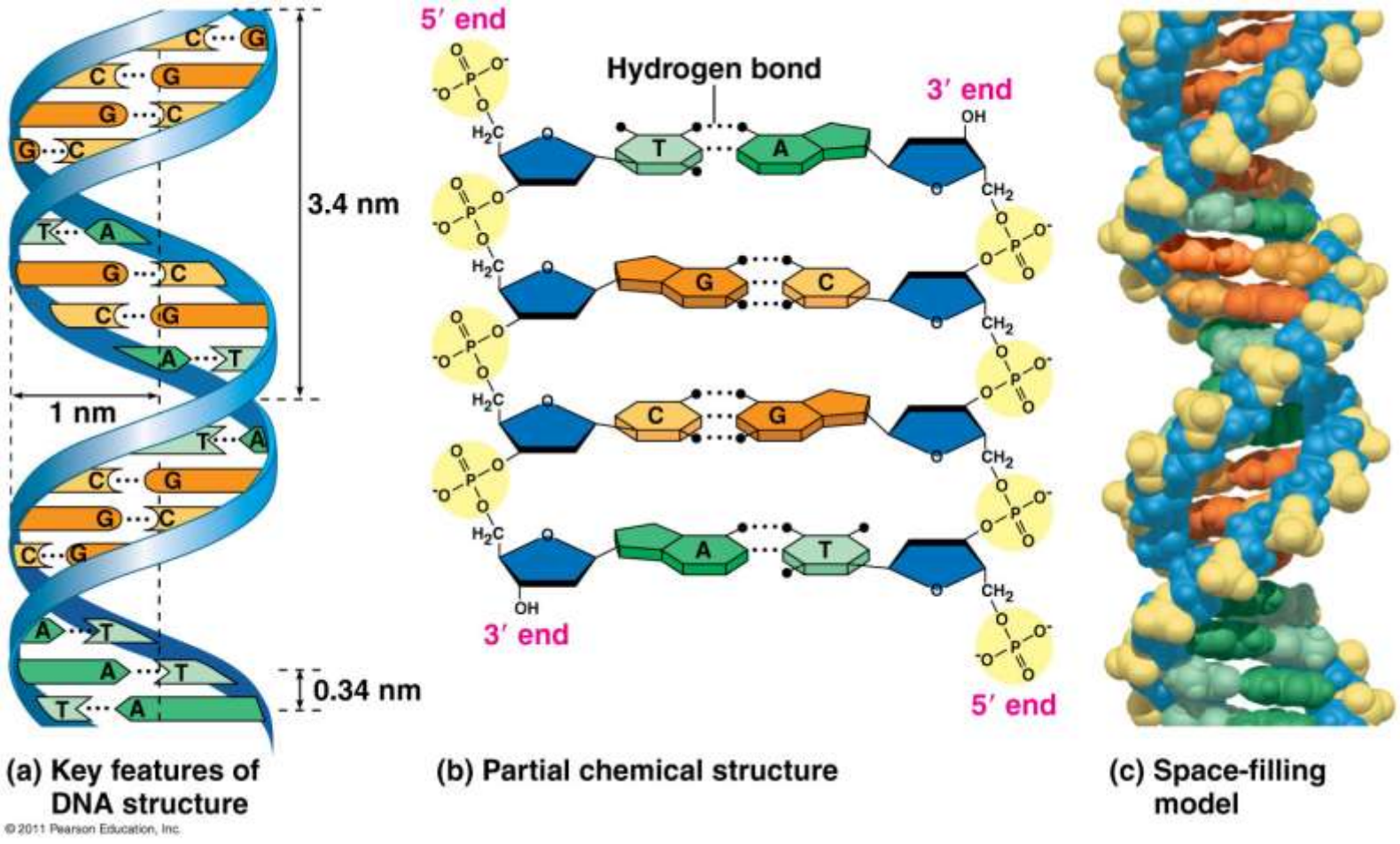
Lecture 15 – The Molecular Basis of Inheritance

In this lecture…

- Structure of DNA and chromatin
- Watson and Crick's experiment
- DNA replication
  - Initiation
  - Elongation
  - Termination
- Mistakes in replication

DNA is the genetic material



Structure of DNA Review

- Antiparallel – 5’ to 3’ pairs with 3’ to 5’
- **Chargaff’s rule** – A pairs with T, C with G
- Each base held together with hydrogen bonds
- Backbone held together with phosphodiester bonds

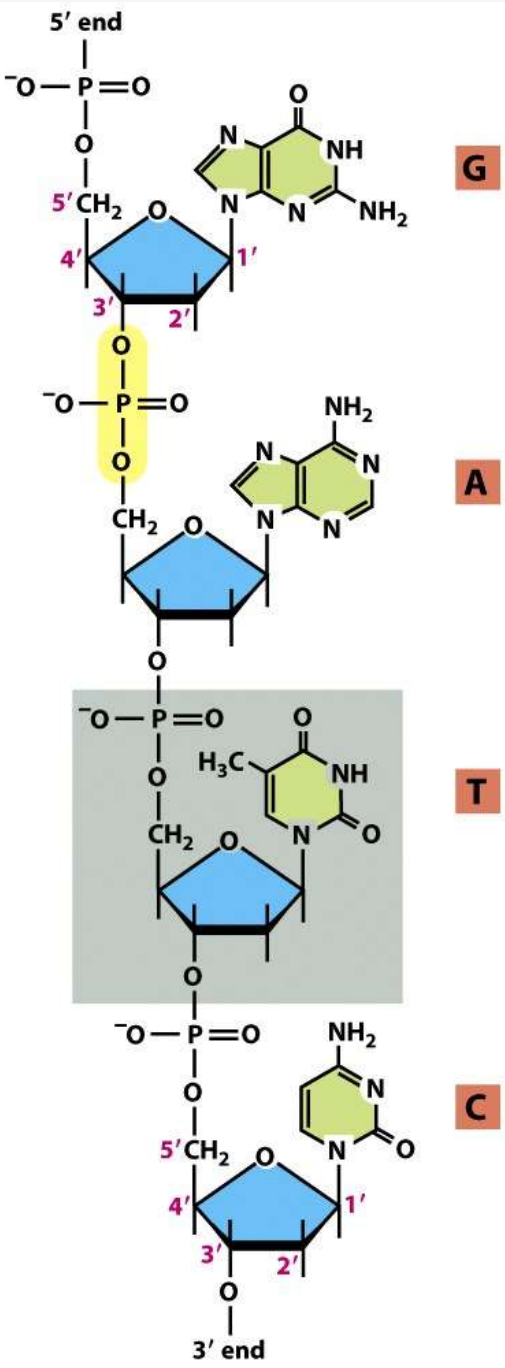


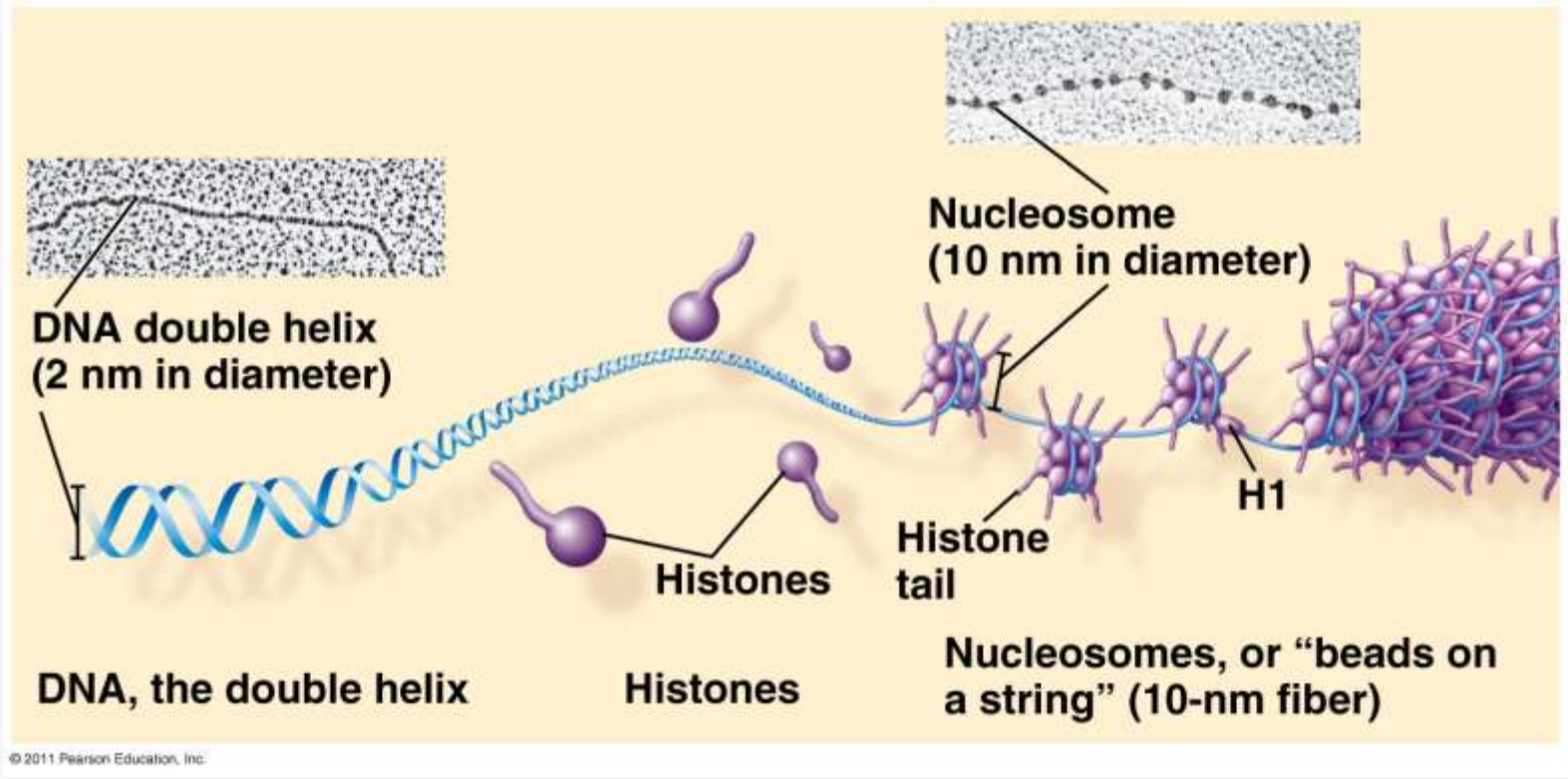
Figure 2-28 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Figure 16.UN04

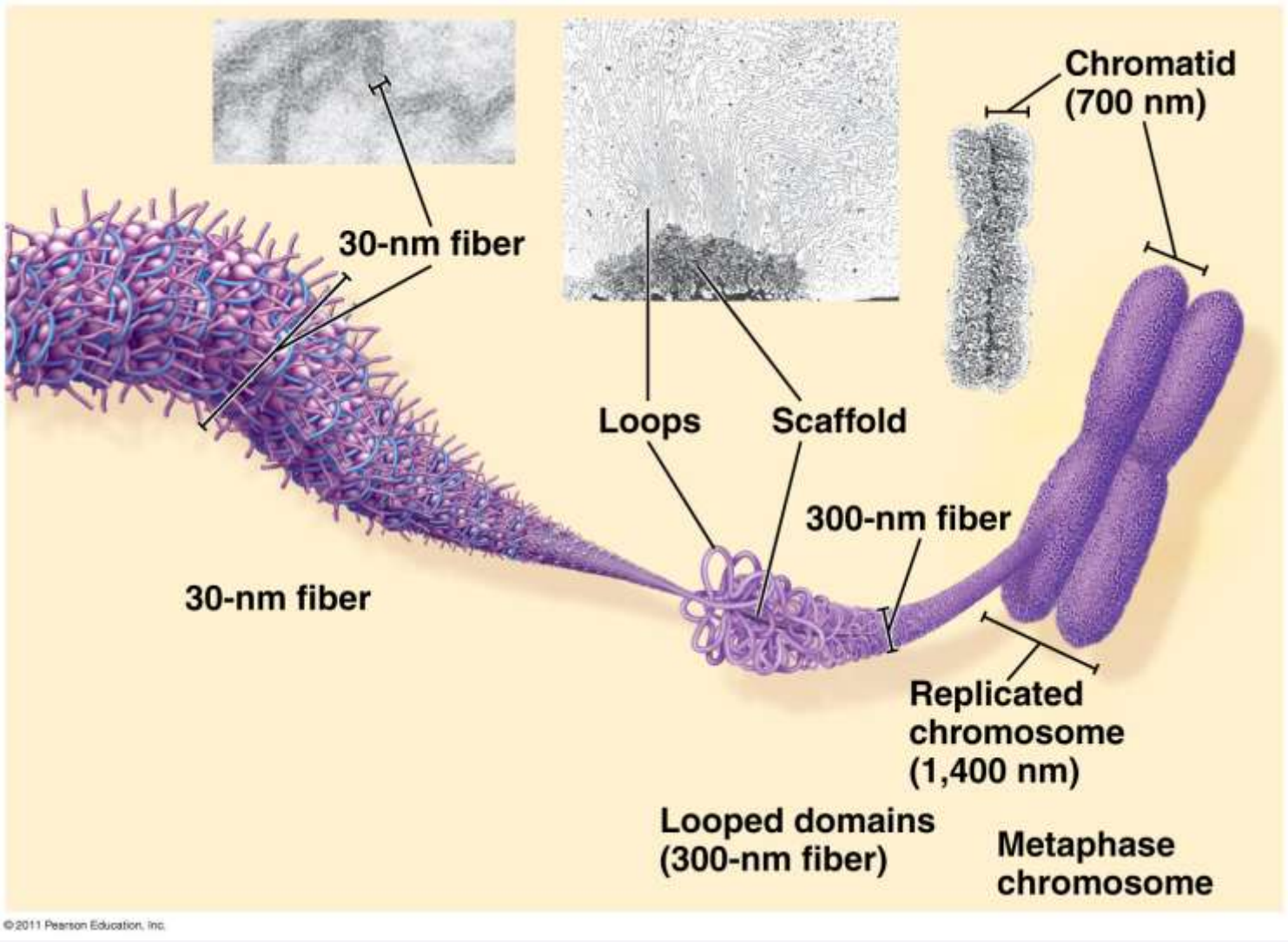
Source	Adenine	Guanine	Cytosine	Thymine
E. coli	24.7%	26.0%	25.7%	23.6%
Wheat	28.1	21.8	22.7	27.4
Sea urchin	32.8	17.7	17.3	32.1
Salmon	29.7	20.8	20.4	29.1
Human	30.4	19.6	19.9	30.1
Ox	29.0	21.2	21.2	28.7

How DNA is compacted

- 6 feet of DNA fits into the nucleus of each cell. How!?



How DNA is compacted



A closer look at histones

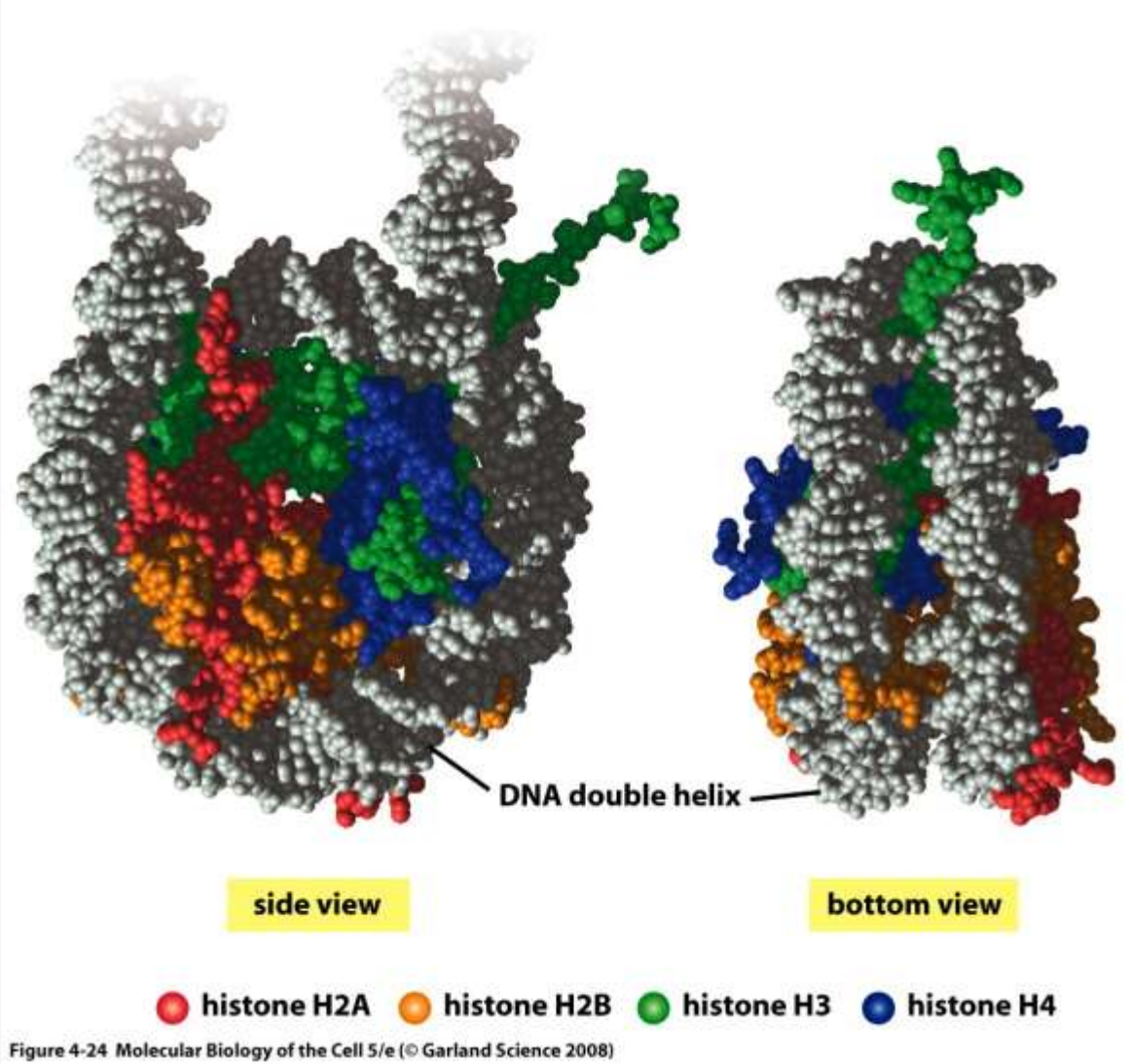
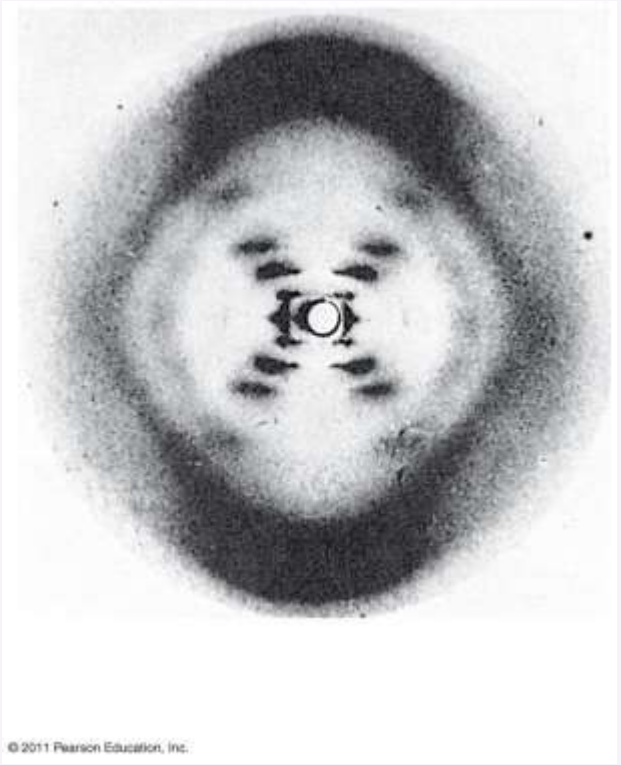


Figure 4-24 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Watson, Crick, and Franklin

- James Watson and Francis Crick are most widely credited with discovering the double-helix structure of DNA in 1953
- They based their structure off of a picture obtained through X-ray crystallography



The controversy

- Rosalind Franklin was left out of the publication and the Nobel Prize
- However, she may have actually been the one to discover the structure
  - Without her picture, Watson and Crick would certainly not have been famous
  - Watson long and viciously denigrated her work

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Yakel, E. B., Corcoran, R., and Jensen, W., *Phil. Mag.*, **40**, 169 (1949).

[2] Longuet-Higgins, H. S., *Mem. Nat. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).

[3] Van Alt, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., **11** (3) (1954).

[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-ester groups joining β-D-deoxy-ribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

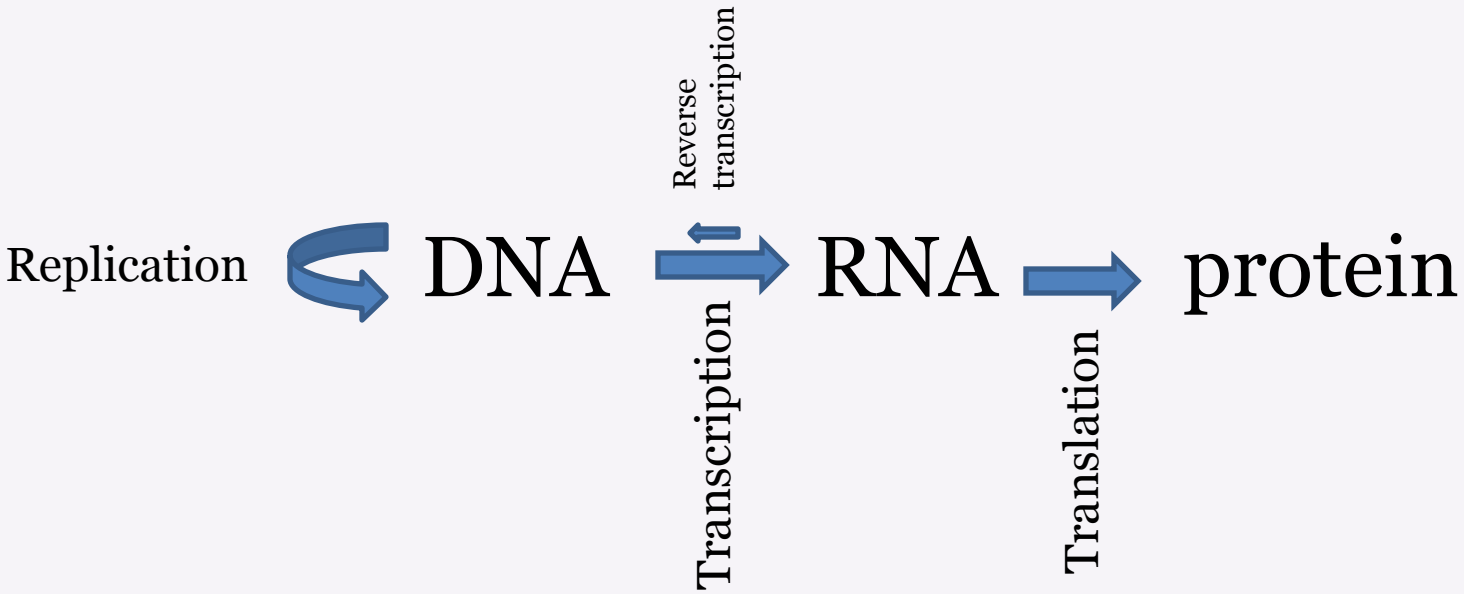
Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

Watson and Crick saw the method of DNA replication coming when they first published the structure of DNA...

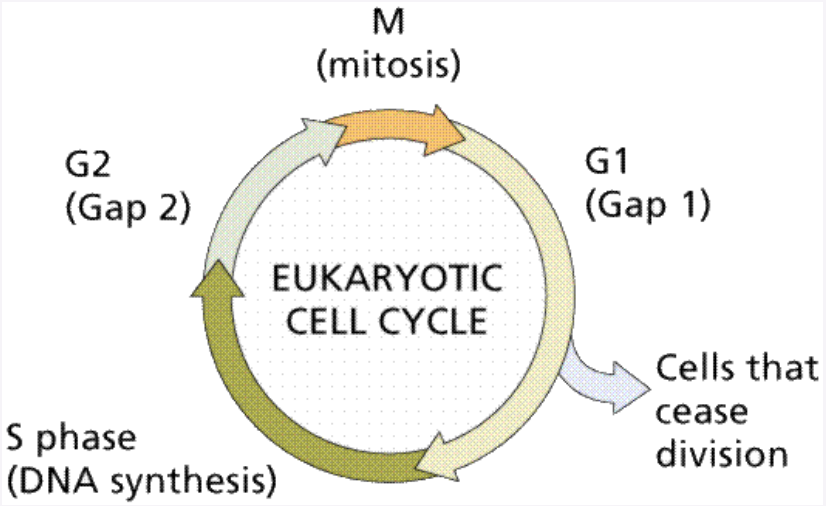
It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

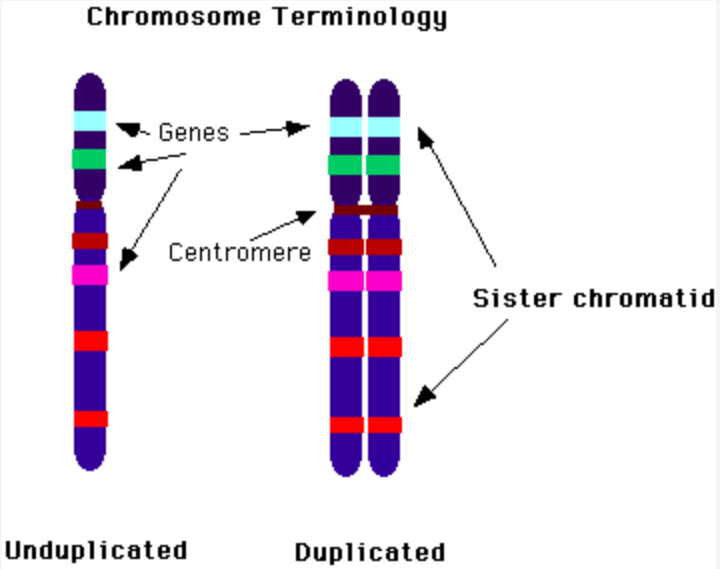
The “Central Dogma” of molecular biology



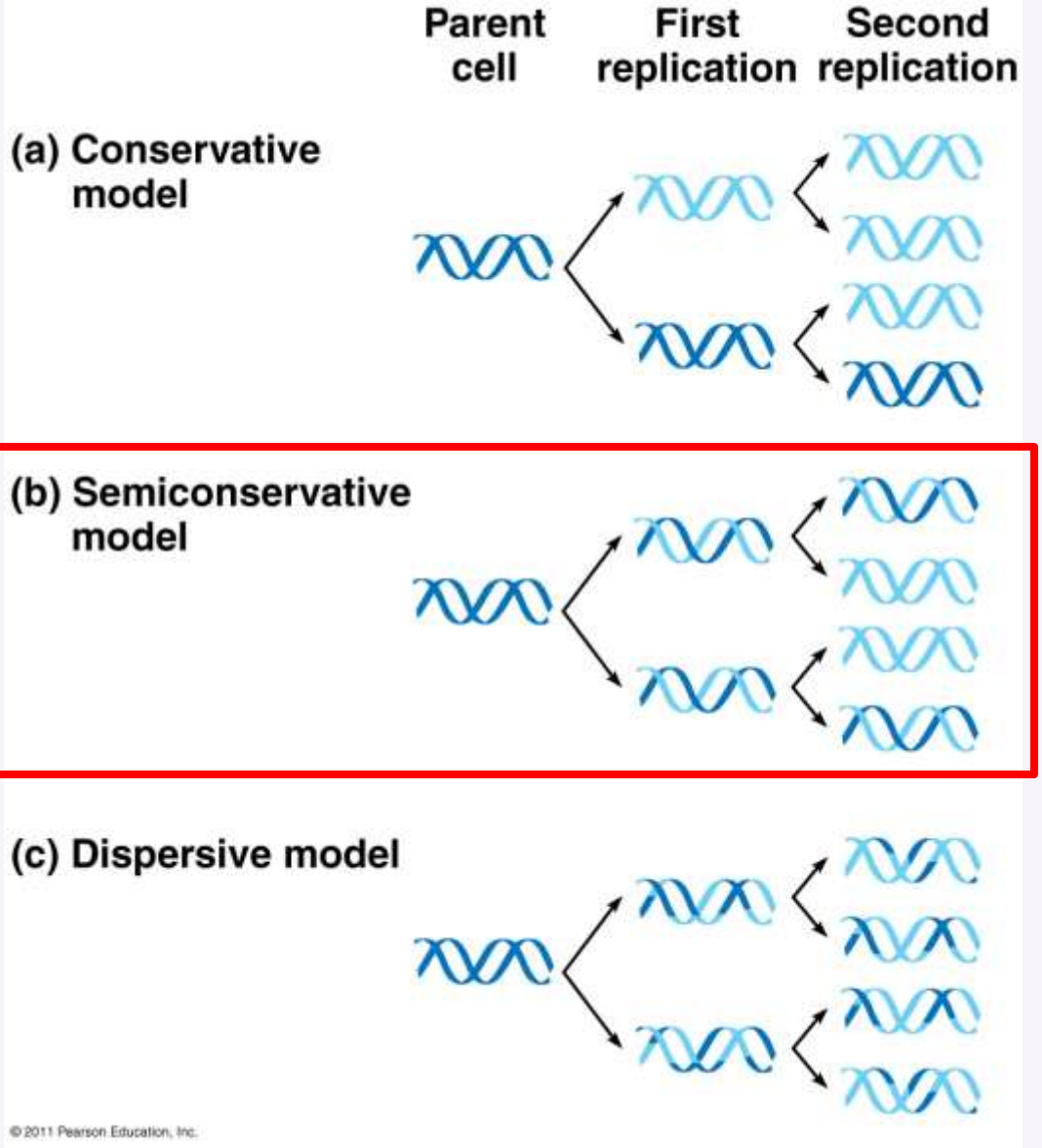
DNA Replication

- DNA replication takes place during S phase
- How does it happen on the molecular level?





Three models of replication



Replication: An overview

- DNA is “unzipped” and base pairs are matched to create two new daughter strands

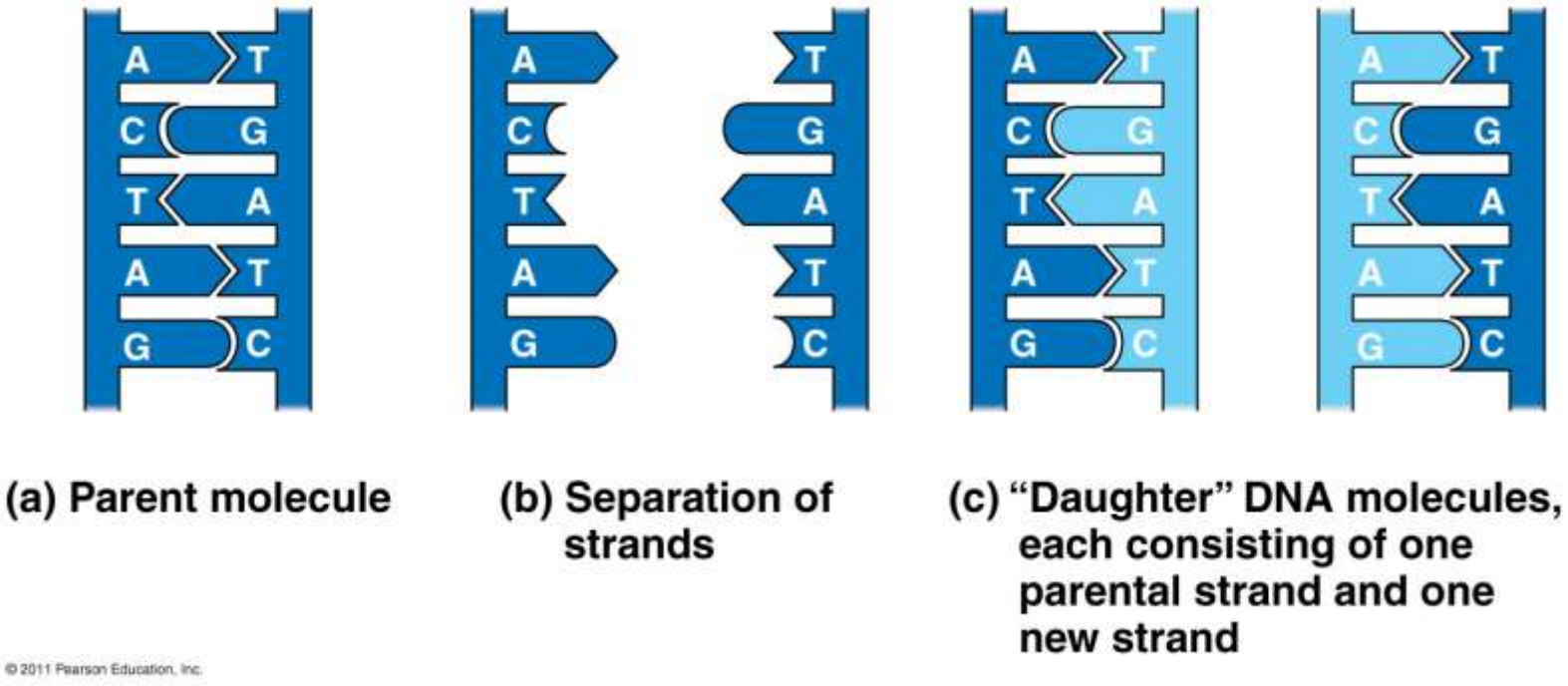
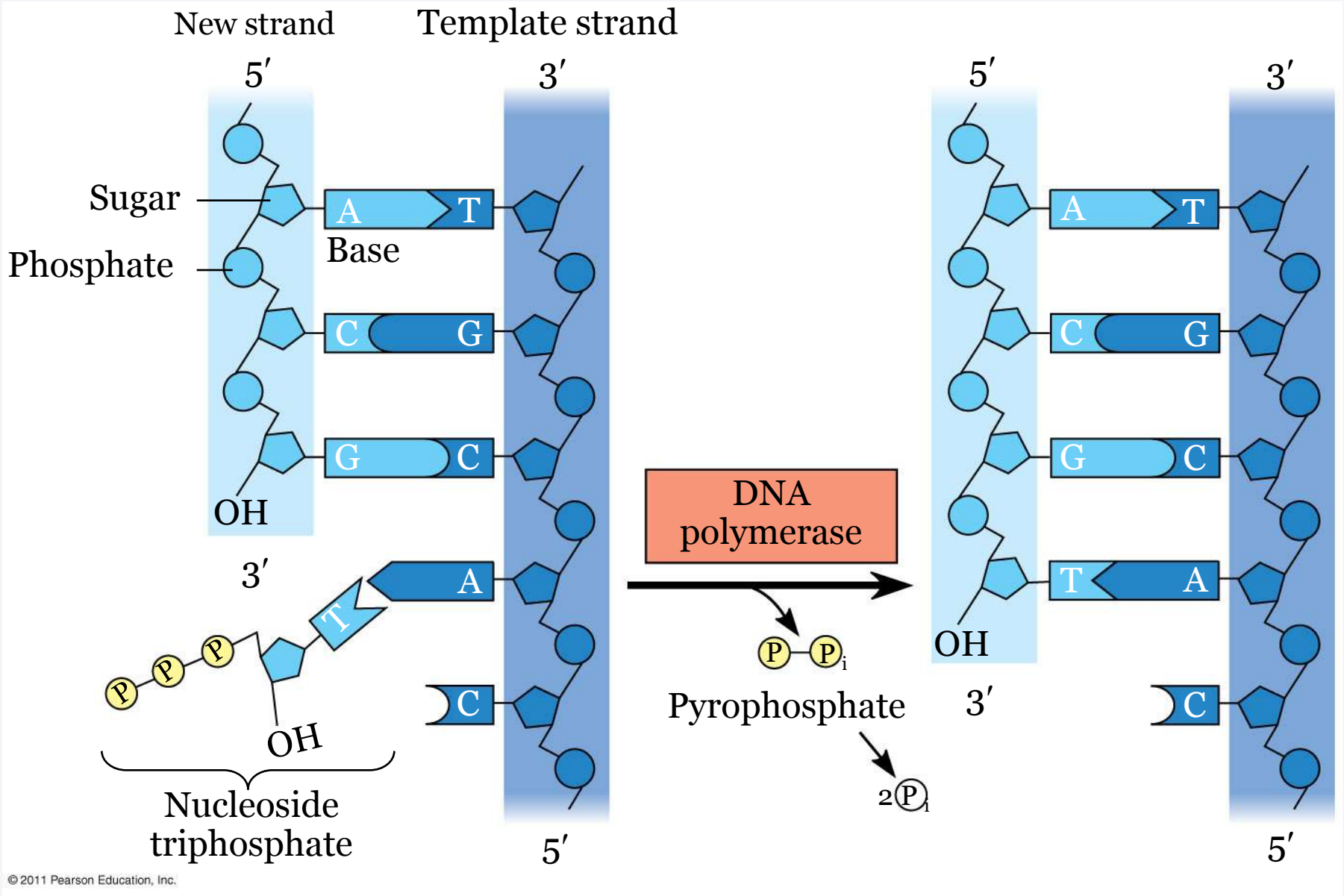


Figure 16.14


New strand
Template strand
5′
3′
Sugar
Phosphate
A
T
Base
C
G
G
C
OH
3′
A
T
P
P
P
OH
C
Nucleoside triphosphate
5′
DNA polymerase
P
Pi
Pyrophosphate
2Pi
5′
3′
A
T
C
G
G
C
T
A
OH
3′
C
5′
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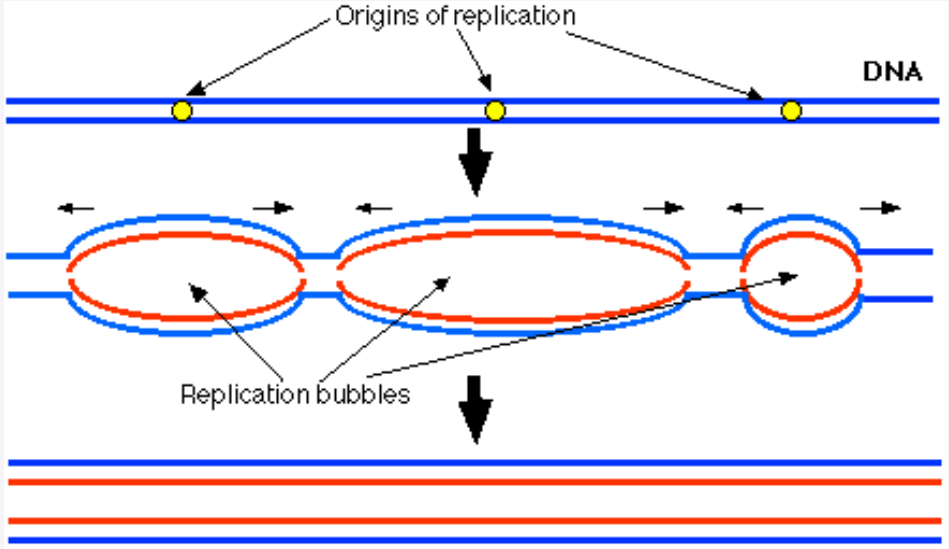
Clock's Ticking…

- Average human chromosome contains 150 x 10^6 base pairs
- Copied at 50 base pairs a second
  - This would normally take over a month to replicate the entire genome!
  - Instead, there are multiple *origins of replication*, which means it actually takes about an hour

Getting started…

- Replication begins at particular sites called **origins of replication**, where the two DNA strands are separated, opening up a replication "bubble"
- A eukaryotic chromosome may have hundreds or even thousands of origins of replication
- Replication proceeds in both directions from each origin, until the entire molecule is copied



Origins of replication

- 10,00 -100,000 ori in a single eukaryotic cell
- Usually only 1 ori in prokaryotic cells
- Ori sequences are A-T rich

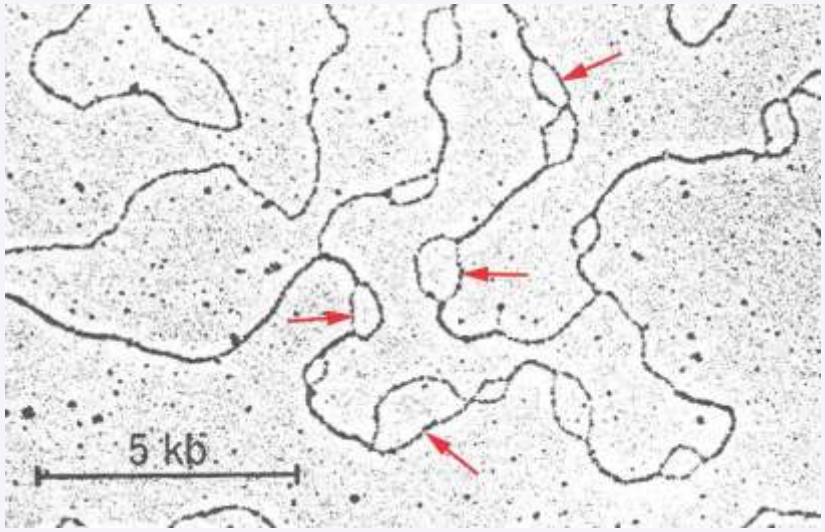
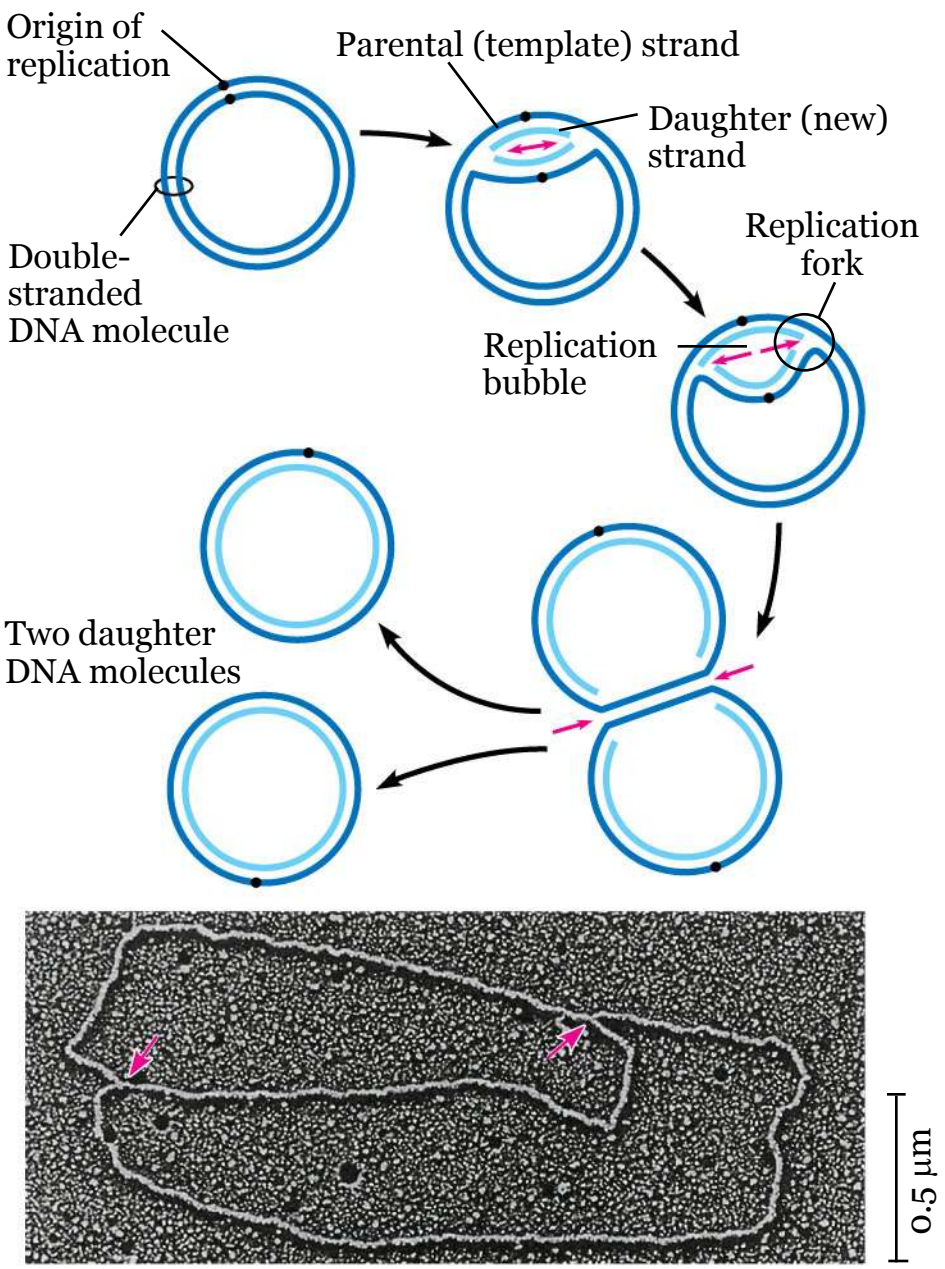
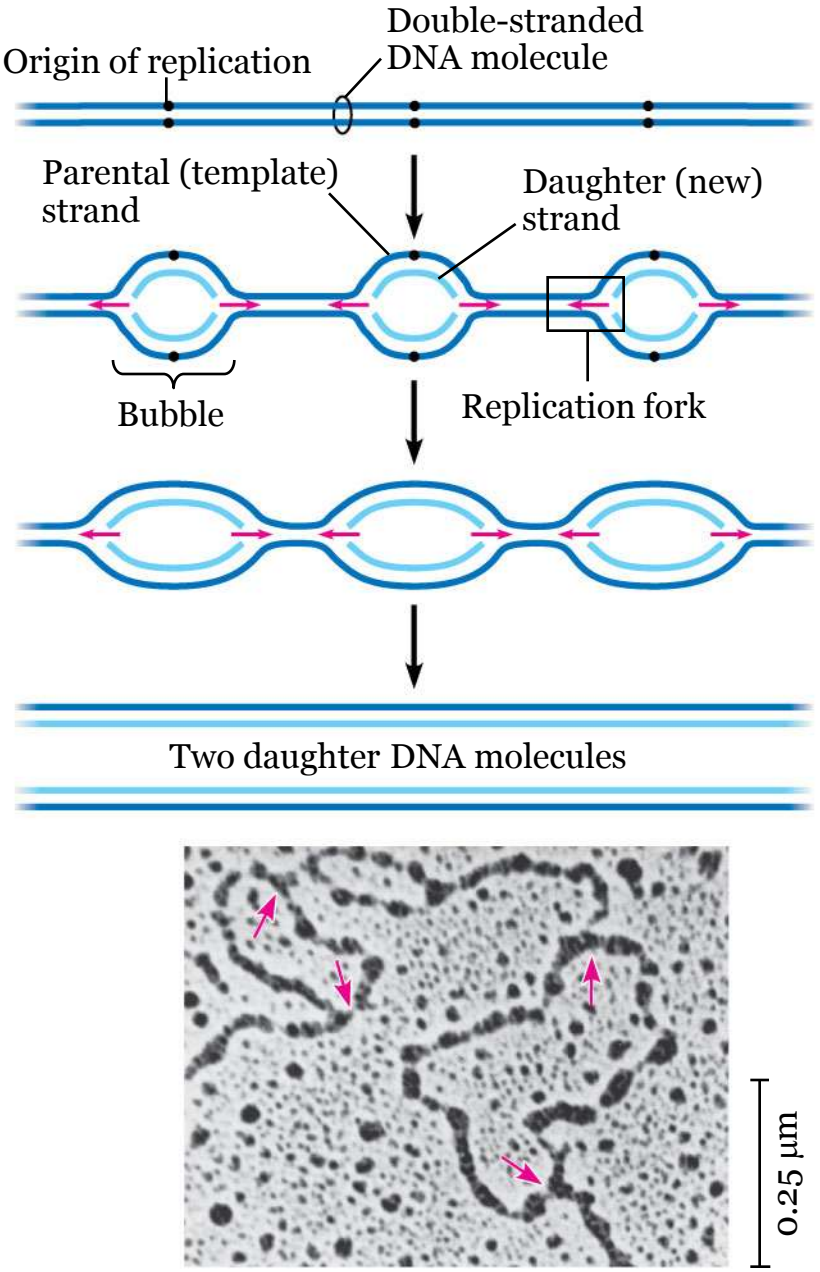


Figure 16.12

(a) Origin of replication in an *E. coli* cell



(b) Origins of replication in a eukaryotic cell



The process in eukaryotes

- Three stages:

  Initiation: Unzipping of the double stranded parent DNA

  Elongation: Complementary base pairing to create daughter strands

  Termination: Fixing gaps and separating the two DNA strands

- However, there are a couple considerations first…

The players

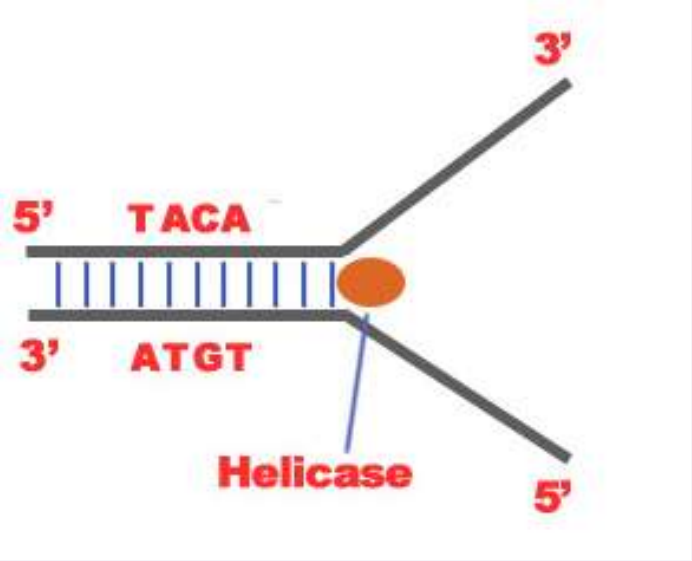
Protein/Enzyme	Where it comes in	Function
Helicase	*Initiation*	Unwinds the parent DNA strand
DNA polymerase	*Elongation*	Reads the parent strand and pairs up nucleotides
RNA primase	*Elongation*	Creates primers on the lagging strand for DNA polymerase to attach to
DNA ligase	*Termination*	Seals the gaps between the Okazaki fragments
Single-stranded binding proteins	*Elongation*	Binds to and stabilizes ssDNA
Topoisomerase	*Initiation, Elongation*	Relieves the torsion caused by helicase activity

Leading vs. lagging strand

- DNA can only be replicated in the 5’-3’ direction
  - The shape of DNA polymerase only allows it to add on nucleotides to the 3’ end of a preexisting chain
  - This produces a **leading strand,** 5’-3’, and a **lagging strand**, 3’-5’

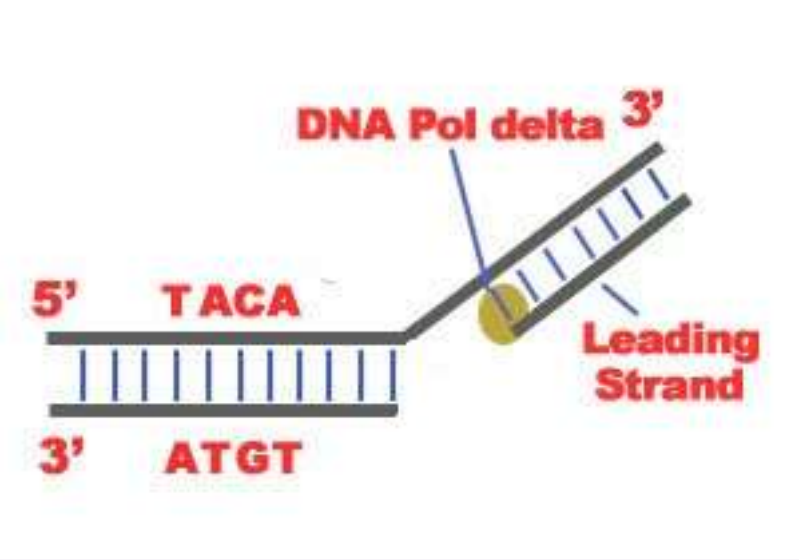
Initiation

- Same process for both leading and lagging strand
- DNA ‘unzips’ starting at the ori
- The enzyme *helicase* breaks the hydrogen bonds between the nucleotides of the parent DNA strand
  - Single-stranded binding proteins bind to and stabilize the newly single-stranded DNA to prevent it from base pairing with itself
- *Topoisomerase* relieves the torsion upstream caused by helicase by breaking and rejoining phosphodiester bonds



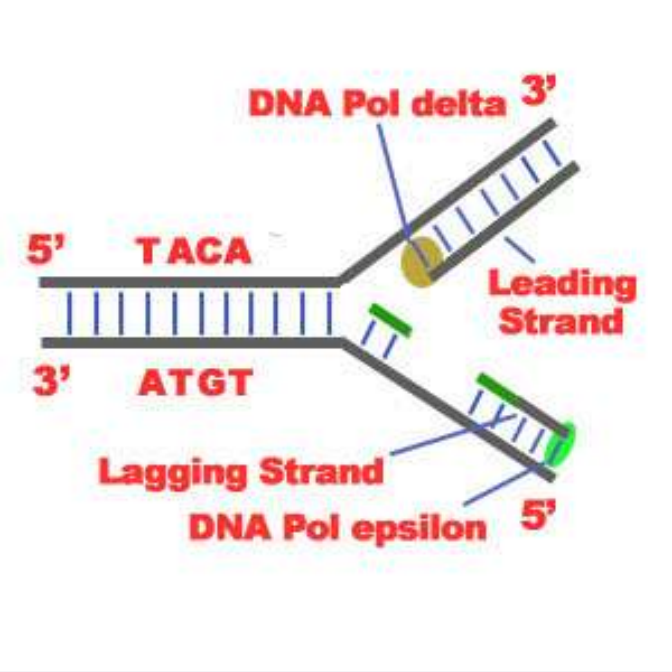
Leading Strand: Elongation

- The cell builds on the 5’ to 3’ strand by creating a complementary daughter strand
- The enzyme *DNA polymerase* does the complementary base pairing
  - DNA polymerase grabs free nucleotides and matches them to the bases on the parent strand



Lagging Strand: Elongation

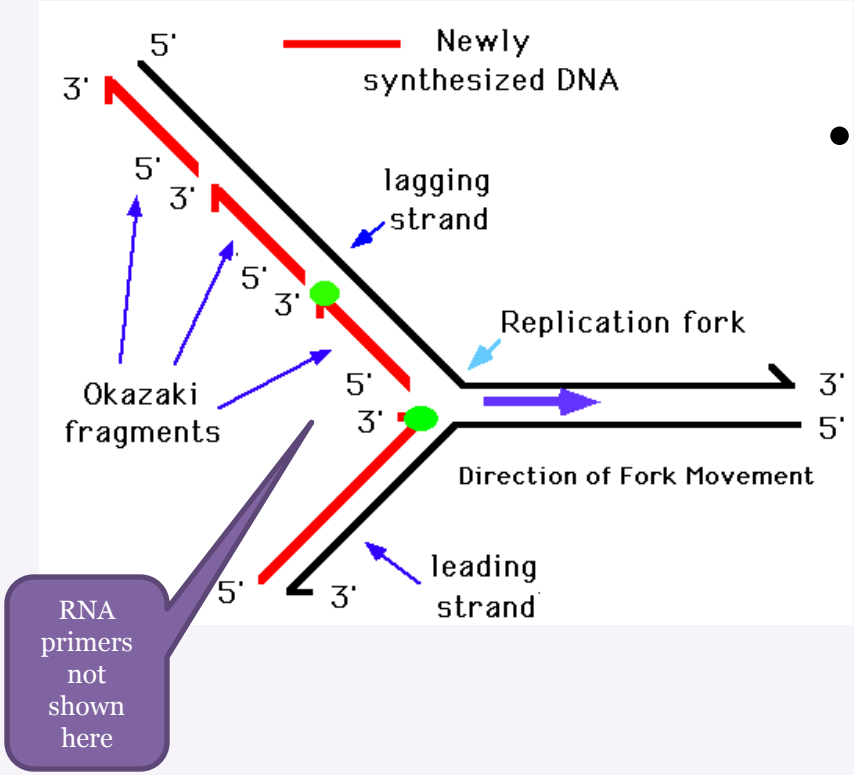
- DNA polymerase cannot synthesize on the 3’-5’ strand by itself
- RNA primers provide a platform for DNA polymerase to synthesize on the 3’-5’ lagging strand
  - *RNA primase* is the enzyme that creates these primers



- DNA polymerase latches onto these primers and can now synthesize in the 5’-3’ direction in short hops

Lagging Strand: Elongation

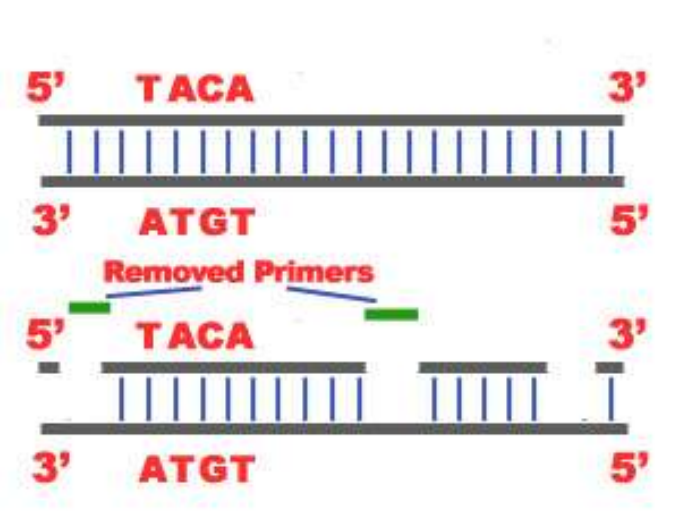
- These hops cause the lagging strand to be broken into short fragments of DNA called **Okazaki fragments**



- RNA primers are replaced with DNA
- Gaps in the backbone between the old and new DNA are sealed by **ligase**

Termination

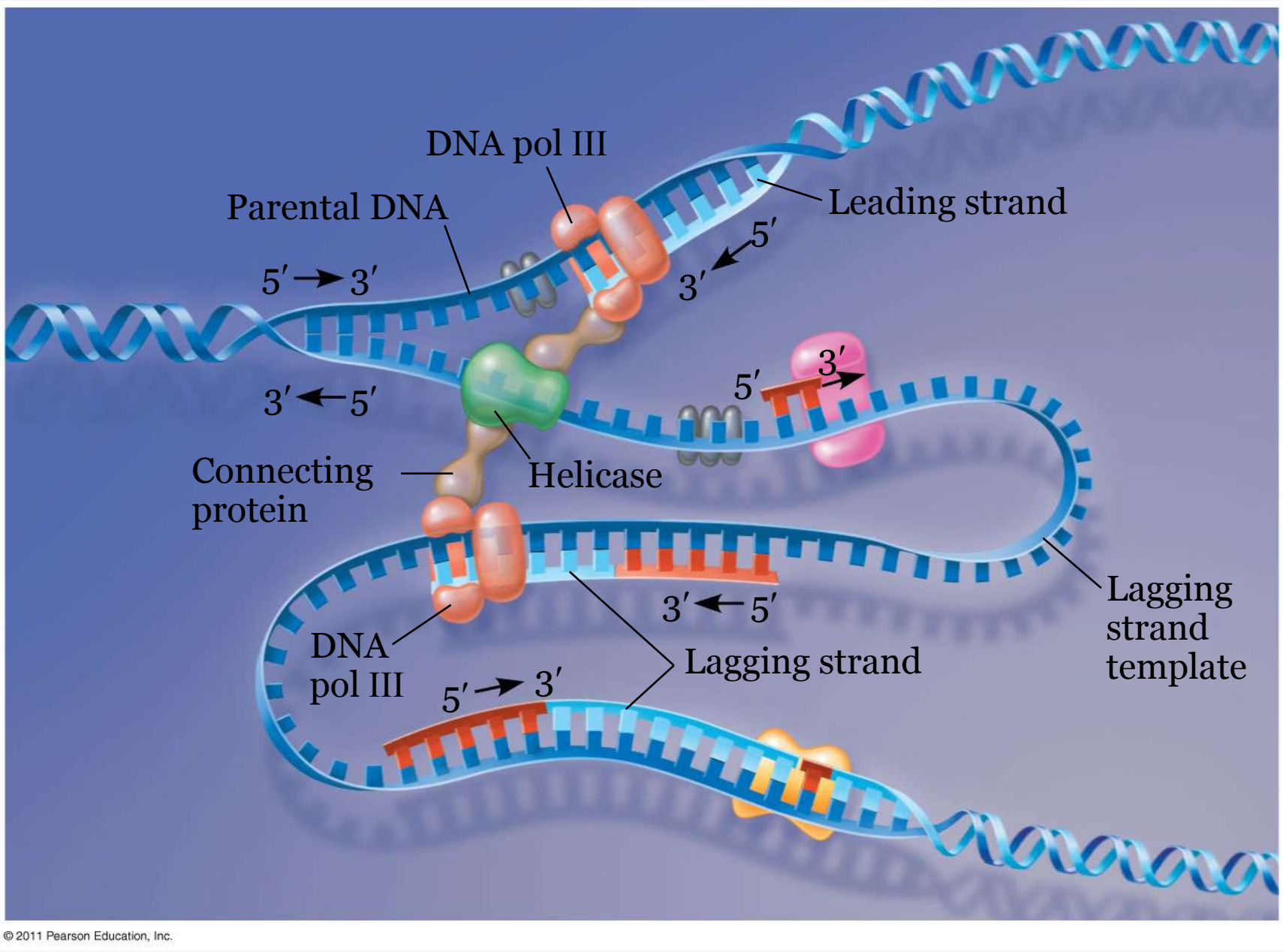
- On the lagging strand:
  - RNA primers are excised by DNA polymerase and replaced with DNA
  - The gaps left over in the phosphodiester backbone are sealed by *DNA ligase*



Leading strand

Lagging strand

Figure 16.18



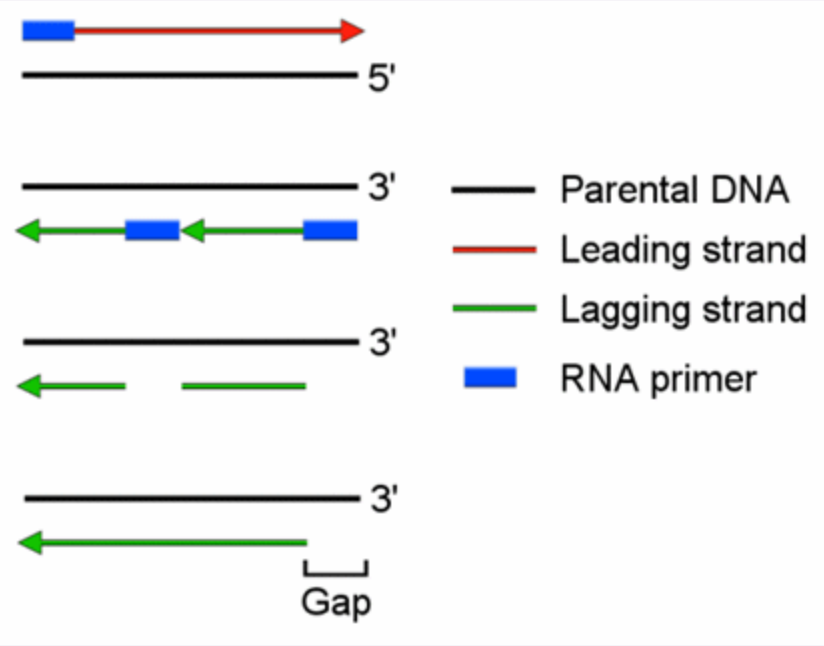
What happens after termination?

- The eukaryotic genome is linear

- The lagging strand can't quite get to the end, so there is a small gap that never gets filled in

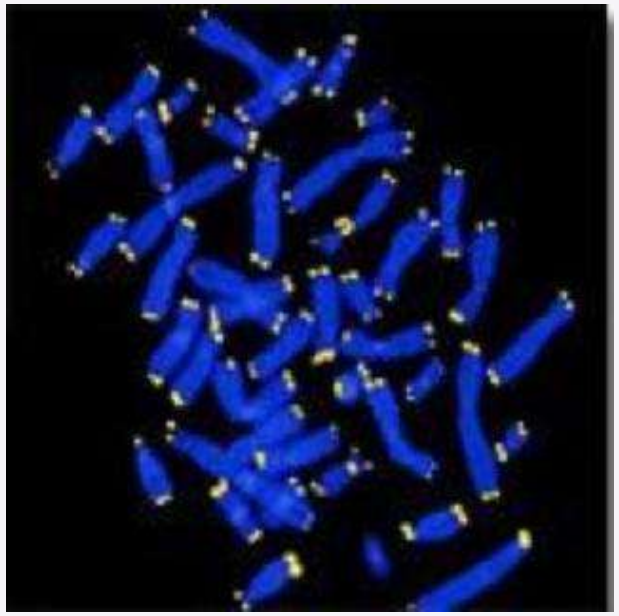
- Over many replications, this leads to the genome actually shortening!

- The regions where this shortening takes place are the telomeres



Telomere shortening and cellular senescence

- Each round of replication shortens telomeres
- Telomere shortening is considered a main cause of aging in cells
  - Cancer cells and stem cells can activate an enzyme that re-lengthens the telomeres
- However, it is not the only nor the ultimate timekeeper of cells
- The shortening of telomeres might protect cells from cancerous growth by limiting the number of cell divisions



Some videos

http://www.youtube.com/watch?v=wkXgwGn_dGU&feature=related

http://www.youtube.com/watch?v=teV62zrm2P0&feature=related

http://www.youtube.com/watch?v=4jtmOZaIvS0&feature=related

Errors with DNA polymerase

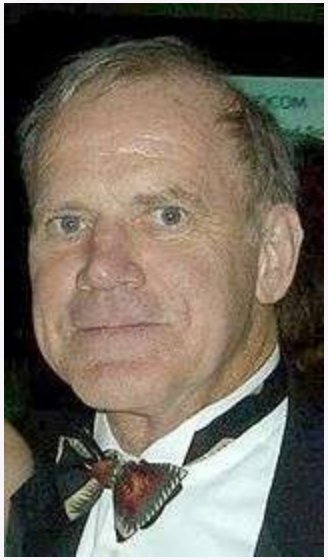
- In general, DNA polymerase makes a mistake on every 1 in 10^7 nucleotides
  - DNA can be damaged by exposure to harmful chemical or physical agents such as cigarette smoke and X-rays; it can also undergo spontaneous changes
- DNA polymerases proofread newly made DNA, replacing any incorrect nucleotides
  - In **mismatch repair** of DNA, repair enzymes correct errors in base pairing
  - In **nucleotide excision repair**, a **nuclease** cuts out and replaces damaged stretches of DNA

HIV: The worst replicator

- HIV DNA polymerase has very low accuracy
- It accumulates mutations faster than any known organism or virus
- Therefore, it evolves the fastest out of an known organism or virus
  - Very easily evolves resistance to drugs

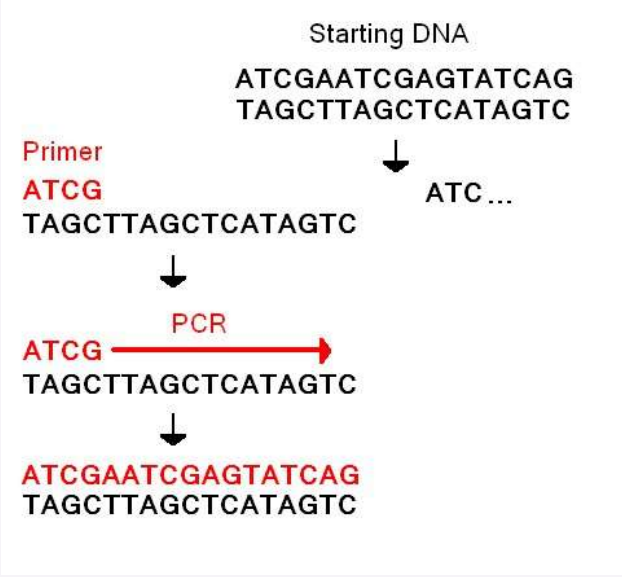
PCR: artificial DNA replication

- Polymerase chain reaction
- Used to amplify specific stretches of DNA
- Sometimes we need to isolate one stretch of DNA from an organisms' genome
  - Presence of a particular gene
  - Gene sequencing
  - Comparing gene sequences



Ingredients for PCR

- Template DNA
  - The genome/sample of interest
- Primers
  - Short DNA sequences complementary to both ends of your sequence of interest
- DNA polymerase
- Buffers
- dNTPs
  - Free nucleotides

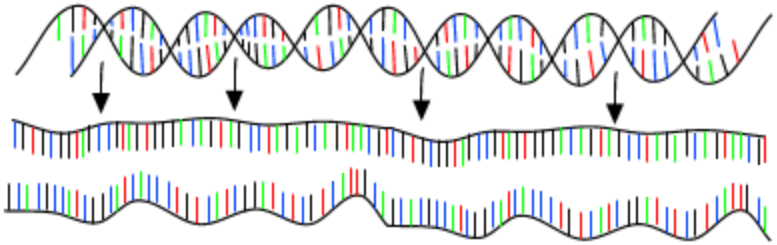


PCR: The process

- Separate strands using heat
- Primers anneal to the newly single-stranded parent DNA
- DNA polymerase recognizes the dsDNA and starts copying
- As soon as the copying is done, apply more heat to separate the strands again
- Repeat

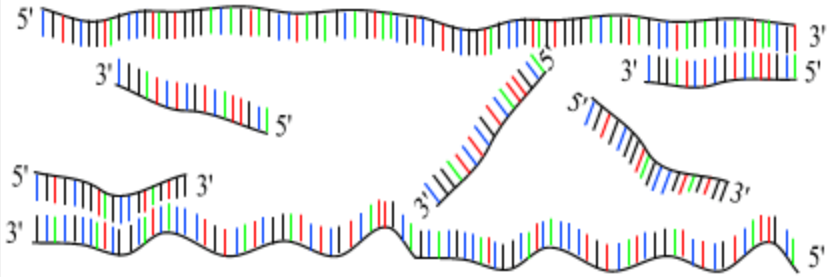
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

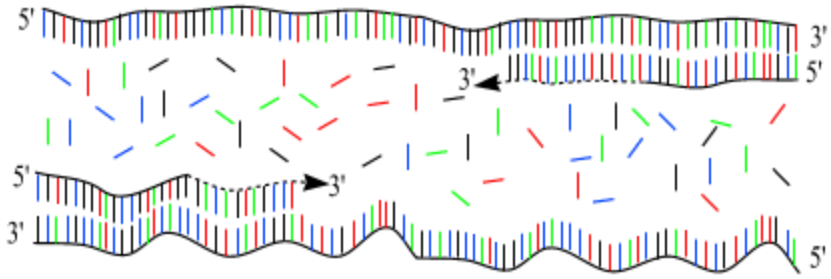
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)

http://learn.genetics.utah.edu/content/labs/pcr/

Vocabulary

- Chargaff's rule
- Antiparallel
- Histone
- Heterochromatin, euchromatin
- Central dogma
- Origin of replication
- DNA polymerase
- Helicase
- Ligase
- Topoisomerase
- Okazaki fragments
- Mismatch repair
- Excision repair