
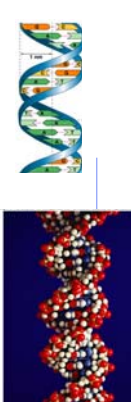


Chapter 16.

DNA
The Genetic Material
Replication




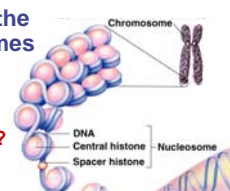

Scientific History

- The march to understanding that DNA is the genetic material
 - ◆ T.H. Morgan (1908)
 - ◆ Frederick Griffith (1928)
 - ◆ Avery, McCarty & MacLeod (1944)
 - ◆ Hershey & Chase (1952)
 - ◆ Watson & Crick (1953)
 - ◆ Meselson & Stahl (1958)

MCC BP Based on work by K. Foglia www.kfoglia.com

Genes are on chromosomes 1908 | 1933

- T.H. Morgan
 - ◆ working with *Drosophila* (fruit flies)
 - ◆ genes are on chromosomes
 - ◆ but is it the **protein** or the **DNA** of the chromosomes that are the genes?
 - through 1940 proteins were thought to be genetic material... **Why?**



The "Transforming Factor" 1928

- Frederick Griffith
 - Streptococcus pneumoniae* bacteria
 - was working to find cure for pneumonia
 - harmless live bacteria mixed with heat-killed infectious bacteria causes disease in mice
 - substance passed from dead bacteria to live bacteria = "**Transforming Factor**"

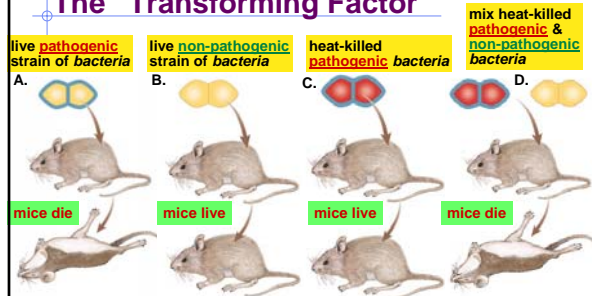


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The "Transforming Factor"

live pathogenic strain of bacteria live non-pathogenic strain of bacteria heat-killed pathogenic bacteria mix heat-killed pathogenic & non-pathogenic bacteria

A. B. C. D.




mice die mice live mice live mice die

Transformation?
something in heat-killed bacteria could still transmit disease-causing properties

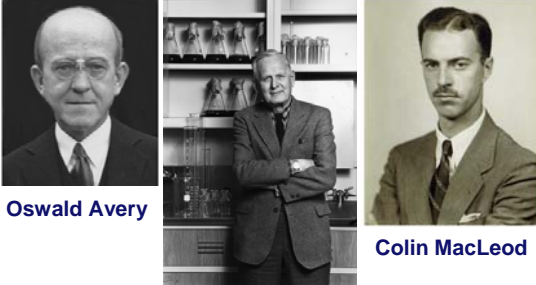
DNA is the "Transforming Factor" 1944

- Avery, McCarty & MacLeod
 - purified both DNA & proteins from *Streptococcus pneumoniae* bacteria
 - which will transform non-pathogenic bacteria?
 - injected **protein** into bacteria
 - no effect
 - injected **DNA** into bacteria
 - transformed harmless bacteria into virulent bacteria



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Avery, McCarty & MacLeod



Oswald Avery

Maclyn McCarty

Colin MacLeod

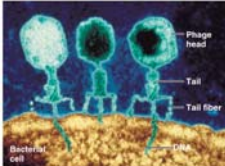
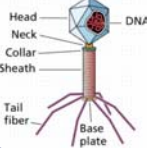
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Confirmation of DNA

1952 | 1969

- **Hershey & Chase**
 - ♦ classic “blender” experiment
 - ♦ worked with **bacteriophage**
 - viruses that infect bacteria
 - ♦ grew phage viruses in 2 media, radioactively labeled with either
 - **³⁵S** in their proteins
 - **³²P** in their DNA
 - ♦ infected bacteria with labeled phages




Why use Sulfur vs. Phosphorus?



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Hershey & Chase

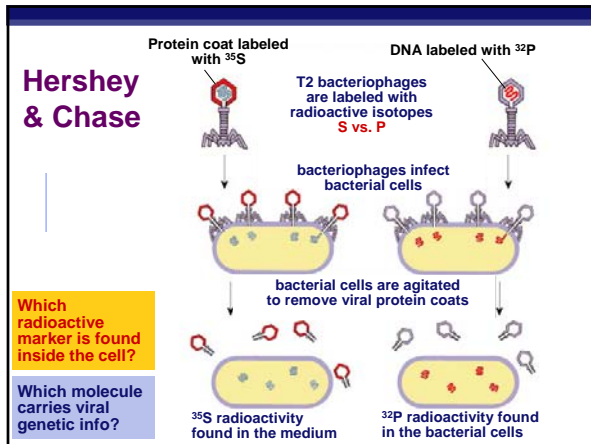


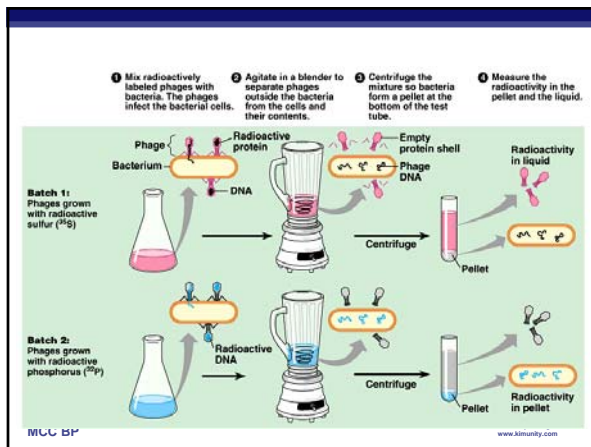
Martha Chase

Alfred Hershey

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Blender experiment

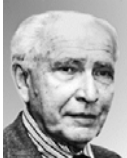
- Radioactive phage & bacteria in blender
 - ^{35}S phage
 - radioactive proteins stayed in supernatant
 - therefore protein **did NOT** enter bacteria
 - ^{32}P phage
 - radioactive DNA stayed in pellet
 - therefore DNA **did** enter bacteria
- Confirmed **DNA** is "transforming factor"

Taaa-Daaa!



Based on work by K. Foglia
www.kimunity.com

Chargaff 1947

- **DNA composition: "Chargaff's rules"**
 - ♦ varies from species to species
 - ♦ all 4 bases not in equal quantity
 - ♦ bases present in characteristic ratio
 - humans:
 - A = 30.9%
 - T = 29.4%
 - G = 19.9%
 - C = 19.8%




Erwin Chargaff

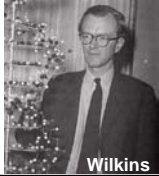


Structure of DNA 1953 | 1962

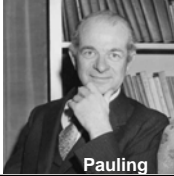
- **Watson & Crick**
 - ♦ developed double helix model of DNA
 - other scientists working on question:
 - ♦ Rosalind Franklin
 - ♦ Maurice Wilkins
 - ♦ Linus Pauling



Franklin






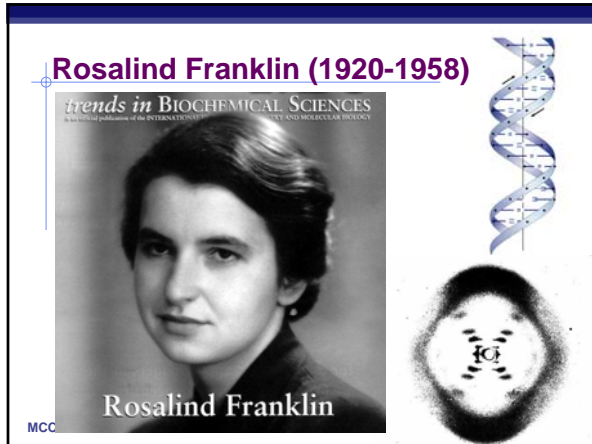
Wilkins

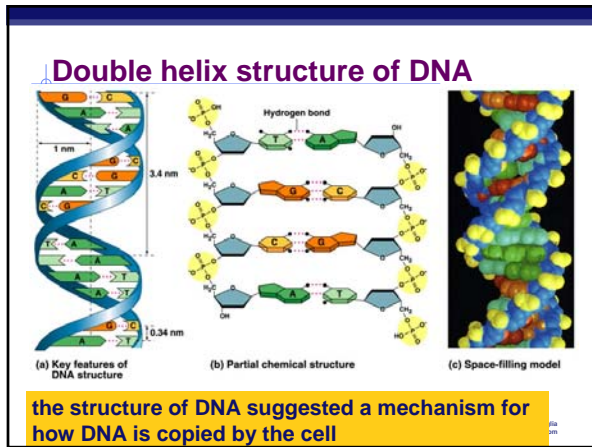


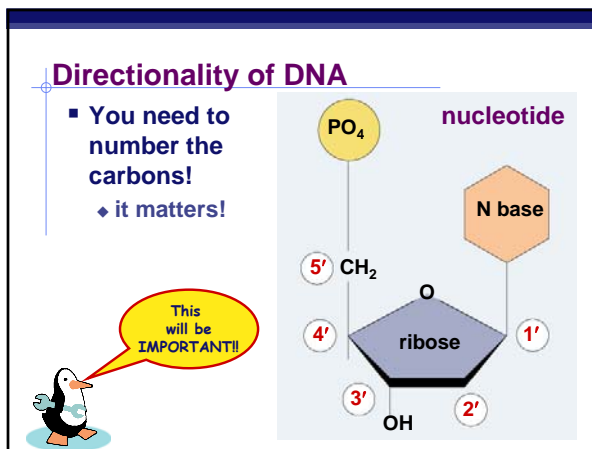
Pauling

Watson and Crick [1953 article in Nature](#)









The DNA backbone

- Putting the DNA backbone together
 - refer to the 3' and 5' ends of the DNA
 - the last trailing carbon

I mean it... This will be IMPORTANT!!

Anti-parallel strands

- Phosphate to sugar bond involves carbons in 3' & 5' positions
 - DNA molecule has "direction"
 - complementary strand runs in opposite direction

"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying 3' hydroxyl mechanism for the genetic material."
 Watson & Crick

Bonding in DNA

....strong or weak bonds?
 How do the bonds fit the mechanism for copying DNA?

Base pairing in DNA

- Purines
 - ◆ adenine (A)
 - ◆ guanine (G)
- Pyrimidines
 - ◆ thymine (T)
 - ◆ cytosine (C)
- Pairing
 - ◆ A : T
 - ◆ C : G

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Based on work by K. Foglia
www.kimunity.com

Copying DNA

- Replication of DNA
 - ◆ base pairing allows each strand to serve as a pattern for a new strand

Based on work by K. Foglia
www.kimunity.com

Models of DNA Replication

- Alternative models
 - ◆ so how is DNA copied?

verify through experiments...

(a) Conservative model. The parental double helix remains intact and an all-new copy is made.

(b) Semiconservative model. The two strands of the parental molecule separate, and each functions as a template for synthesis of a new complementary strand.

(c) Dispersive model. Each strand of both daughter molecules contains a mixture of old and newly synthesized parts.

1958

Semi-conservative replication

- **Meselson & Stahl**
 - ◆ label nucleotides of "parent" DNA strands with heavy nitrogen = ^{15}N
 - ◆ label new nucleotides with lighter isotope = ^{14}N

"The Most Beautiful Experiment in Biology"

make predictions...

1 Bacteria cultured in medium containing ^{15}N

2 Bacteria transferred to medium containing ^{14}N

3 DNA sample centrifuged after 20 min

4 DNA sample centrifuged after 40 min

1958

Semi-conservative replication

- **Make predictions...**
 - ◆ ^{15}N strands replicated in ^{14}N medium
 - ◆ 1st round of replication?
 - ◆ 2nd round?

	Predictions		
	Conservative	Semiconservative	Dispersive
3 DNA sample centrifuged after 20 min First replication			
4 DNA sample centrifuged after 40 min Second replication			

DNA Replication

- **Large team of enzymes coordinates replication**

let's meet the team...

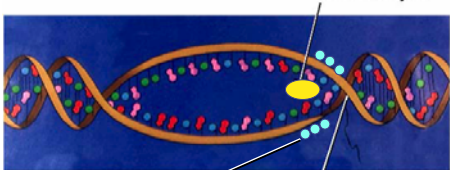
(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

(b) In this micrograph, three replication bubbles are visible along the DNA of cultured Chinese hamster cells. The arrows indicate the direction of DNA replication at the two ends of each bubble (TEM).

MCC | K. Foglia unity.com

Replication: 1st step

- Unwind DNA
 - helicase enzyme
 - unwinds part of DNA helix
 - stabilized by single-stranded binding proteins

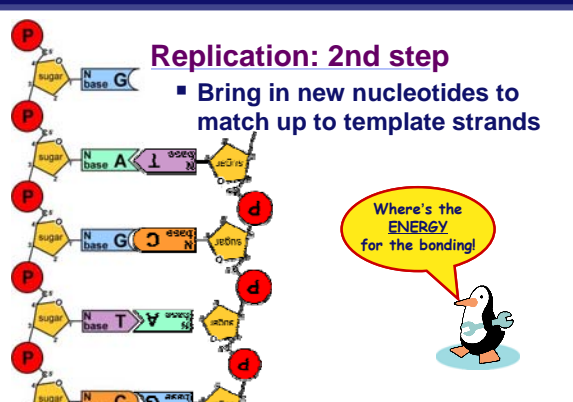


MCC single-stranded binding proteins replication fork helicase enzyme

K. Foglia
university.com

Replication: 2nd step

- Bring in new nucleotides to match up to template strands

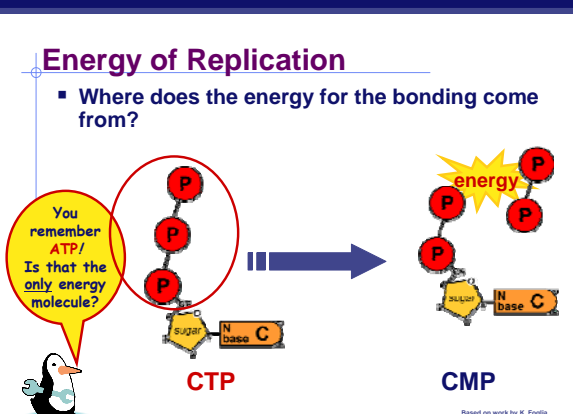


Where's the ENERGY for the bonding!

Based on work by K. Foglia
www.kfoggia.com

Energy of Replication

- Where does the energy for the bonding come from?



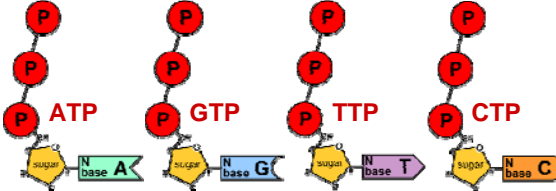
You remember ATP! Is that the only energy molecule?

CTP CMP

Based on work by K. Foglia
www.kfoggia.com

Energy of Replication

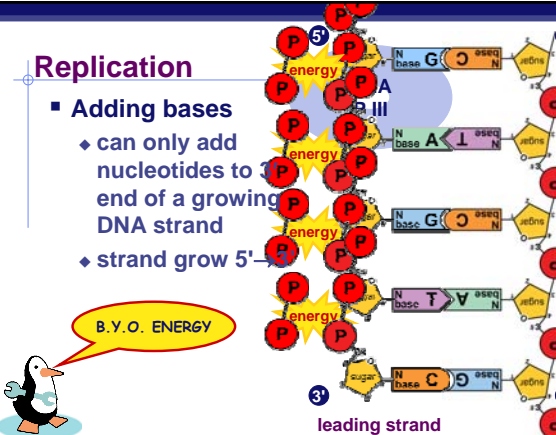
- The nucleotides arrive as **nucleosides**
 - DNA bases with **P-P-P**
 - DNA bases arrive with **their own energy** source for bonding
 - bonded by **DNA polymerase III**



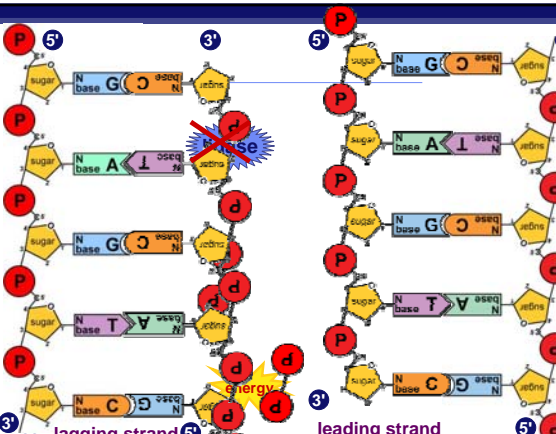
Replication

- Adding bases
 - can only add nucleotides to the **end** of a growing DNA strand
 - strand grow 5'→3'

B.Y.O. ENERGY



leading strand



lagging strand leading strand

Leading & Lagging strands

Leading strand
- continuous synthesis

Lagging strand
- Okazaki fragments
- joined by **ligase**
- "spot welder" enzyme

Okazaki fragments

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Priming DNA synthesis

- DNA polymerase III can only extend an existing DNA molecule
 - ♦ cannot start new one
 - cannot place first base
 - ♦ short **RNA primer** is built first by **primase**
 - starter sequences
 - DNA polymerase III can now add nucleotides to RNA primer

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Cleaning up primers

DNA polymerase I removes sections of RNA primer and replaces with DNA nucleotides

Single-stranded region of parental DNA

Primase joins RNA nucleotides into primer.

DNA polymerase III adds DNA nucleotides to primer.

RNA primer

Newly made DNA

DNA polymerase I replaces the RNA with DNA.

Newest DNA

DNA polymerase III

This daughter strand is now complete.

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Replication fork

DNA polymerase I

DNA polymerase III

lagging strand

Okazaki fragments

primase

SSB

helicase

DNA polymerase III

ligase

5' 3'

5' 3'

5' 3'

5' 3'

direction of replication

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Based on work by K. Foglia
www.khanacademy.com

And in the end...

- Ends of chromosomes are eroded with each replication
 - an issue in aging?
 - ends of chromosomes are protected by telomeres

Parental DNA

RNA priming and DNA synthesis

Leading strand

Lagging strand

RNA primer

Removal of primers and filling of gaps with DNA where a 3' end is available

Gap remains unfilled

Further rounds of replication

Shorter and shorter daughter molecules

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Telomeres

- Expendable, non-coding sequences at ends of DNA
 - short sequence of bases repeated 1000s times
 - TTAGGG in humans
- Telomerase enzyme in certain cells
 - enzyme extends telomeres
 - prevalent in cancers
 - Why?

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Replication bubble

Adds 1000 bases/second!

→ Which direction does DNA build?
→ List the enzymes & their role

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Replication enzymes

- helicase
- DNA polymerase III
- primase
- DNA polymerase I
- ligase
- single-stranded binding proteins

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DNA polymerases

- DNA polymerase III
 - ◆ 1000 bases/second
 - ◆ main DNA building enzyme
- DNA polymerase I
 - ◆ 20 bases/second
 - ◆ editing, repair & primer removal

DNA polymerase III enzyme

(a) (b) (c)

Editing & proofreading DNA

- 1000 bases/second = lots of typos!
- DNA polymerase I
 - ◆ **proofreads** & corrects typos
 - ◆ repairs **mismatched** bases
 - ◆ excises **abnormal** bases
 - repairs damage throughout life
 - ◆ reduces error rate from 1 in 10,000 to 1 in 100 million bases

1 Thymine dimer distorts DNA molecule
2 A nuclease enzyme cuts the damaged DNA strand at two points
3 Repair synthesis by DNA polymerase fills the gap
4 DNA ligase seals the remaining nick

MCC BP Based on work by K. Foglia www.kimunity.com

Fast & accurate!

- It takes *E. coli* <1 hour to copy 5 million base pairs in its single chromosome
 - ◆ divide to form 2 identical daughter cells
- Human cell copies its 6 billion bases & divide into daughter cells in only few hours
 - ◆ remarkably accurate
 - ◆ only ~1 error per 100 million bases
 - ◆ ~30 errors per cell cycle

MCC BP Based on work by K. Foglia www.kimunity.com

