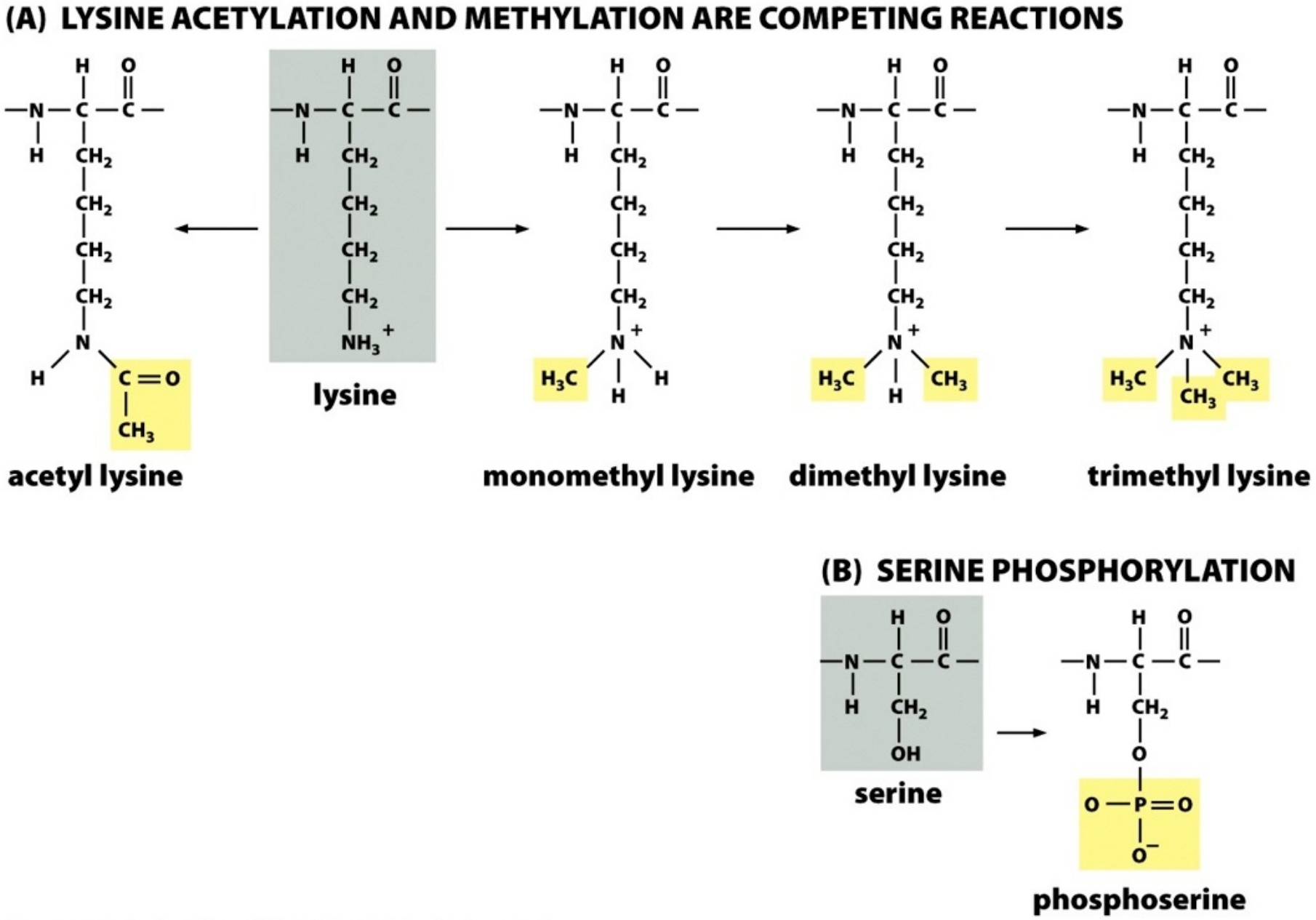


Figure 4-38 Molecular Biology of the Cell 5/e (© Garland Science 2008)

The covalent modification of core histone tails

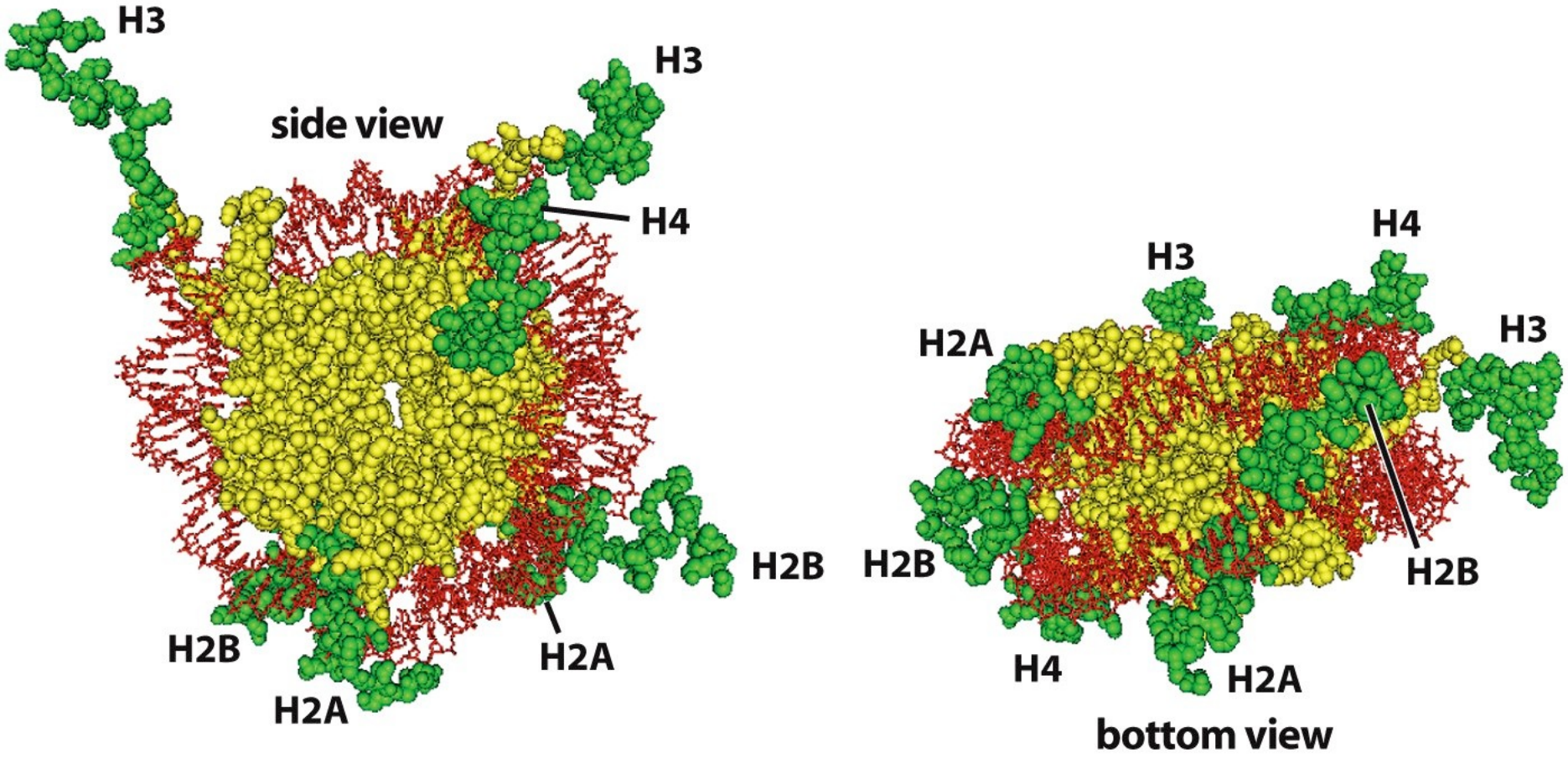


Figure 4-39a Molecular Biology of the Cell 5/e (© Garland Science 2008)

A map of histone modifications on the surface of the nucleosome core particle

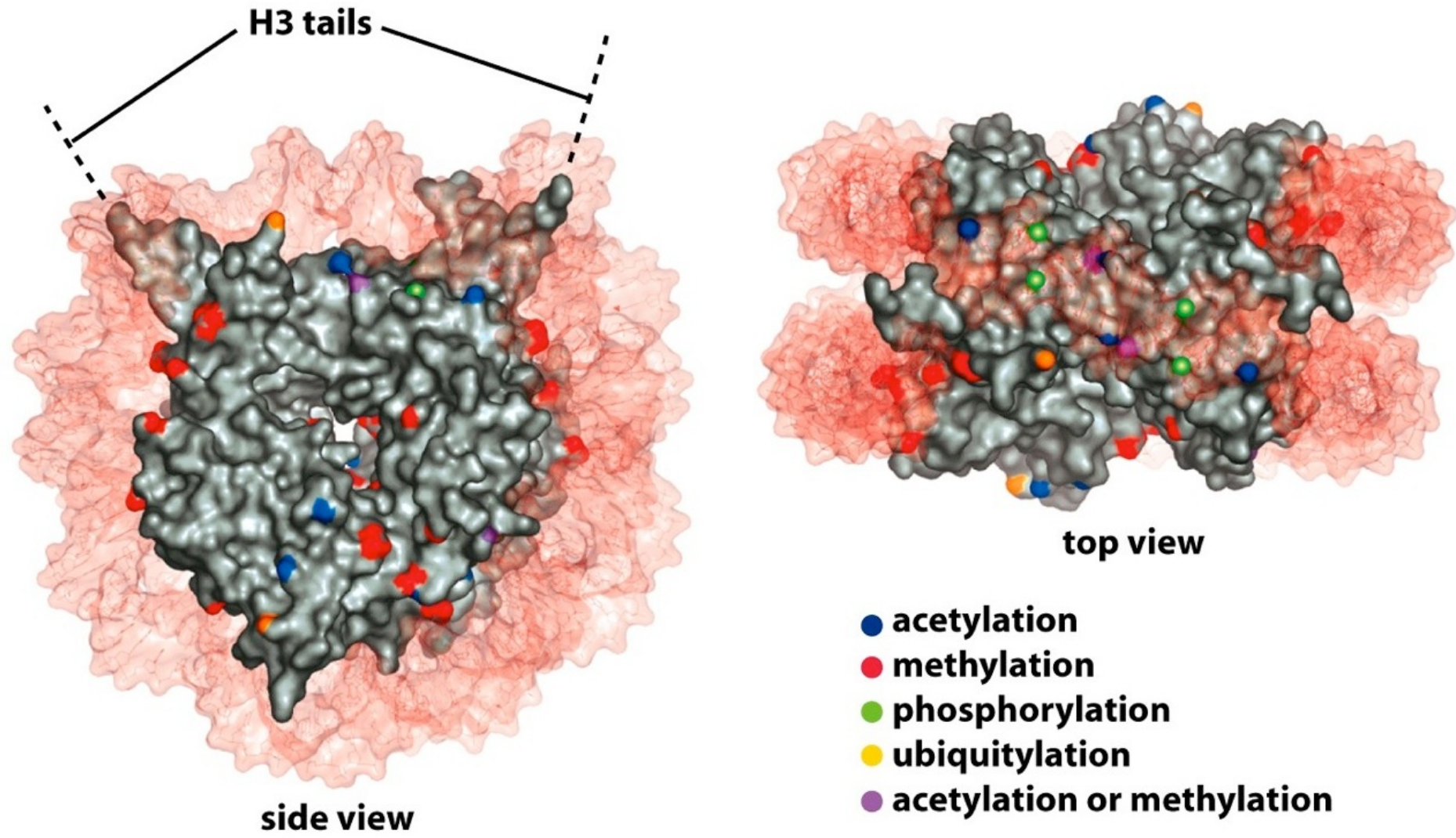


Figure 4-40 Molecular Biology of the Cell 5/e (© Garland Science 2008)

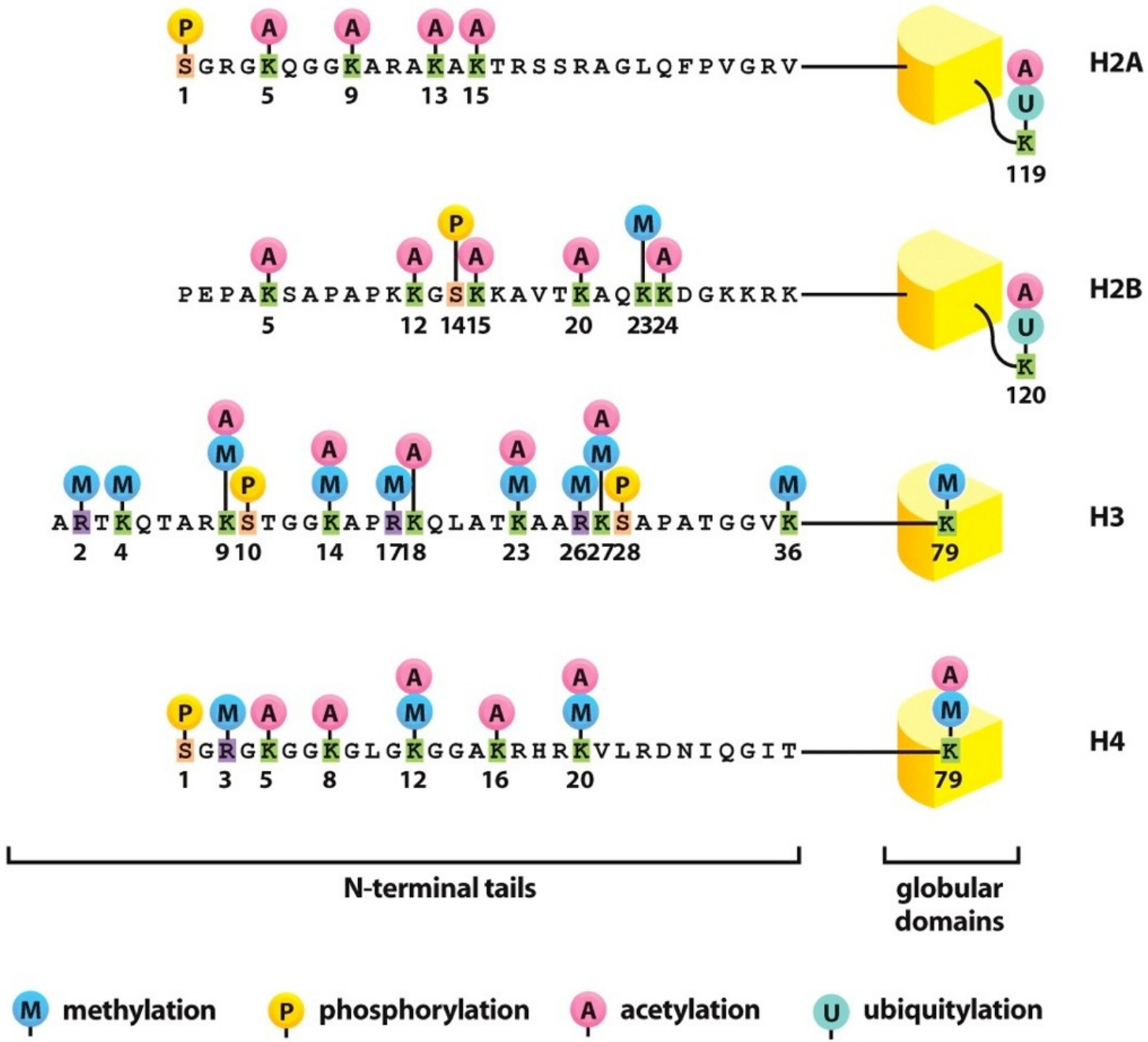


Figure 4-39b Molecular Biology of the Cell 5/e (© Garland Science 2008)

Some specific meanings of the histone code

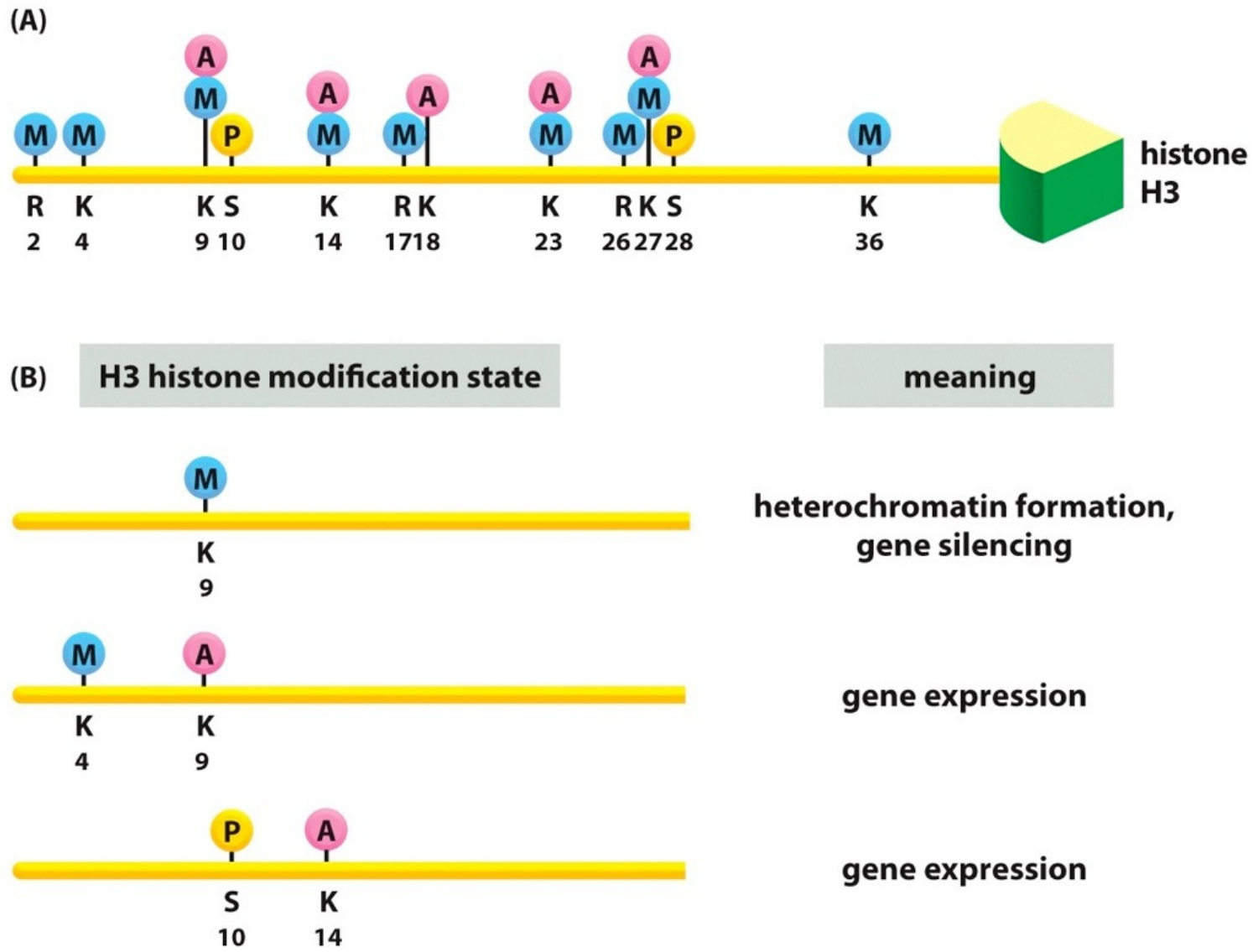


Figure 5-28 Essential Cell Biology 3/e (© Garland Science 2010)

Chemical Modifications of the Histones

Tails of all four of the core histones.

Covalent modifications (ac, ph, me, or ub).

Affect the ability of the *histone tails to bind specific proteins* and therefore *recruit* them to particular stretches of chromatin.

Different patterns attract *different proteins*.

Specific combinations have *different meanings* for the cell.

Further (de)condensation of chromatin.

The enzymes that modify histone tails are *tightly regulated*.

The histone-modifying enzymes **work in concert with (Team work)** the *chromatin-remodeling complexes* to (de)condense stretches of chromatin.

결론: *Rapidly change local chromatin structure* according to the needs of the cell.

Heterochromatin

How is the formation of heterochromatin *induced*?

A particular set of histone tail modifications (ex. H3K9me, Fig. 5-28B).

H3K9me attracts a set of *heterochromatin-specific proteins*.

These specific proteins then *induce the same H3K9me* in adjacent nucleosomes.

Conclusion

A spreading wave of *condensed chromatin*.

In this manner, *an extended region of heterochromatin* is established along the DNA.

Example #1

Position effect *Variegation*

(see Figure legend 5-29, p190)

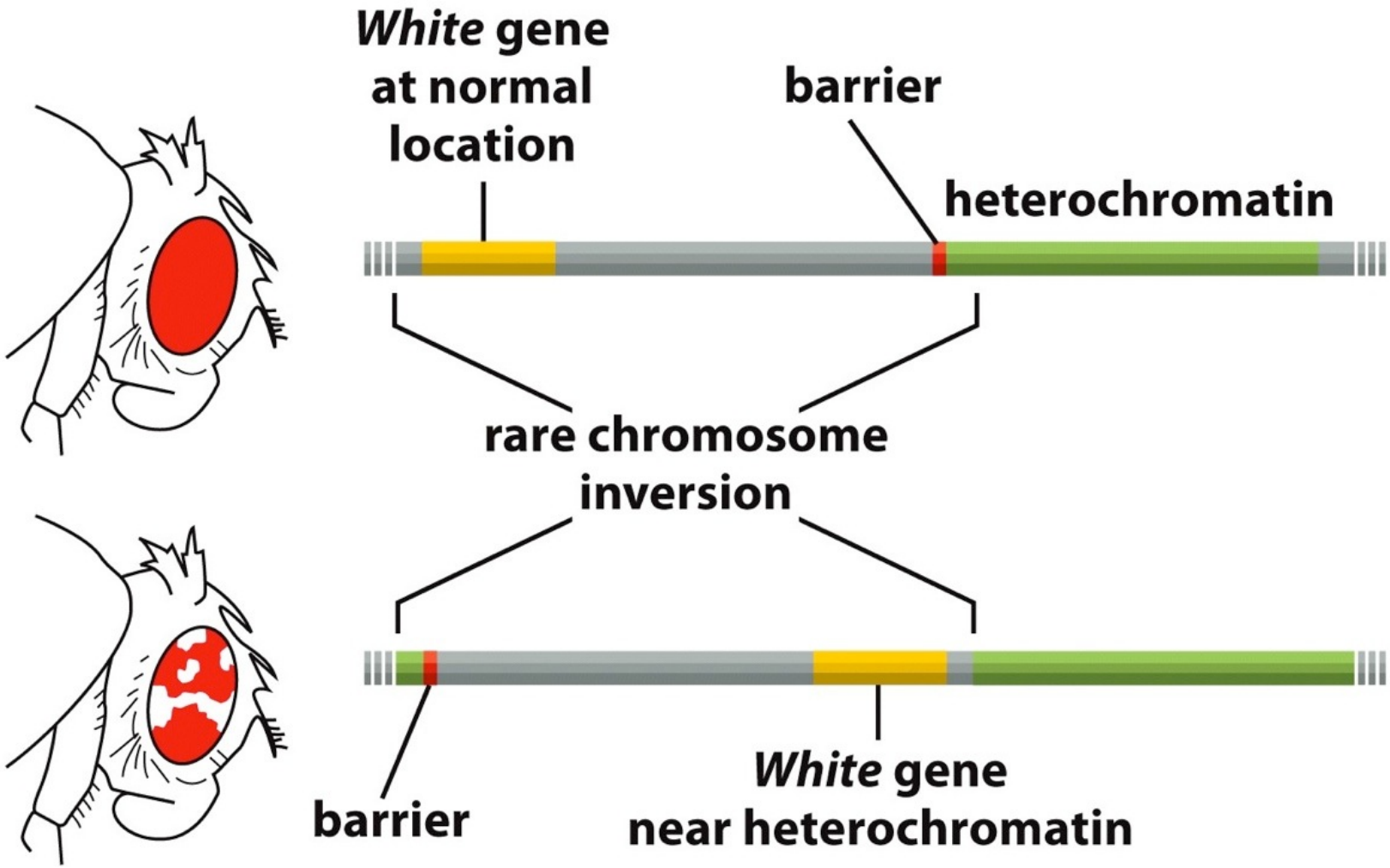


Figure 5-29 Essential Cell Biology 3/e (© Garland Science 2010)

I. *Position Effect Variegation*

The activity of a gene depends on its ***position*** along a chromosome (Fig. 5-29).

Genes that do become packaged into heterochromatin usually *become resistant to being expressed* because heterochromatin is unusually compact.

Example #2

Inappropriate packing of genes in heterochromatin can cause *disease*.

β-*globin* is situated next to heterochromatin.
If, DNA is deleted, the region of heterochromatin is spreads, the the gene is poorly expressed.

A severe form of *anemia*.

Example #3

X chromosome inactivation

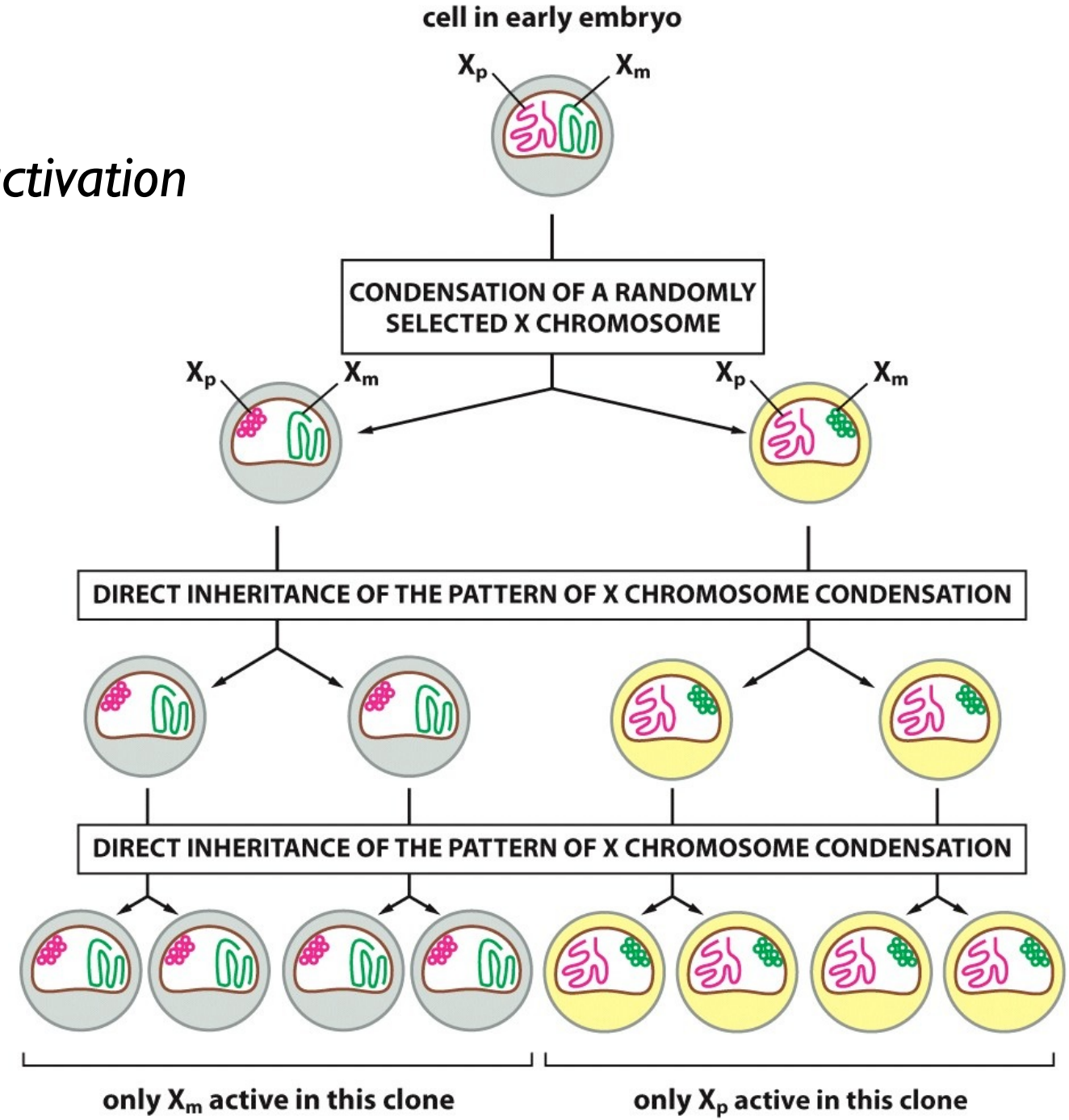


Figure 5-30 Essential Cell Biology 3/e (© Garland Science 2010)

3. *X chromosome inactivation*

The *interphase X chromosomes* of female mammals.

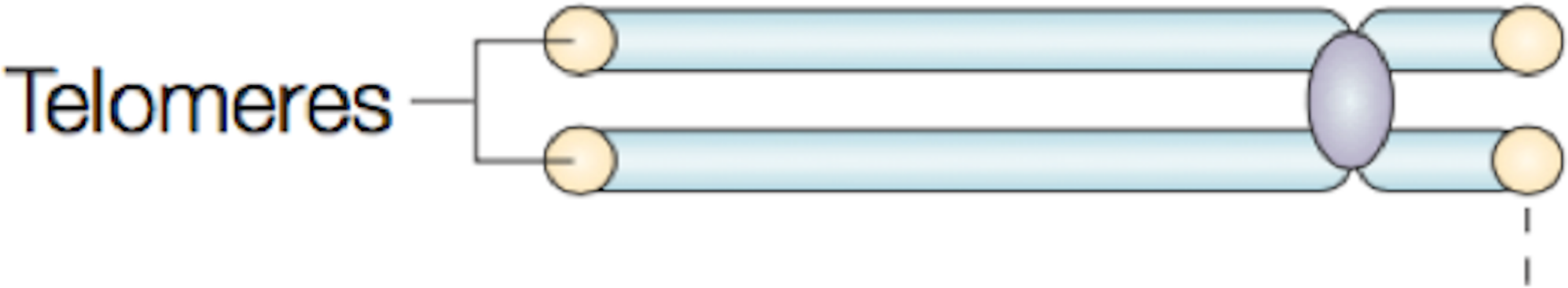
A double dose of X-chromosome products would be *lethal*.

One of the two X chromosomes in each cell is *permanently inactivated*.

Completely inactivated by heterochromatin formation *in early embryo* (see fig 5-28).

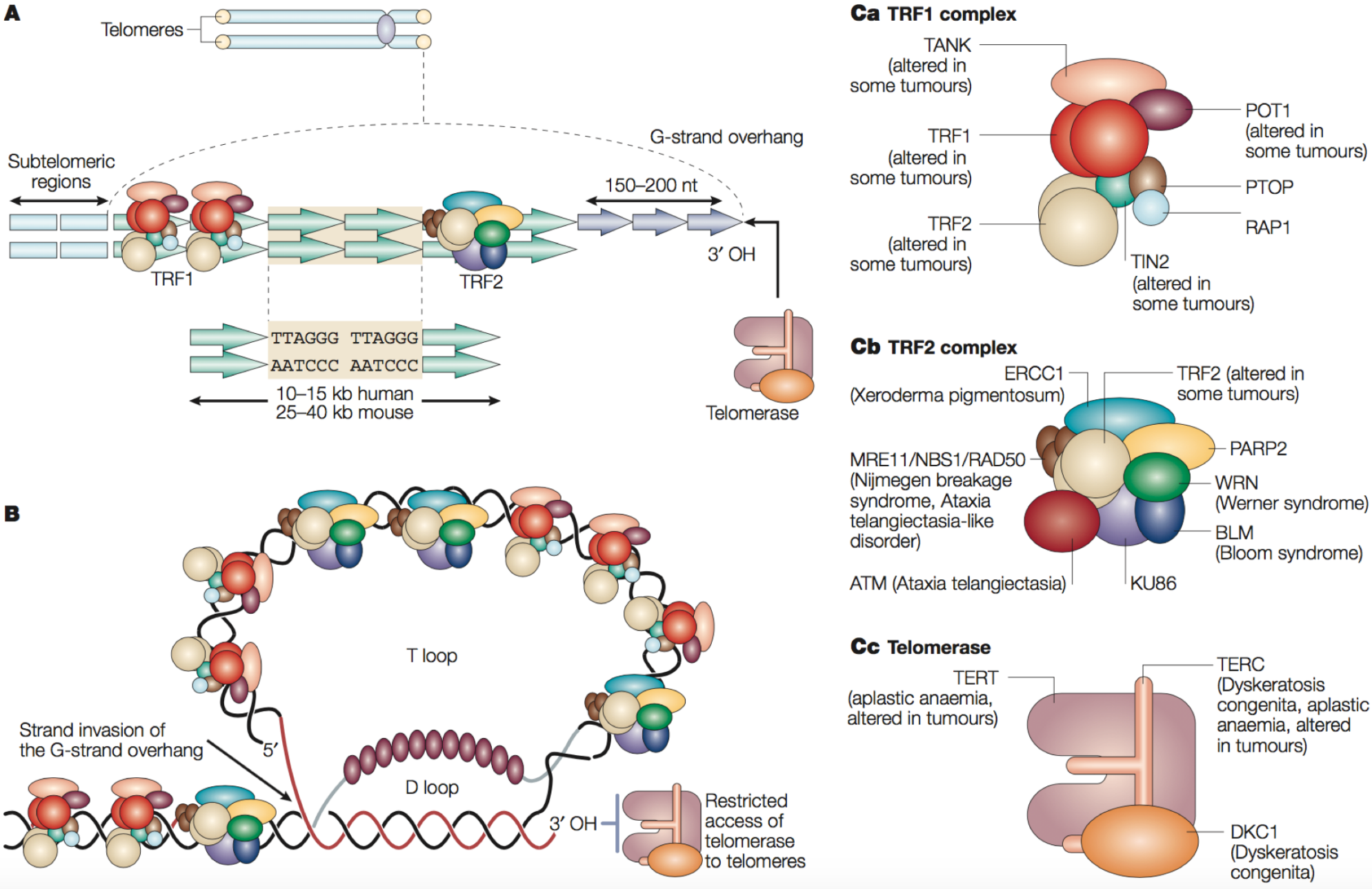
Inactive state of X-chromosome is *inherited*.

Telomeres Have a Special Form of Heterochromatin



TELOMERES AND HUMAN DISEASE: AGEING, CANCER AND BEYOND

Maria A. Blasco


A
Telomeres
G-strand overhang
Subtelomeric regions
150–200 nt
3′ OH
TRF1
TRF2
TTAGGG TTAGGG
AATCCC AATCCC
10–15 kb human
25–40 kb mouse
Telomerase
B
T loop
Strand invasion of the G-strand overhang
5′
D loop
3′ OH
Restricted access of telomerase to telomeres
Ca TRF1 complex
TANK (altered in some tumours)
TRF1 (altered in some tumours)
TRF2 (altered in some tumours)
POT1 (altered in some tumours)
PTOP
RAP1
TIN2 (altered in some tumours)
Cb TRF2 complex
ERCC1 (Xeroderma pigmentosum)
TRF2 (altered in some tumours)
PARP2
MRE11/NBS1/RAD50 (Nijmegen breakage syndrome, Ataxia telangiectasia-like disorder)
WRN (Werner syndrome)
BLM (Bloom syndrome)
ATM (Ataxia telangiectasia)
KU86
Cc Telomerase
TERT (aplastic anaemia, altered in tumours)
TERC (Dyskeratosis congenita, aplastic anaemia, altered in tumours)
DKC1 (Dyskeratosis congenita)

Telomeres Have a Special Form of Heterochromatin

Heterochromatin involves an additional level of folding and requires many proteins in addition to histones.

*S*ilent *i*nformation *r*egulator (Sir) proteins induce the silencing of genes located near telomeres.

A telomere-bound Sir protein complex that recognizes underacetylated N-terminal tails of selected histones.

Sir2 is a highly conserved histone deacetylase (HDAC).

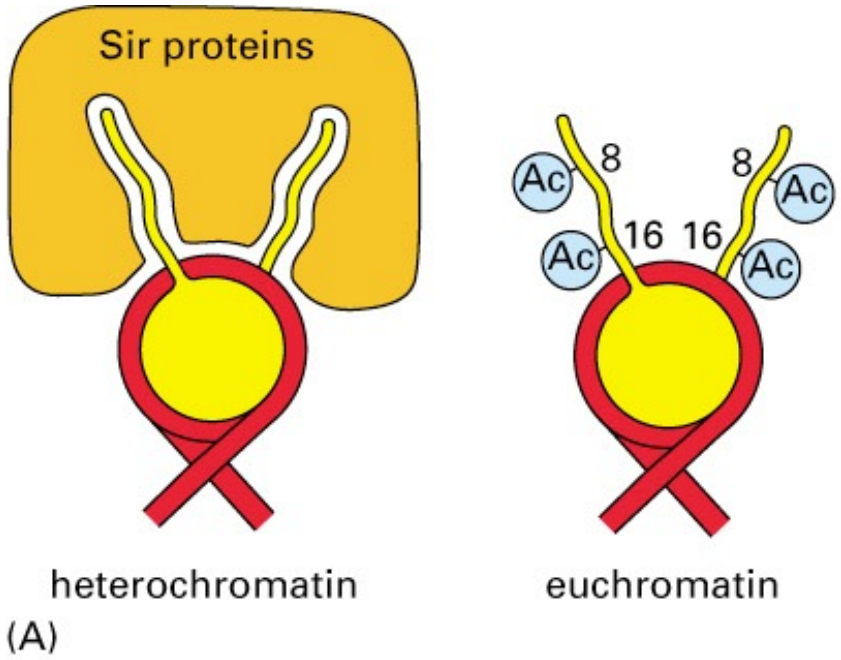


Figure 4–47 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

- Heterochromatin is generally underacetylated.
- Underacetylated tails of histone H4 are proposed to interact with a complex of Sir proteins, thus stabilizing the association of these proteins with nucleosomes.

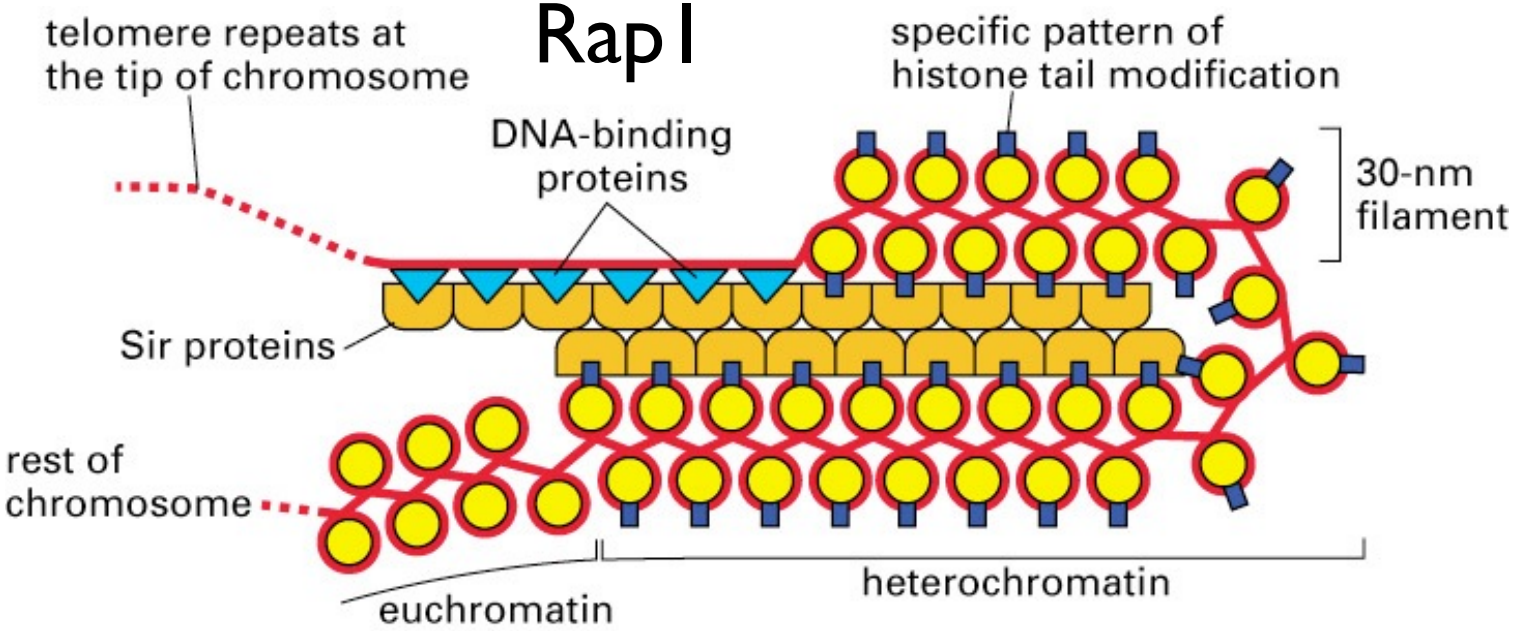


Figure 4–47 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Specialized DNA-binding proteins (blue triangles) recognize DNA sequences near the ends of chromosomes and attract the Sir proteins (Sir2). This then leads to the cooperative spreading of the Sir protein complex down the chromosome.