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- **Introduction: biology and medicine, two separated compartments**
- **What we need to know:**

  - boring basics in DNA/RNA structure and overview of particular aspects of molecular biology techniques

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- **Molecular diagnostics of cardiovascular diseases**

  - Mutations in Factor V

  - Mutations in Factor II

  - Mutations in MTHFR gene
- **Breast cancer and BRCA1 and 2 genes**

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  - Breast cencer genes

  - sequence in selected areas

  - p53 and breast cancer
- **Pharmacogenomics: finding the right drug for a patient**

  - ADR: an emerging problem

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  - Example 1: TPMT-enzyme and the metabolism of azathioprines

  - Example 2: Clozapine in the treatment of psychiatric diseases

  - CXP3A4 and the metabolism of anti-coagulant drugs

What is about molecular biology?

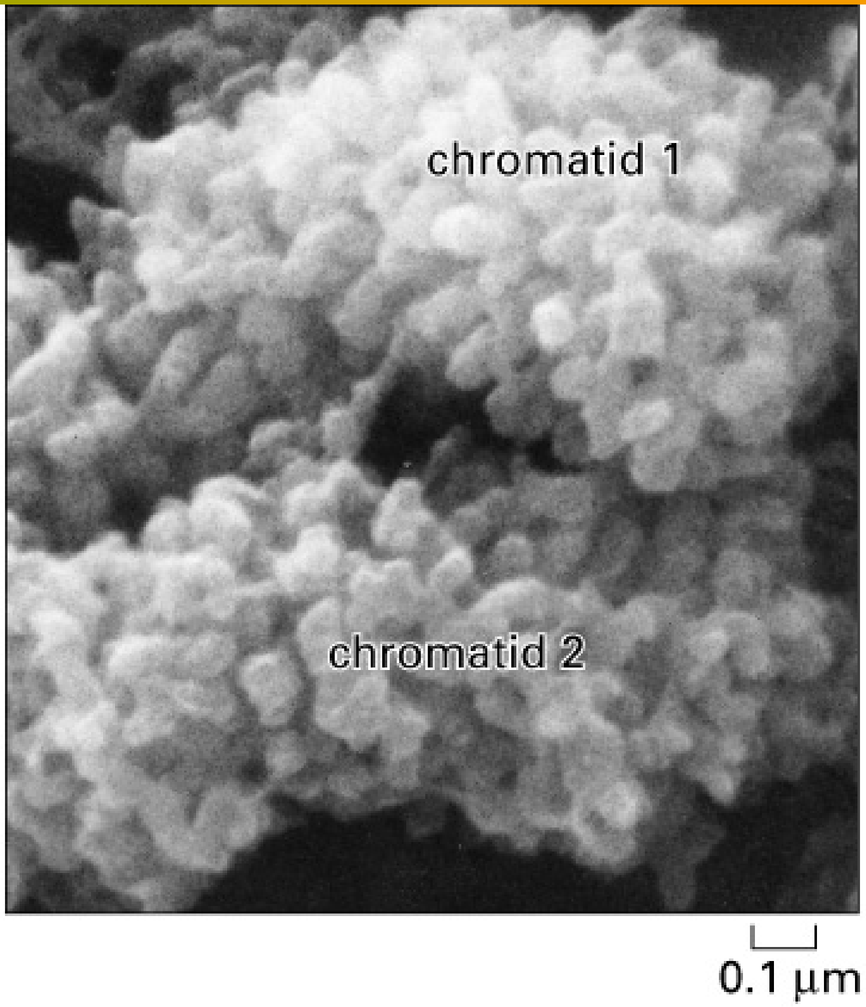
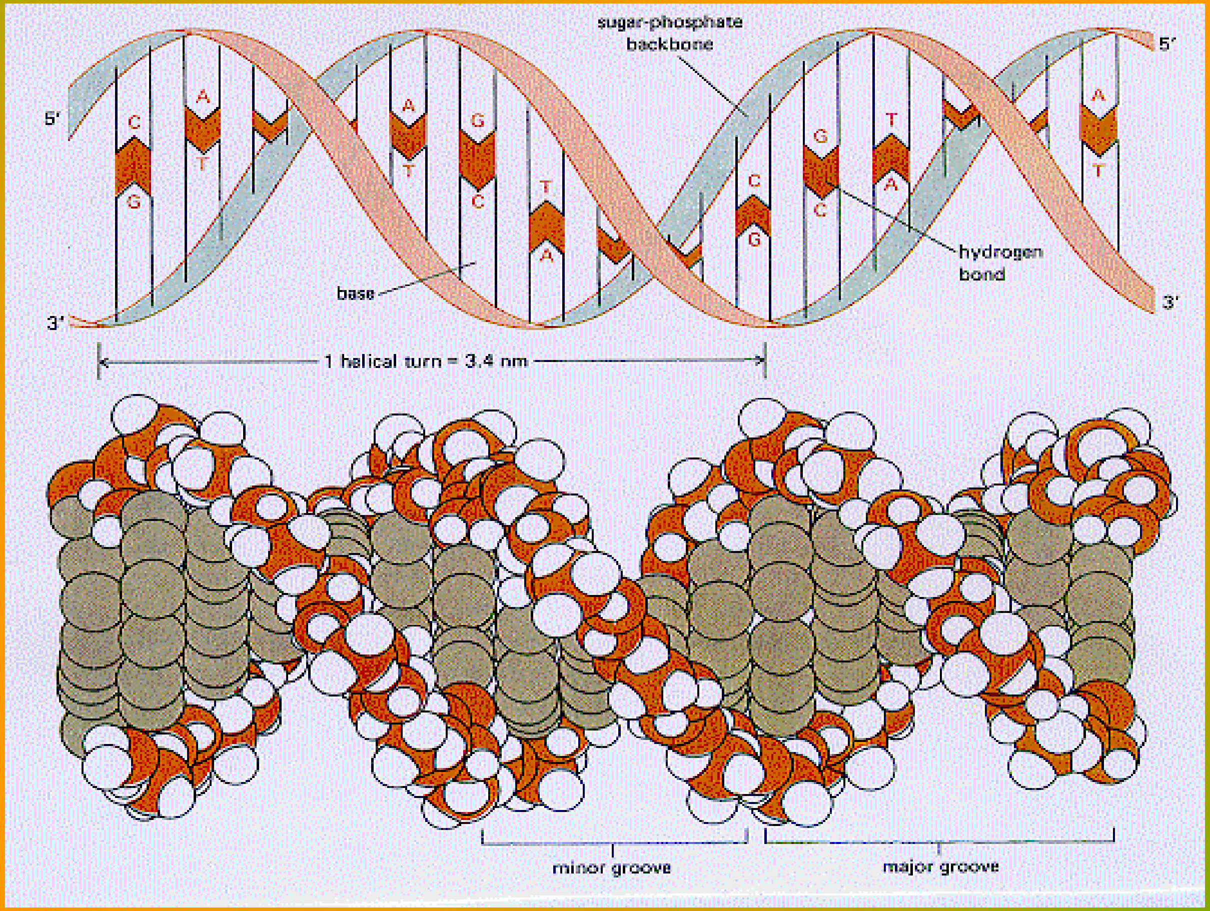


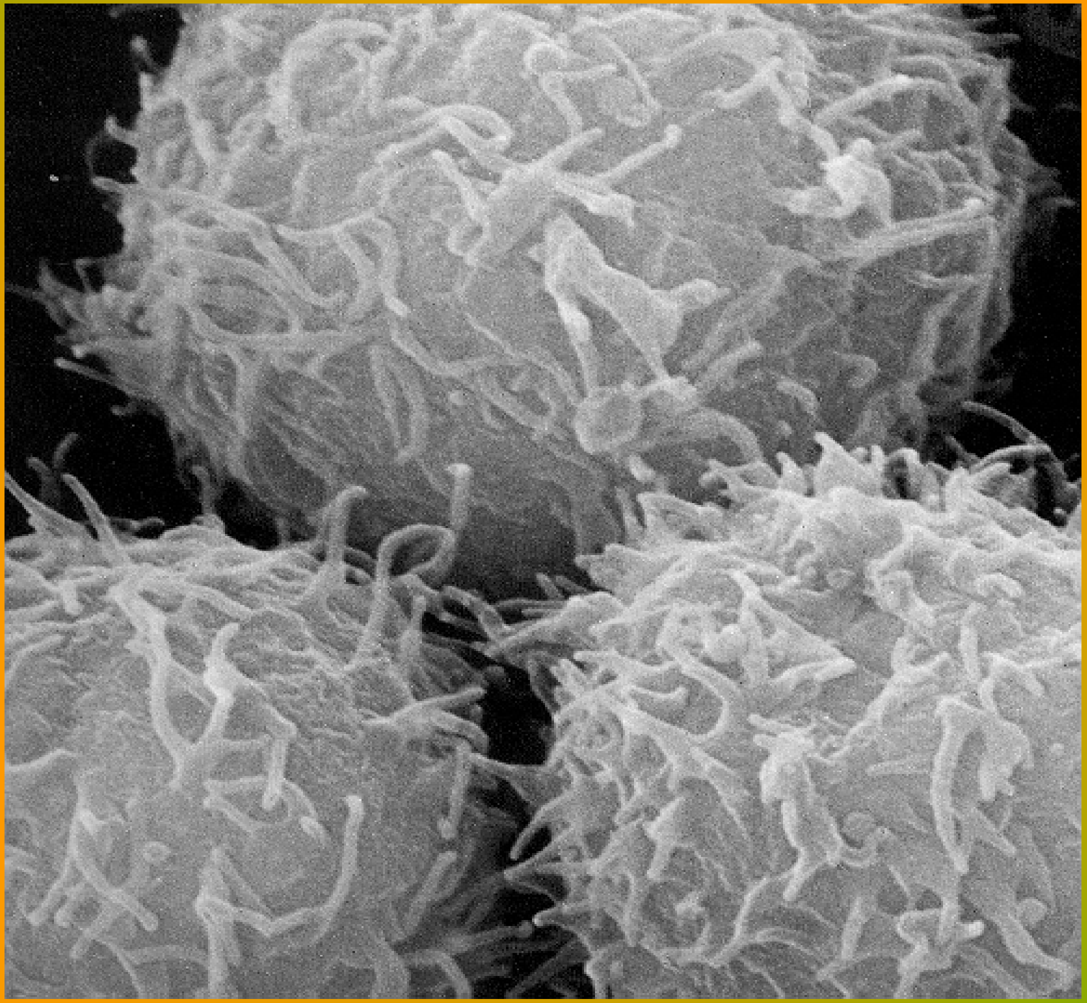
Figure 4–53. Molecular Biology of the Cell, 4th Edition.

Molecular biology is about DNA

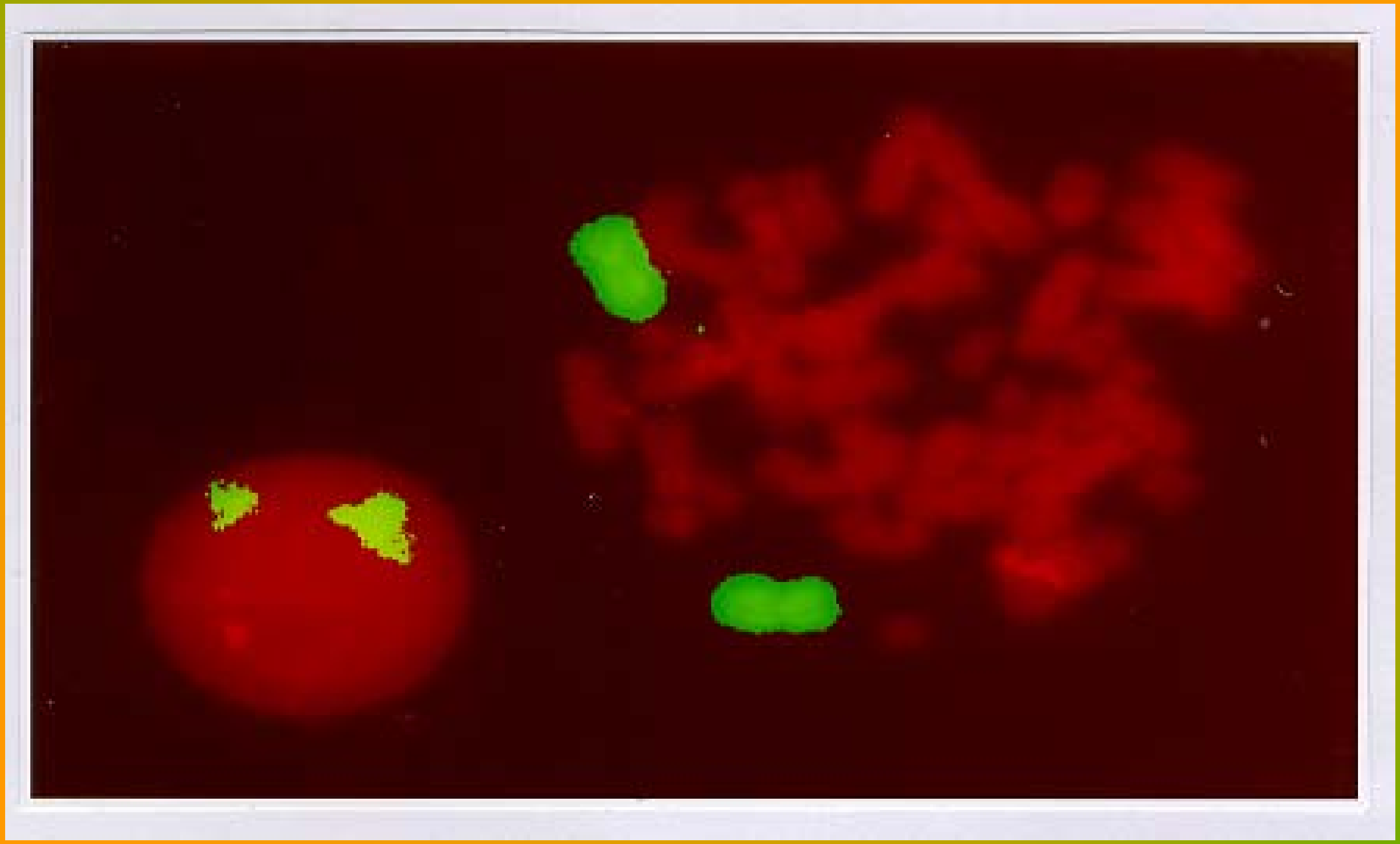


DNA containers

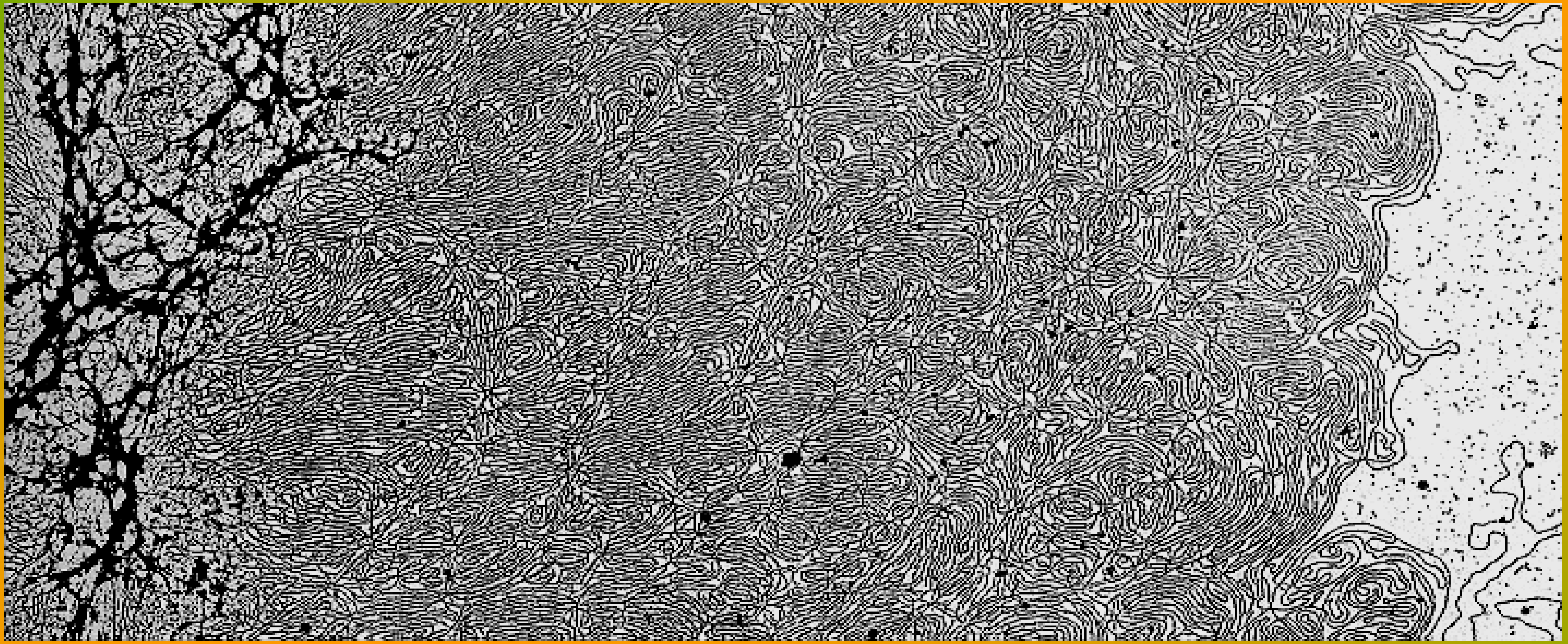
And DNA containers



And inside cells...nuclei



And inside nuclei...DNA



Inside every cell we can find more than 2 meters of DNA

Chromosomes

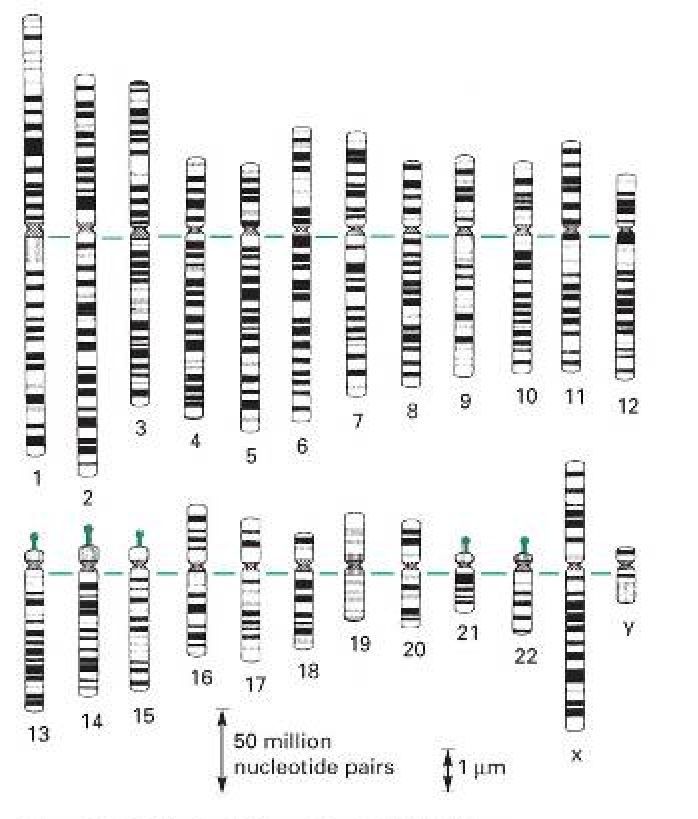
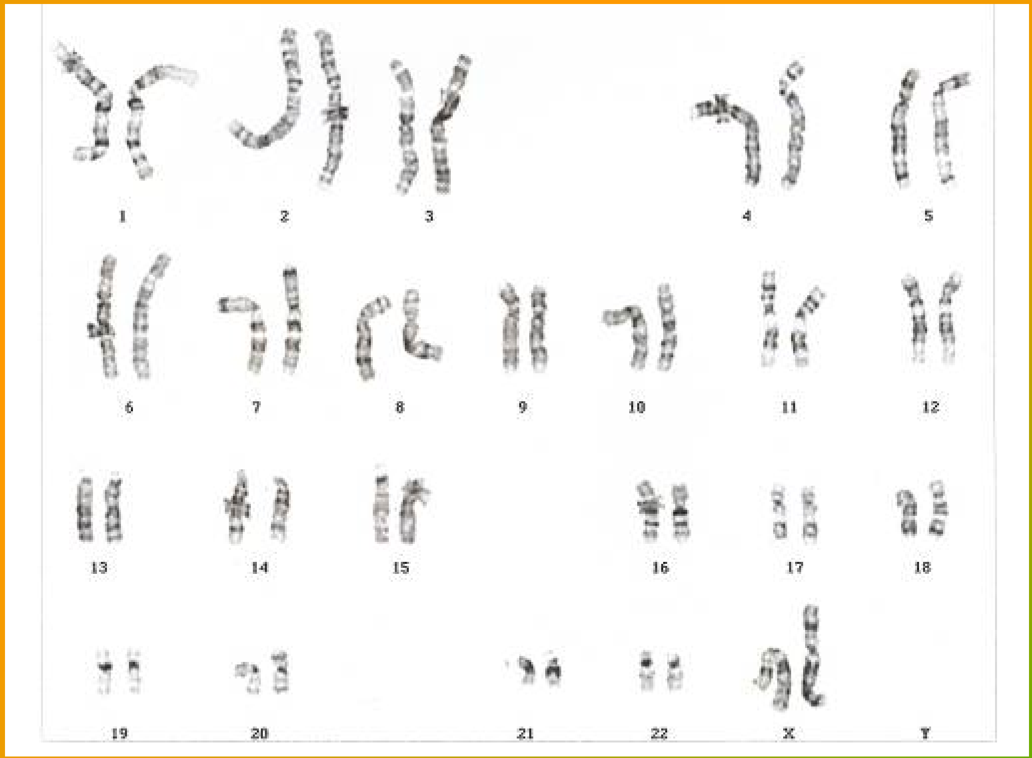
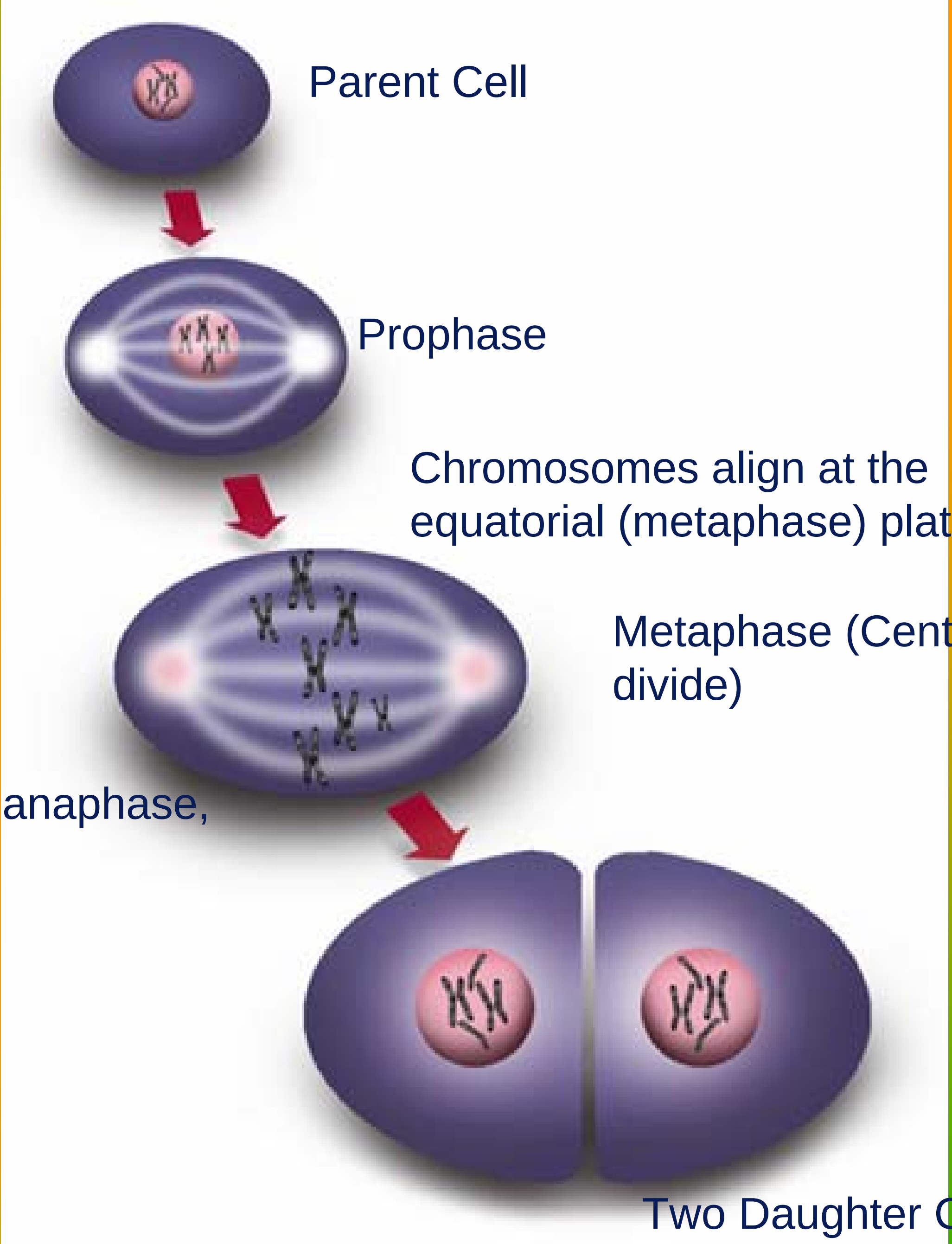


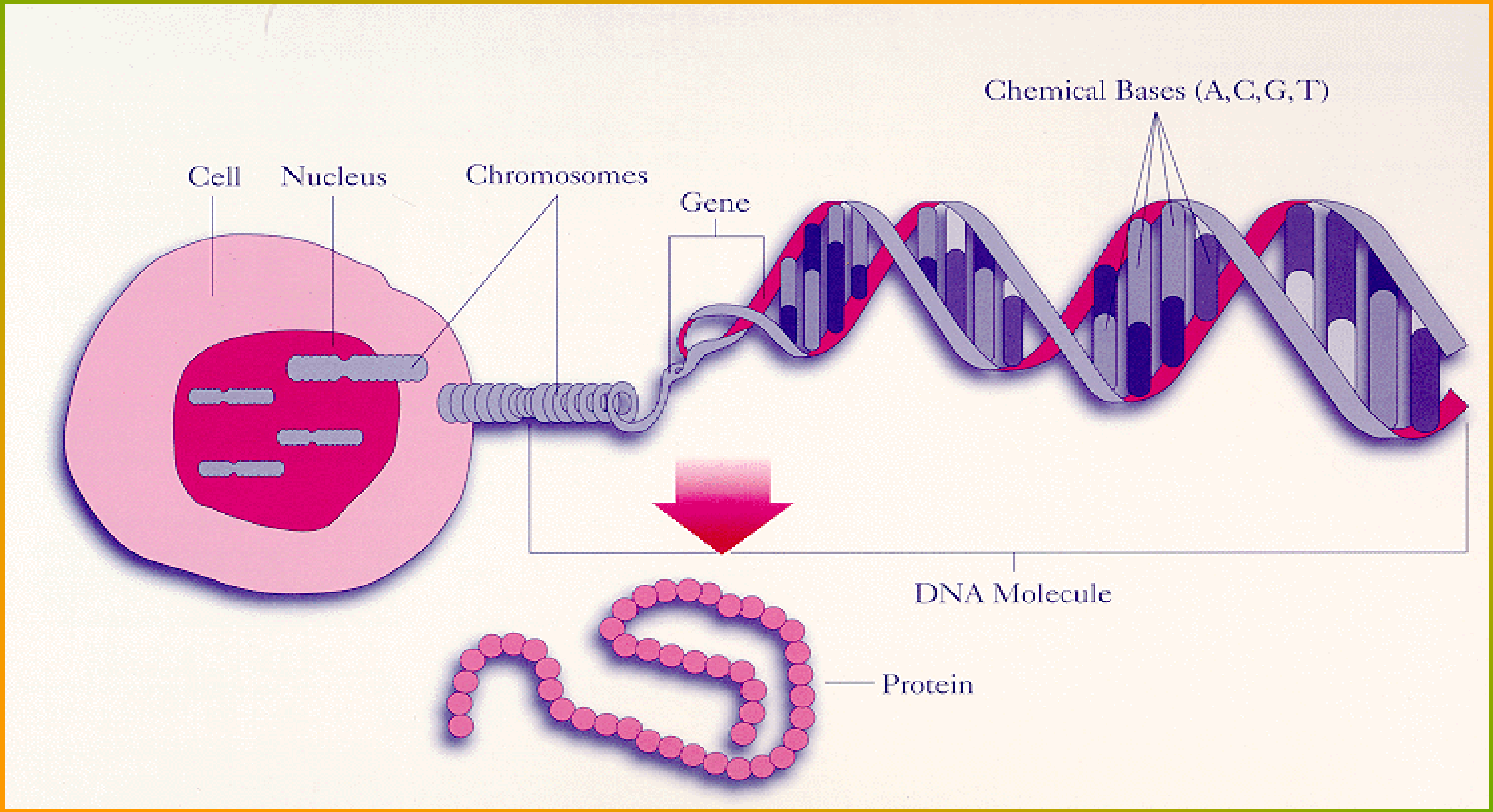
Figure 4–11. Molecular Biology of the Cell, 4th Edition.



Every time a cell divides its DNA is duplicated



And here is how we explain it



DNA structure

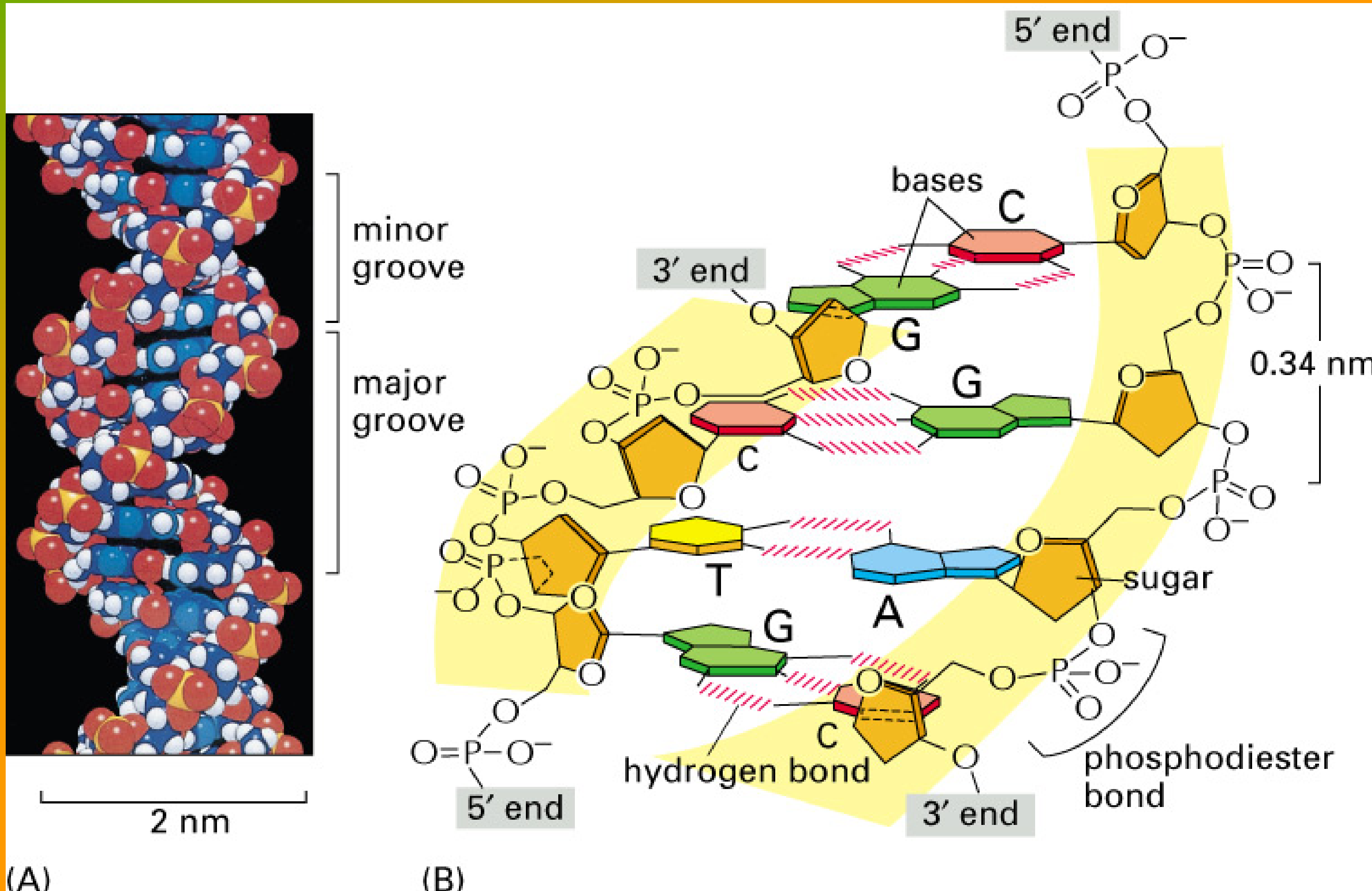
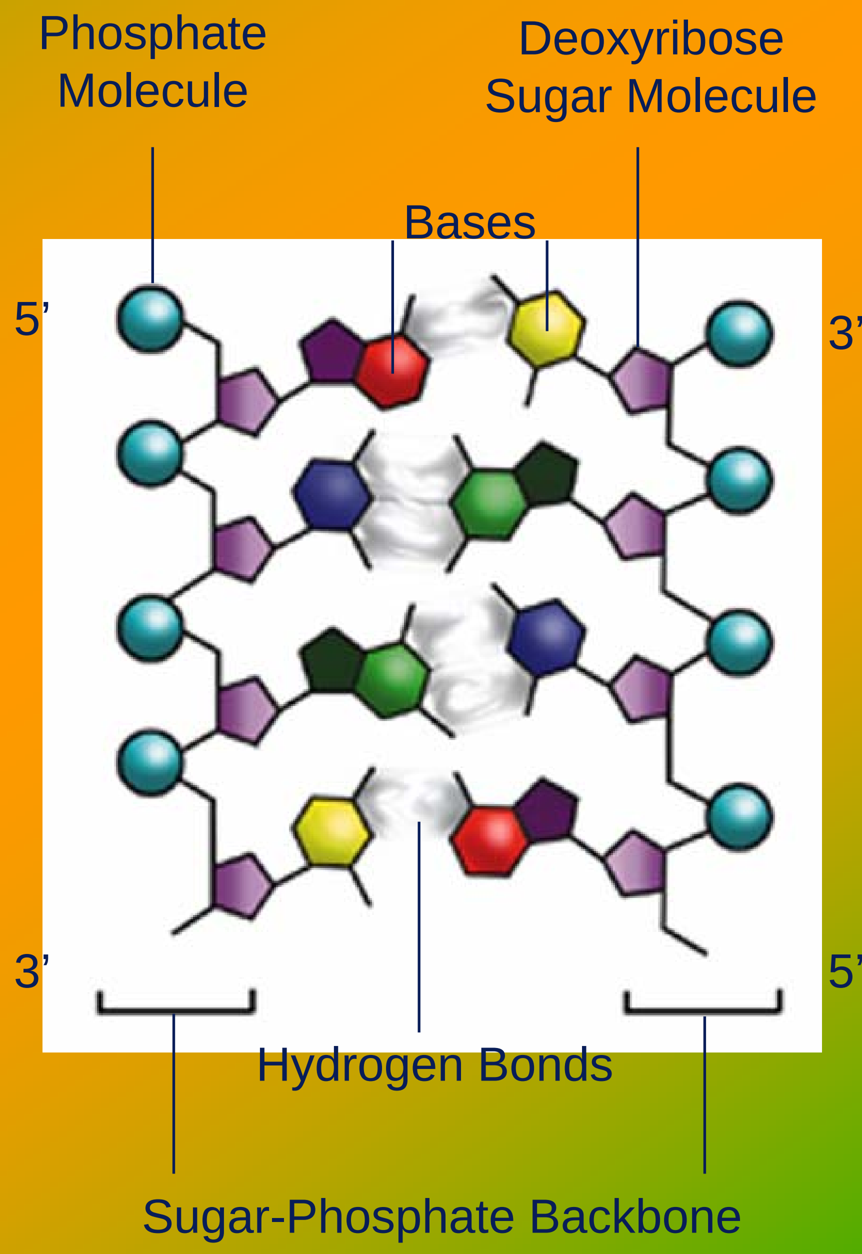


Figure 4–5. Molecular Biology of the Cell, 4th Edition.

Bonding of Bases, Sugar and Phosphate Groups



Hydrogen bonding

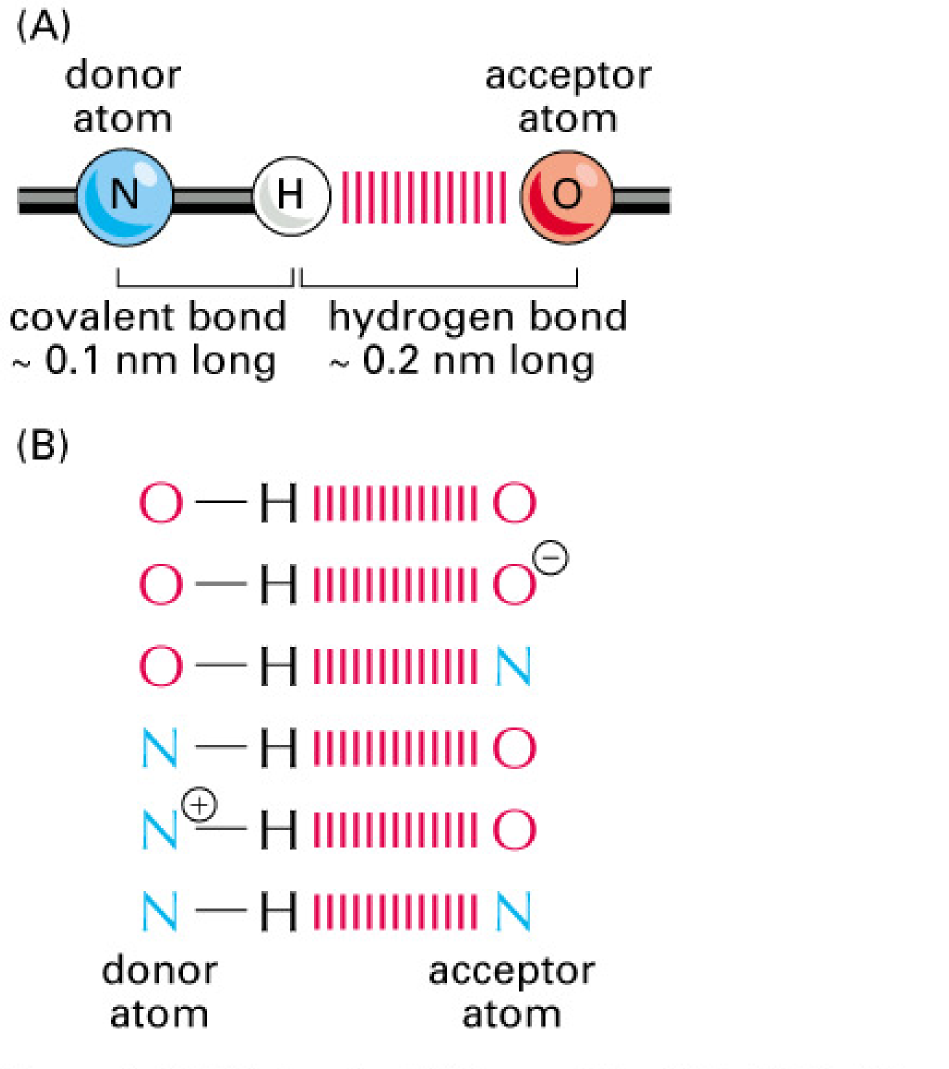


Figure 2–15. Molecular Biology of the Cell, 4th Edition.

Hydrogen bonding in DNA

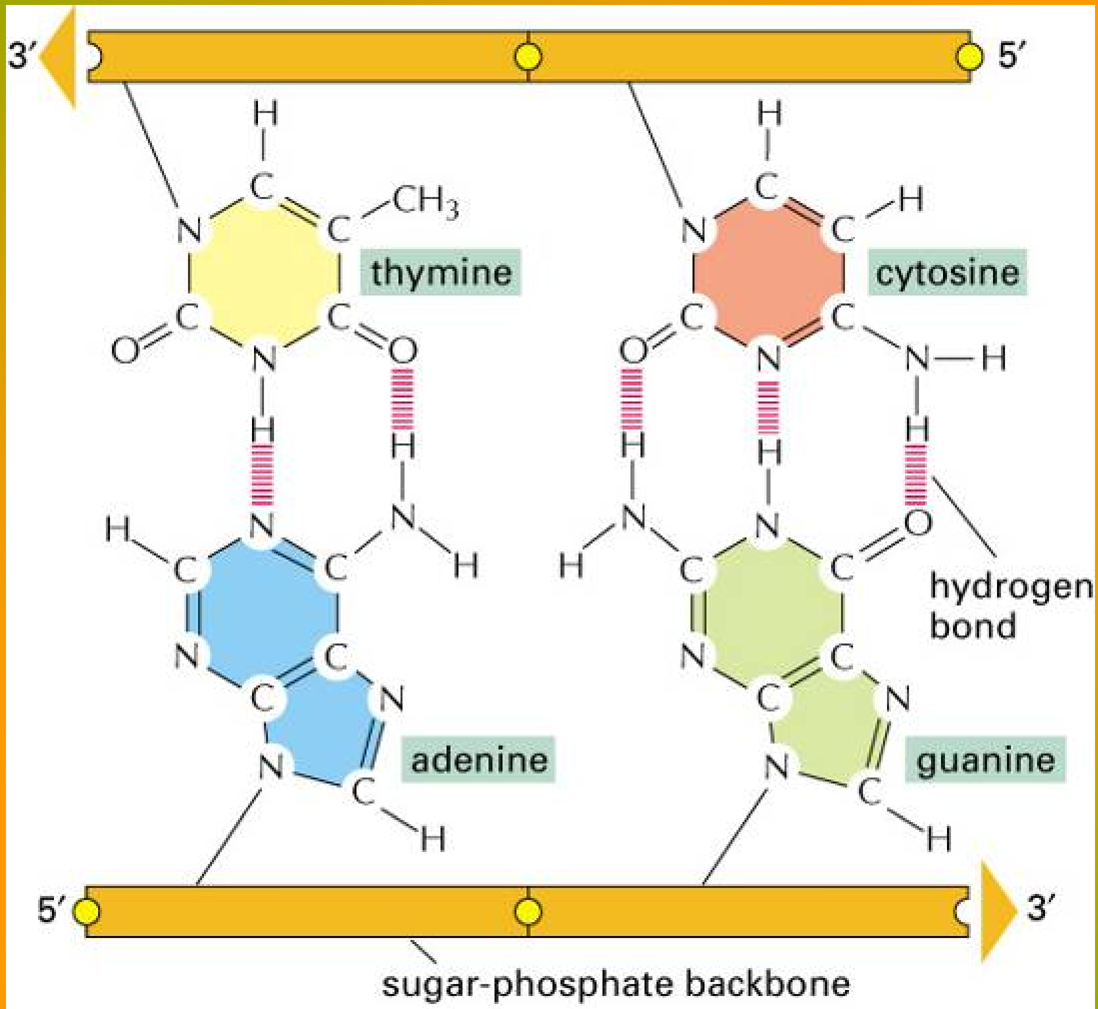
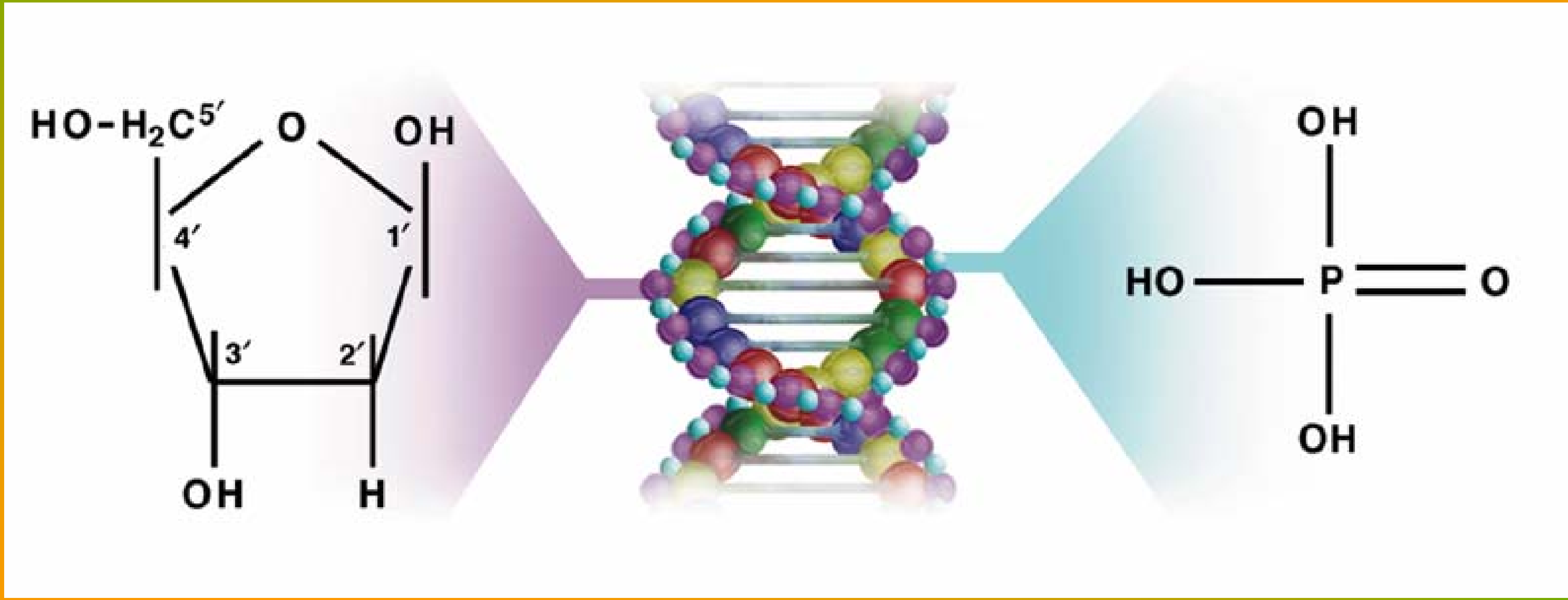


Figure 4–4. Molecular Biology of the Cell, 4th Edition.

Deoxyribose and Phosphoric Acid



Deoxyribose

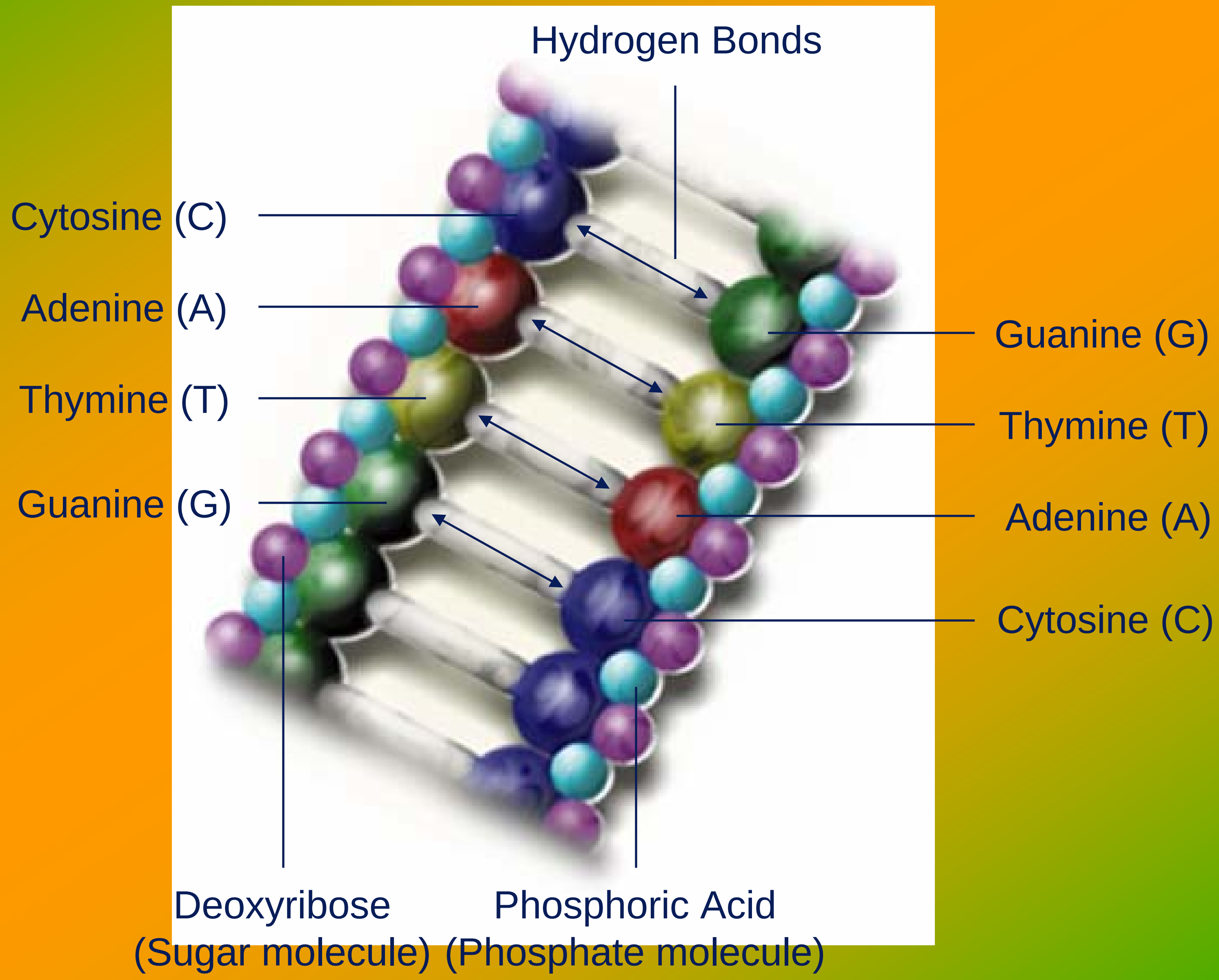
Phosphoric Acid

DNA Base Nomenclature

DNA Base Nomenclature

Base	Nucleoside	Nucleotide	Abbreviation	Base Ring Structure
Adenine (A)	Adenosine	Adenosine Triphosphate	dATP	Purine
Guanine (G)	Guanosine	Guanosine Triphosphate	dGTP	Purine
Thymine (T)	Thymidine	Thymidine Triphosphate	dTTP	Pyrimidine
Cytosine (C)	Cytidine	Cytidine Triphosphate	dCTP	Pyrimidine

The Nucleotide Sequence



Genomes are very similar but very different

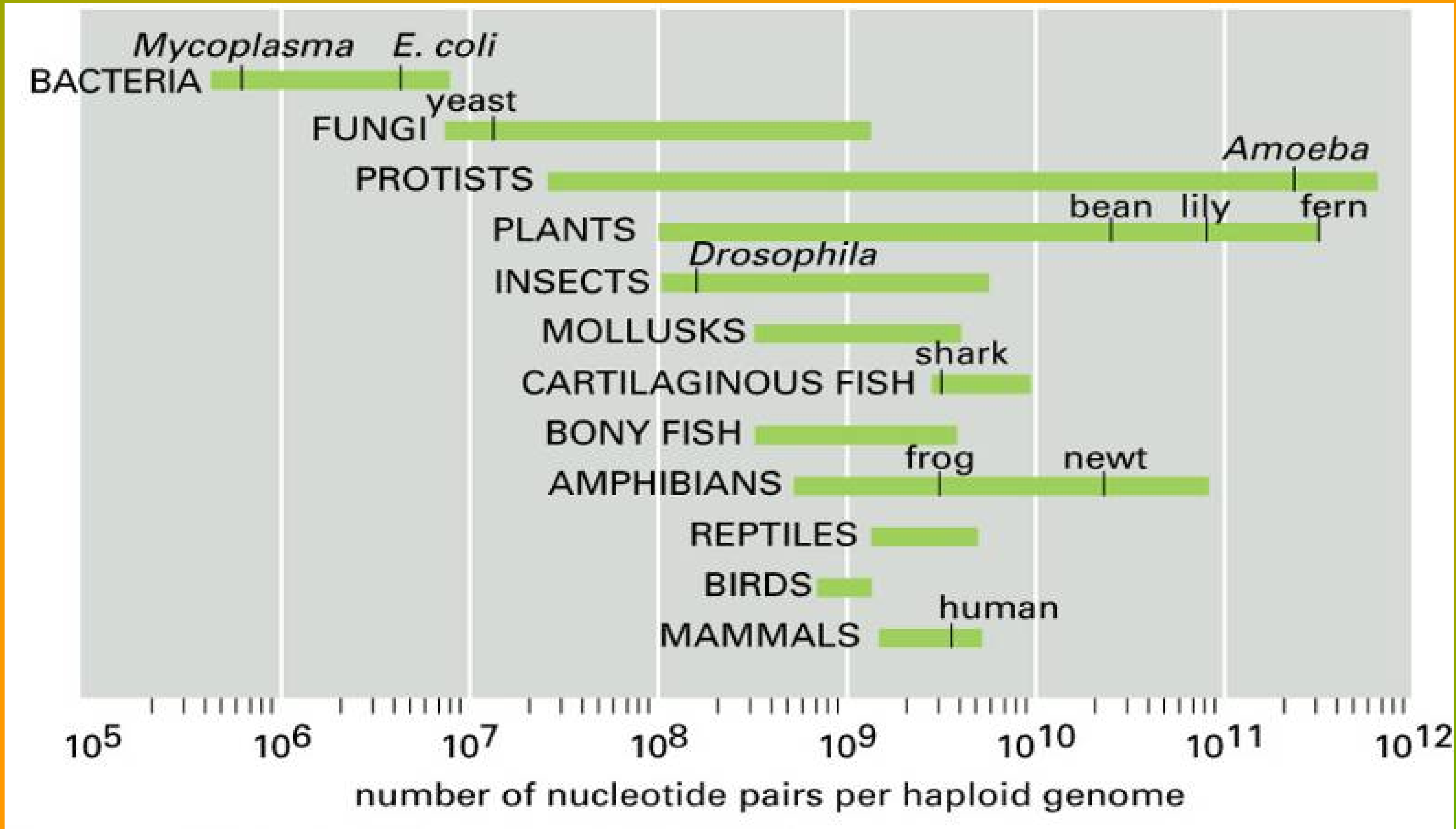


Figure 1–38. Molecular Biology of the Cell, 4th Edition.

How DNA is organized

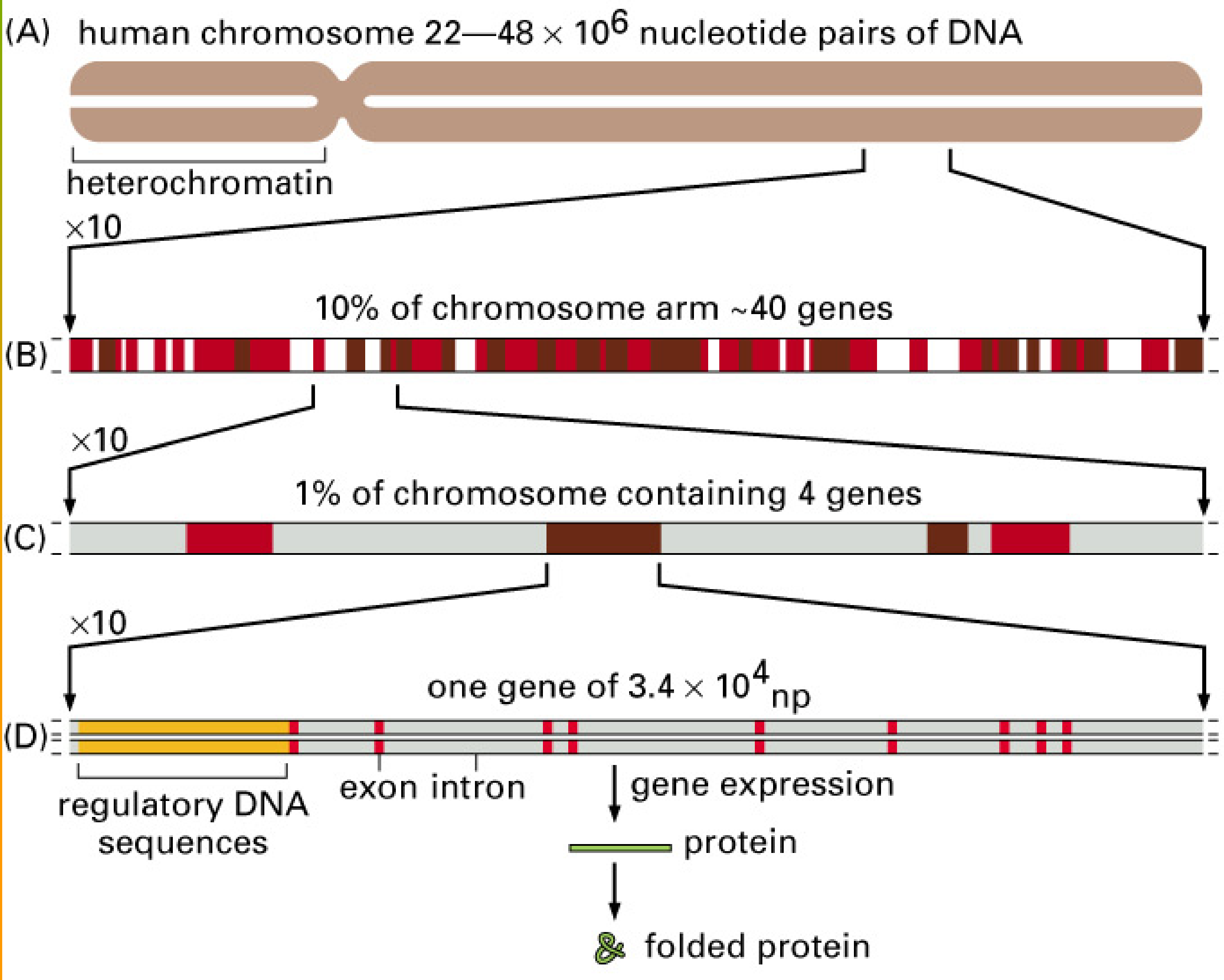


Figure 4–15. Molecular Biology of the Cell, 4th Edition.

How DNA is organized

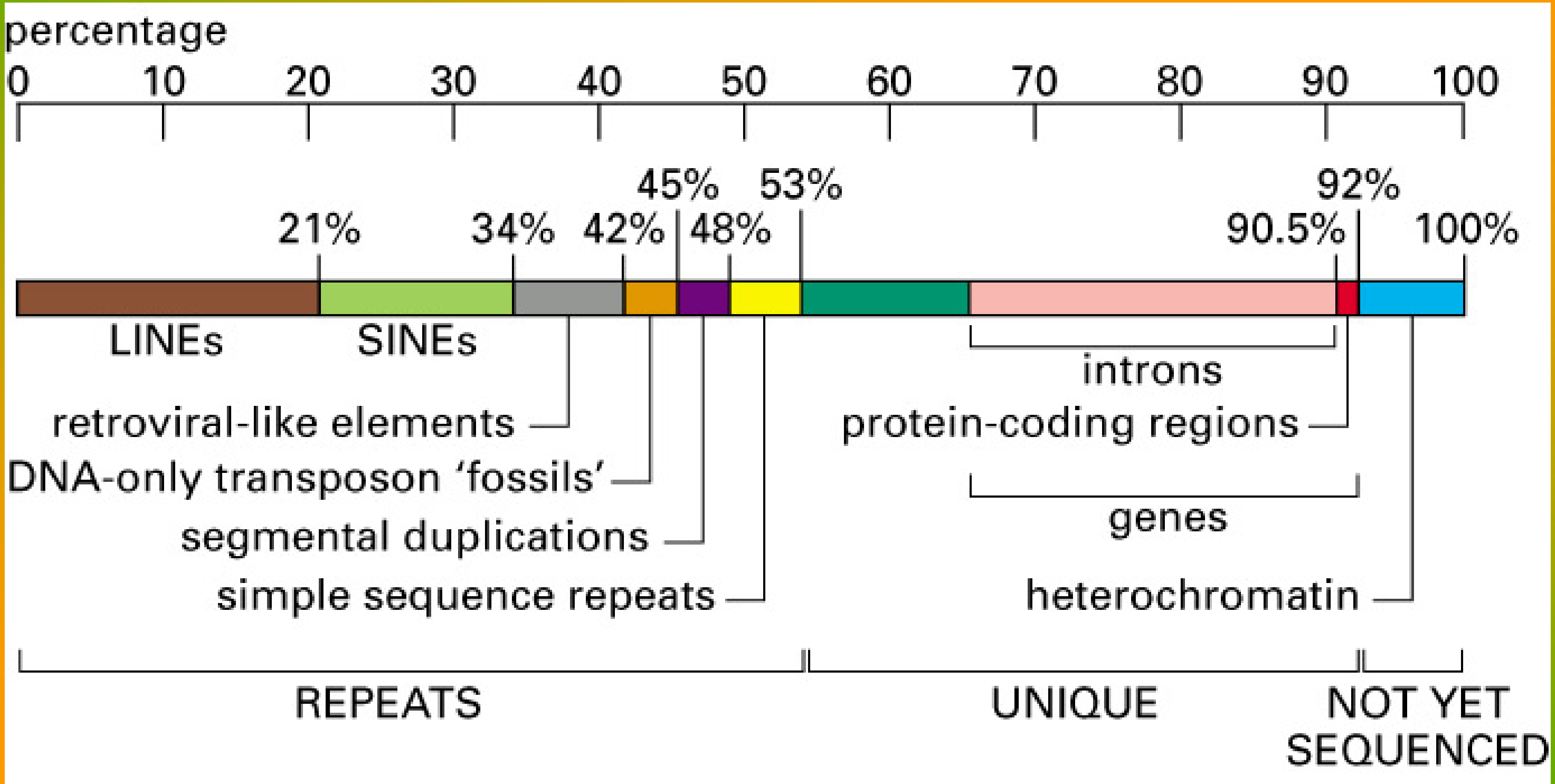


Figure 4–17. Molecular Biology of the Cell, 4th Edition.

DNA is compacted into chromatin

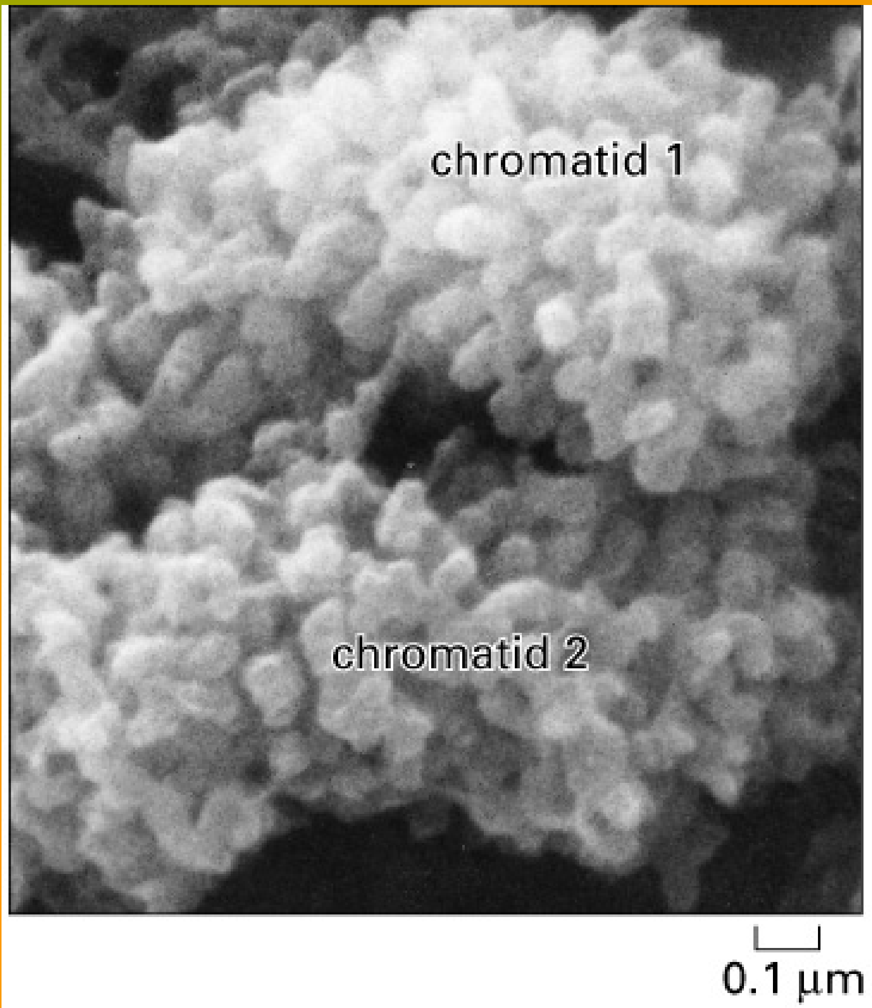


Figure 4–53. Molecular Biology of the Cell, 4th Edition.

DNA package

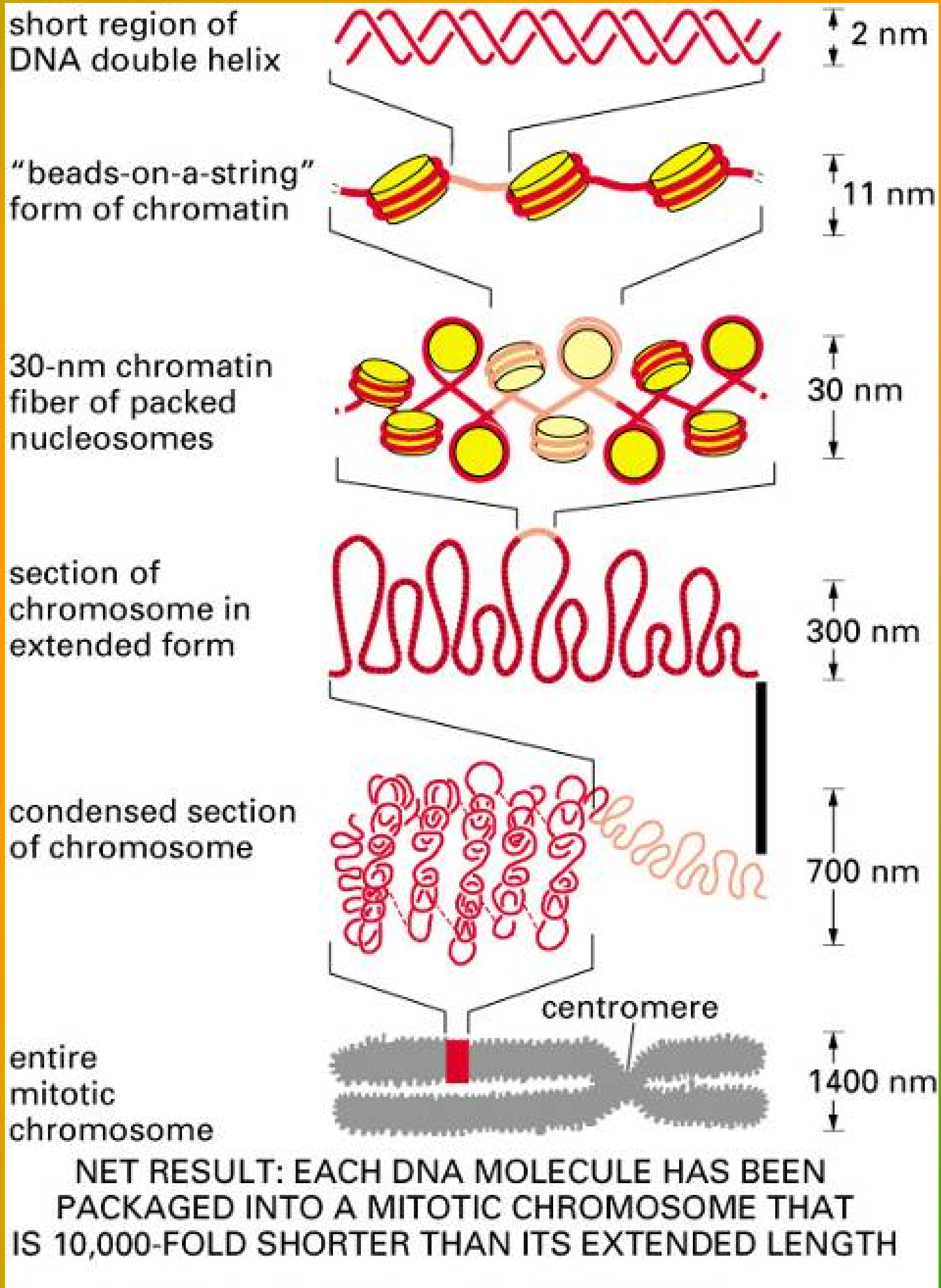


Figure 4–55. Molecular Biology of the Cell, 4th Edition.

From chromatin observation to modeling

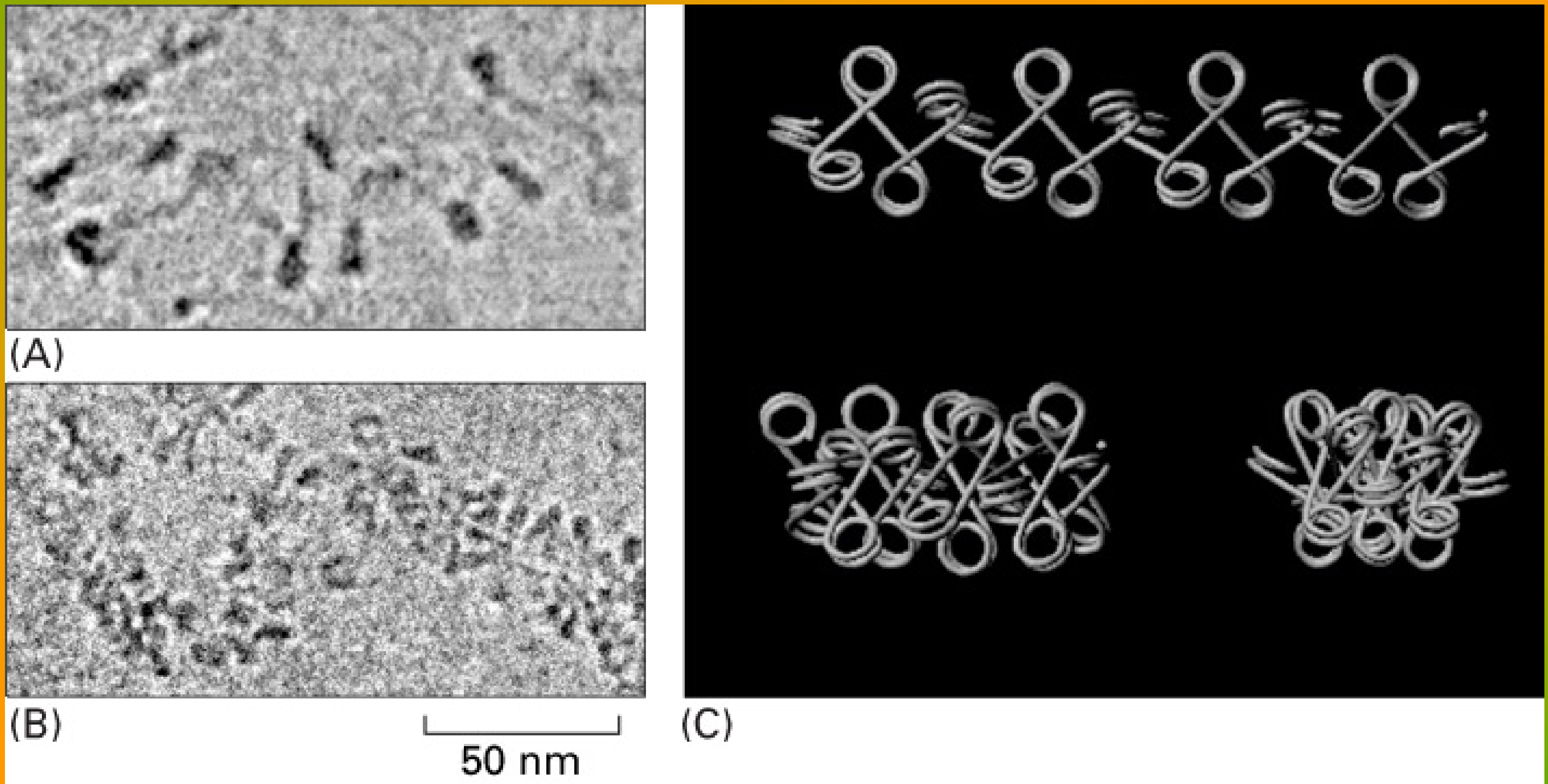


Figure 4–29. Molecular Biology of the Cell, 4th Edition.

DNA is packaged in nucleosomes

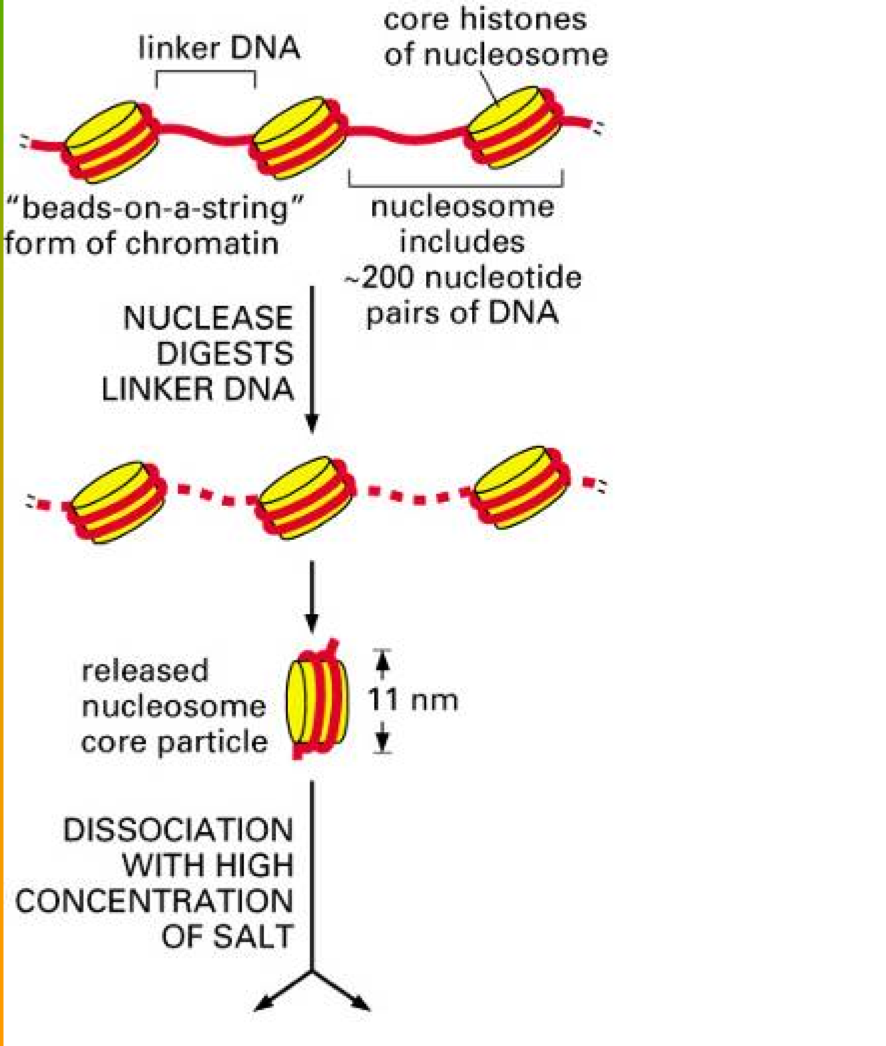


Figure 4–24 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

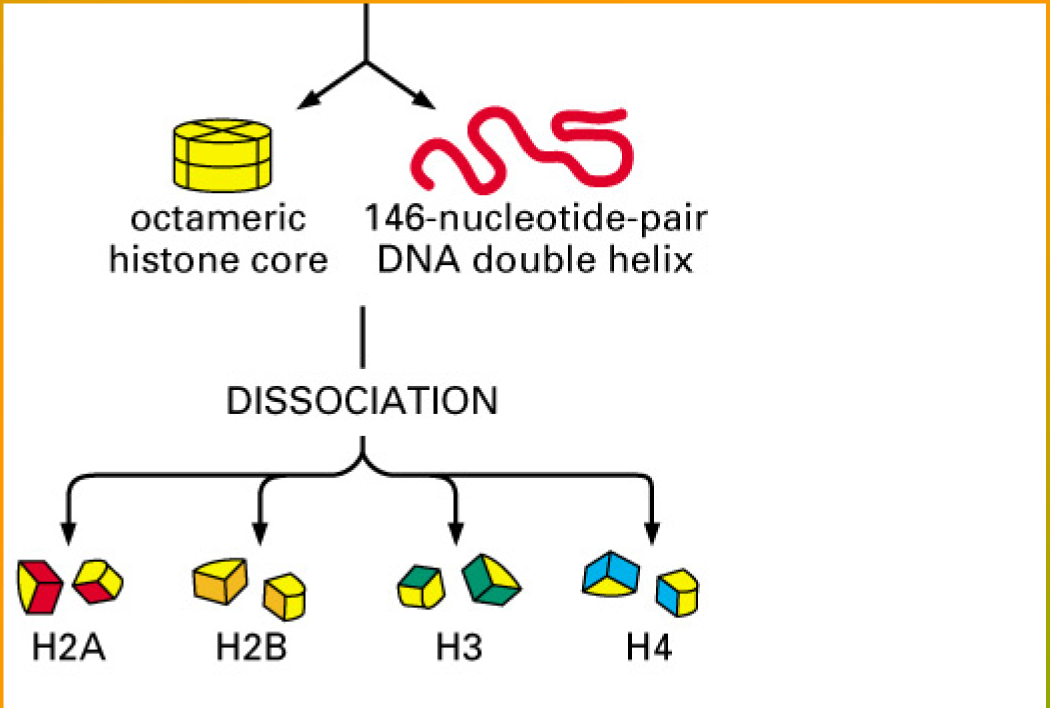


Figure 4–24 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Histones are very conserved proteins

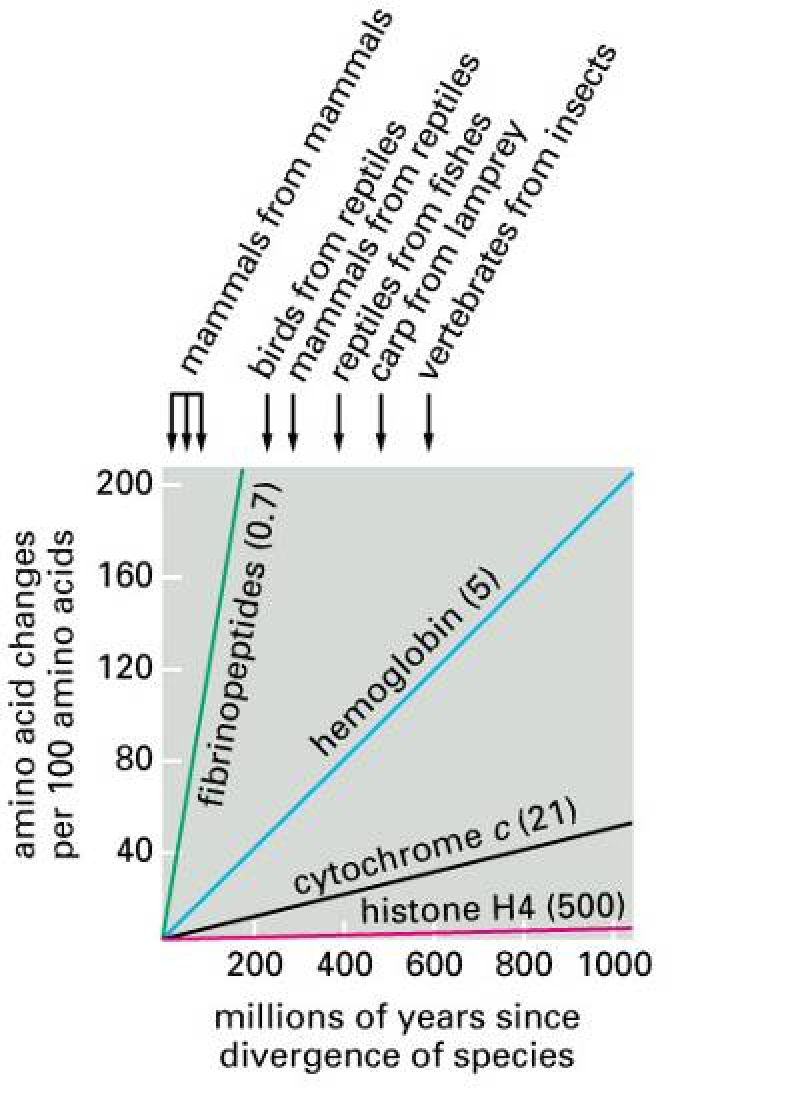


Figure 5–1. Molecular Biology of the Cell, 4th Edition.

The central dogma

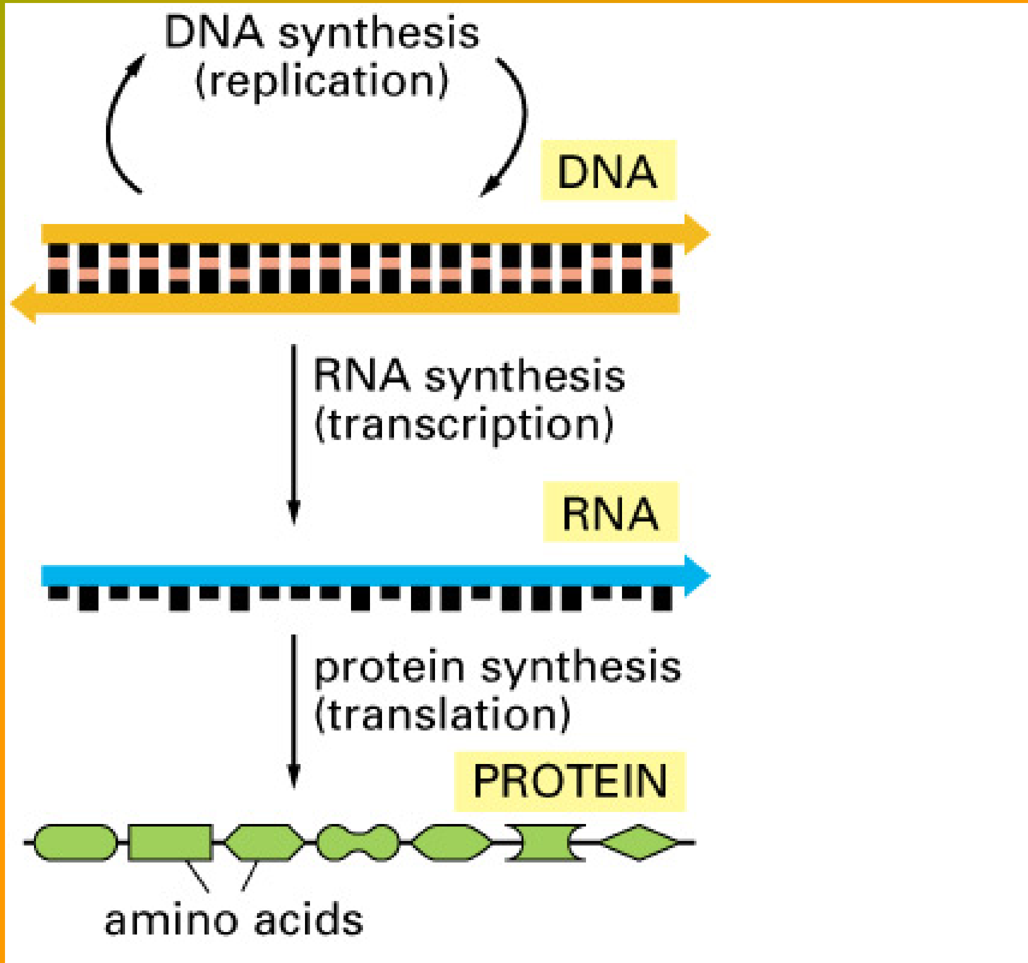


Figure 1–4. Molecular Biology of the Cell, 4th Edition.

Where protein translation is the final aim

To build a new organism

Like many other molecules DNA works on a polymeric basis

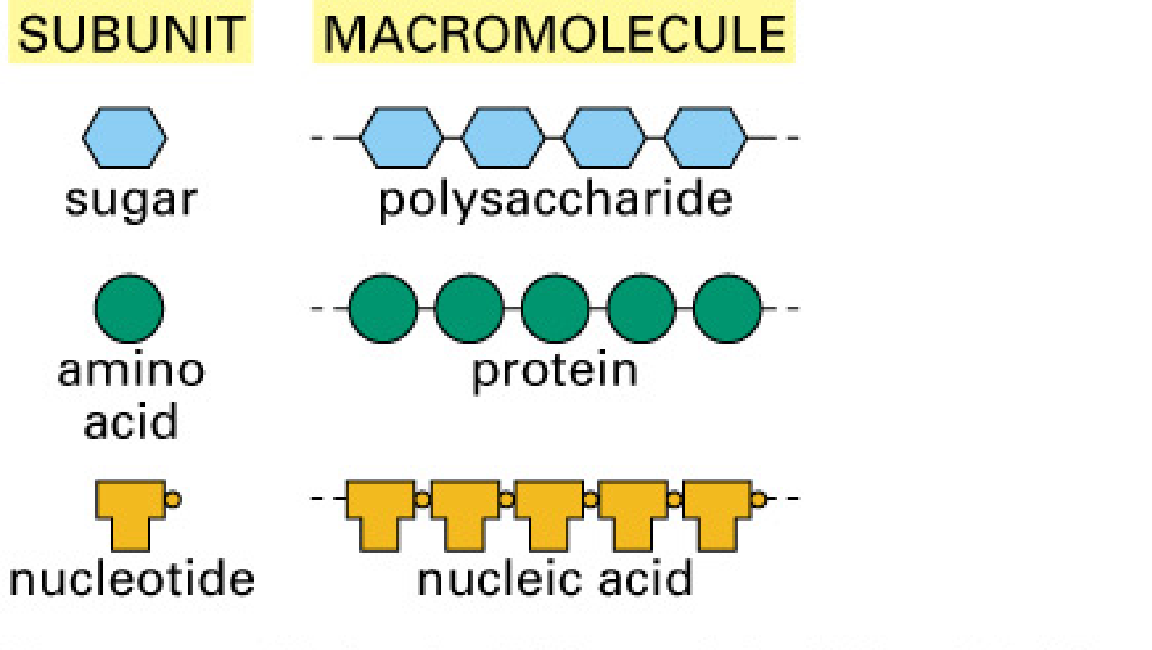
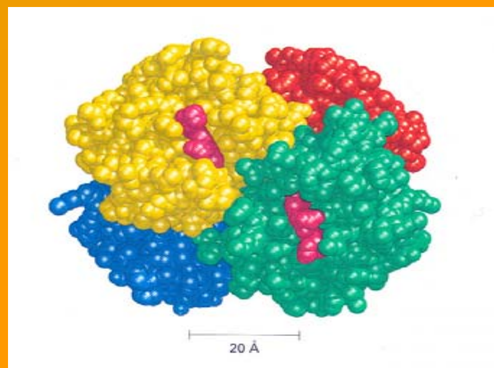


Figure 2–30. Molecular Biology of the Cell, 4th Edition.

There is a direct relationship between DNA sequence and protein



DNA Protein Organisms

This direct relationship is the genetic code

GCA GCC GCG GCU	AGA AGG CGA CGC CGG CGU	GAC GAU	AAC AAU	UGC UGU	GAA GAG	CAA CAG	GGA GGC GGG GGU	CAC CAU	AUA AUC AUU
Ala	Arg	Asp	Asn	Cys	Glu	Gln	Gly	His	Ile
A	R	D	N	C	E	Q	G	H	I

UUA UUG CUA CUC CUG CUU	AAA AAG	AUG	UUC UUU	CCA CCC CCG CCU	AGC AGU UCA UCC UCG UCU	ACA ACC ACG ACU	UGG	UAC UAU	GUA GUC GUG GUU	UAA UAG UGA
Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	stop
L	K	M	F	P	S	T	W	Y	V	

Figure 6–50. Molecular Biology of the Cell, 4th Edition.

The genetic code allows the translation of the DNA sequence into functional proteins

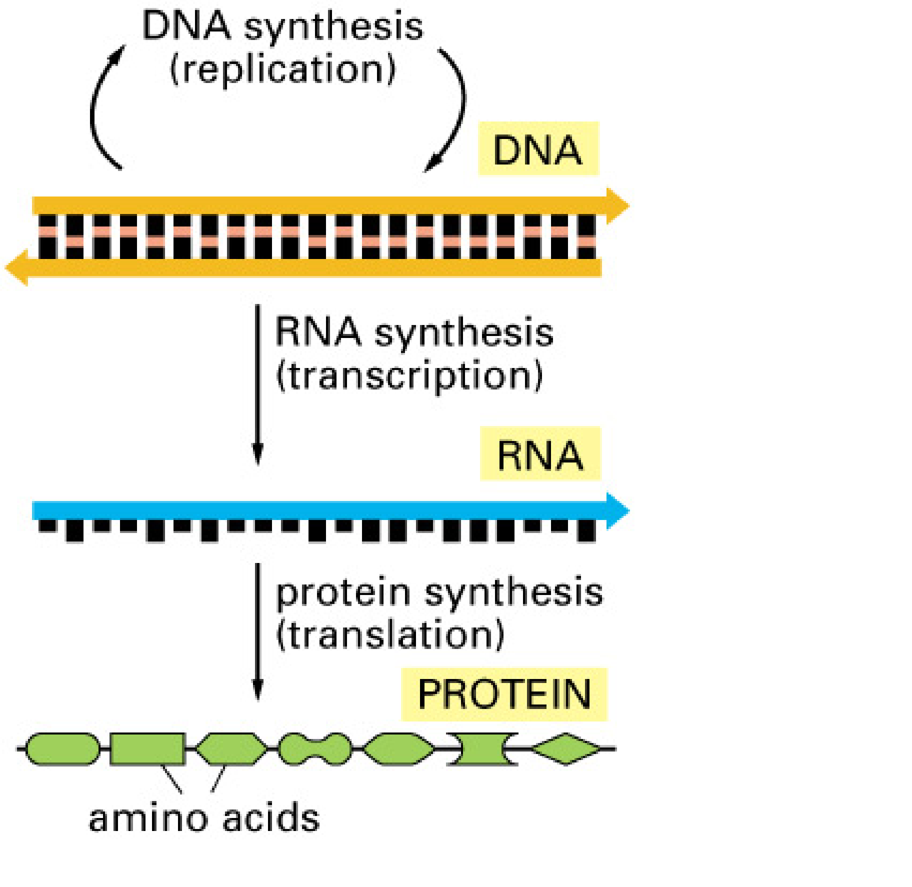
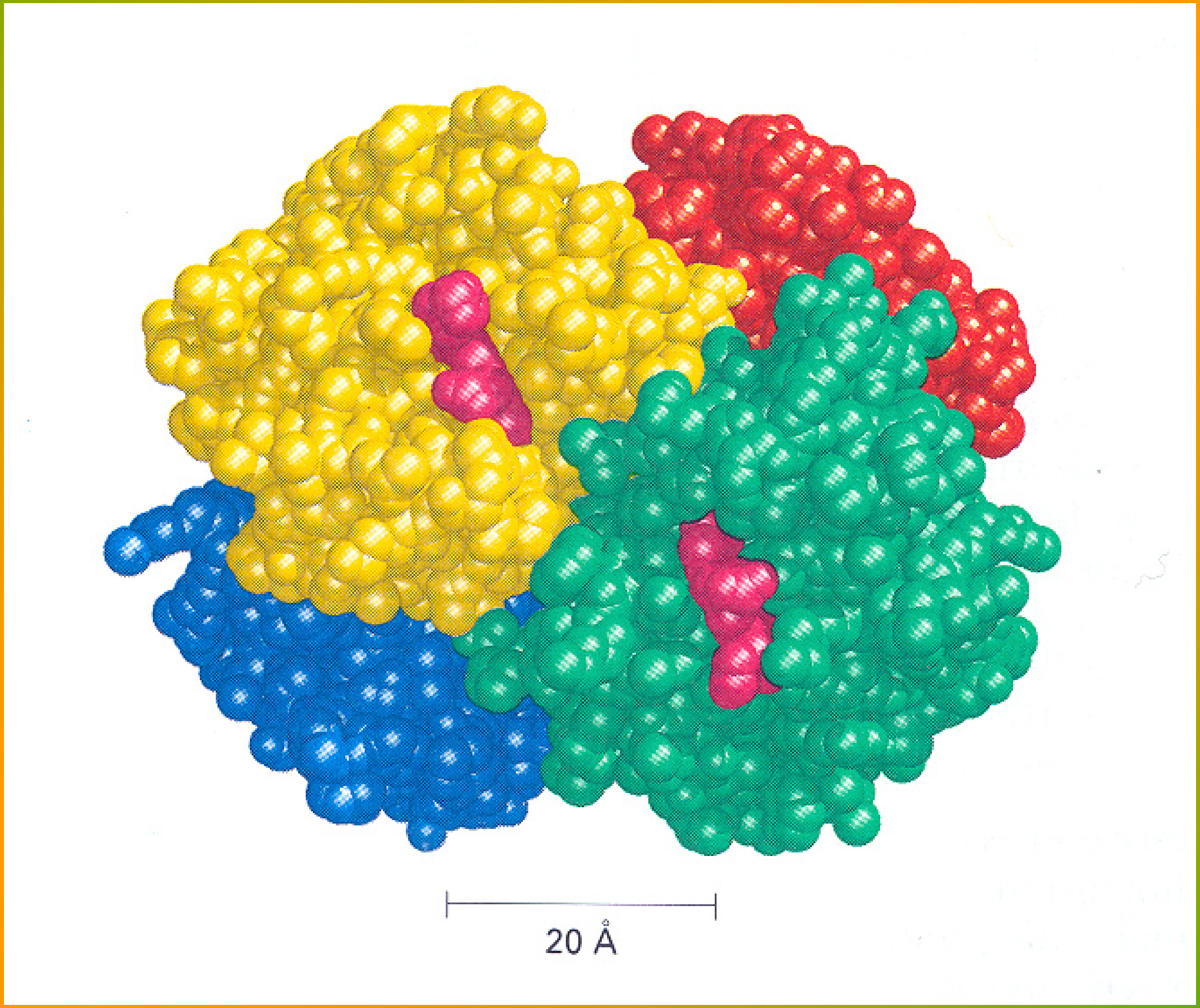
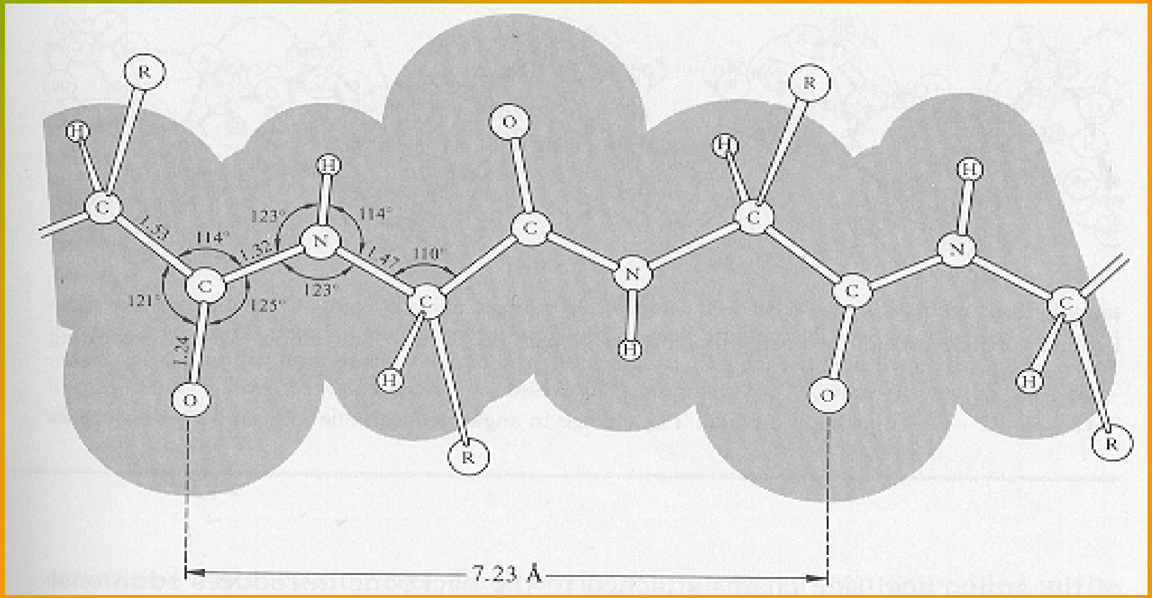


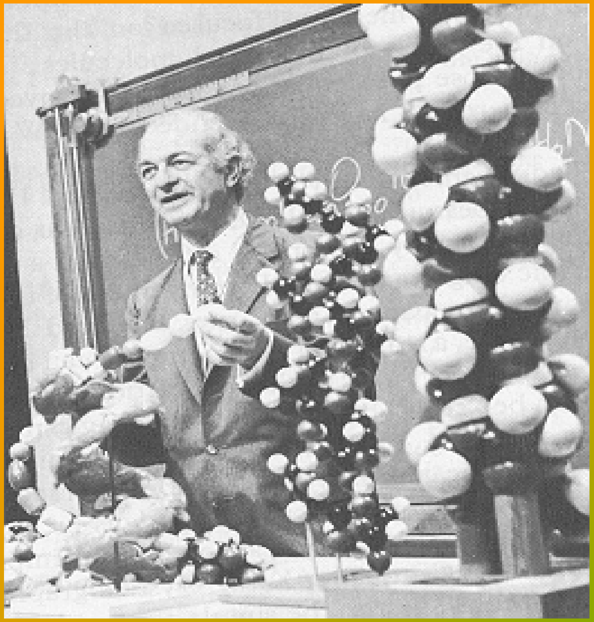
Figure 1–4. Molecular Biology of the Cell, 4th Edition.

Proteins



1951: Linus Pauling and the secondary structure of proteins





Pauling worked out the secondary structure of proteins by cristallographic analysis. From these data he constructed a model of a regular peptide backbone: the alpha-helix.

Secondary structure of proteins

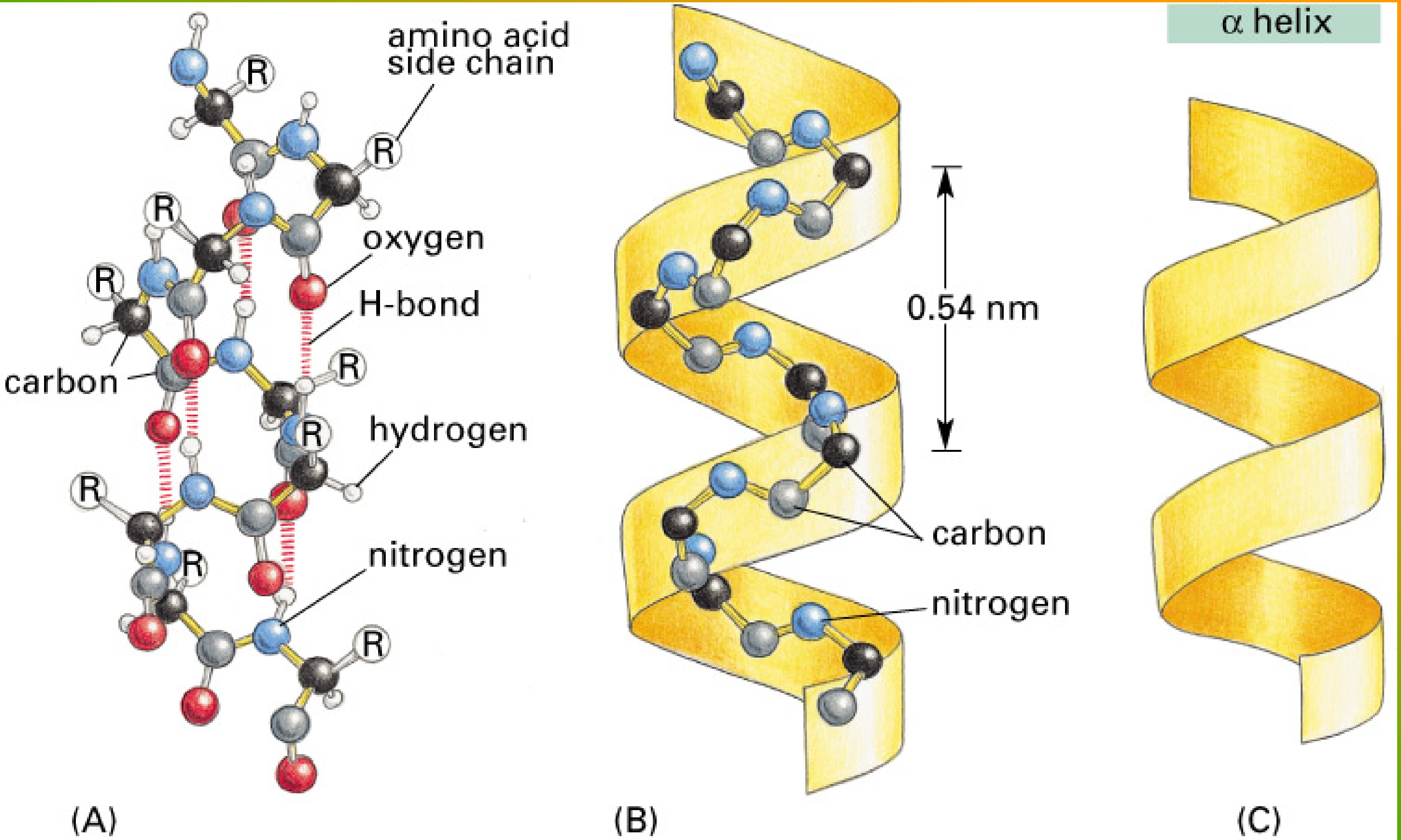
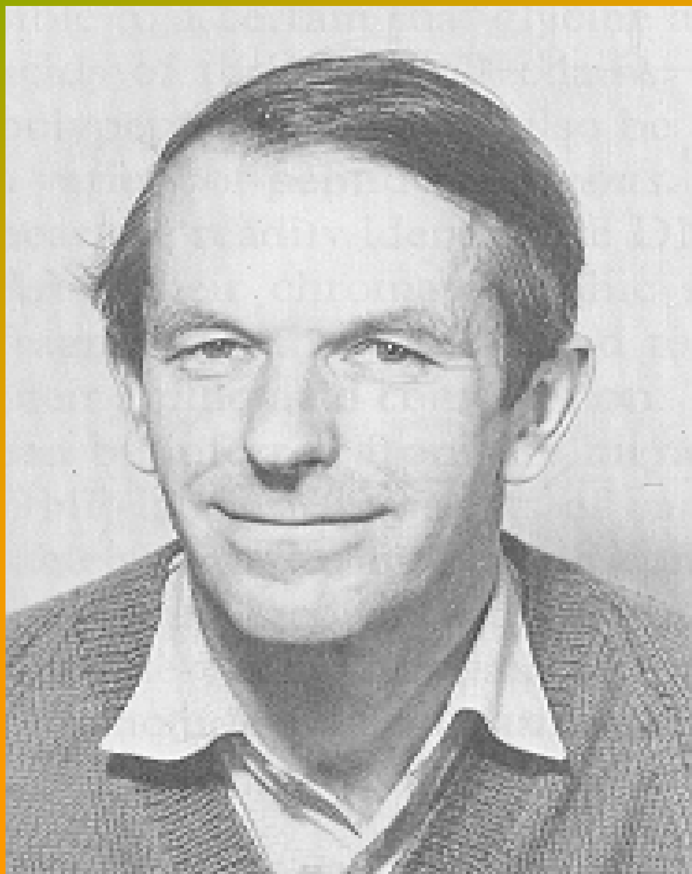
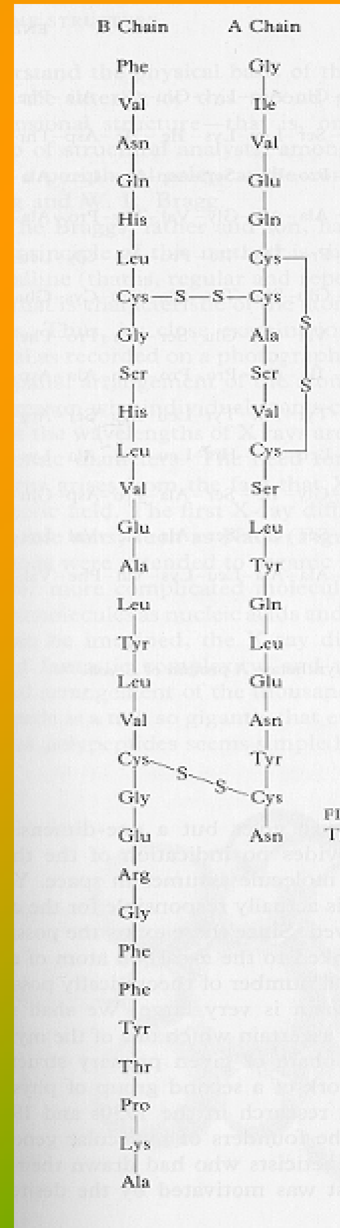


Figure 3–9 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

1955: Frederick Sanger



Reconstruction of the exact aminoacid sequence of the whole insulin molecule



Proteins are monomeric units bound together

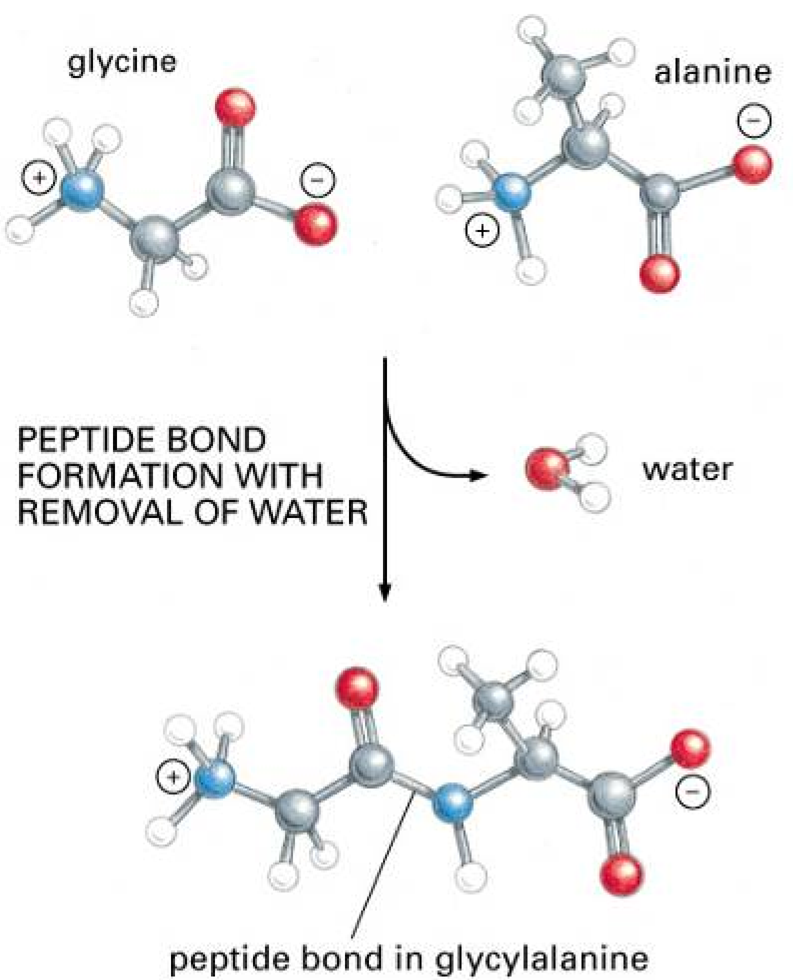


Figure 3–1. Molecular Biology of the Cell, 4th Edition.

Getting particular shape and function at pH 7.4

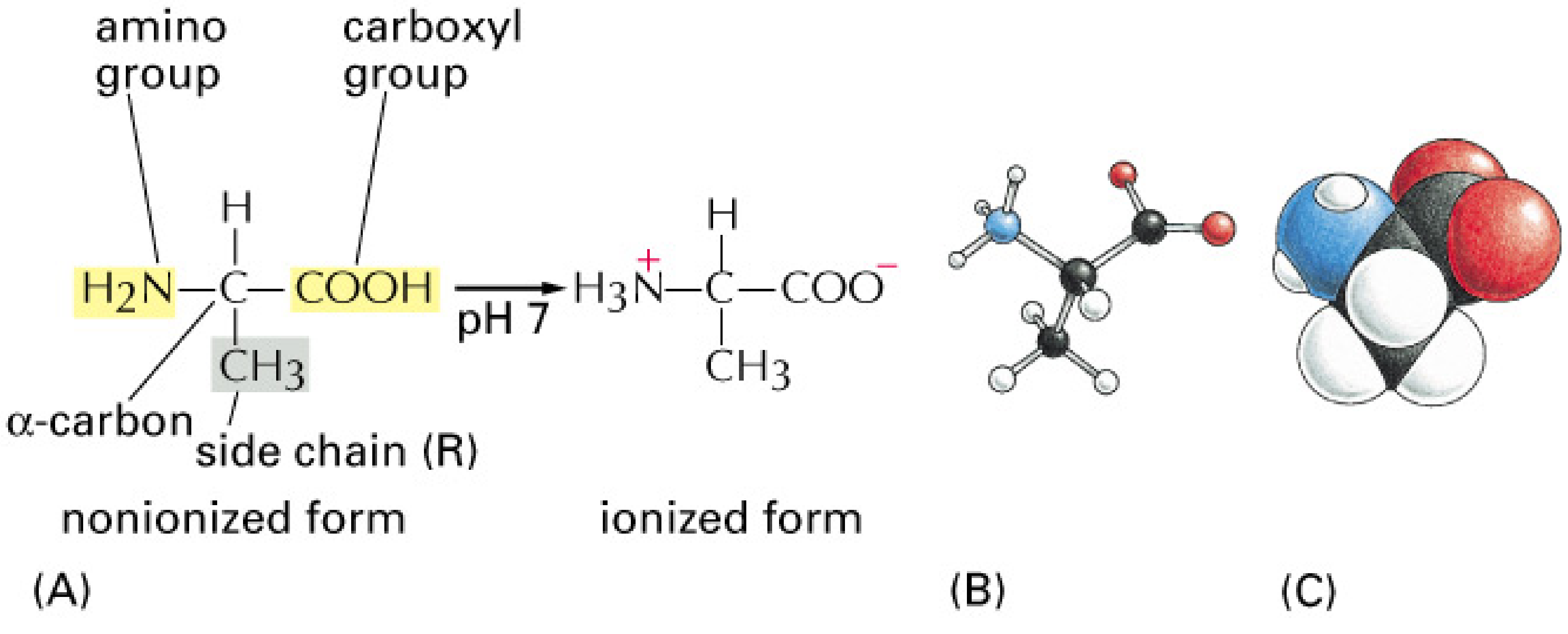
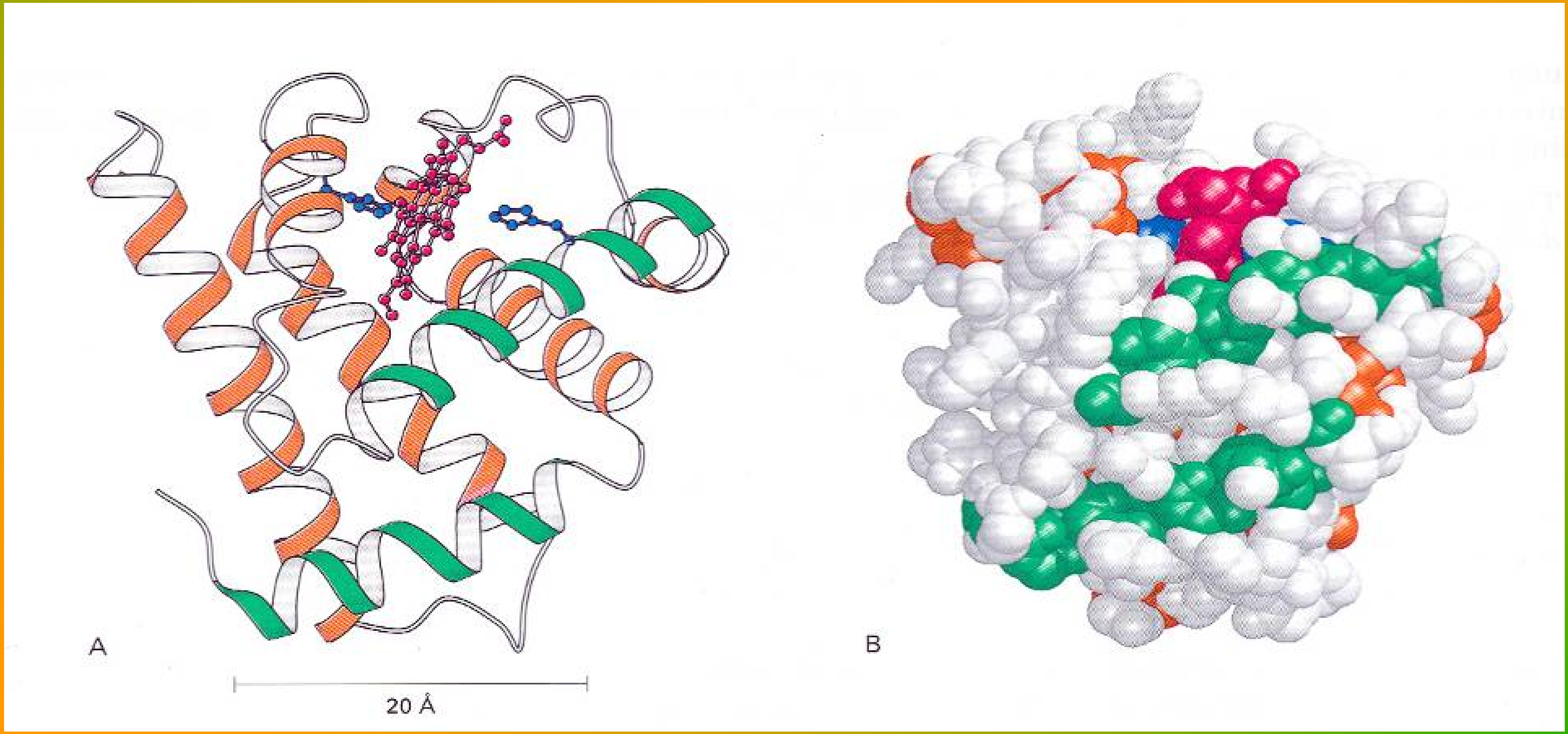


Figure 2–23. Molecular Biology of the Cell, 4th Edition.

The tridimensional structure of the protein is the direct consequence of its aminoacid sequence



And proteins shape our body

DNA is at every time accessible to DNA-binding proteins (regulation of gene expression)

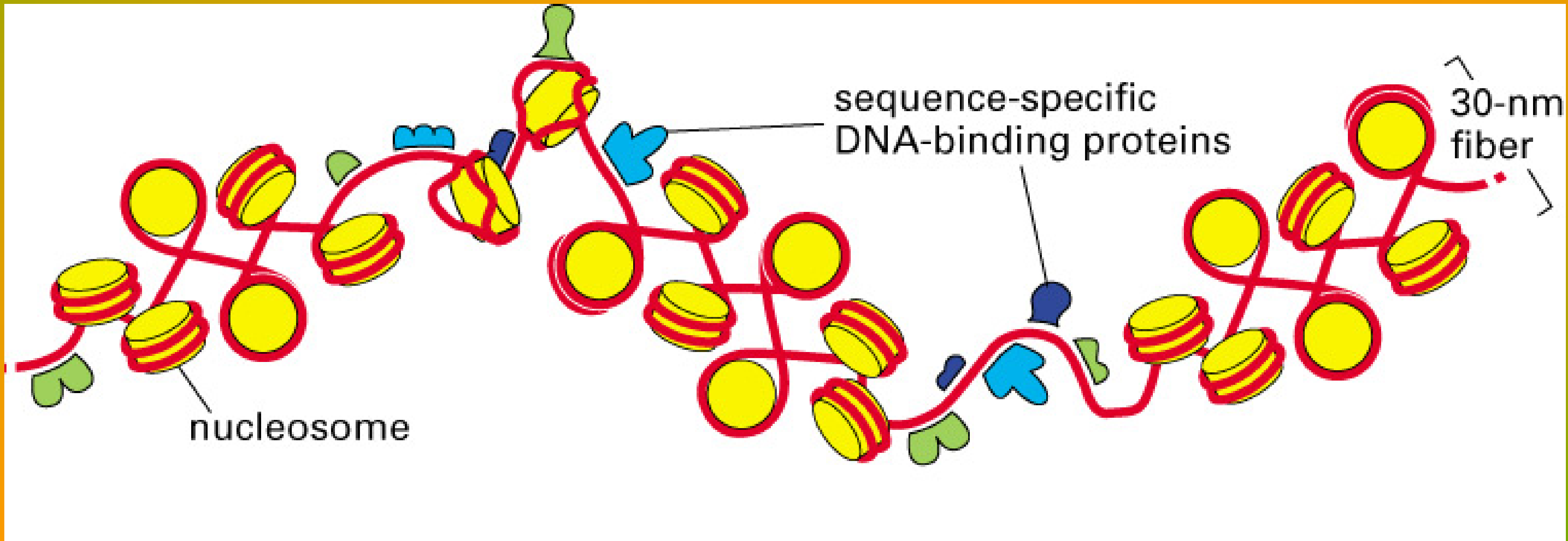


Figure 4–30. Molecular Biology of the Cell, 4th Edition.

Proteins and DNA interact continuosly

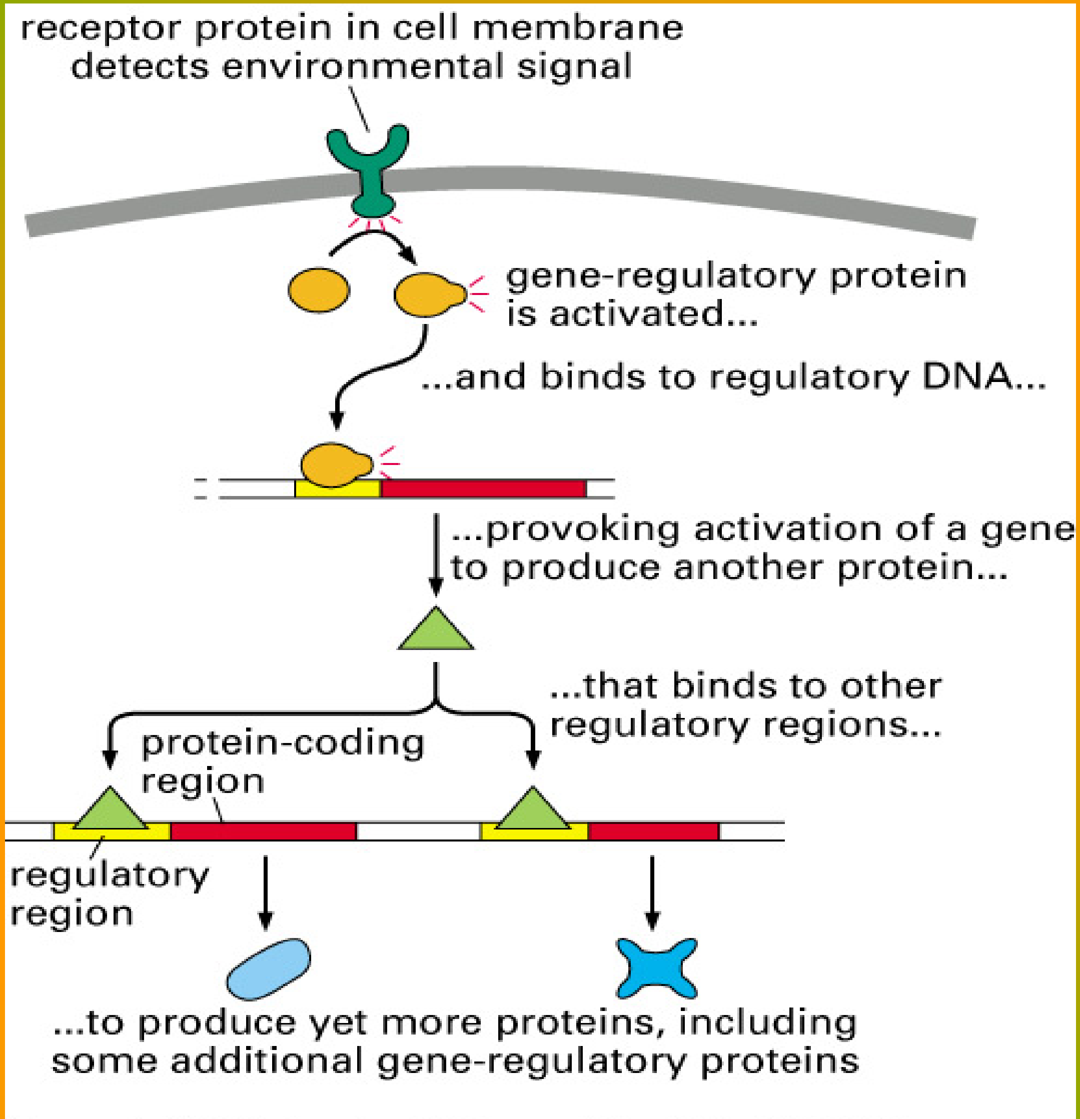
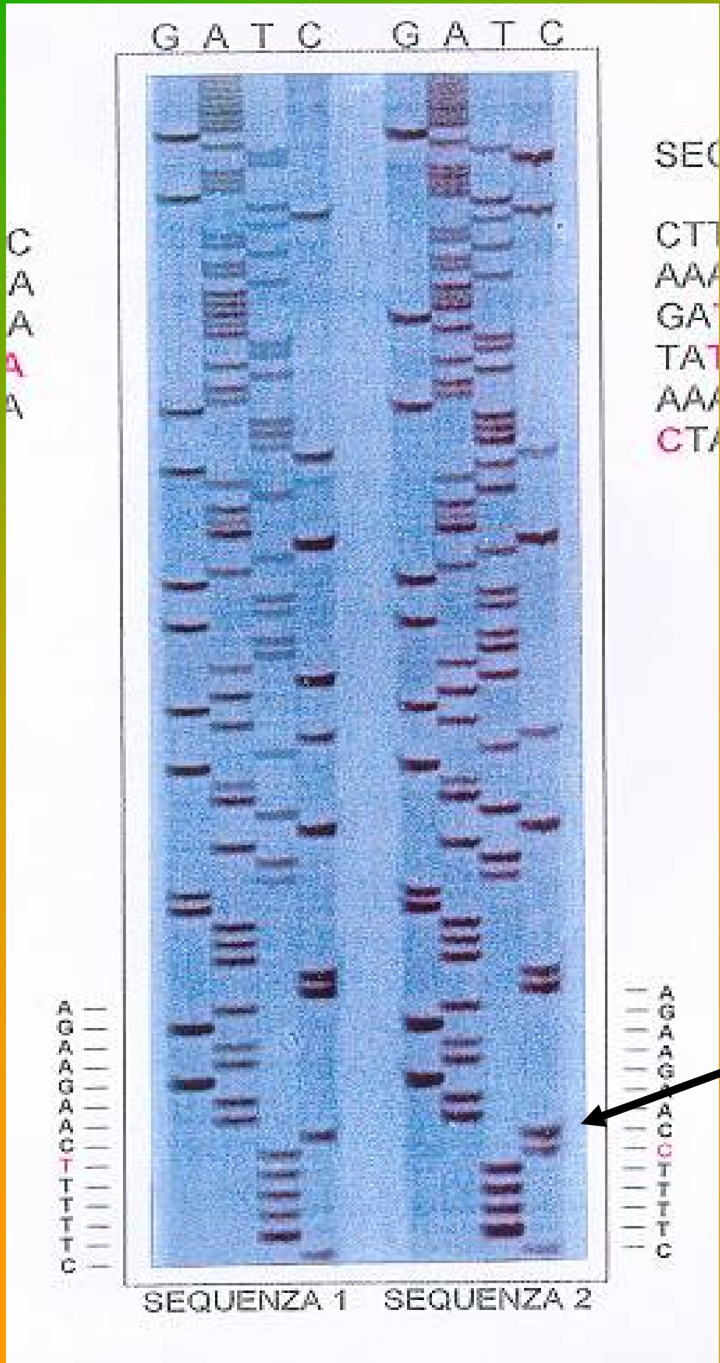


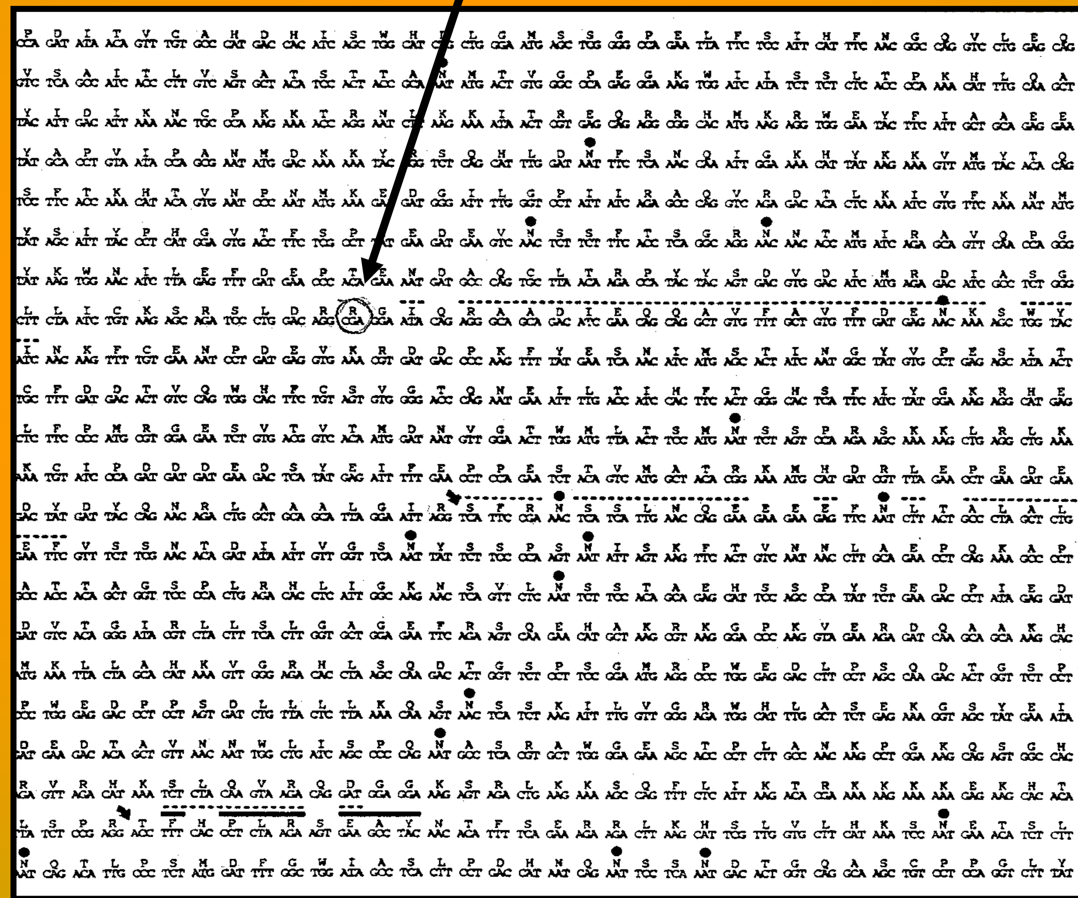
Figure 1–40. Molecular Biology of the Cell, 4th Edition.

DNA variation is at the origin of evolution

Figure 1–50. Molecular Biology of the Cell, 4th Edition.

Genes can mutate



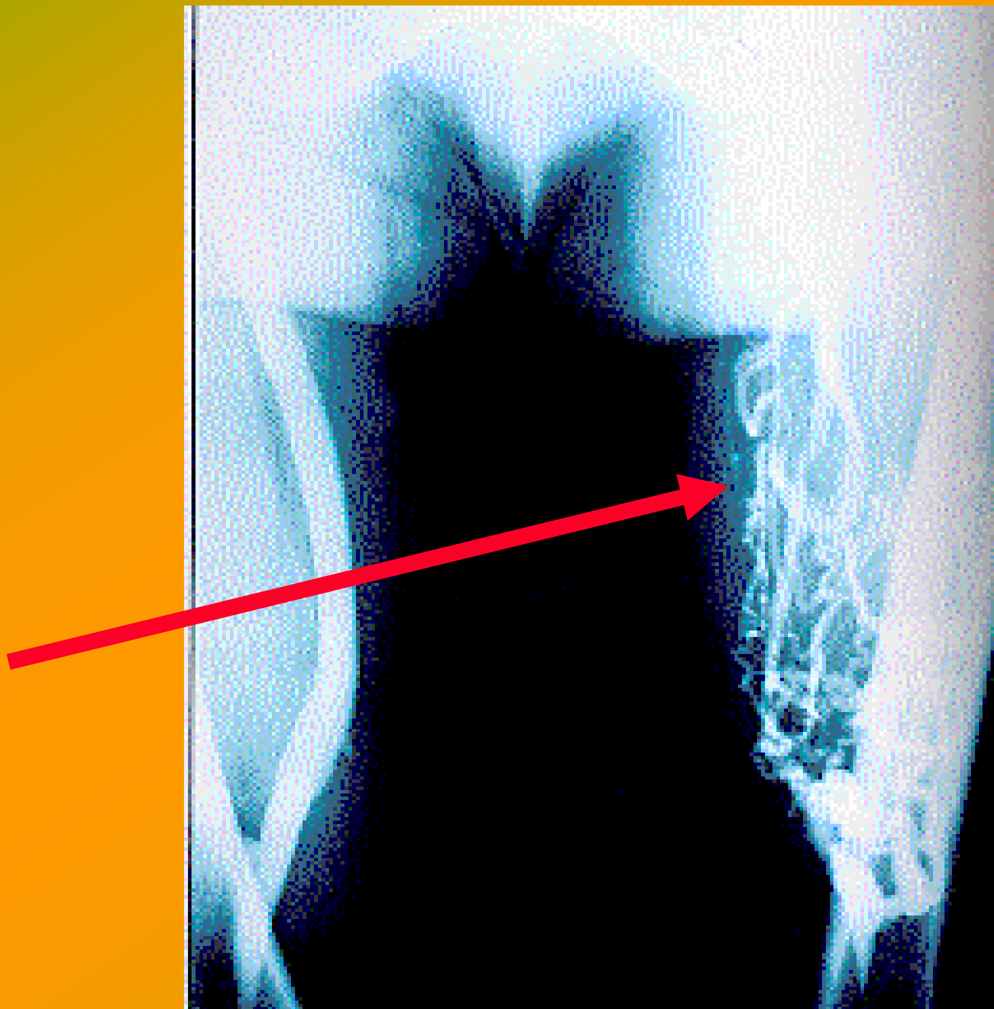


Mutation is different from SNP (Single Nucleotide Polymorphism)

- Mutazione: rare change in DNA sequence with deleterious effects

- SNP
  - more common genetic variation (>1% of the population)
  - actually more than 4 million SNPs are registered in DNA banks
  - they determine susceptibility to a particular pathology
  - they are responsible for a differentiated metabolism of drugs
  - they have no clinical significance (present in non-coding regions of DNA or do not induce any difference at the protein level)

Mutation is a change in DNA coding sequence leading to protein disfunction



Polymorphism is a more common change in DNA coding sequence leading to a change in protein function

Example the cytochrome 2D6 enzyme responsible for the metabolism of many dugs

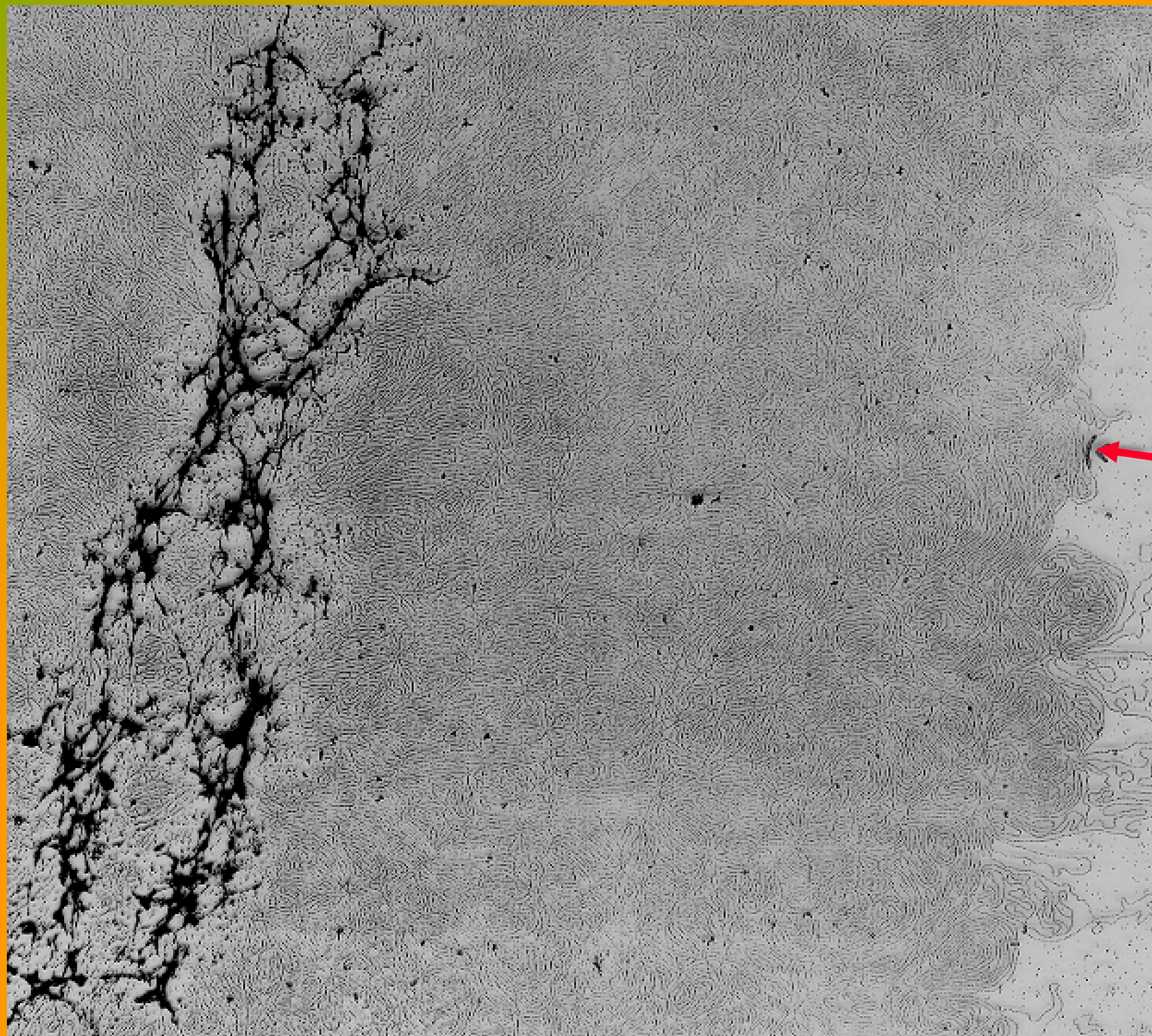
<u>CYP2D6*1 (WT with normal activity)</u>

...CATCTCCCACCCCCA<u>A</u>GGACGCCCCTTTCGC...

<u>CYP2D6*4 (with reduced activity)</u>

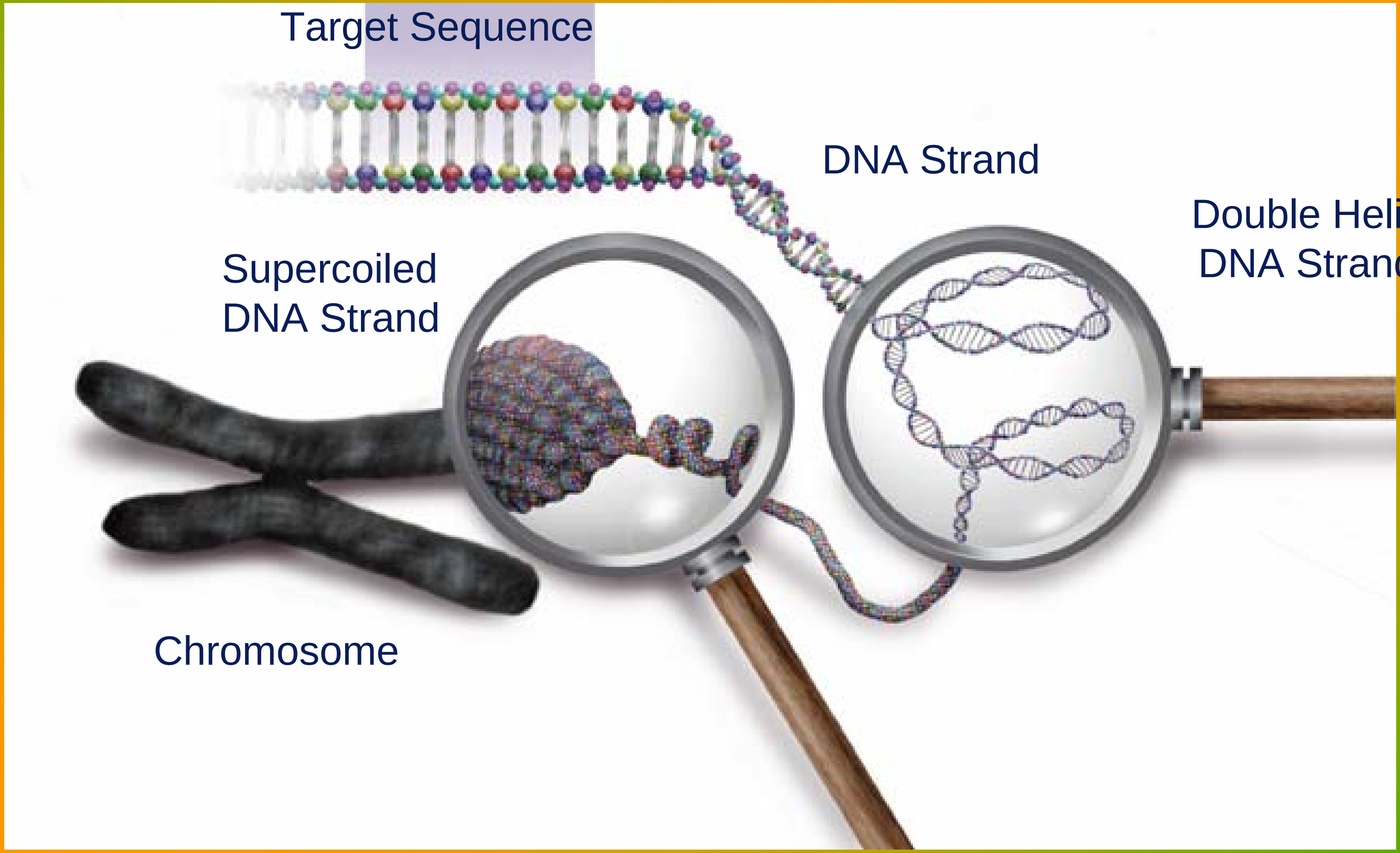
...CATCTCCCACCCCC<u>G</u>GGACGCCCCTTTCGC...

The game is…find the gene!

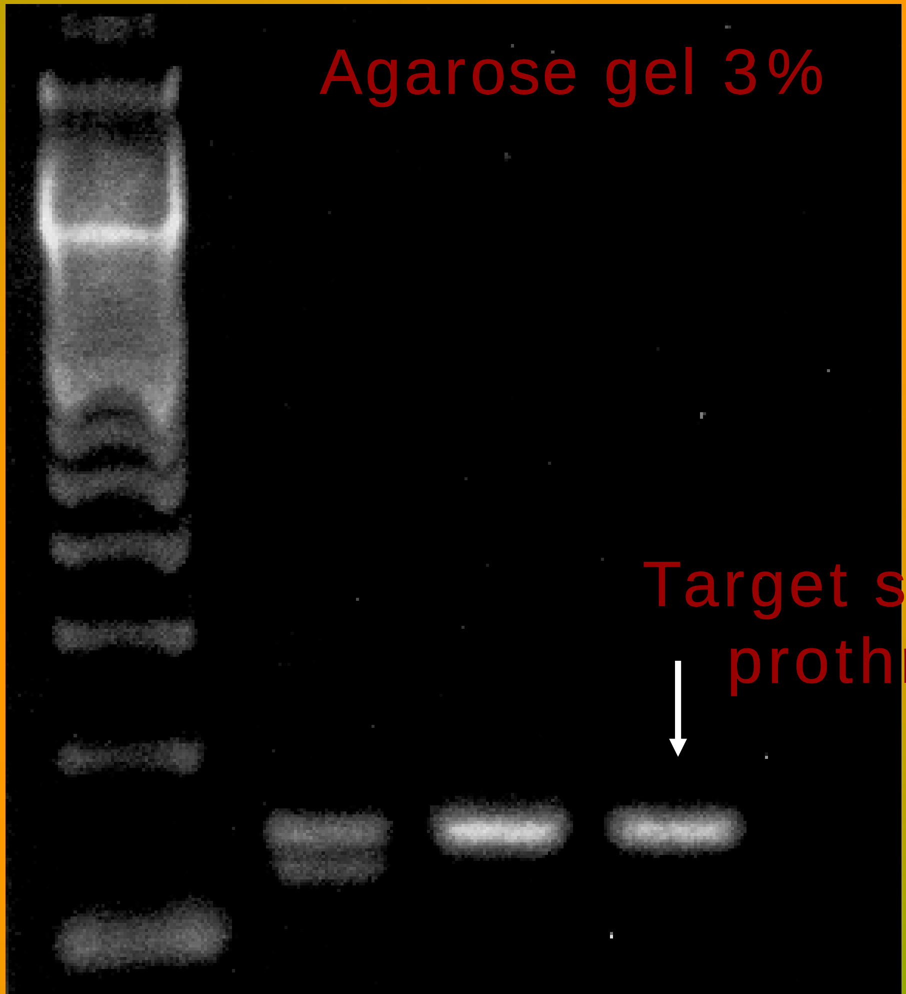


Here's one!

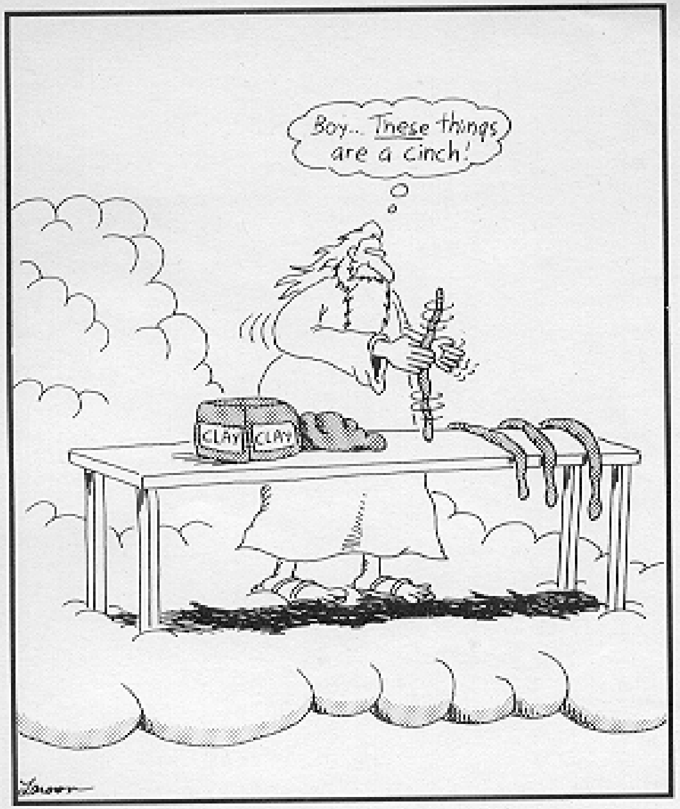
PCR Amplifies Targeted DNA Sequences



In billions of copies to render them visible by eye

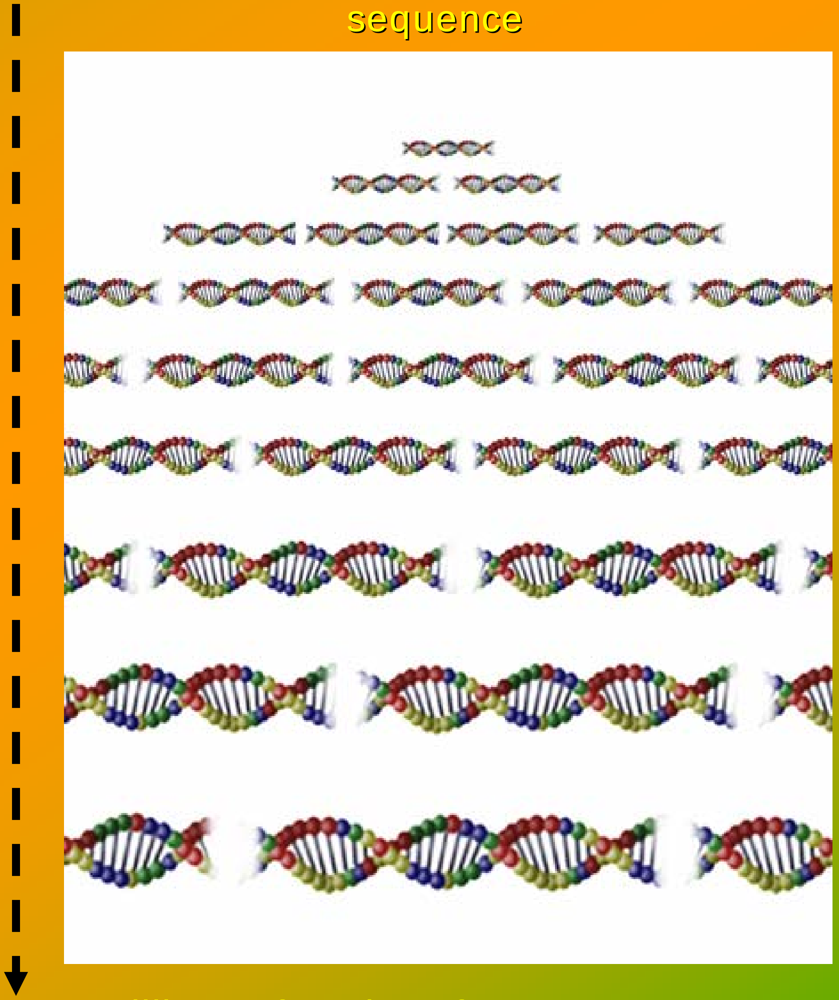


But how does PCR work?



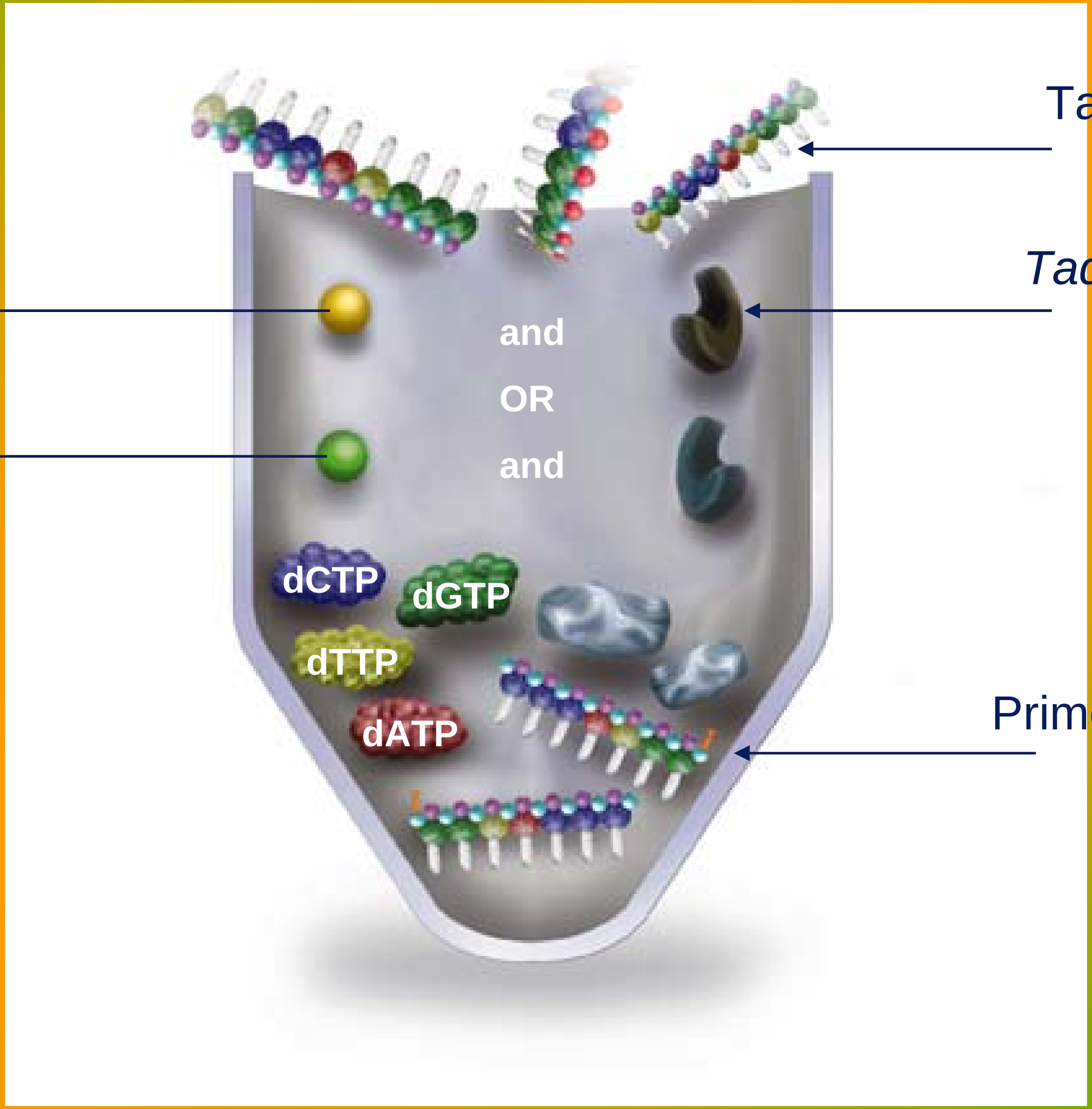
God makes the snake

1 copy of target sequence

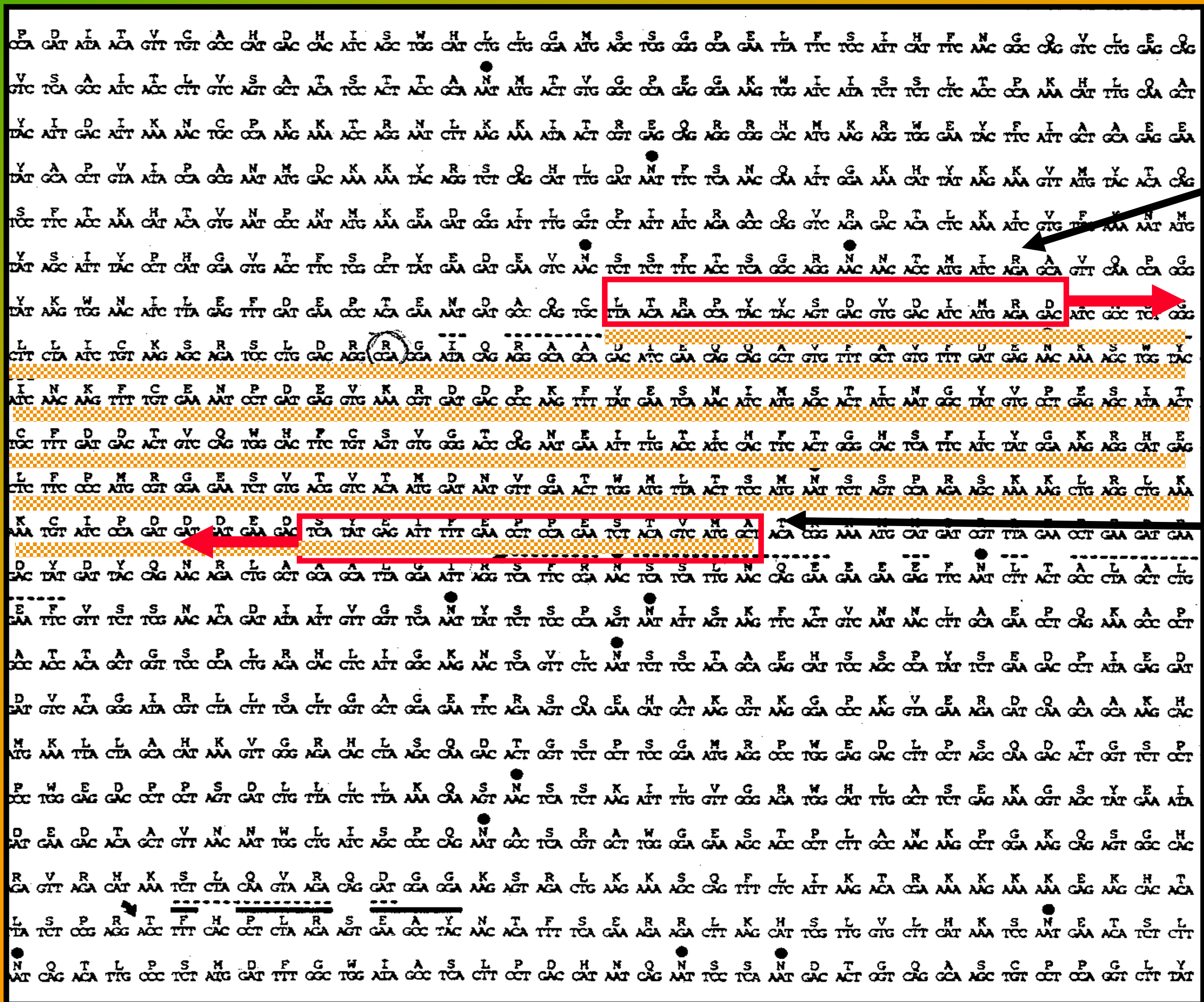


Billions of copies of target sequence

Cooking PCR: Master Mix Components



PRIMERS identify the sequence that will be amplified



A forward primer

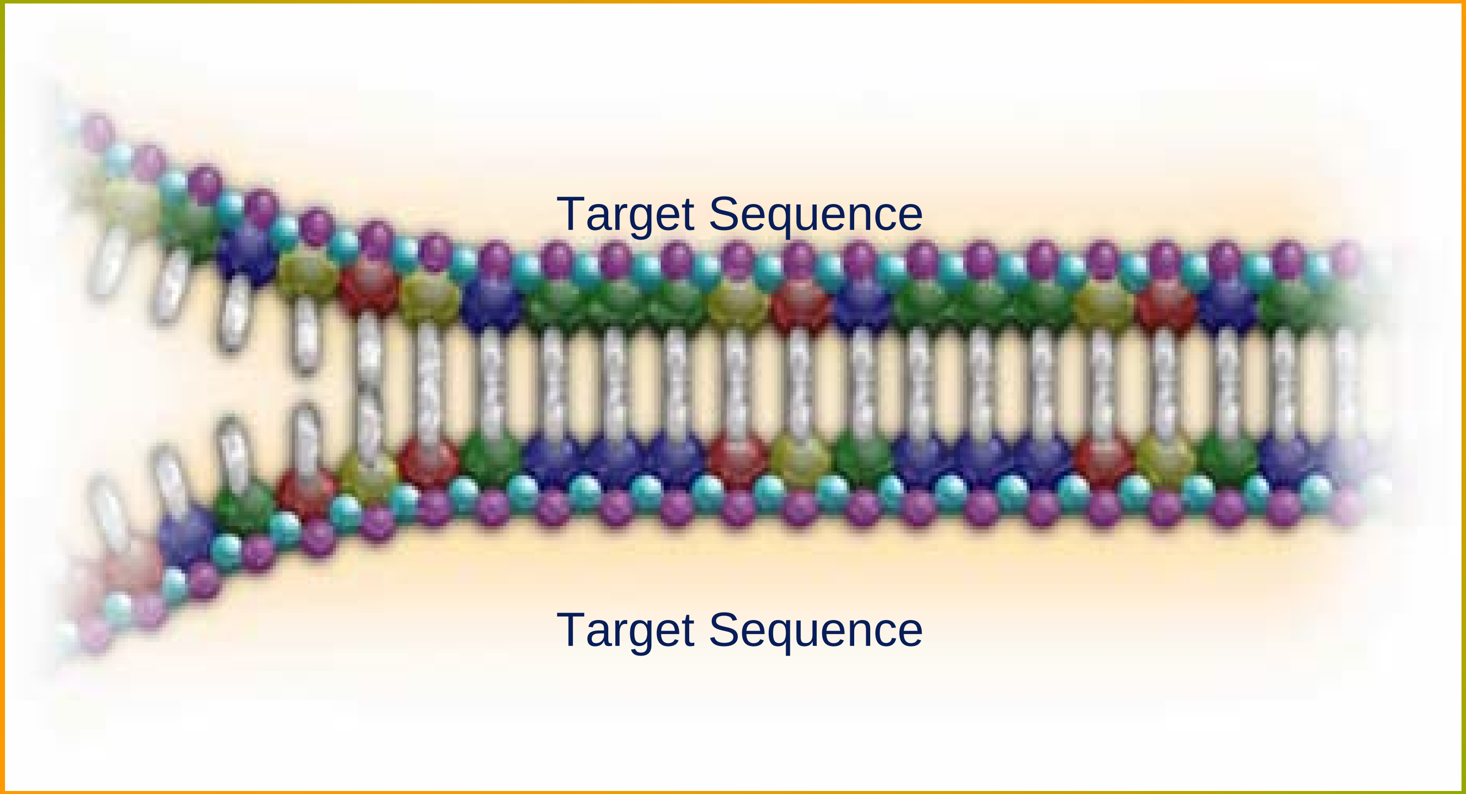
And a reverse primer

Taq Polymerase adds nucleotides to the growing amplicon

	AmpliTaq	Stoffel	Vent	Pfu
Molecular weight	94kD	61kD	?	92kD
Processivity	50-60 nt	5-10 nt	30-40 nt	?
Extension rate	75nt/sec	>50nt/sec	>80nt/sec	60nt/sec
Thermostability half-life time at - 97.5°C	10min	20min	130min	>3h
- 95.0°C	40min	90min	360min	>2h
- 92.5°C	130min	?	?	?
5'-3'-exonuclease activity (pmol/sec/pmol enzyme)	0.3pmol	<0.00001pmol	?	?
3'-5'-exonuclease activity	no	no	yes	yes
Mg^{2+} optimum	1.5mM	3.0mM	2.0mM	1.5-2.0mM
KCl optimum	50mM	10mM	10mM	10mM
Reverse transcriptase activity	(yes)	?	?	?

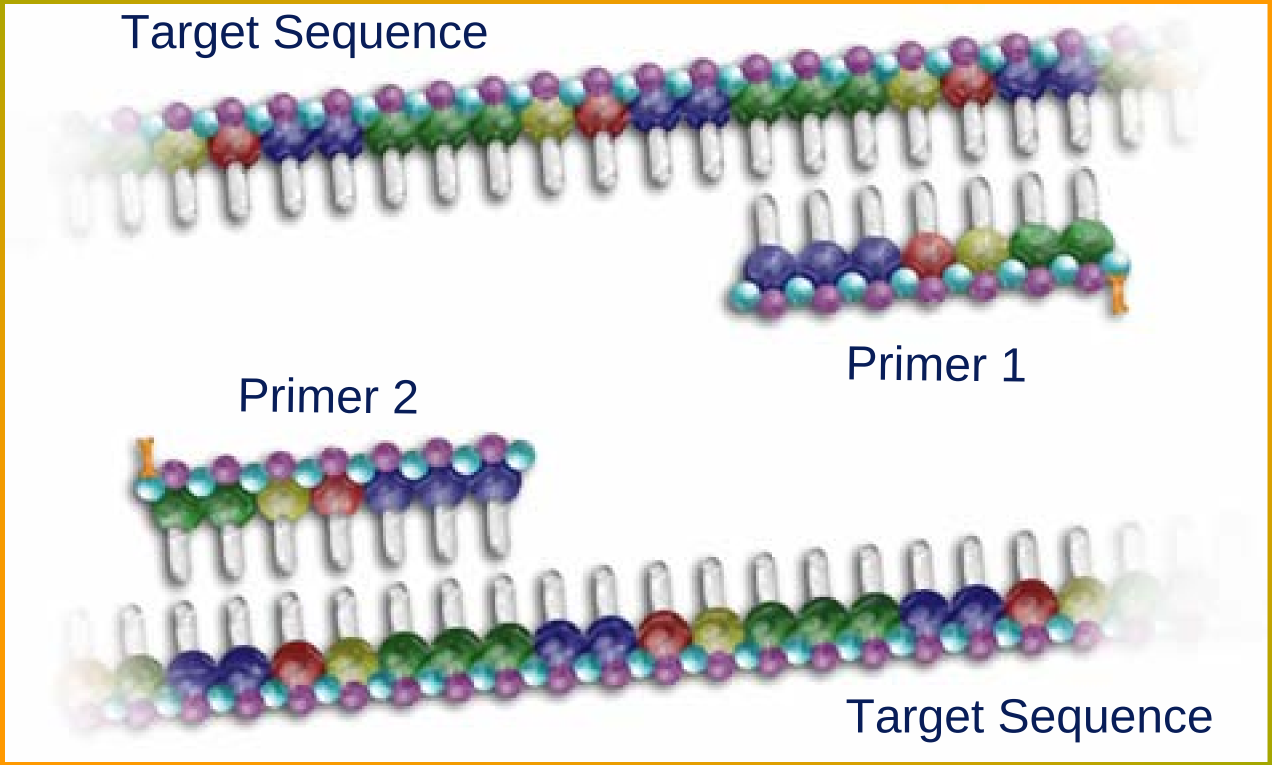
Table 21.2: Some characteristics of different polymerases

PCR Cycle - Step 1 - Denaturation by Heat



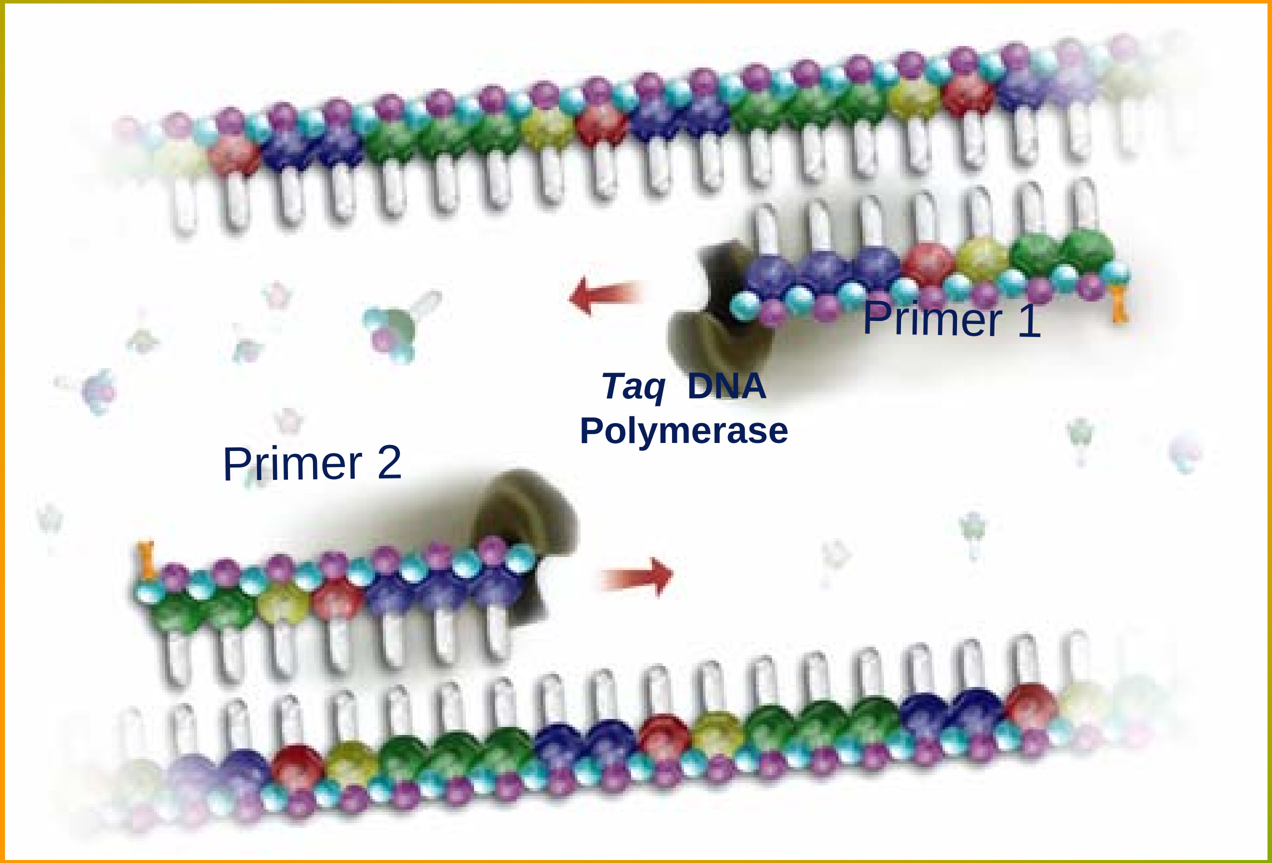
Hydrogen bond denaturation (94°C)

PCR Cycle - Step 2 - Primer pair anneals to ends of target sequence



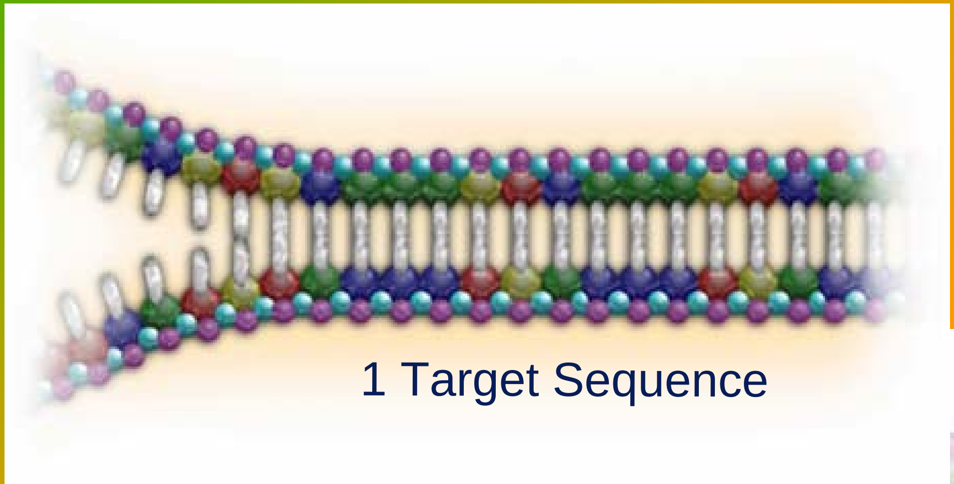
Annealing of primers (58-68°C)

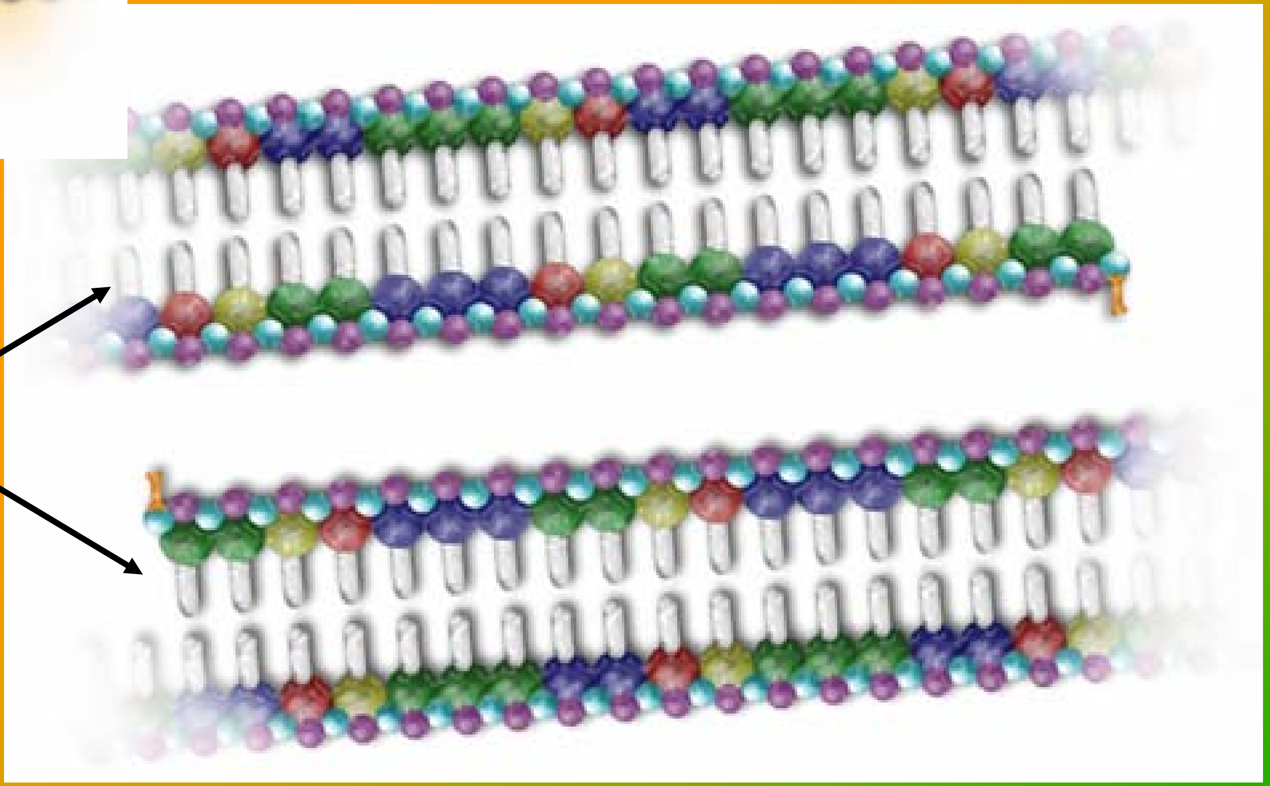
PCR Cycle - Step 3 - *Taq* DNA Polymerase Catalyses Primer Extension as Complementary Nucleotides are Incorporated



Extension of the target sequence copy (72°C)

End of the 1st PCR cycle results in two copies of the target sequence





2 identical Target Sequences

PCR: the first cycle

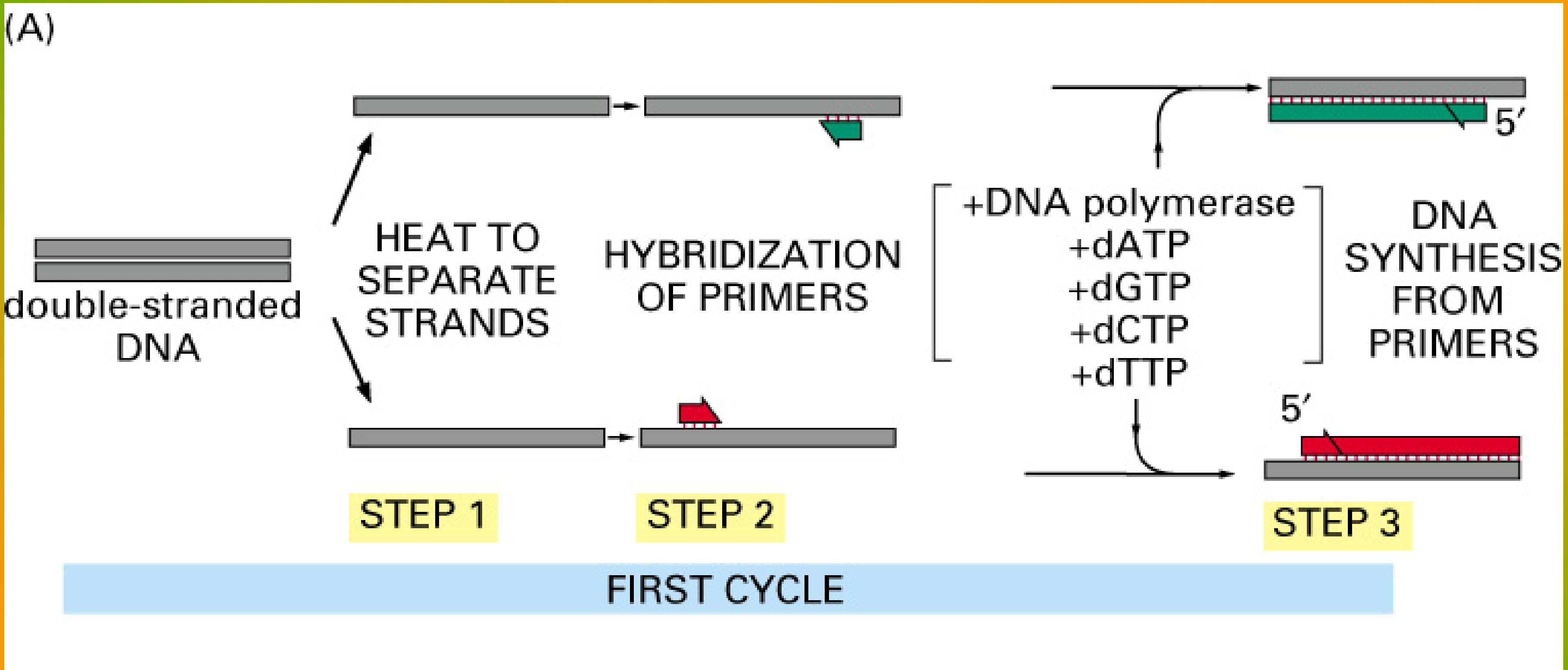


Figure 8–39 part 1 of 3. Molecular Biology of the Cell, 4th Edition.

PCR: the second cycle

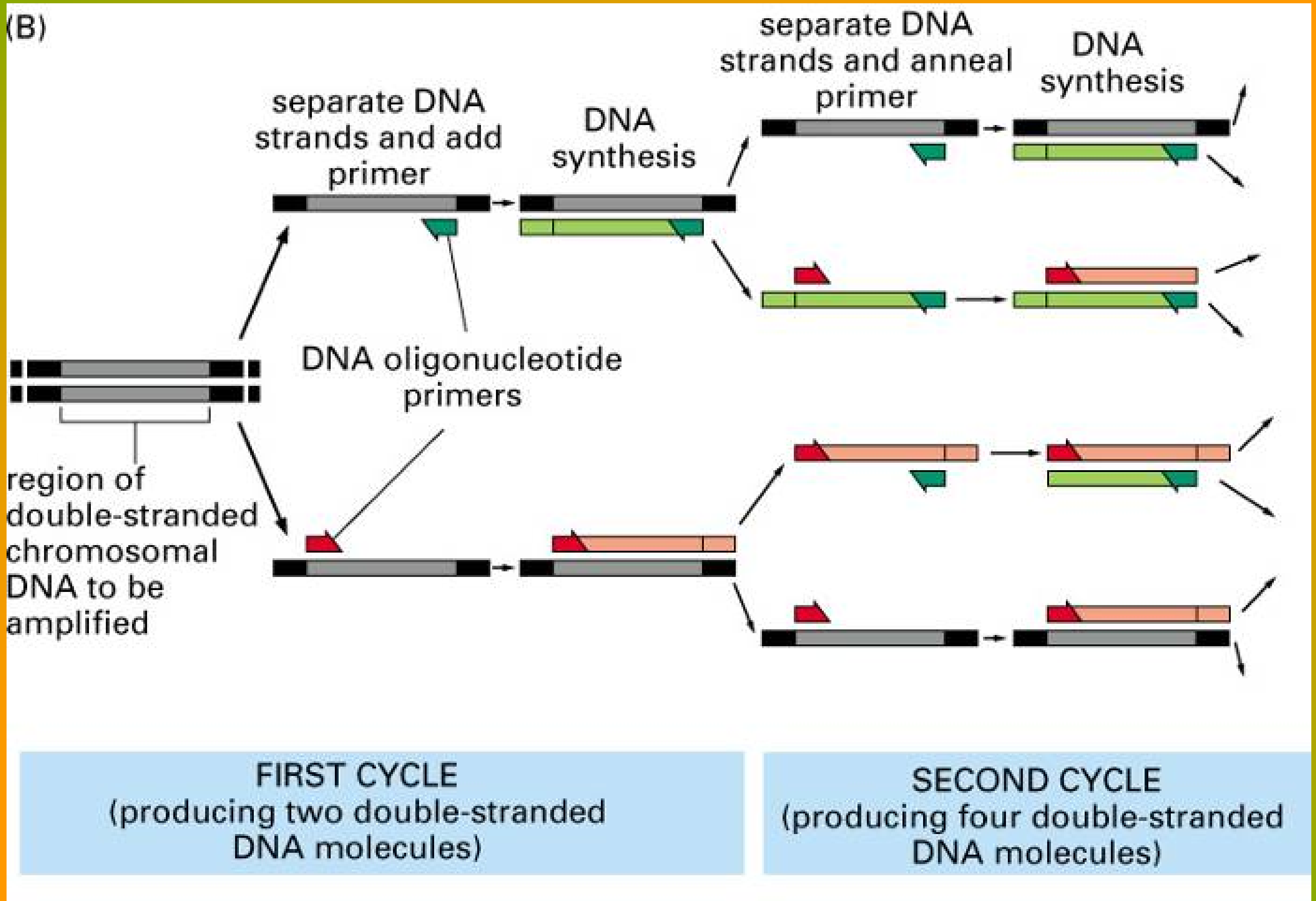


Figure 8–39 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

PCR: the third cycle

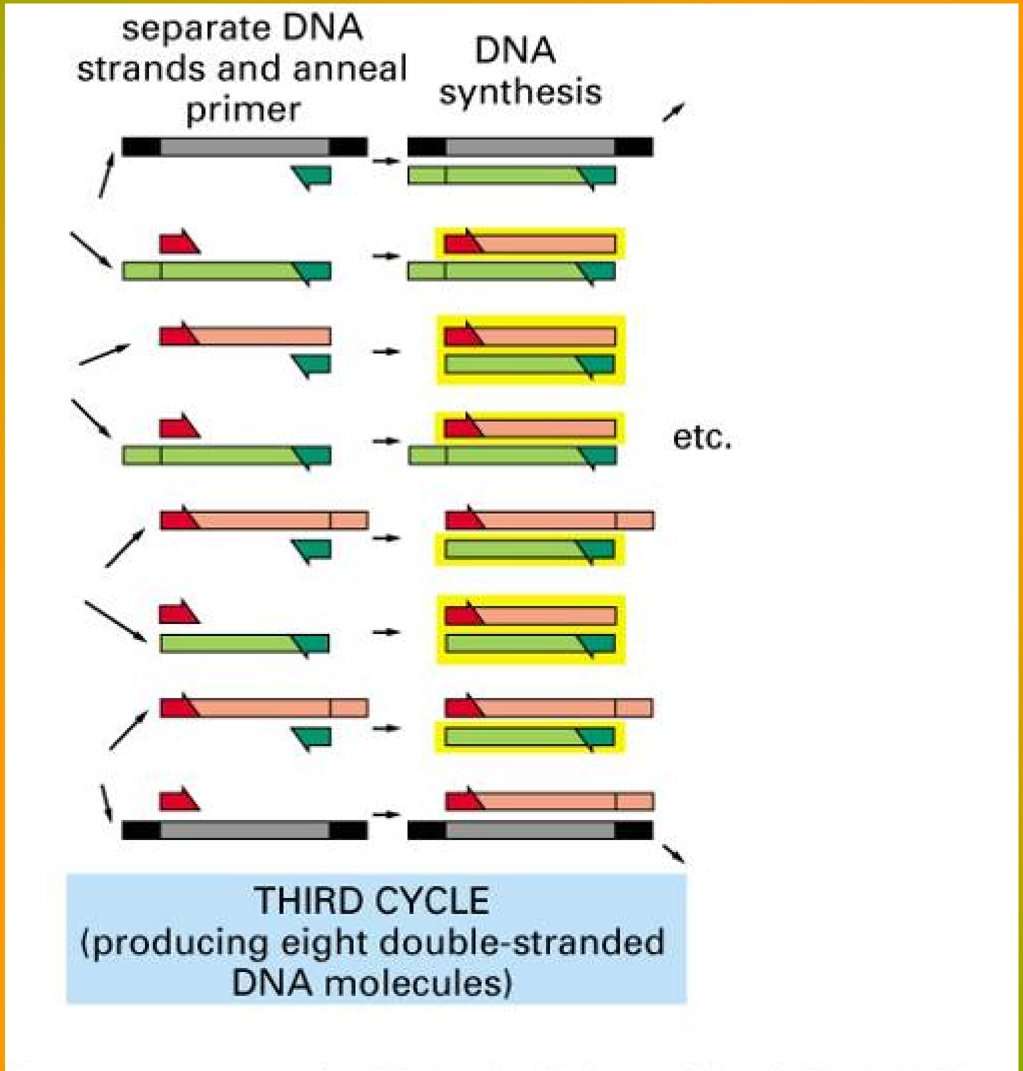
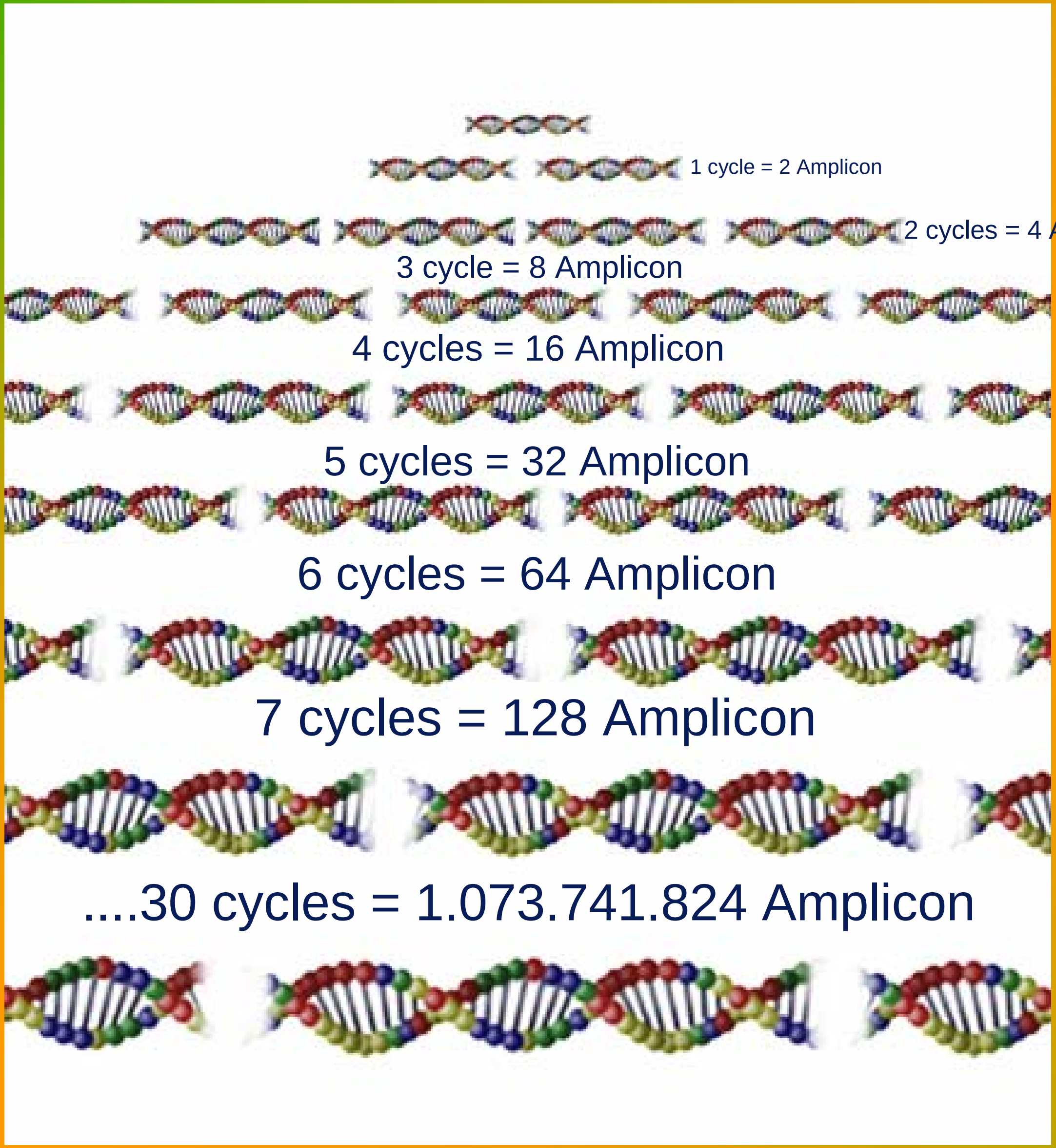


Figure 8–39 part 3 of 3. Molecular Biology of the Cell, 4th Edition.

Target Amplification



$$2^n$$

No. Of cycles (n)	No. Of amplicon copies of the target
1	2
2	4
3	8
4	16
5	32
6	64
20	1,048,576
30	1,073,741,824

Using PCR in diagnostics. An example: Hemochromatosis

- Autosomal recessive disease of iron overload due to intestinal hyperabsorption
- Common genetic disorder: approximately 1 in 200/300 humans
- The mutation C282Y that causes iron overload is relatively frequent (1 to 5 % of the general population)
- Storage of iron in the liver and other tissues cause a number of symptoms
- The symptoms appear frequently above age of 40

Design of primers in the HFE gene outside the C282Y mutation (expected amplicon lenght = 307 bp)

```
1   ttttctgaaa agggtatttc cttcctccaa cctatagaag gaagtgaaag ttccagtctt
61  cctggcaagg gtaaacagat cccctctcct catccttcct ctttcctgtc aagtgcctcc
121 tttggtgaag gtgacacatc atgtgacctc ttcagtgacc actctacggt gtcgggcctt
181 gaactactac ccccagaaca tcaccatgaa gtggctgaag gataagcagc caatggatgc
241 caaggagttc gaacctaaag acgtattgcc caatggggat gggacctacc agggctggat
301 aaccttggct gtaccccctg gggaagagca gagatatacg tgccaggtgg agcacccagg
361 cctggatcag cccctcattg tgatctgggg tatgtgactg atgagagcca ggagctgaga
421 aaatctattg gggttgaga ggagtgcctg aggaggtaat tatggcagtg agatgaggat
481 ctgctctttg ttagggggtg ggccgagggt ggcaatcaaa ggctttaact tgcttttttct
541 gttttagagc cctcaccgtc tggcacccta gtcattggag tcatcagtgg aattgctgtt
601 tttgtcgtca tcttgttcat tggaattttg ttcataatat taaggaagag gcagggttca
661 agtgagtagg aacaaggggg aagtctctta gtacctctgc cccagggcac agtgggaaga
721 ggggcagagg gga
```

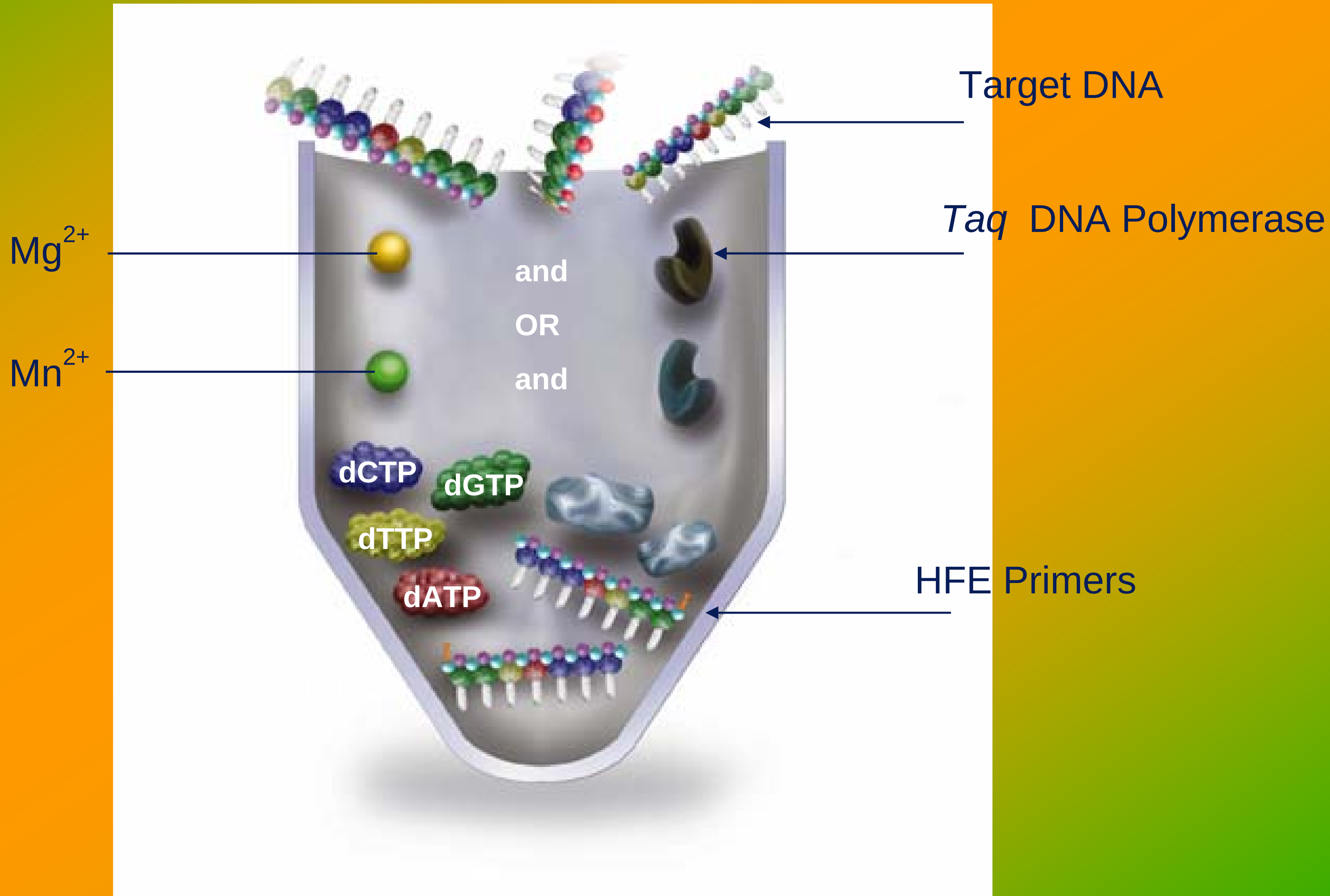
Forward primer

Site of mutation

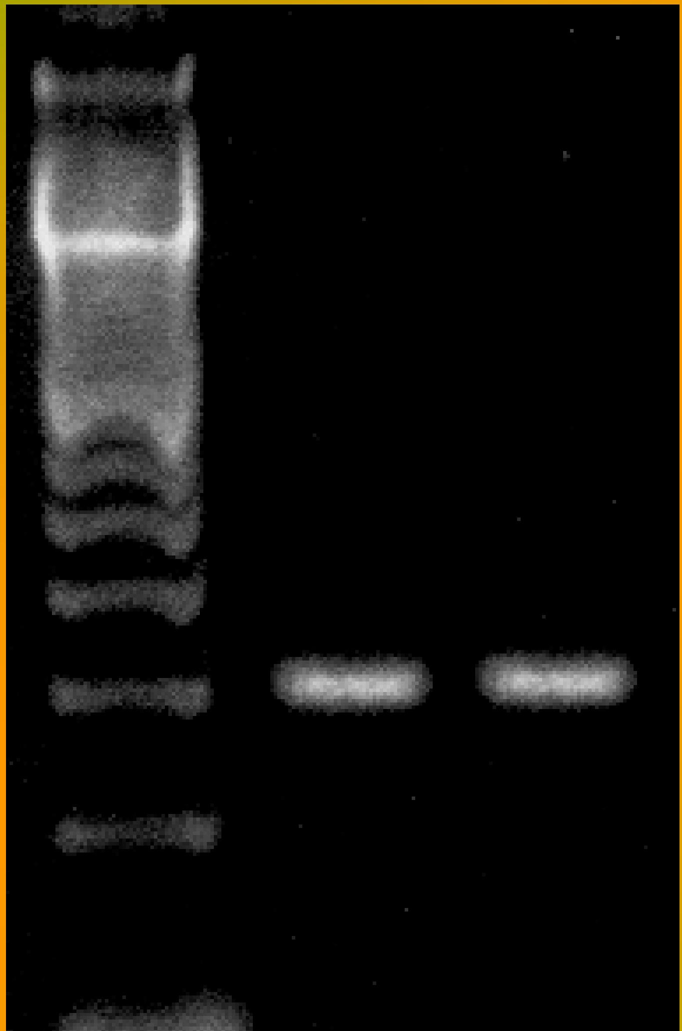
Reverse primer

TGC = Cys —C282Y→ TAC = Tyr

PCR for the amplification of the HFE gene

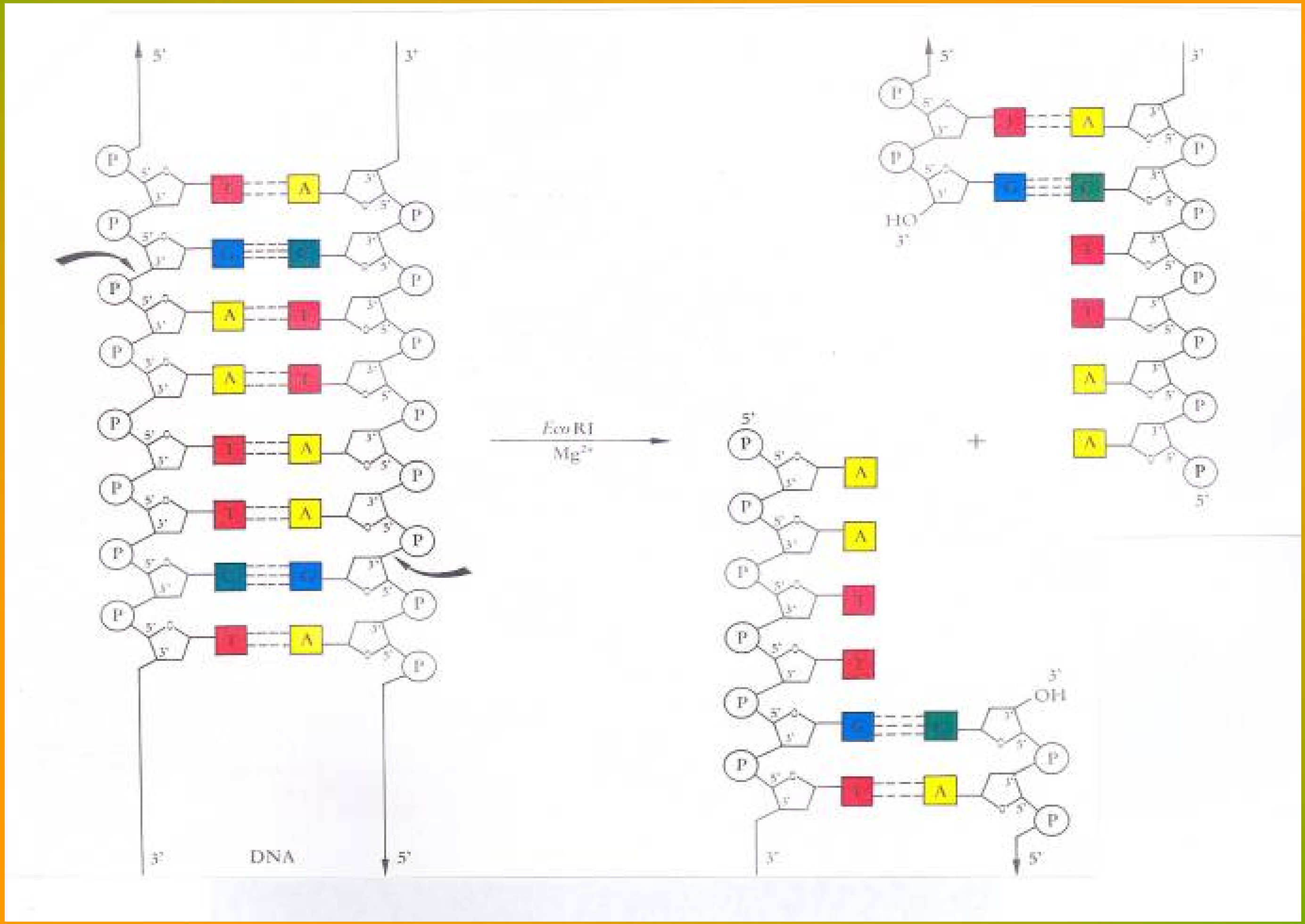


And expected PCR product



307 bp

Find a restriction enzyme that cuts the mutated base (C282Y)



Restriction sites in the HFE gene amplicon

```
1   ttttctgaaa agggtatttc cttcctccaa cctatagaag gaagtgaaag ttccagtctt
61  cctggcaagg gtaaacagat cccctctcct catccttcct ctttcctgtc aagtgcctcc
121 tttggtgaag gtgacacatc atgtgacctc ttcagtgacc actctacggt gtcgggcctt
181 gaactactac ccccagaaca tcaccatgaa gtggctgaag gataagcagc caatggatgc
241 caaggagttc gaacctaaag acgtattgcc caatggggat gggacctacc agggctggat
301 aaccttggct gtaccccctg gggaagagca gagatatacg tgccaggtgg agcacccagg
361 cctggatcag cccctcattg tgatctgggg tatgtgactg atgagagcca ggagctgaga
421 aaatctattg ggggttgaga ggagtgcctg aggaggtaat tatggcagtg agatgaggat
481 ctgctctttg ttagggggtg ggccgagggt ggcaatcaaa ggctttaact tgctttttct
541 gttttagagc cctcaccgtc tggcacccta gtcattggag tcatcagtgg aattgctgtt
601 tttgtcgtca tcttgttcat tggaattttg ttcataatat taaggaagag gcagggttca
661 agtgagtagg aacaaggggg aagtctctta gtacctctgc cccagggcac agtgggaaga
721 ggggcagagg gga
```

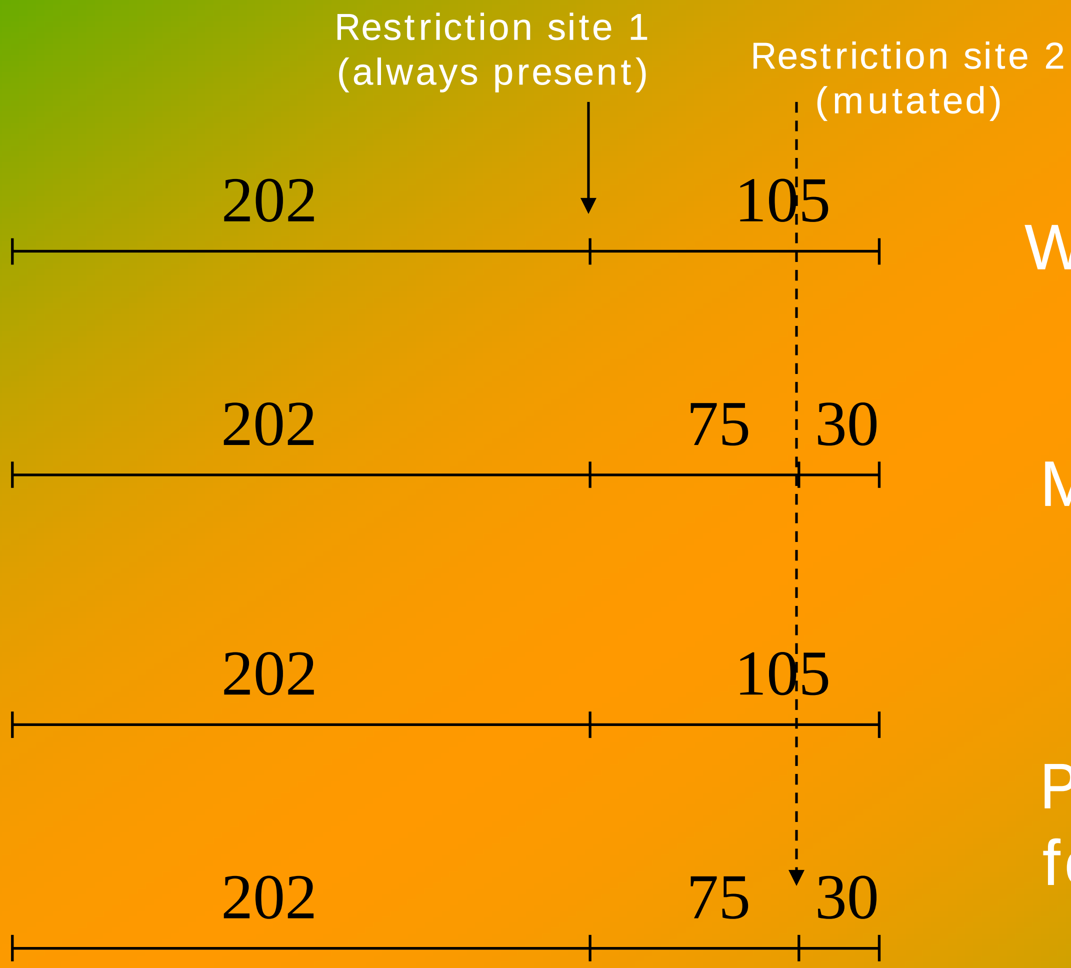
Restriction site 1 (always present)

Restriction site 2 (present only if mutated)

C282Y

TGC = Cys → TAC = Tyr

Enzimatic restriction (Rsa I) of the PCR product



Wild-type gene

Mutated gene

Pazient heterozygous
for the mutation

Results