

Science of Living System

BS20001

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Lecture	Date	Topic
1	02/01/2019	Nucleic acids – 1
2	09/01/2019	Nucleic acids – 2
3	16/01/2019	Transcription and Translation – 1
4	23/01/2019	Transcription and Translation – 2 / Protein structure – 1
5	30/01/2019	Protein structure – 2
6	06/02/2019	Enzymes
**	13/02/2019	CLASS TEST - 1
7	20/02/2019	
8	27/02/2019	Cellular architecture
9	06/03/2019	Cell division and apoptosis
10	13/03/2019	Host defense/vaccines
11	20/03/2019	Genetic Engineering & its impact
**	04/04/2019	CLASS TEST - 2
**	11/04/2019	DISCUSSION AND REVISION

Why study Living System?



“I was impressed by astonishing functions that live organisms are capable to develop...Since then, learning from nature was a common thing for me.” – Eiji Nakatsu



STORAGE LIMITS

Estimates based on bacterial genetics suggest that digital DNA could one day rival or exceed today's storage technology.

	Hard disk	Flash memory	Bacterial DNA	WEIGHT OF DNA NEEDED TO STORE WORLD'S DATA
Read-write speed (μs per bit)	~3,000–5,000	~100	<100	
Data retention (years)	>10	>10	>100	
Power usage (watts per gigabyte)	~0.04	~0.01–0.04	<10 ⁻¹⁰	
Data density (bits per cm ³)	~10 ¹³	~10 ¹⁶	~10 ¹⁹	 ~1 kg

©nature

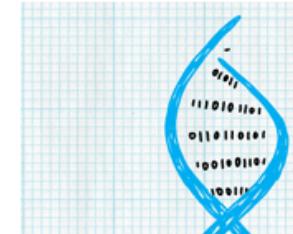
Microsoft Corporation – DNA Storage Research

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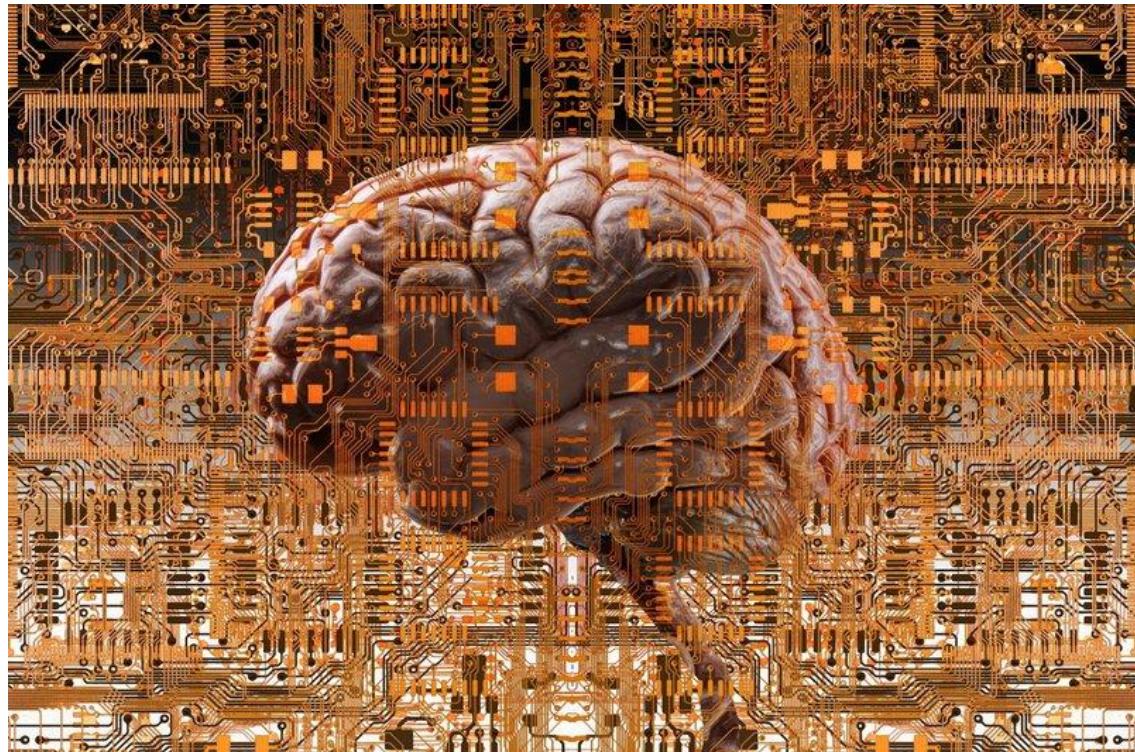
DNA Storage

Established: January 1, 2015

The amount of digital data produced has long been outpacing the amount of storage available. This project enables molecular-level data storage into DNA molecules by leveraging biotechnology advances in synthesizing, manipulating and sequencing DNA to develop archival storage. Microsoft and University of Washington researchers are collaborating to use DNA as a high density, durable and easy-to-manipulate storage medium.



Brain vs Computer



NIH Director Dr. Francis Collins and
President Barack Obama announcing the
BRAIN Initiative 2013

What are the characteristics of a Living System?

- High degree of **complexity**
- Mechanisms for **sensing** and **responding** to alterations in surroundings
- Systems for extracting, transforming and using **energy** from the environment
- Ability to **adapt** and **evolve**
- Ability to develop and **grow**
- Capacity for precise **self-replication** and **self-assembly**, known as reproduction



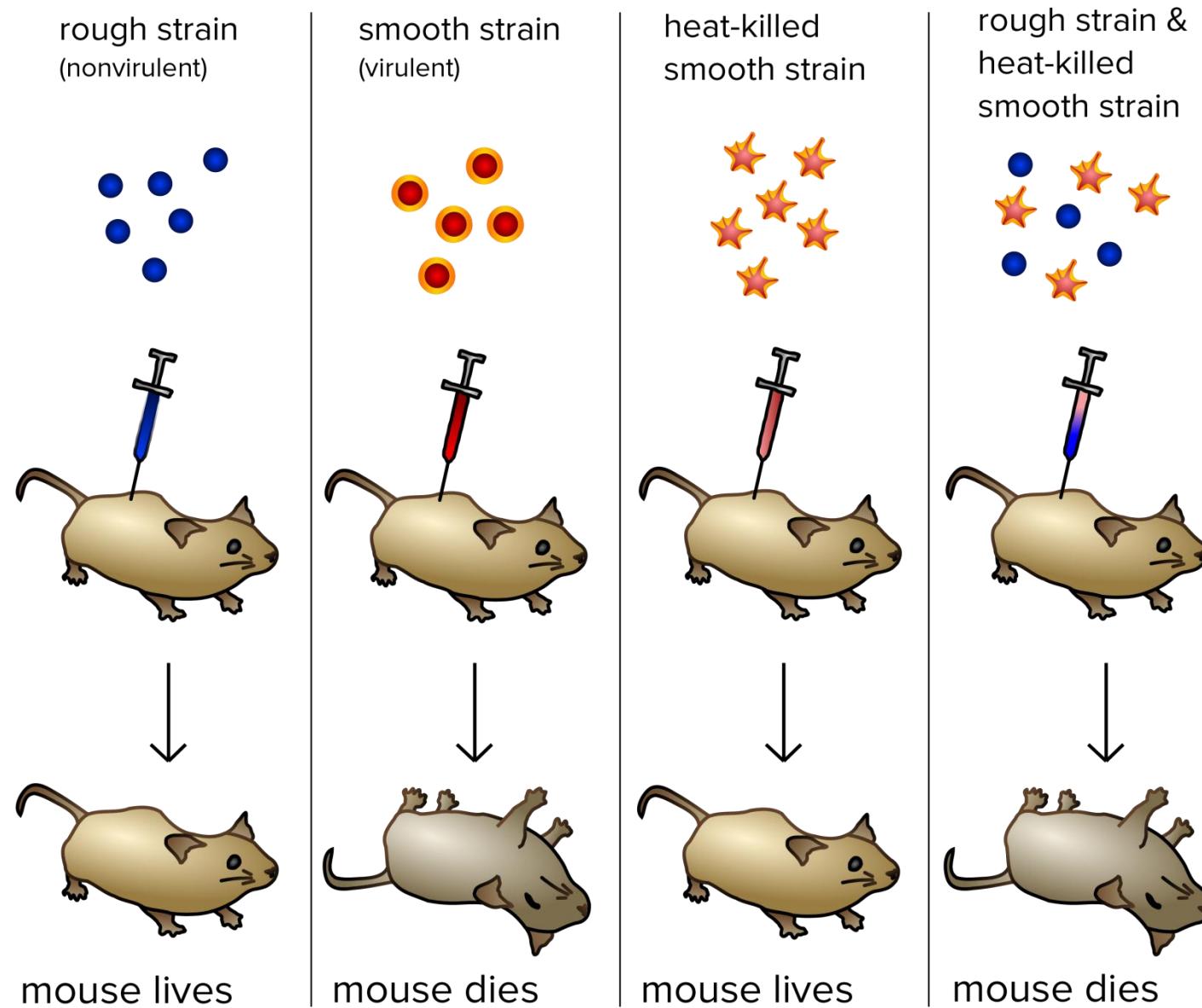
Nucleic Acid

Nucleic Acid

RNA: Ribonucleic Acid

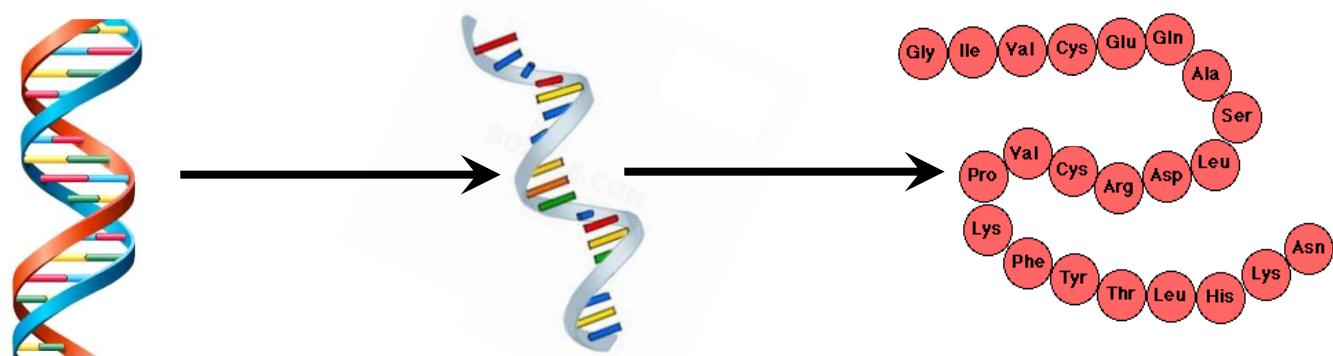
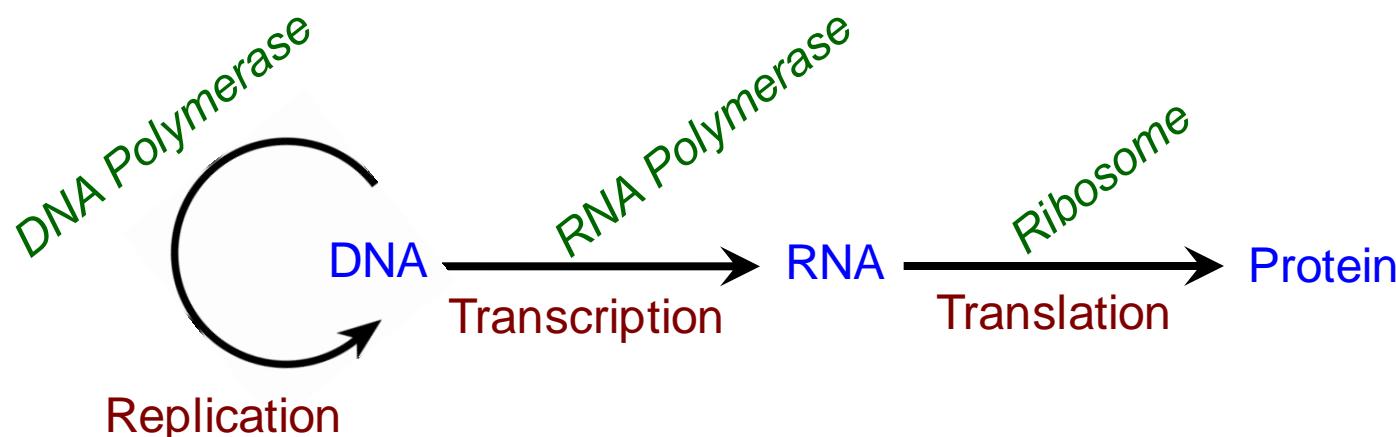
DNA: Deoxyribonucleic Acid

Experiment that Proves DNA is Our Genetic Material



Frederick Griffith's experiment with bacteria (*Streptococcus pneumoniae*) (1928).

Flow of Genetic Information: The Central Dogma of Molecular Biology



Polymer of
nucleotides

Polymer of
nucleotides

Polymer of
amino acids

Nucleic Acid

- Nucleic acids are polymers
- Monomer---nucleotides

- Nitrogenous bases
 - Purines
 - Pyrimidines

- Sugar
 - Ribose
 - Deoxyribose

- Phosphates

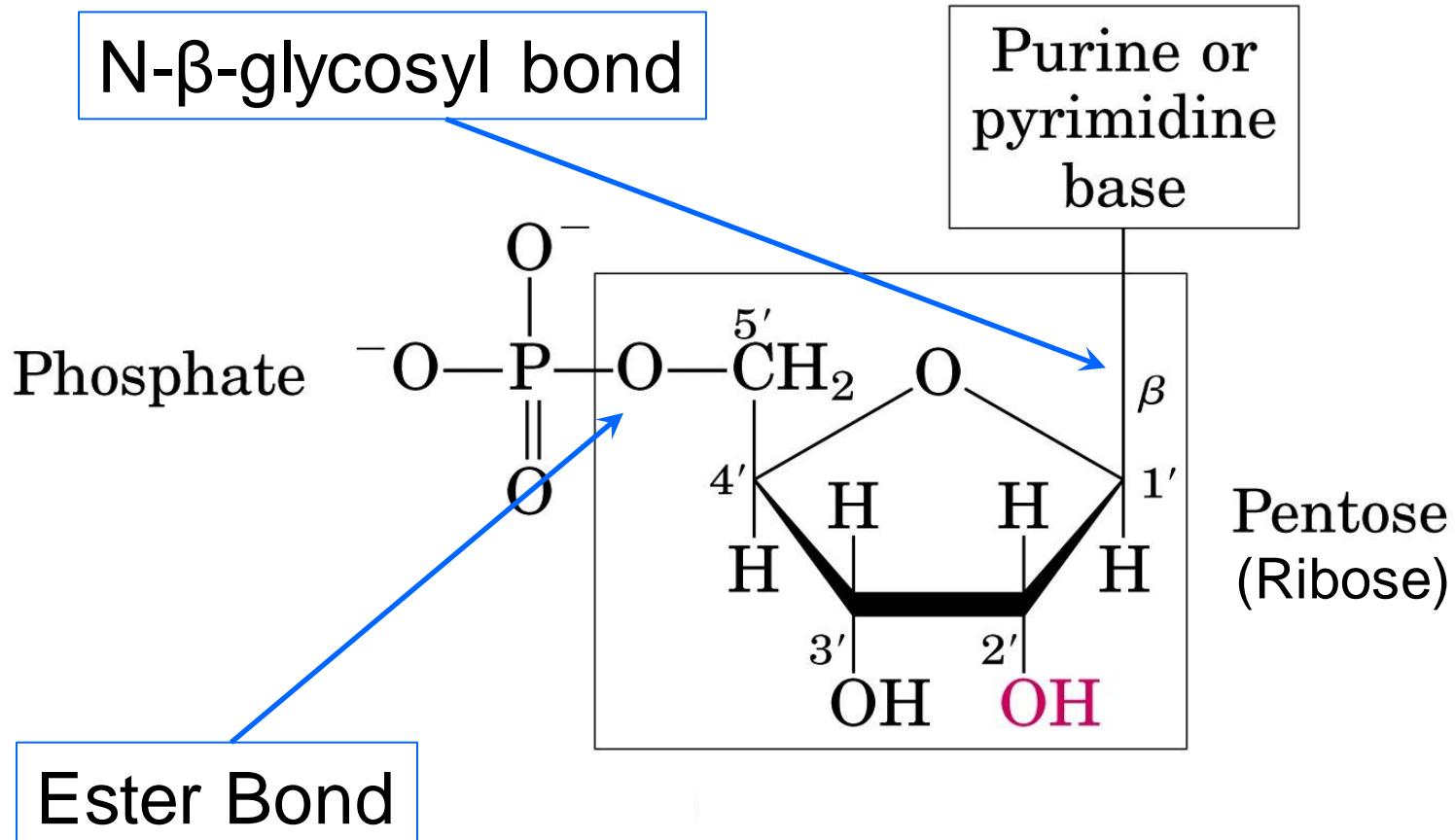
Nucleosides

Nucleotides

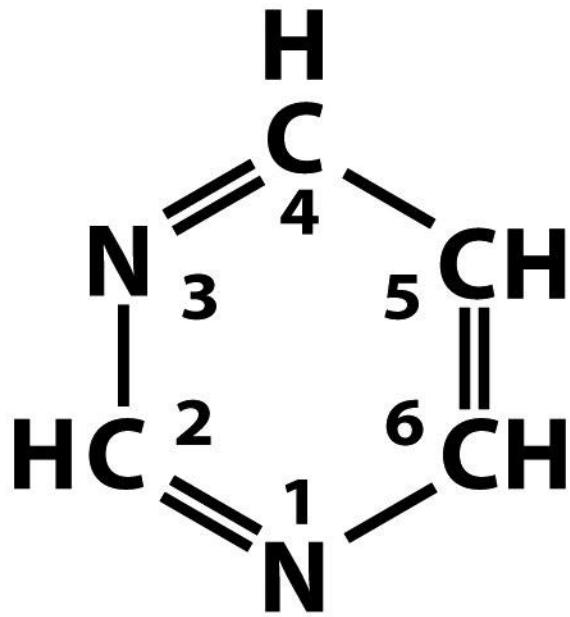
RNA vs DNA – Sugar

RNA - Ribonucleic Acid (OH)

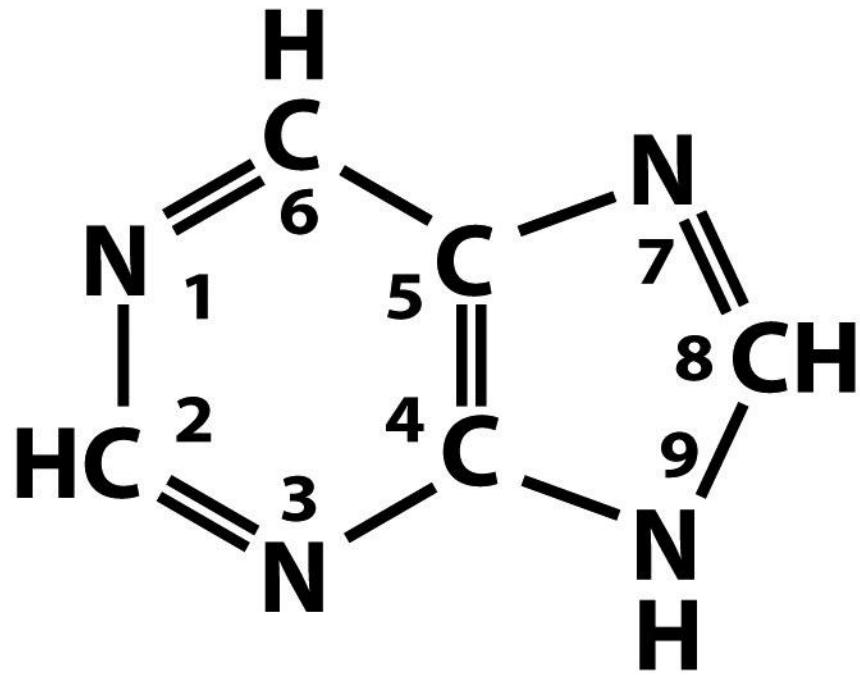
DNA - Deoxyribonucleic Acid (H)



Two Types of Nitrogenous Bases



Pyrimidine



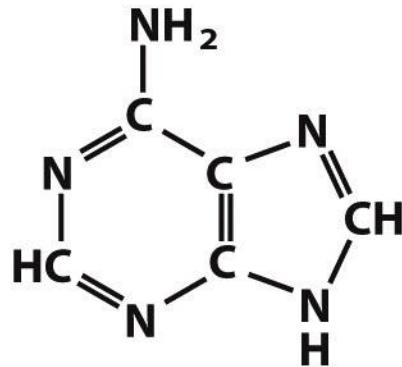
Purine

Figure 8-1b

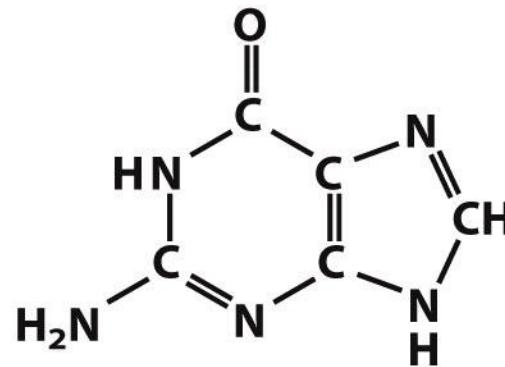
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Purine and Pyrimidine Bases

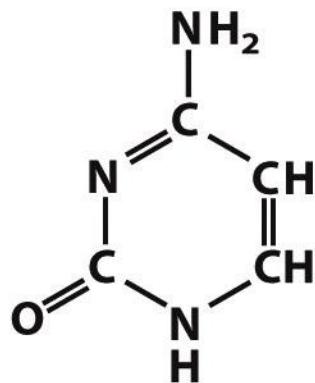


Adenine

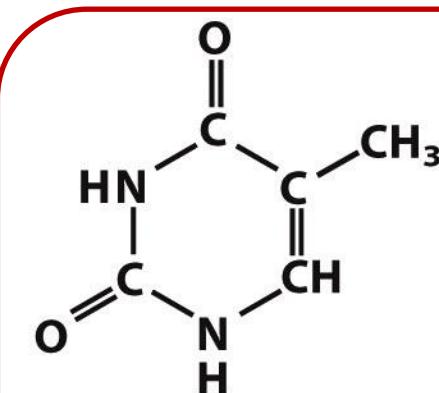


Guanine

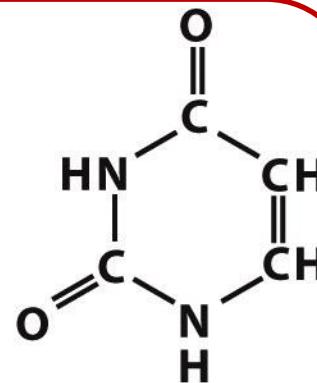
Purines



Cytosine



Thymine
(DNA)

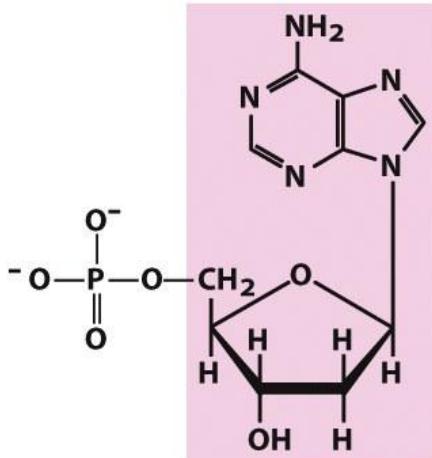


Uracil
(RNA)

Pyrimidines

Nucleotide = Nucleoside + Phosphate

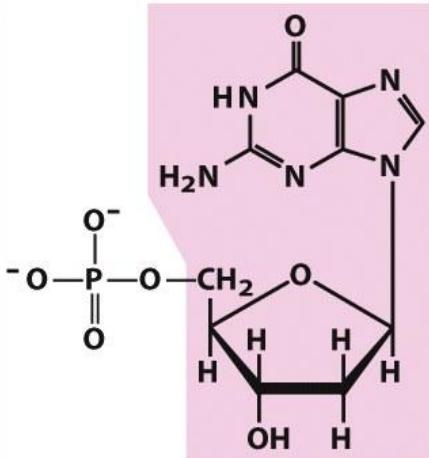
(Nucleoside = Sugar + Base)



Nucleotide: Deoxyadenylate
(deoxyadenosine
5'-monophosphate)

Symbols: A, dA, dAMP

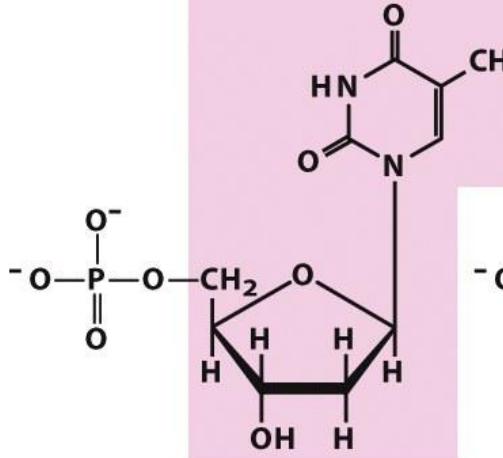
Nucleoside: Deoxyadenosine



Nucleotide: Deoxyguanylate
(deoxyguanosine
5'-monophosphate)

Symbols: G, dG, dGMP

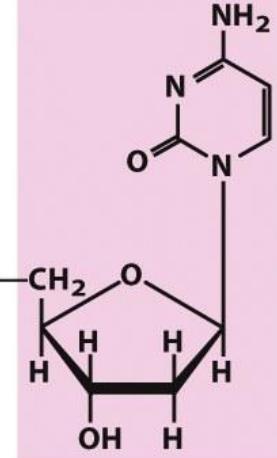
Nucleoside: Deoxyguanosine



Nucleotide: Deoxythymidylate
(deoxythymidine
5'-monophosphate)

Symbols: T, dT, dTMP

Nucleoside: Deoxythymidine



Nucleotide: Deoxycytidylate
(deoxycytidine
5'-monophosphate)

Symbols: C, dC, dCMP

Nucleoside: Deoxycytidine

Deoxyribonucleotides

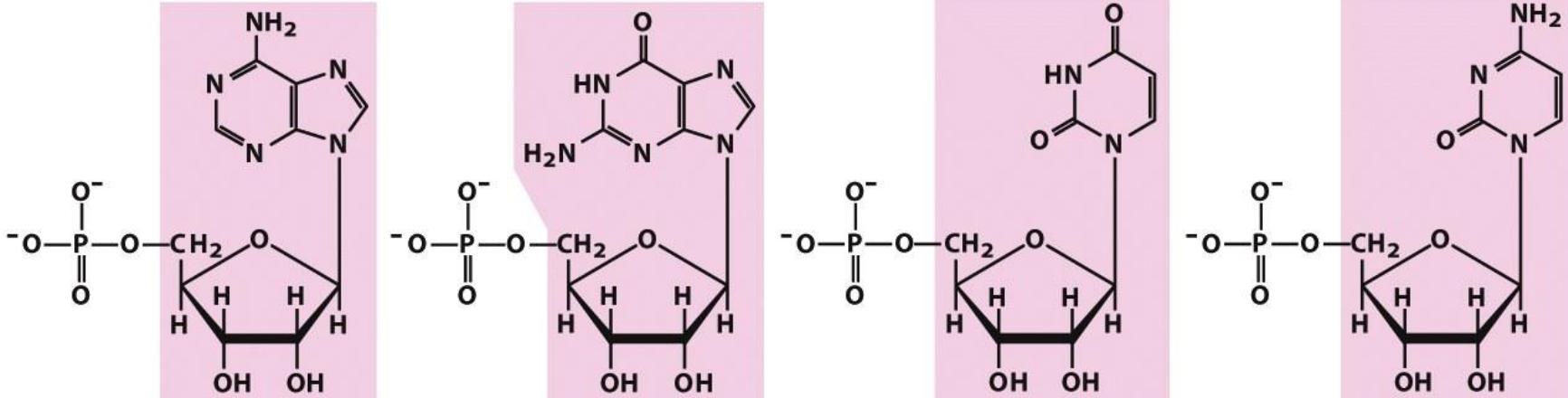
Figure 8-4a

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Nucleotide = Nucleoside + Phosphate

(Nucleoside = Sugar + Base)



Nucleotide: Adenylate (adenosine 5'-monophosphate)

Symbols: A, AMP

Nucleoside: Adenosine

Guanylate (guanosine 5'-monophosphate)

G, GMP

Guanosine

Uridylate (uridine 5'-monophosphate)

U, UMP

Uridine

Cytidylate (cytidine 5'-monophosphate)

C, CMP

Cytidine

Ribonucleotides

Figure 8-4b

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Phosphodiester Linkages in the Covalent Backbone of Nucleic Acid

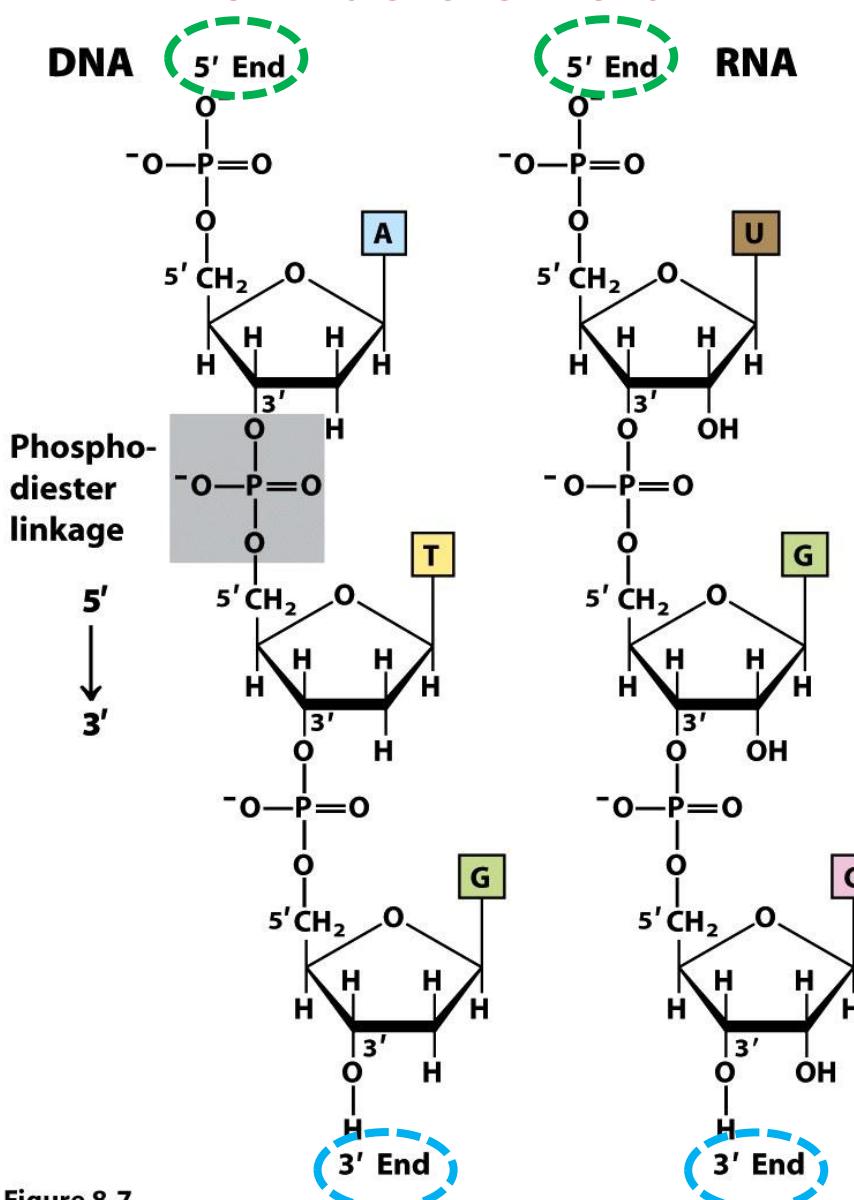
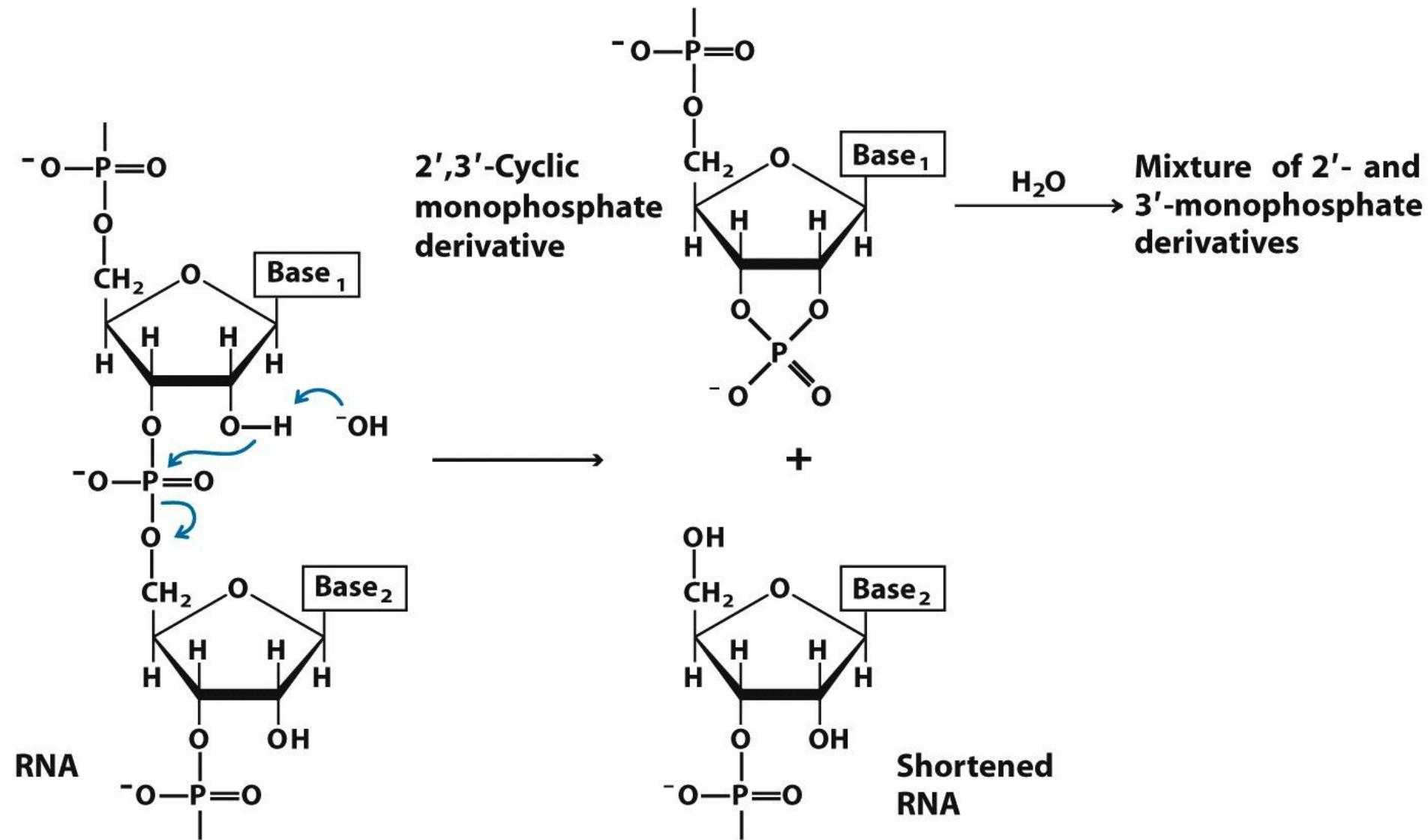


Figure 8-7

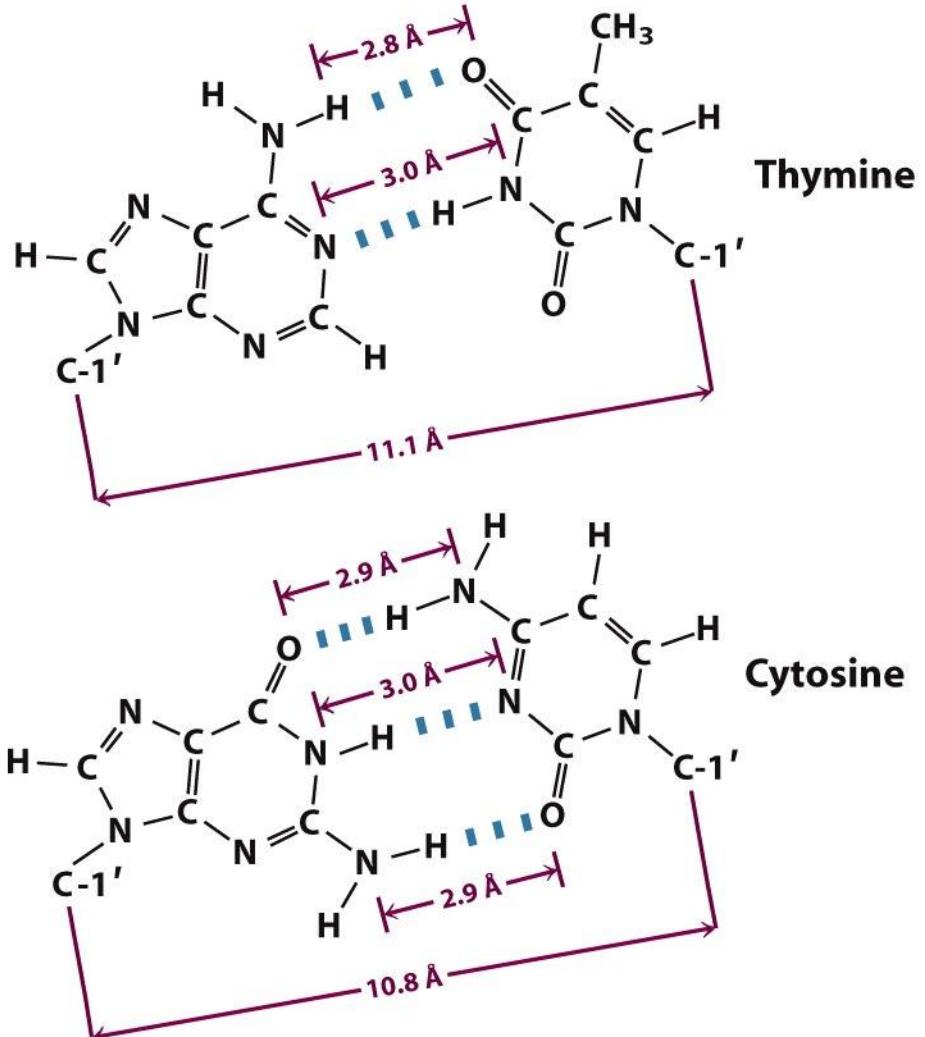
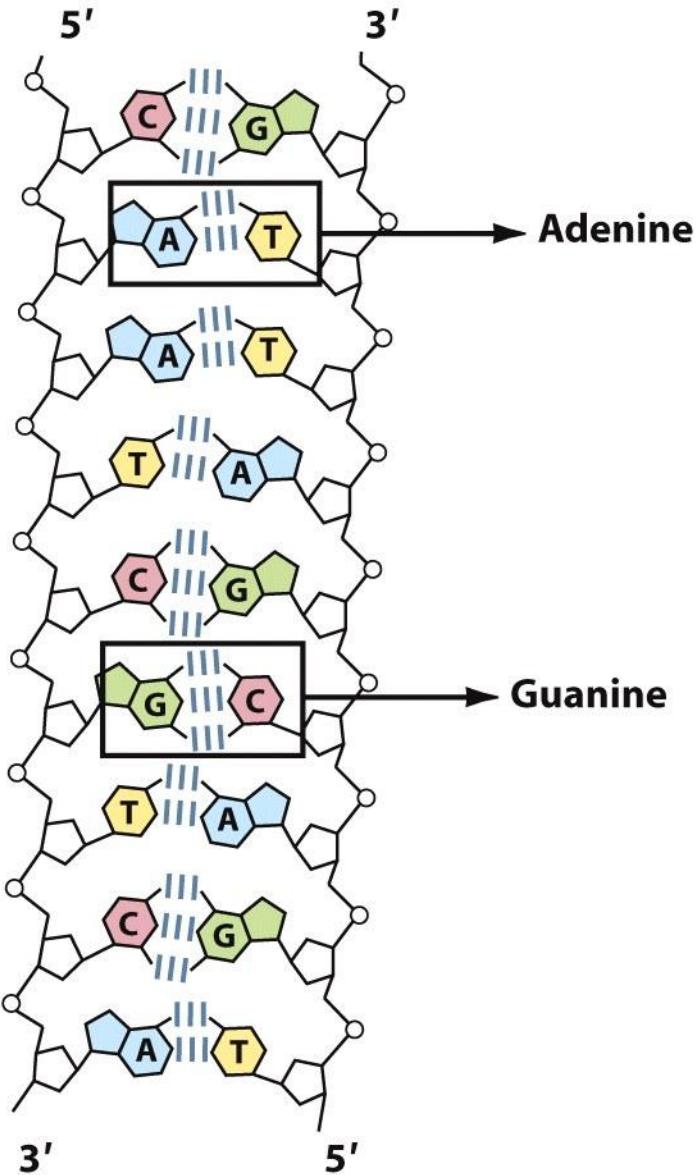
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RNA is Less Stable than DNA



DNA: Deoxyribonucleic Acid



Discovery of the DNA Structure

- Structure was discovered in 1953 by James Watson and Francis Crick
- Awarded Nobel Prize in 1962

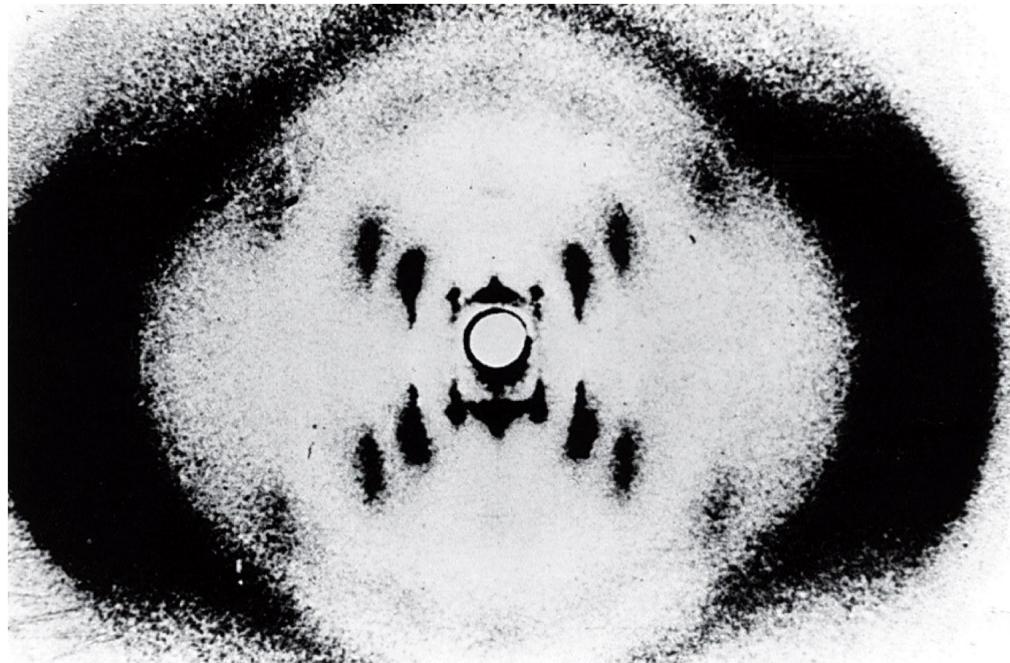
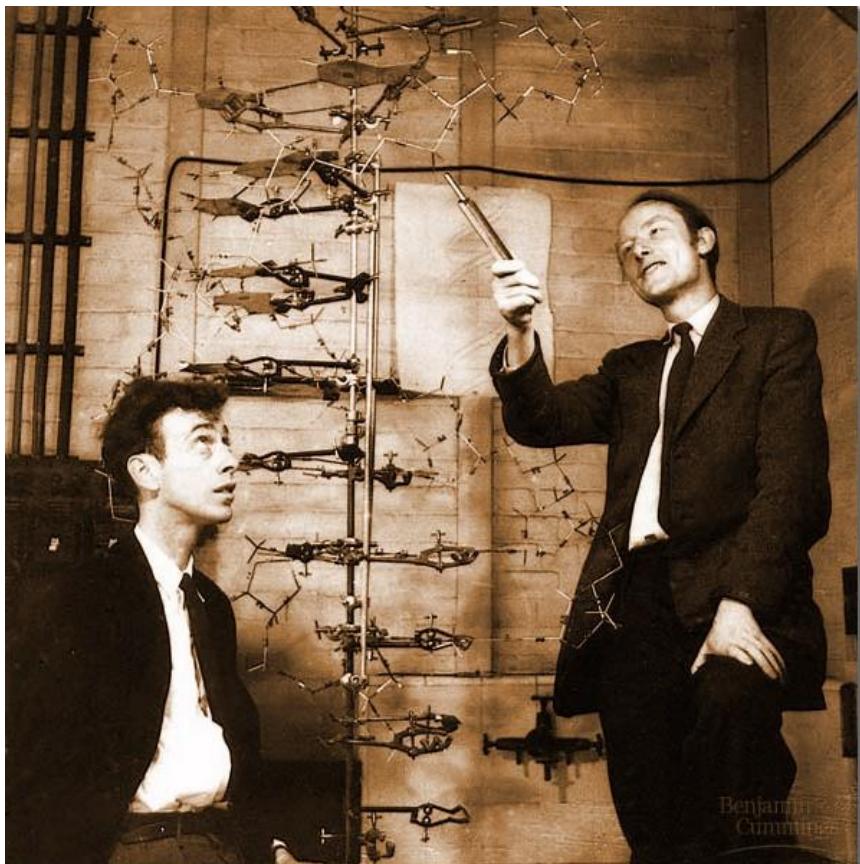
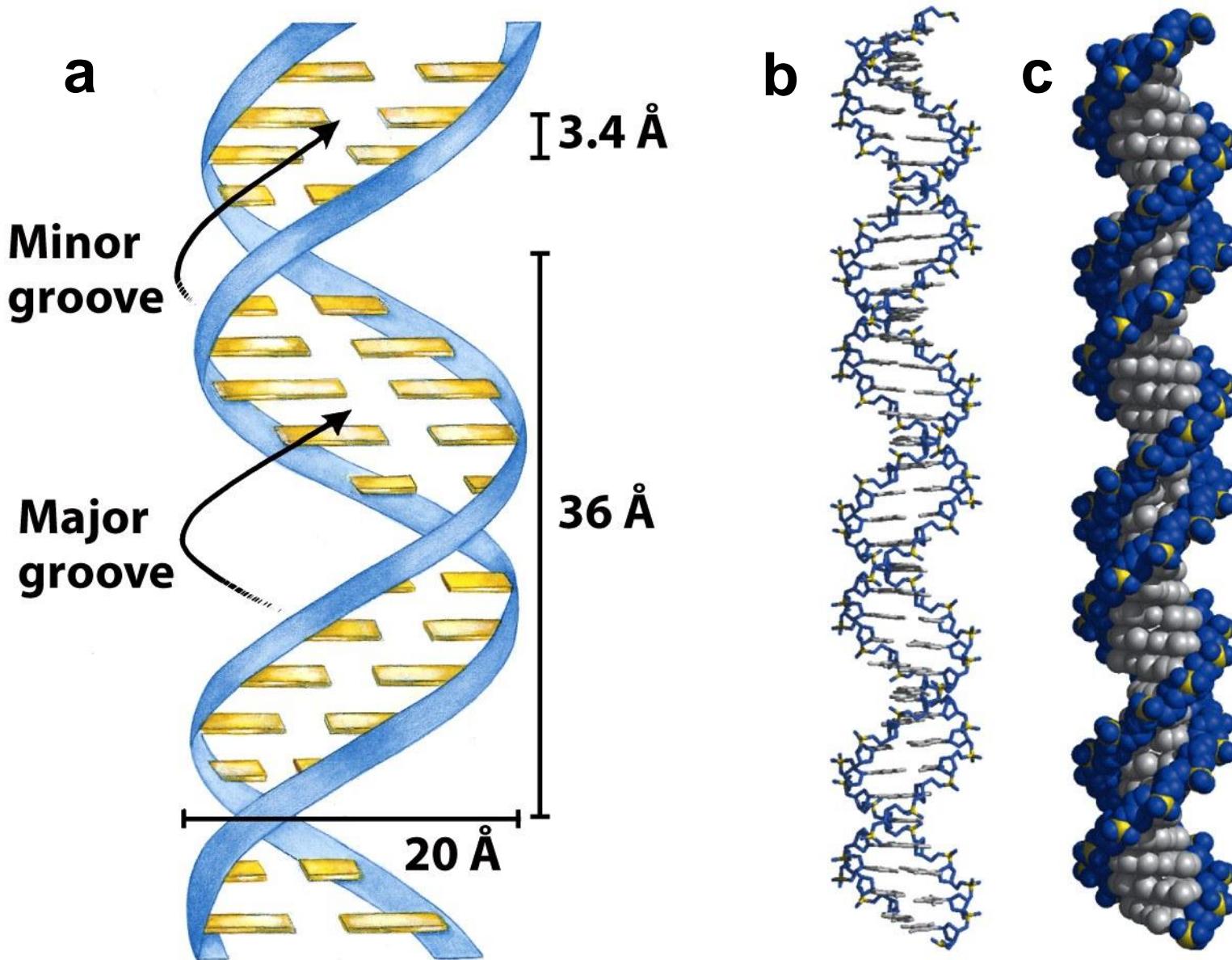


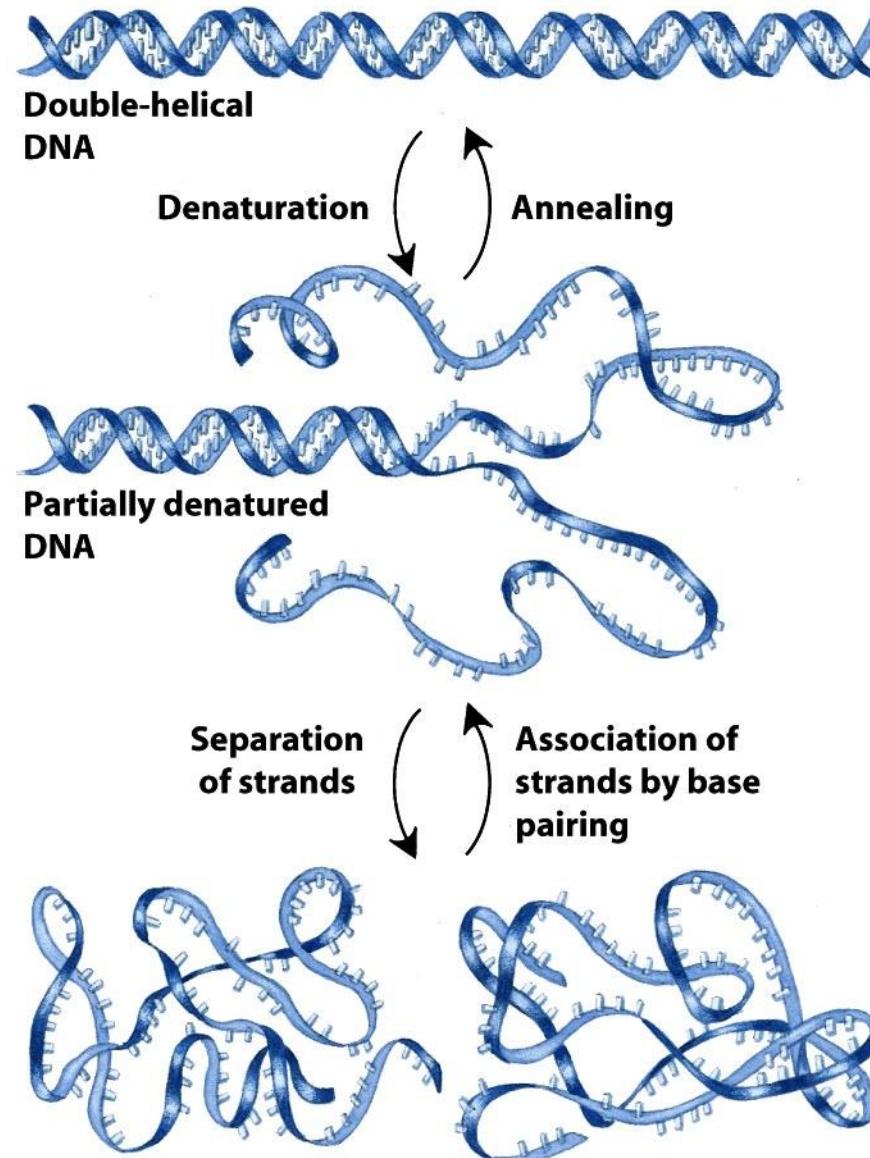
Figure 8-12
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Rosalind Franklin

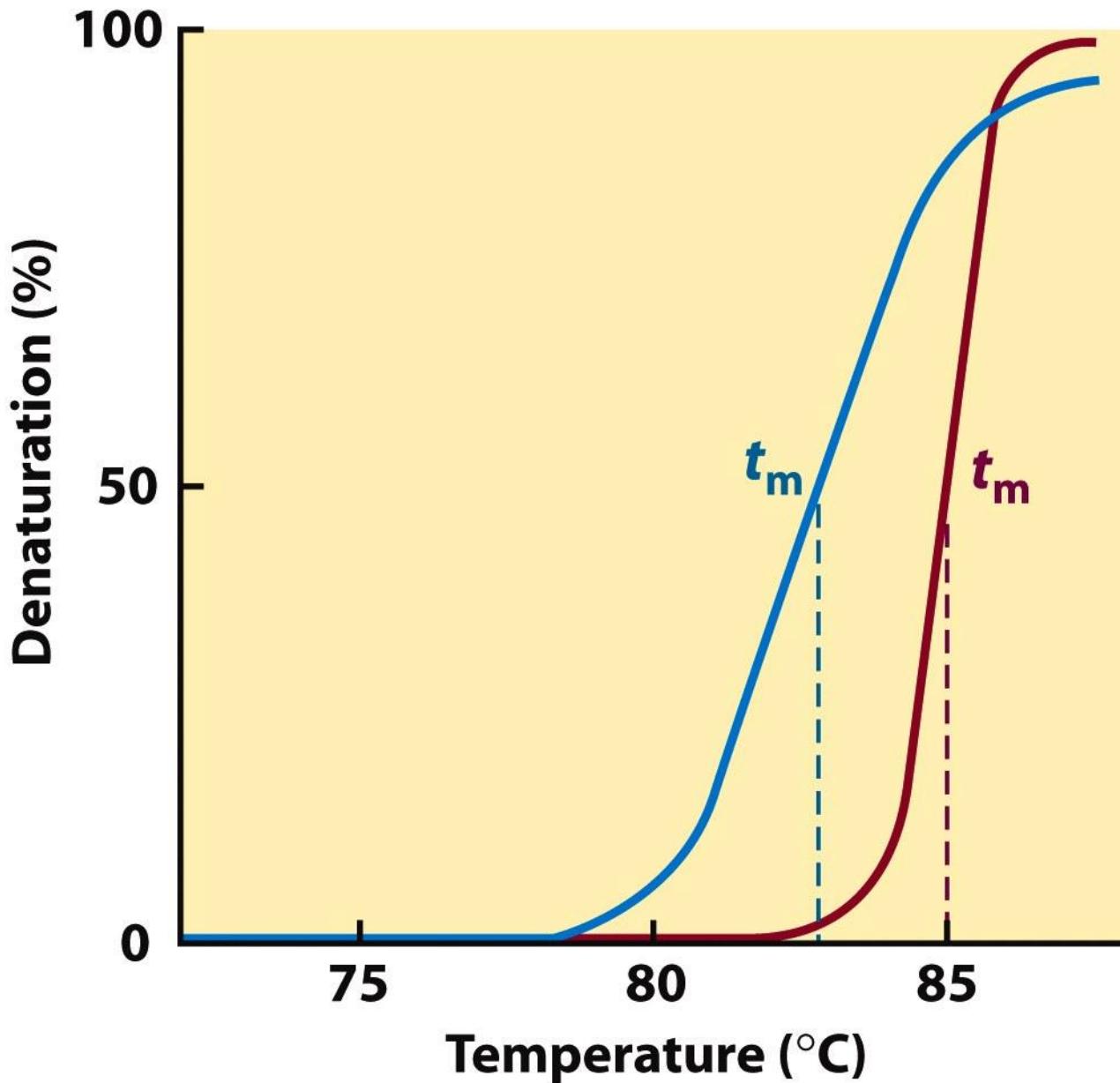
Watson-Crick Model for the Structure of DNA



Reversible Denaturation and Annealing (Renaturation) of DNA

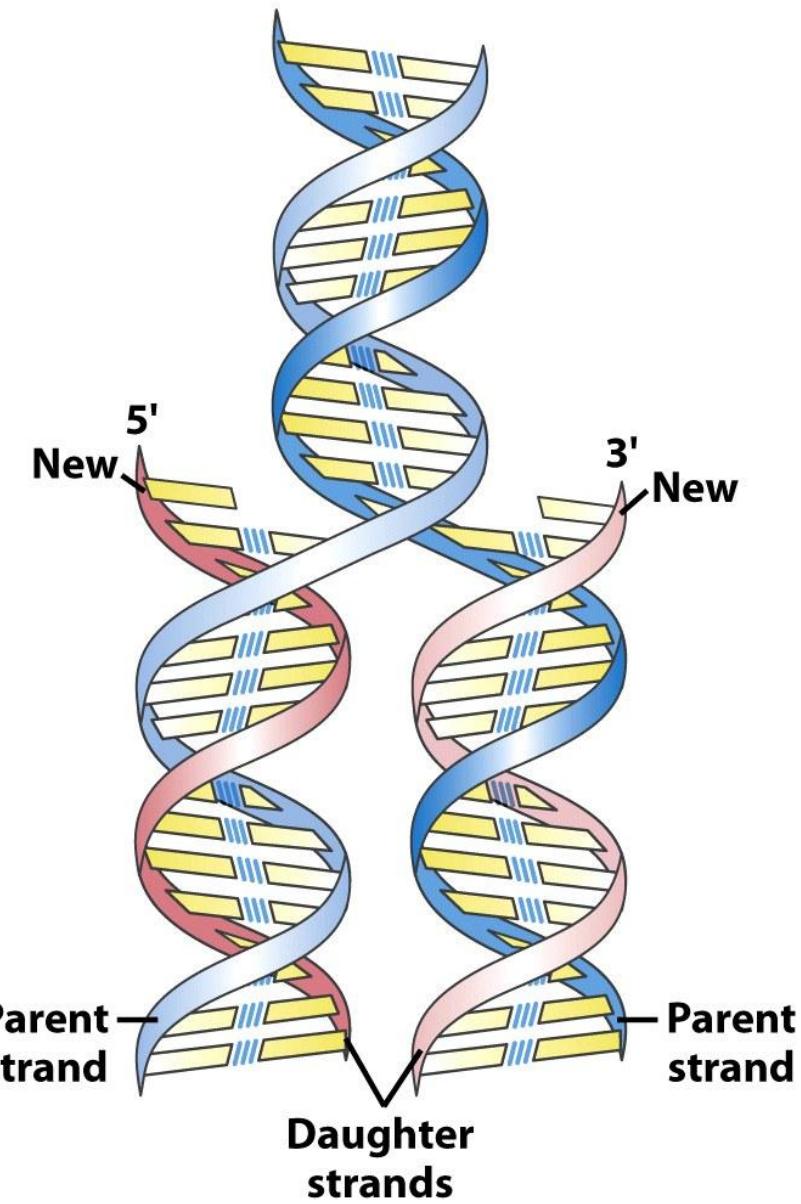
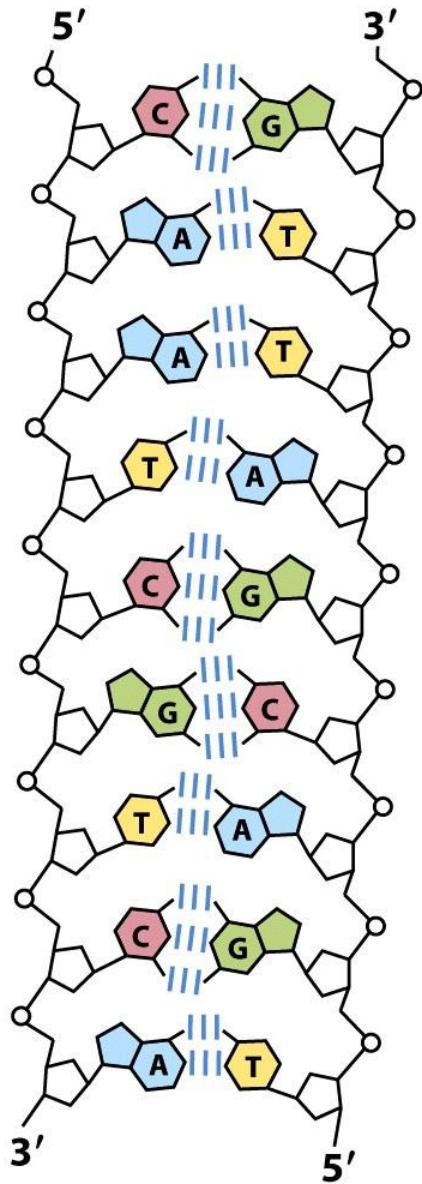


Heat Denaturation of DNA

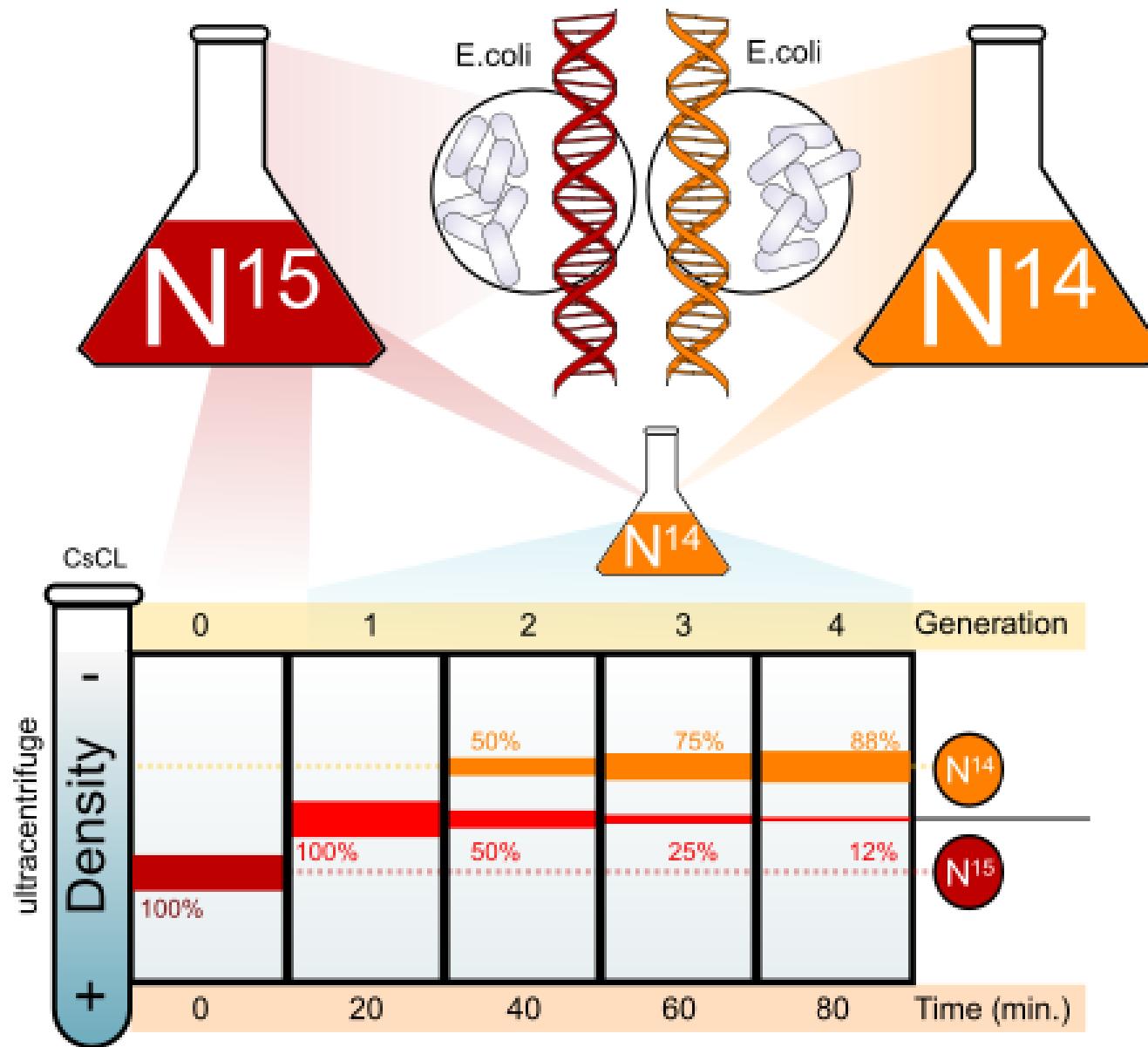


DNA Replication: An Overview

Semiconservative Replication of DNA as suggested by Watson and Crick

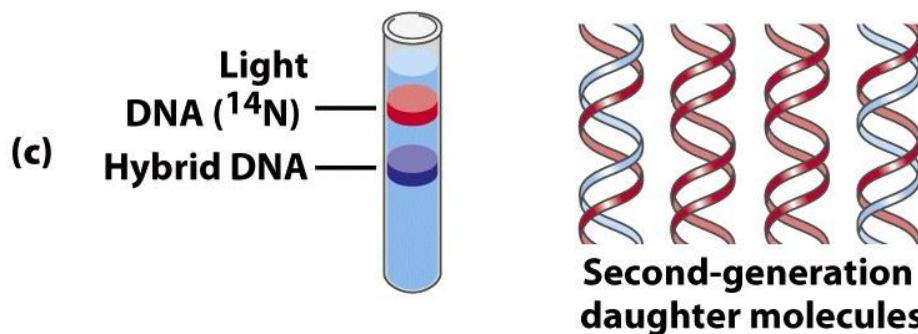
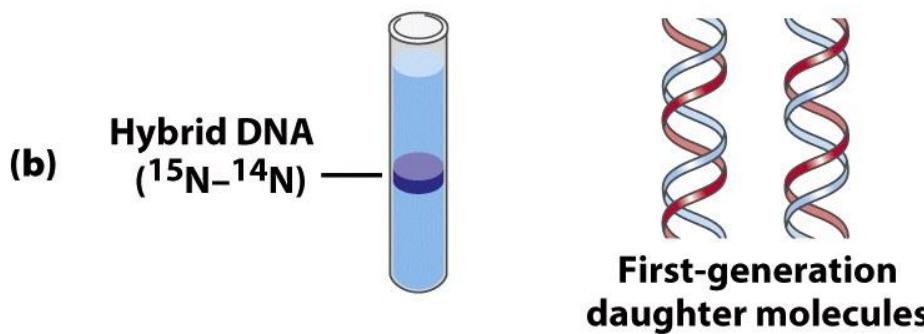
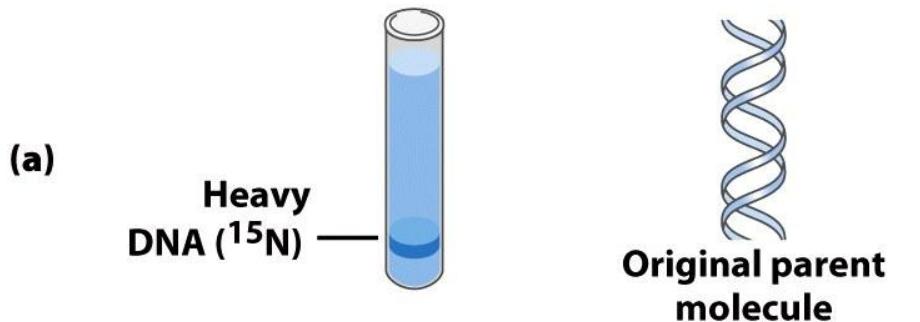


The Meselson-Stahl Experiment (1958)



The Meselson-Stahl Experiment Supports Semiconservative Replication

DNA extracted and centrifuged
to equilibrium in CsCl density gradient

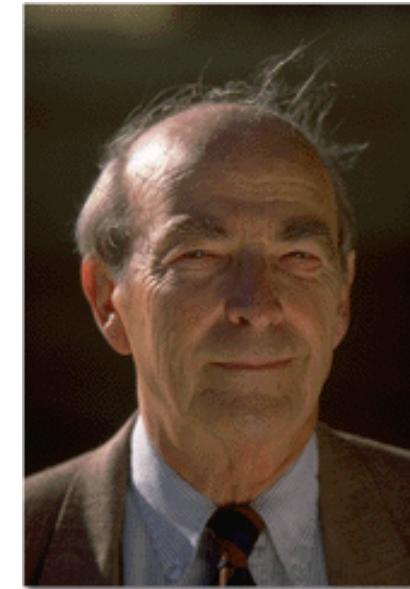


Outline for Replication

- A. Initiation
- B. Priming
- C. Elongation
- D. Proofreading and Termination



Arthur Kornberg Nobel Prize in 1959



Worked with *E. coli*. Discovered the mechanisms of DNA synthesis in 1956.

Four components are required:

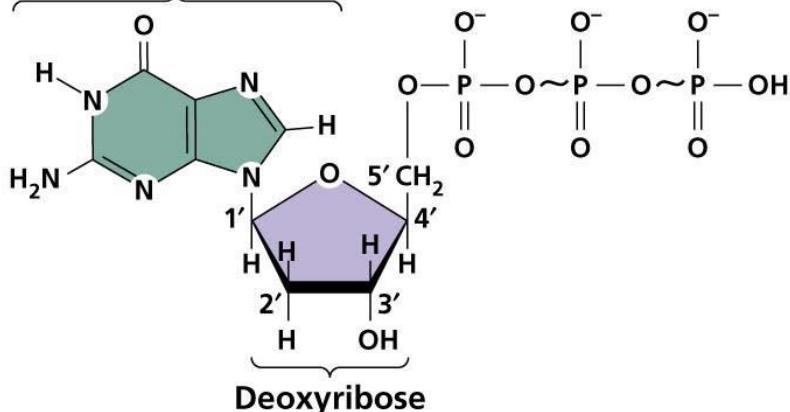
1. dNTPs: dATP, dTTP, dGTP, dCTP
 - (deoxyribonucleoside 5'-triphosphates)
 - (sugar-base + 3 phosphates)
2. DNA template
3. DNA polymerase (*Kornberg enzyme*)
4. Mg²⁺ (optimizes DNA polymerase activity)

Polymerization of nucleotide triphosphates by DNA polymerase – needs free 3'OH

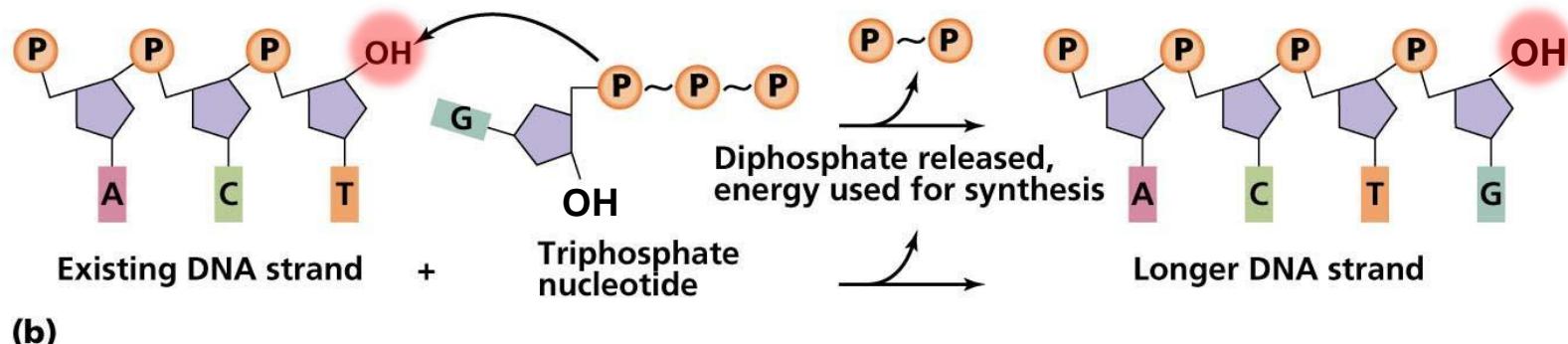
Guanosine triphosphate deoxyribonucleotide (dGTP)

Guanine nucleotide (dGMP)

Guanine base



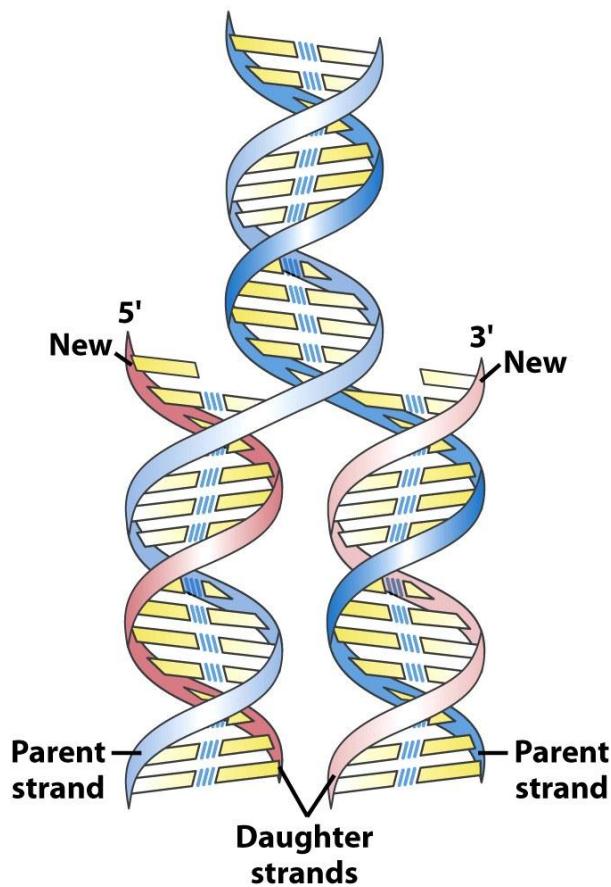
(a)



(b)

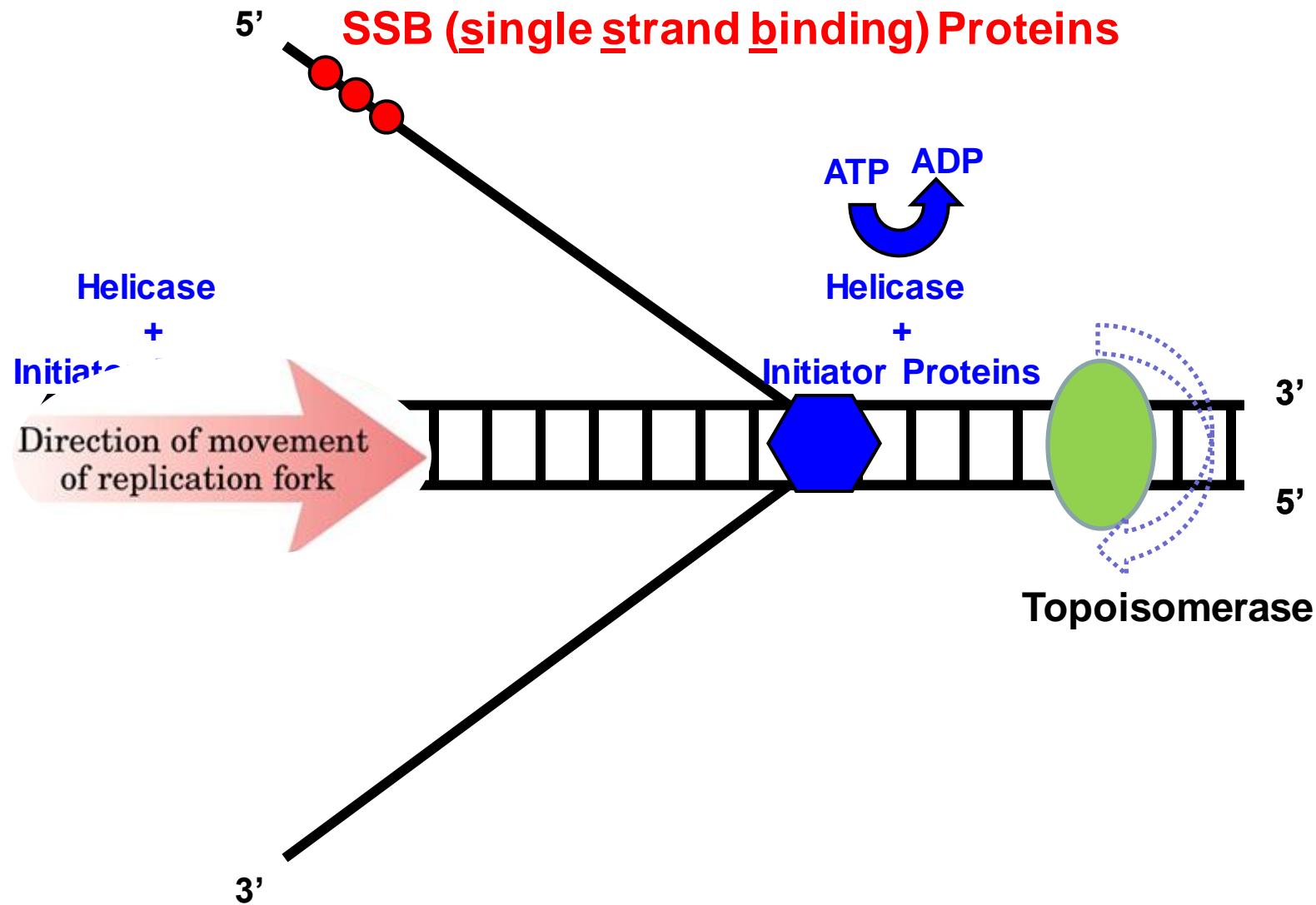
Problem -1

DNA is double helical. Hence the two strands have to be separated from each other



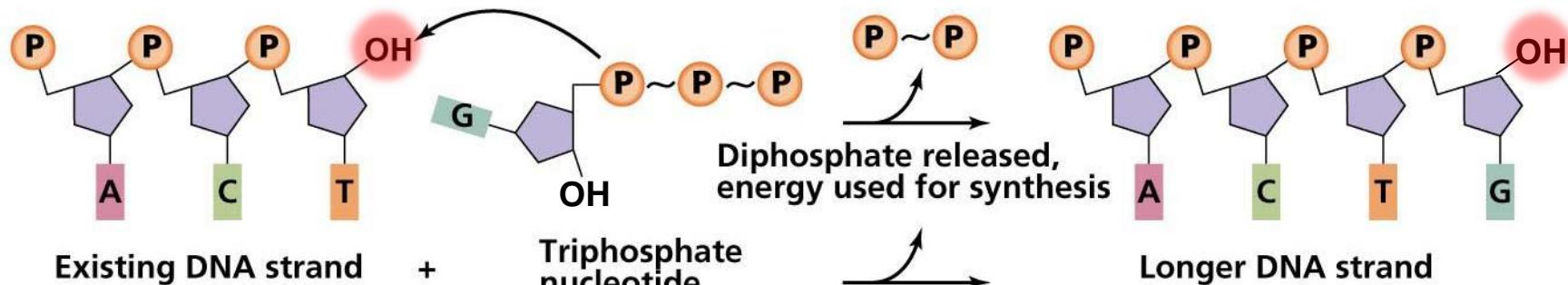
Problem - 1 : DNA unwinding

DNA is unwound by initiator protein + helicase, stabilized by SSB proteins; supercoiling is relaxed by Topoisomerase



Problem - 2

DNA polymerase needs a free 3'-OH of a pre-existing nucleotide to extend the chain

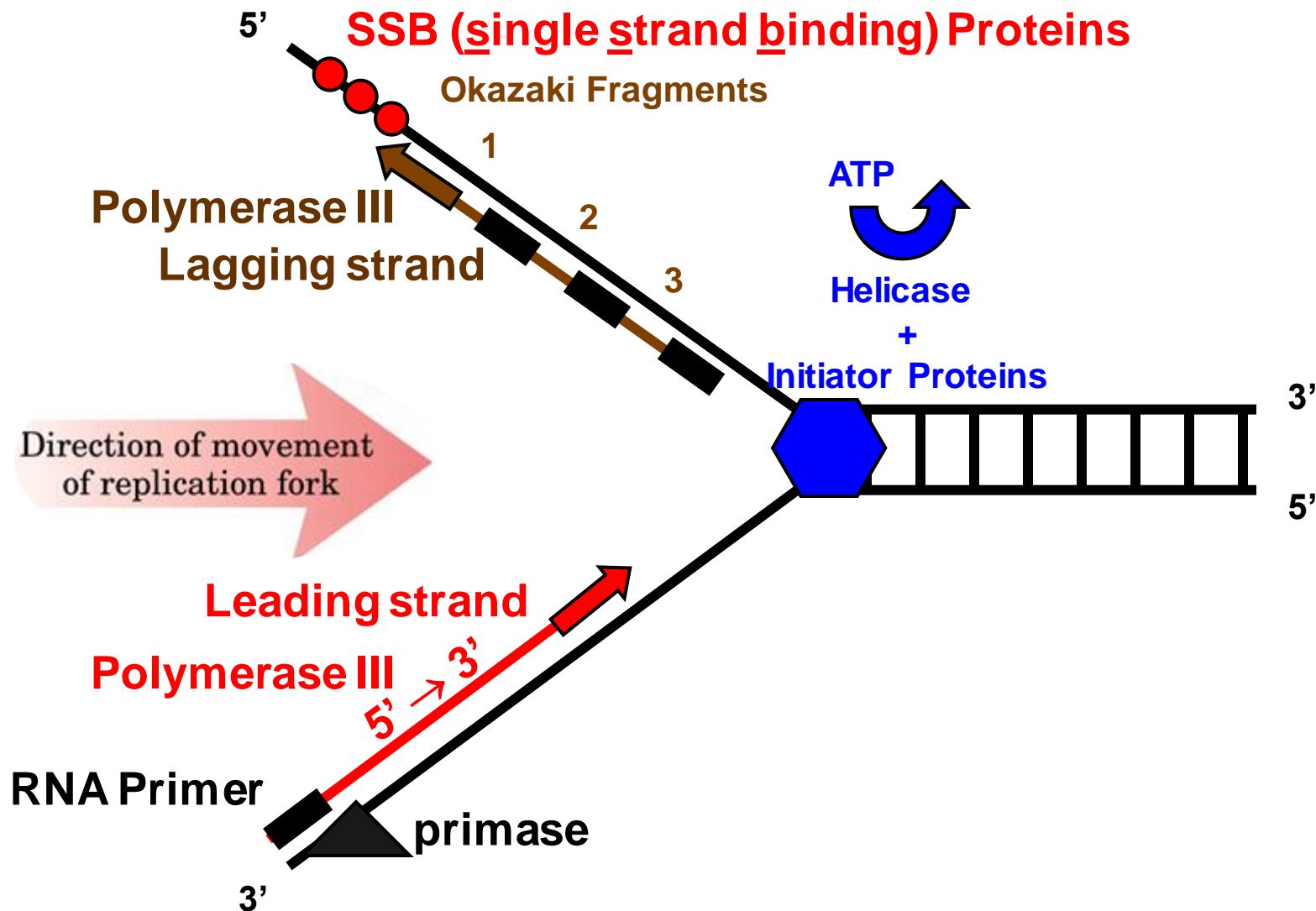


(b)

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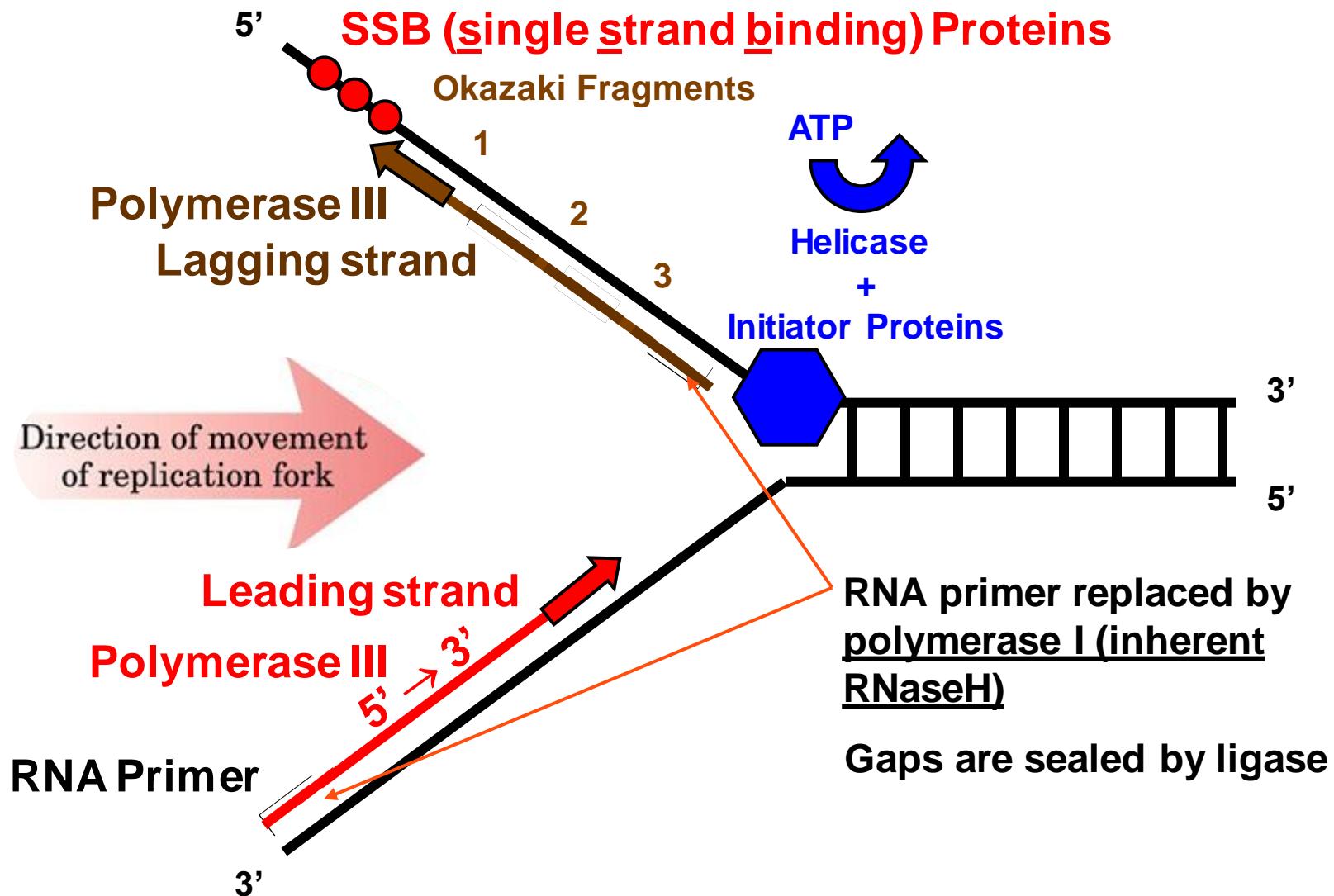
Problem – 2 : Free 3' OH group required

Primase synthesizes small RNA primers,
DNA Polymerase III extends them



Problem -3 : RNA primers need to be removed

RNA primers are replaced with DNA by DNA Polymerase I, which has both RNaseH and DNA Polymerase activity. The gaps are sealed by Ligase

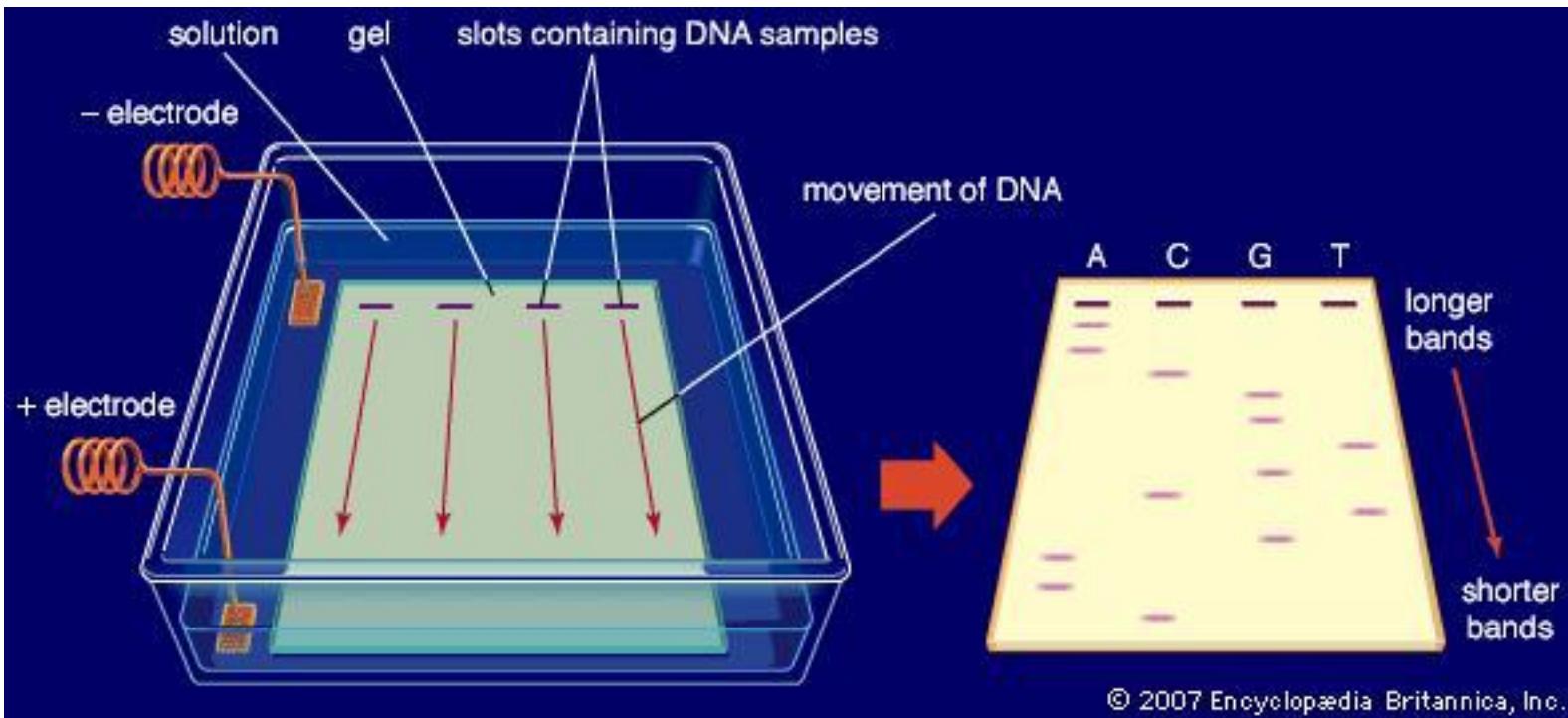


DNA Replication: Summary

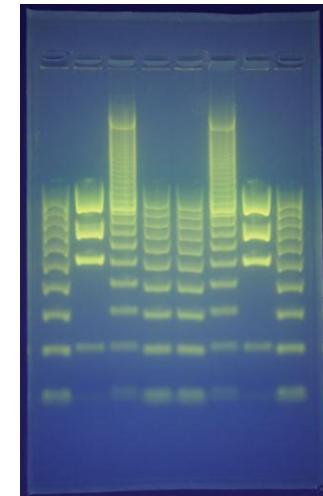
- DNA is unwound by initiator protein + helicase
- Single stranded DNA is stabilized by single stranded DNA binding proteins (SSB)
- Supercoiling is relaxed by Topoisomerase
- Small RNA primers are added by Primase (RNA polymerase)
- DNA Polymerase III extends from 3' ends of primer sequences
 - DNA Polymerase can only extend from pre-existing 3'OH group
 - Hence synthesis is always from 5'-3' direction
 - It needs DNA template, dNTPs and Mg²⁺ ions
- Replication is continuous in leading strand and discontinuous in lagging strands. Small newly synthesized DNA fragments in lagging strand are called Okazaki fragments
- RNA primers are replaced with DNA by DNA Polymerase I, which has both RNaseH and DNA Polymerase activity
- The gaps are sealed by Ligase

Laboratory methods for nucleic acid analysis

DNA Gel Electrophoresis



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DNA Gel Electrophoresis

- Agarose gel is used to analyze a mixture of DNA.
- DNA is negatively charged and hence migrates towards the positive terminal in the applied electric field gradient.
- Different DNA molecules separate according to mass.
- Smaller molecules migrate faster.
- DNA is visualized in the gel by staining with ethidium bromide, which fluoresces under UV light.
- UV light at 302 nm or 365 nm wavelengths are used.

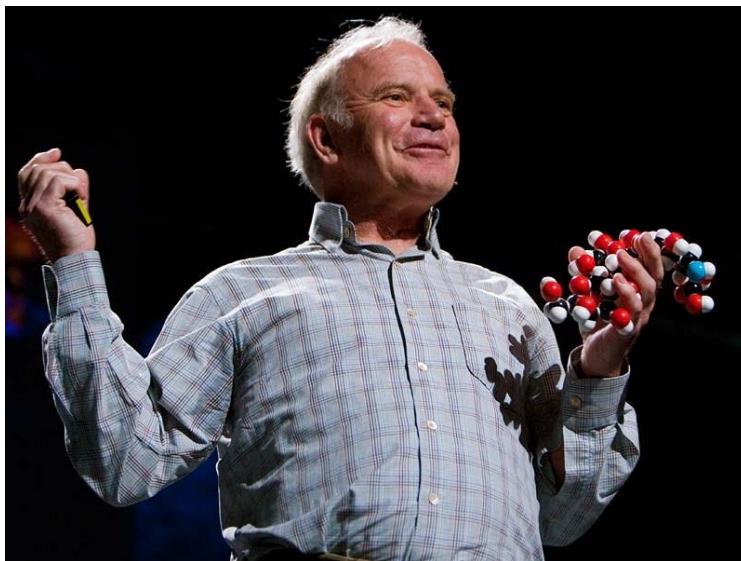
Polymerase Chain Reaction (PCR) and Its Applications

What is PCR?

PCR is an exponentially progressing synthesis of the defined target DNA sequences in vitro.

It was invented in 1983 by Dr. Kary Mullis

Nobel Prize in 1993



Polymerase Chain Reaction (PCR)

Why “Polymerase” ?

It is called “polymerase” because the only enzyme used in this reaction is DNA polymerase.

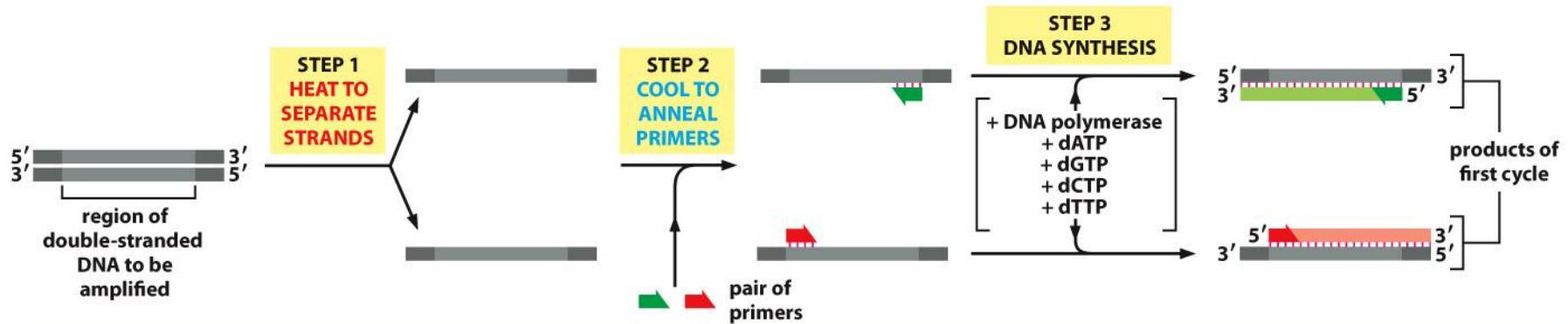
Why “Chain” ?

It is called “chain” because the products of the first reaction become substrates of the following one, and so on.

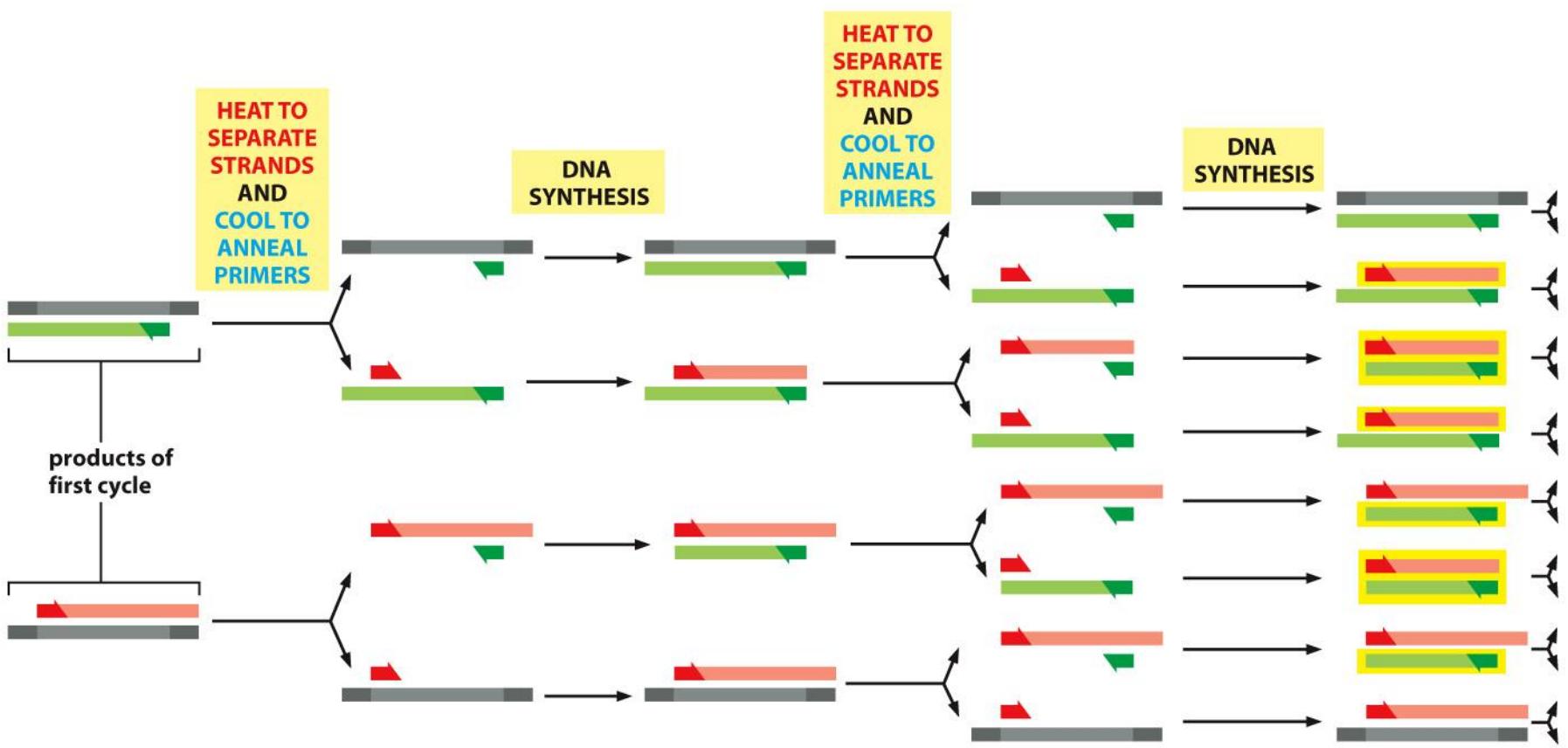
Polymerase Chain Reaction (PCR)

The “Reaction” Components

- 1) Target DNA - contains the sequence to be amplified.
- 2) Pair of Primers - oligonucleotides that define the sequence to be amplified.
- 3) dNTPs - deoxynucleotidetriphosphates: DNA building blocks.
- 4) Thermostable DNA Polymerase - enzyme that catalyzes the reaction
- 5) Mg⁺⁺ ions - cofactor of the enzyme
- 6) Buffer solution - maintains pH and ionic strength of the reaction solution suitable for the activity of the enzyme



FIRST CYCLE OF AMPLIFICATION

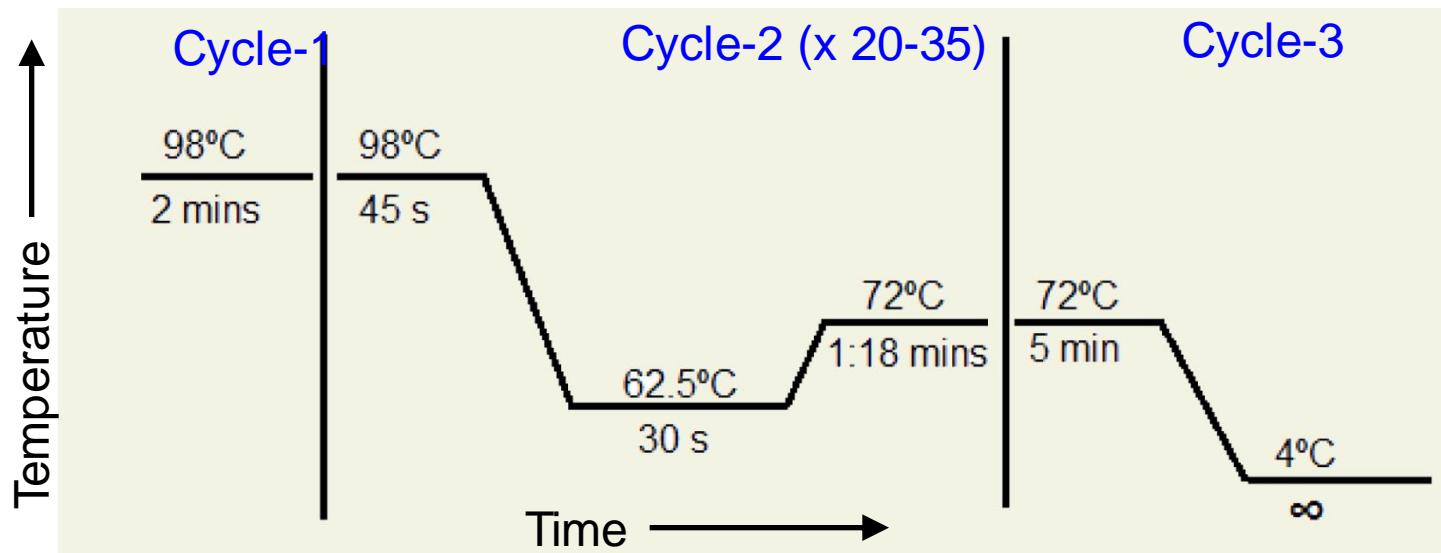


END OF FIRST CYCLE

SECOND CYCLE
(produces four double-stranded DNA molecules)

THIRD CYCLE
(produces eight double-stranded DNA molecules)

Reaction Cycle and the Machine



30 cycles = 2^{30} i.e. 1,073,741,824 copies



Thermocycler

DNA analysis in forensic science



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Enhancing Forensics in India
Scientific Examination, Research
and Analysis

011-26320016 (Head Office)
098714 10821 (Delhi-NCR)
098192 88253 (Mumbai)
098396 62999 (Rest of India)
email: investigation@ifsr.in



Computer
Forensics



Document Fraud
& Handwriting



Fingerprint
Forensics



Biology, DNA
Toxicology



- DNA analysis has become an important tool in forensic science in the past decade.
- The innocence project (<http://www.innoscenceproject.org>) in the USA has used DNA testing to free 343 wrongfully convicted people and finding of 147 real perpetrators.

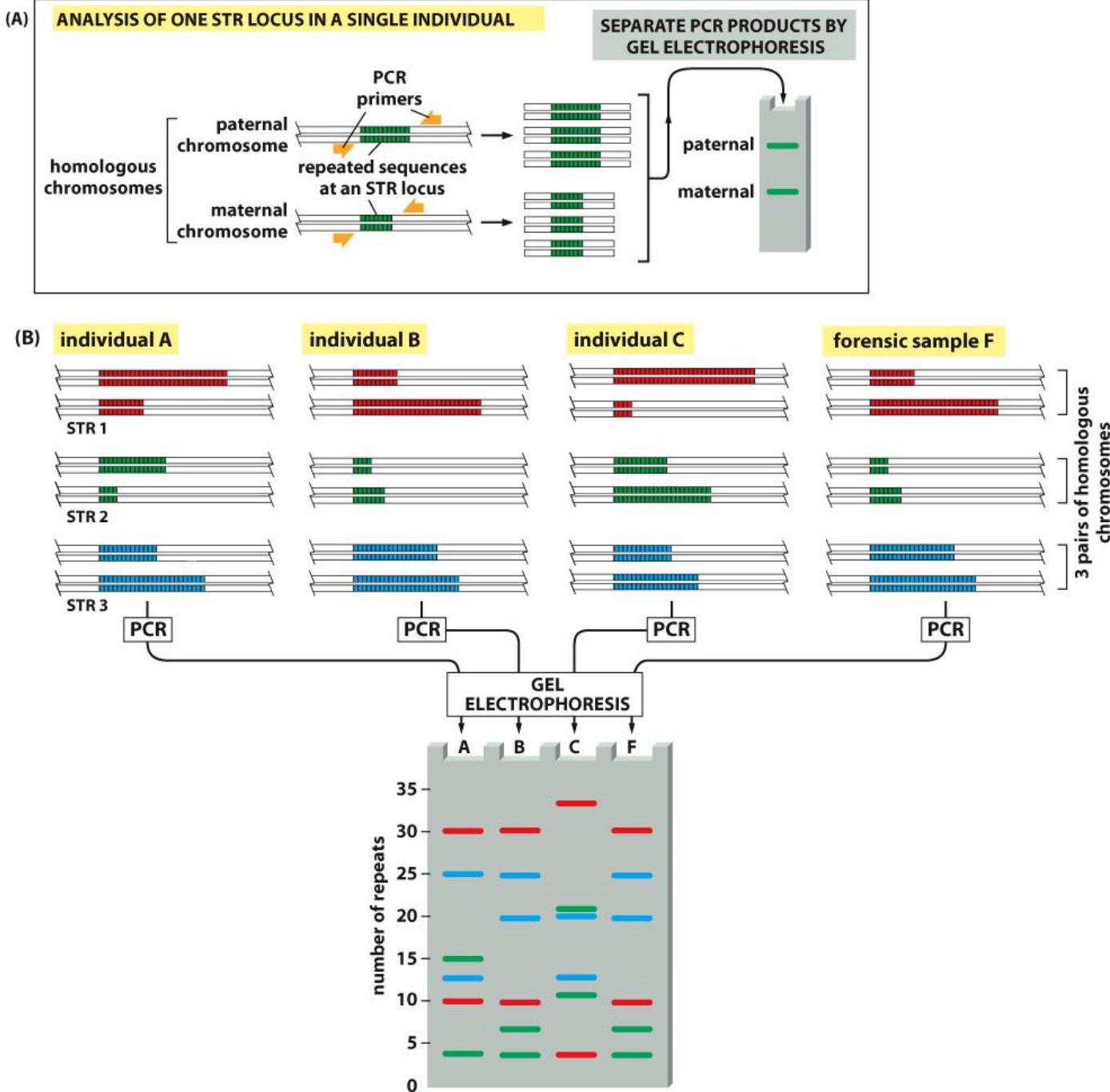


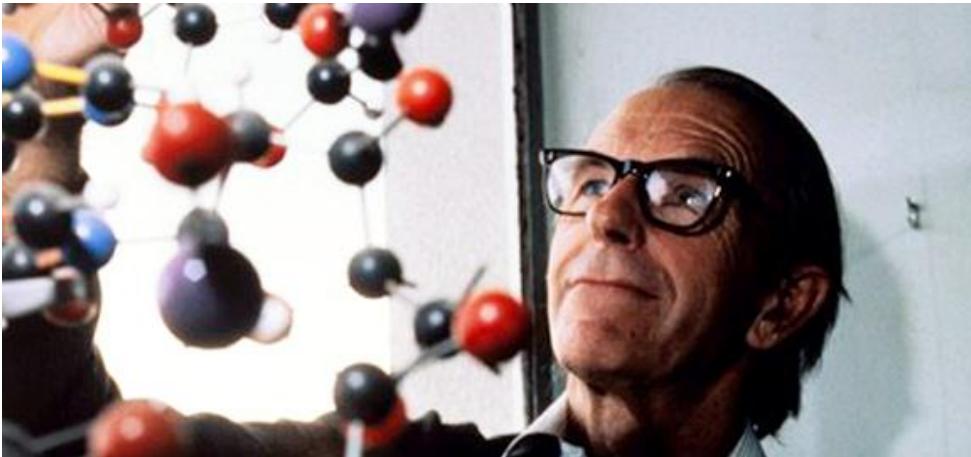
Figure 8-39 Molecular Biology of the Cell 6e (© Garland Science 2015)

How PCR and gel electrophoresis is used in forensic science?

(A) The DNA sequence that create the variability used in this analysis contain runs of short, repeated sequences, such as CACACA ..., which are found in various positions (loci) in the human genome. The number of repeats in each run can be highly variable in the population ranging from 4 to 40 in different individuals. A run of repeated nucleotides of this type is commonly referred to as a hypervariable microsatellite-sequence also known as a VNTR (variable number of tandem repeat) sequence. Because of the variability in these sequences at each locus, individuals usually inherit a different variant from their mother and from their father; two unrelated individuals therefore do not usually contain the same pair of sequences. A PCR analysis using primers that bracket the locus produces a pair of bands of amplified DNA from each individual, one band representing the maternal variant and the other representing the paternal variant. The length of the amplified DNA, and thus the position of the band it produces after electrophoresis, depends on the exact number of repeats at the locus.

(B) In the schematic example shown here, the same three VNTR loci are analyzed (requiring three different pairs of specially selected oligonucleotide primers) from three Suspects (individuals A, B and C), producing six DNA bands for each person after polyacrylamide-gel electrophoresis. Although some individuals have several bands in common, the overall pattern is quite distinctive for each. The band pattern can therefore serve as a "fingerprint" to identify an individual nearly uniquely. The fourth lane (F) contains the products of the same reactions carried out on a forensic sample. The starting material for such a PCR can be a single hair or a tiny sample of blood that was left at the crime scene. When examining the variability at 5-10 different VNTR loci, the odds that two random individuals would share the same genetic pattern by chance can be approximately 1 in 10 billion. In the case shown here, individuals A and C can be eliminated from further enquiries whereas individual B remains a clear suspect for committing the crime. A similar approach is now routinely used for paternity testing.

DNA Sequencing



Frederick Sanger
Nobel Prize in 1980

Sequencing: The process by which you determine the exact order of the nucleotides in a given region of DNA

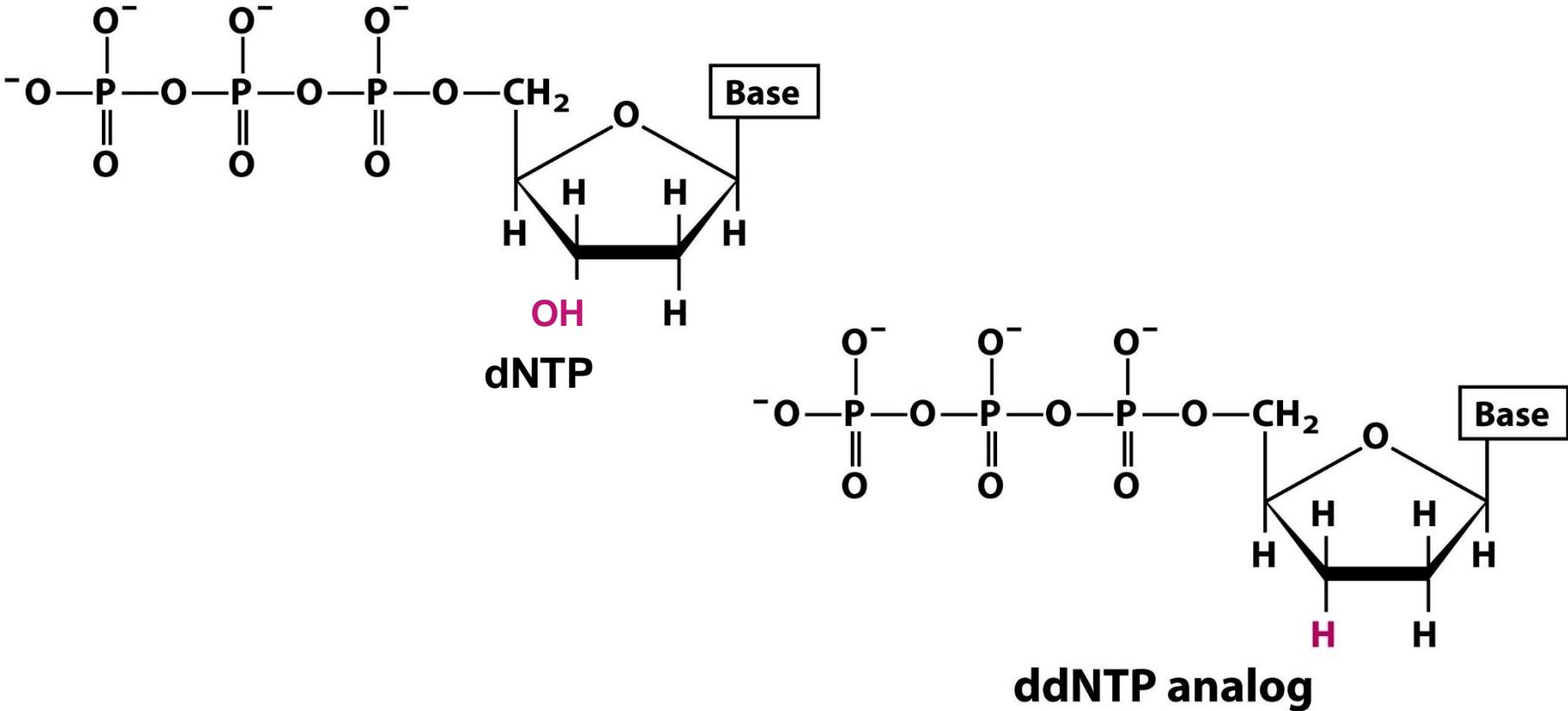
Why? To decode the genetic information embedded in DNA

How? Through complementary chain synthesis and early termination

The synthesized chains are visualized by methods using:

- Radioactive labels
- Nonradioactive labels

Dideoxynucleotides (ddNTPs)



The 3' hydroxyl has been changed to a hydrogen in ddNTP's, which terminates a DNA chain because a phosphodiester bond cannot form at this 3' location

Requirements for Sanger-Coulson Sequencing

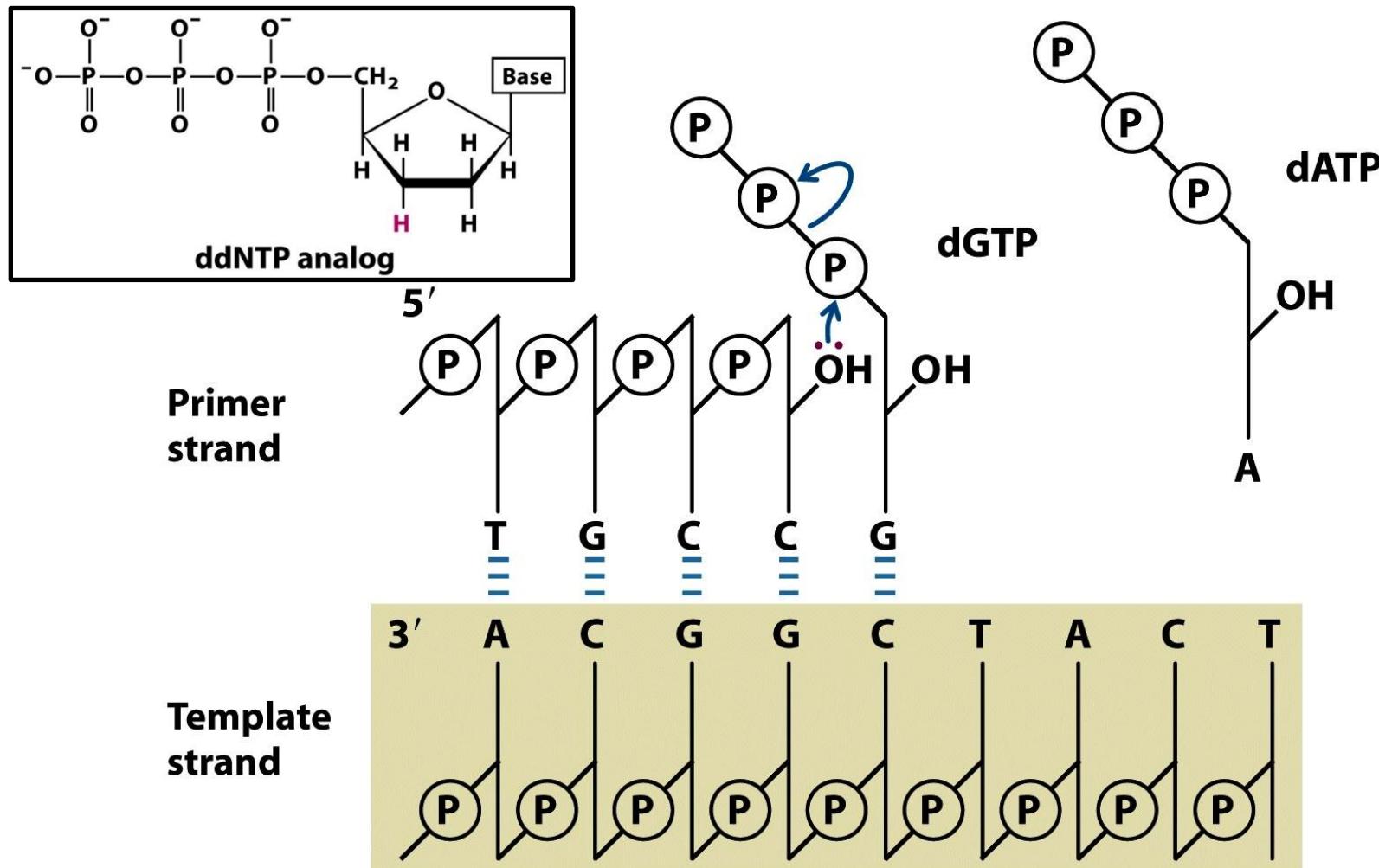
DNA to be sequenced must be in single strand form

The region to be sequenced must be flanked by a stretch of 3' known sequence

Reagents needed are:

- A primer complementary to the known region to direct chain synthesis.**
- DNA polymerase.**
- 4 deoxynucleotide triphosphates (dNTPs).**
- 4 dideoxynucleotide triphosphates (ddNTPs) (one for each set)**

Mechanism of DNA polymerization



Since the 3' –OH is changed to a –H in ddNTPs, it is unable to form a phosphodiester bond by nucleophilic attack on the phosphate, and it will cause a termination in the DNA chain

Sequencing using denaturing gel-electrophoresis

Template	3' – A T A T C T G T A C C T A G G T G A G T C A G T A C C -5'
Primer	5' – T A T A G A C
Differentially elongated fragments	5' – T A T A G A C A 5' – T A T A G A C A T G G A 5' – T A T A G A C A T G G A T C C A 5' – T A T A G A C A T G G A T C C A C T C A 5' – T A T A G A C A T G G A T C C A C T C A G T C A

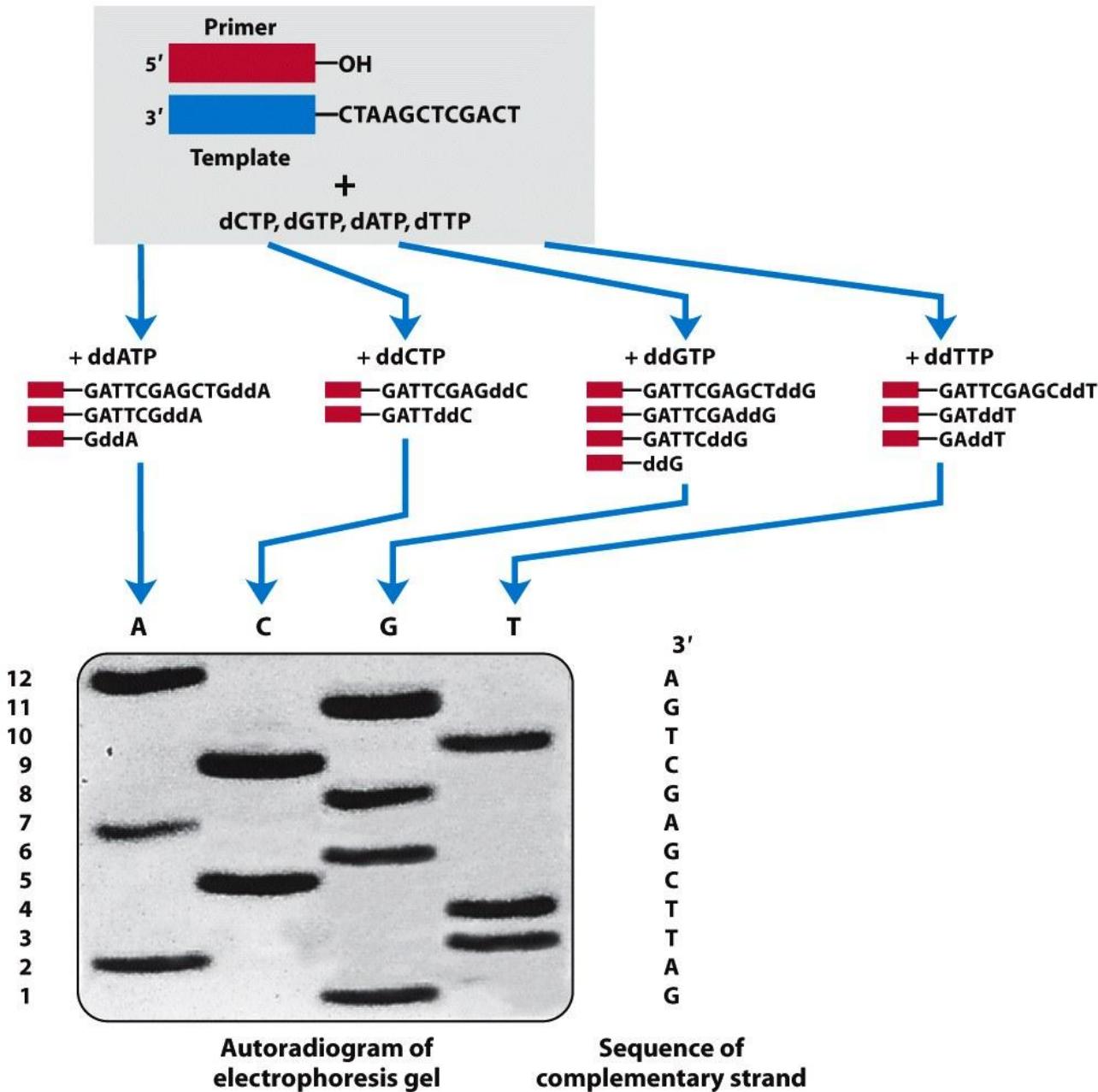
To each reaction along with the four regular dNTPs, only one of the four dideoxynucleotides (**ddATP in the above reaction**) are added

The ddNTP is added to be approximately 100-fold lower in concentration than the corresponding dNTP

A series of differentially elongated DNA fragments (hence of different length) will be produced

Can be visualized by labelling the primers with radioactive phosphate (^{32}P or ^{33}P)

Sequencing using denaturing gel-electrophoresis



Automated sequencing

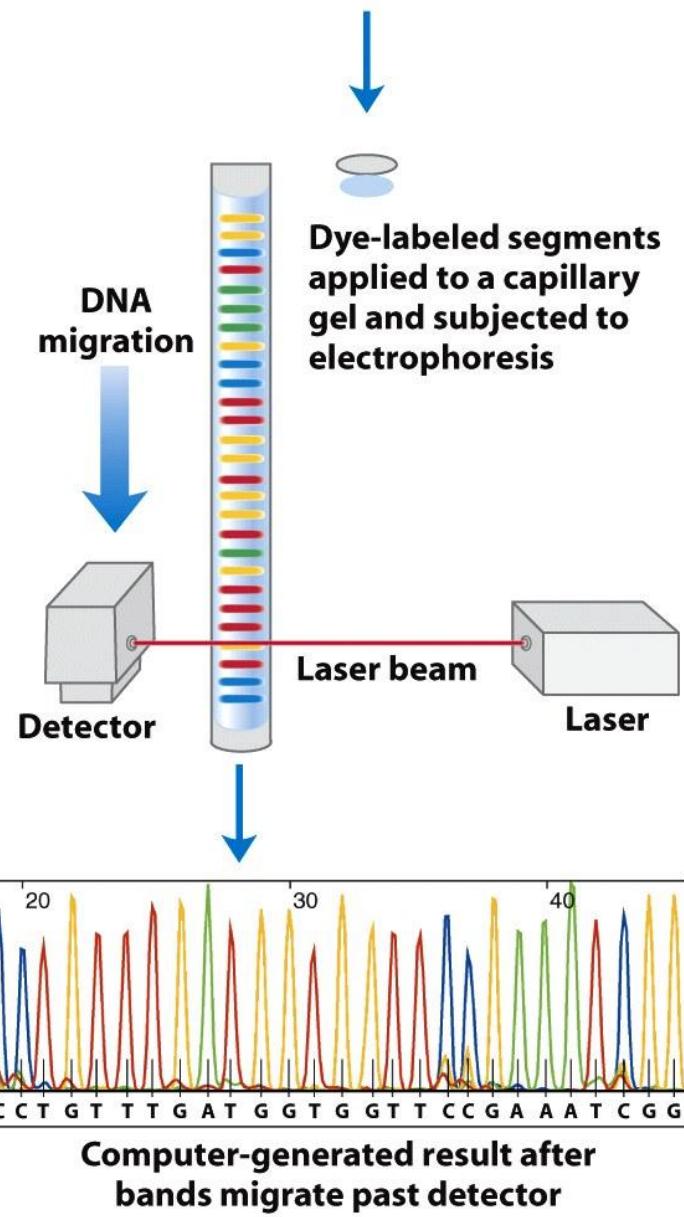
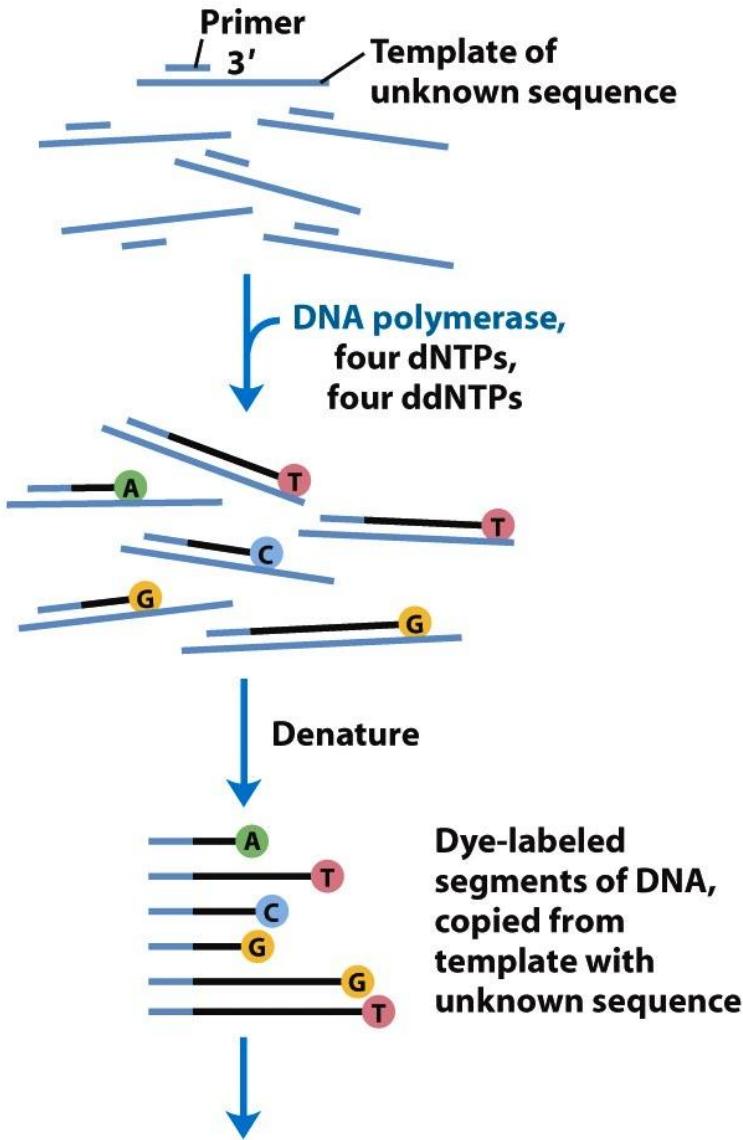


Figure 8-34

Lehninger Principles of Biochemistry, Fifth Edition

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Automated sequencing

Each dideoxynucleotide used are linked to a fluorescent molecule that gives all the fragments terminating in that nucleotide a particular color.

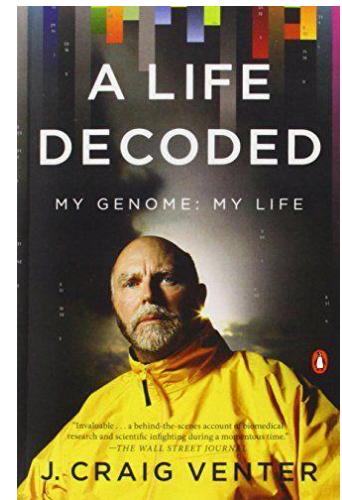
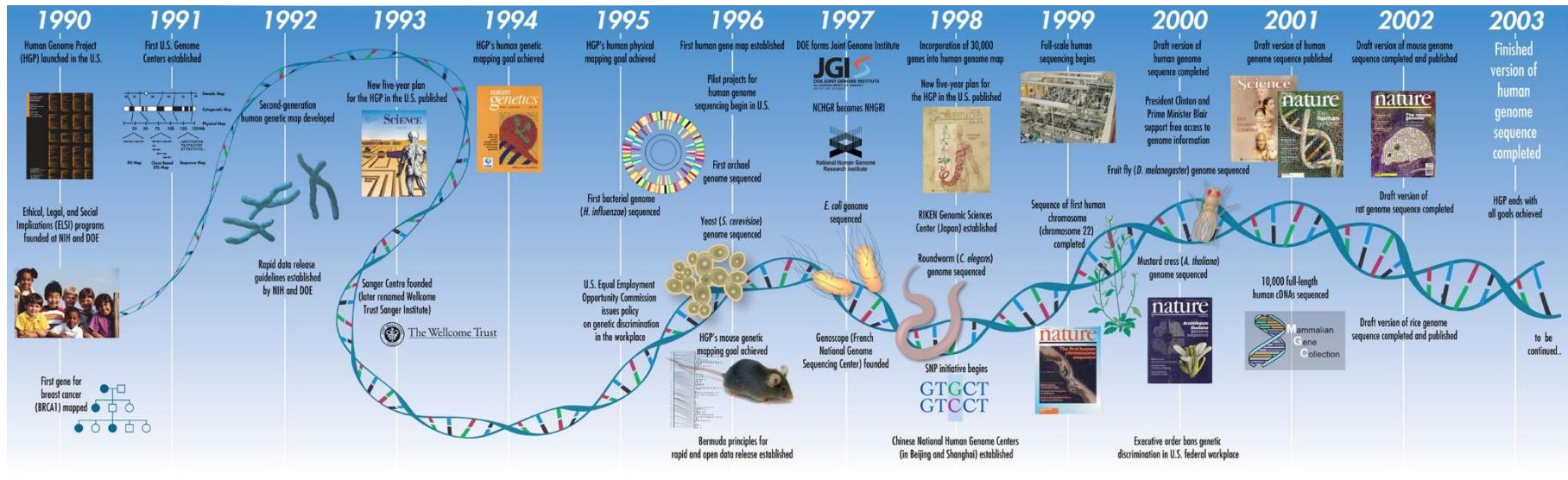
All four labelled ddNTPs are added to a single tube.

The resulting colored DNA fragments are then separated by capillary gel electrophoresis

The color associated with each peak is detected using a laser beam.

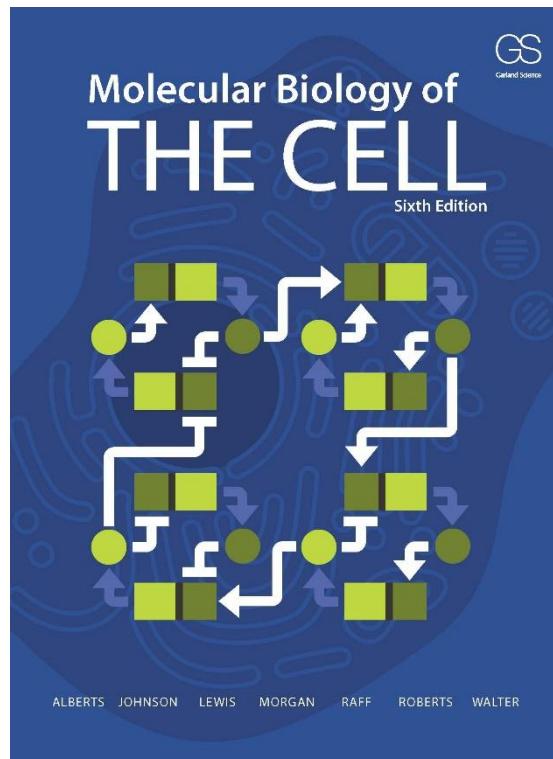
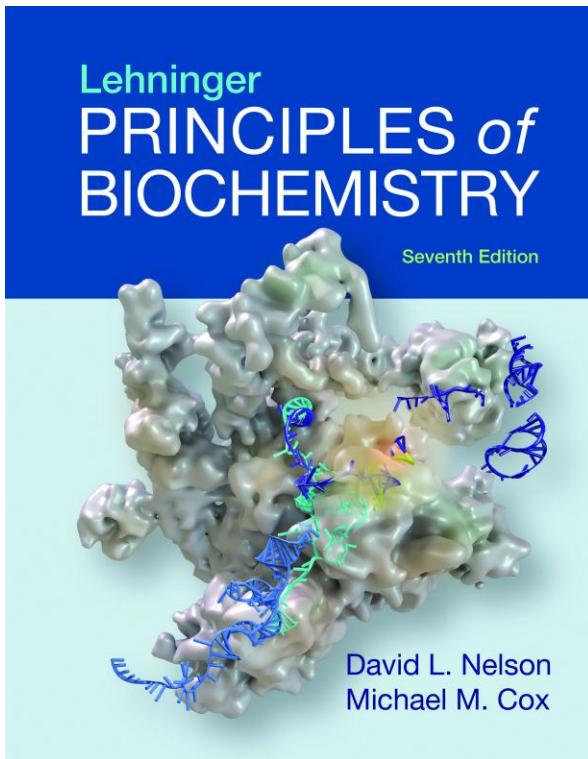
The DNA sequence is read by determining the sequence of colors in the peaks as they pass the detector.

This information is fed directly to a computer, which determines the sequence.



An individual somatic (**diploid**) cell contains about 6.2 billion base pairs.
2.7 billion US dollar spend for 12 years to complete human genome sequencing..

References



Extra resources

Book:

The Double Helix: A Personal Account of the Discovery of the Structure of DNA - By James D Watson

Videos:

DNA replication

<https://www.youtube.com/watch?v=TNKWgcFPHqw>

DNA sequencing:

<https://www.youtube.com/watch?v=vK-HIMaitnE>

Polymerase Chain Reaction (PCR)

<http://video.mit.edu/watch/pcr-polymerase-chain-reaction-10948/>

<https://www.dnalc.org/view/15475-The-cycles-of-the-polymerase-chain-reaction-PCR-3D-animation.html>