It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

Comparing Mitosis and Meiosis

- Mitosis is a *conservative* process that maintains the genetic status quo

IN CONTRAST,

- Meiosis generates *combinatorial variation* through independent assortment and crossing-over (recombination)

Comparing Mitosis and Meiosis

Mitosis: One cell division resulting in *two diploid* daughter cells

Meiosis: Two cell divisions resulting in *four haploid* products

…..

Mitosis: One S phase per cell division

Meiosis: One S phase for two cell divisions

DNA Structure and Analysis

Central Dogma

DNA is the genetic material. It is used to make RNA, the “transport form” of genetic information, which travels to the ribosome. “Reading” the information in RNA, the ribosome synthesizes protein, which goes on to form or do the work of the cell.

DNA-RNA-Protein

Who figured it all out?

1927: Griffith described transmission of virulence from dead virulent bacteria to live avirulent bacteria

1944: Avery, McCleod and McCarty demonstrate DNA is the transforming principle in bacteria.

1952: Hershey and Chase tracked radiolabeled DNA and protein as viruses infected proteins.

Phage T2

Phage (virus) T2 infects a bacterial cell, takes over and forces the bacterial cell to reproduce viral particles. The bacteria ultimately lyses, releasing the viral particles.

Phage T2 is composed simply of a protein coat surrounding a core of DNA.

Hershey and Chase

^{32}P to label viral DNA

^{35}S to label viral protein

Let the virus infect the bacteria and see where the radioactivity goes.

It's a quiz… what was their hypothesis?

If DNA is the genetic material, ***then*** the ^{32}P will move into the bacterial cell. Alternatively, ***if*** protein is the genetic material, ***then*** the ^{35}S will move into the bacterial cell.

So what happened?

Most of the ^{32}P-DNA transferred into the bacteria following viral adsorption, while most of the ^{35}S-protein stayed outside the bacteria and was recovered in the empty phage coats stripped off the infected bacteria.

The viruses that were produced inside the bacteria contained ^{32}P but not ^{35}S.

Hershey and Chase

Conclusion:

DNA is responsible for directing viral reproduction; therefore, DNA is the information storage molecule.

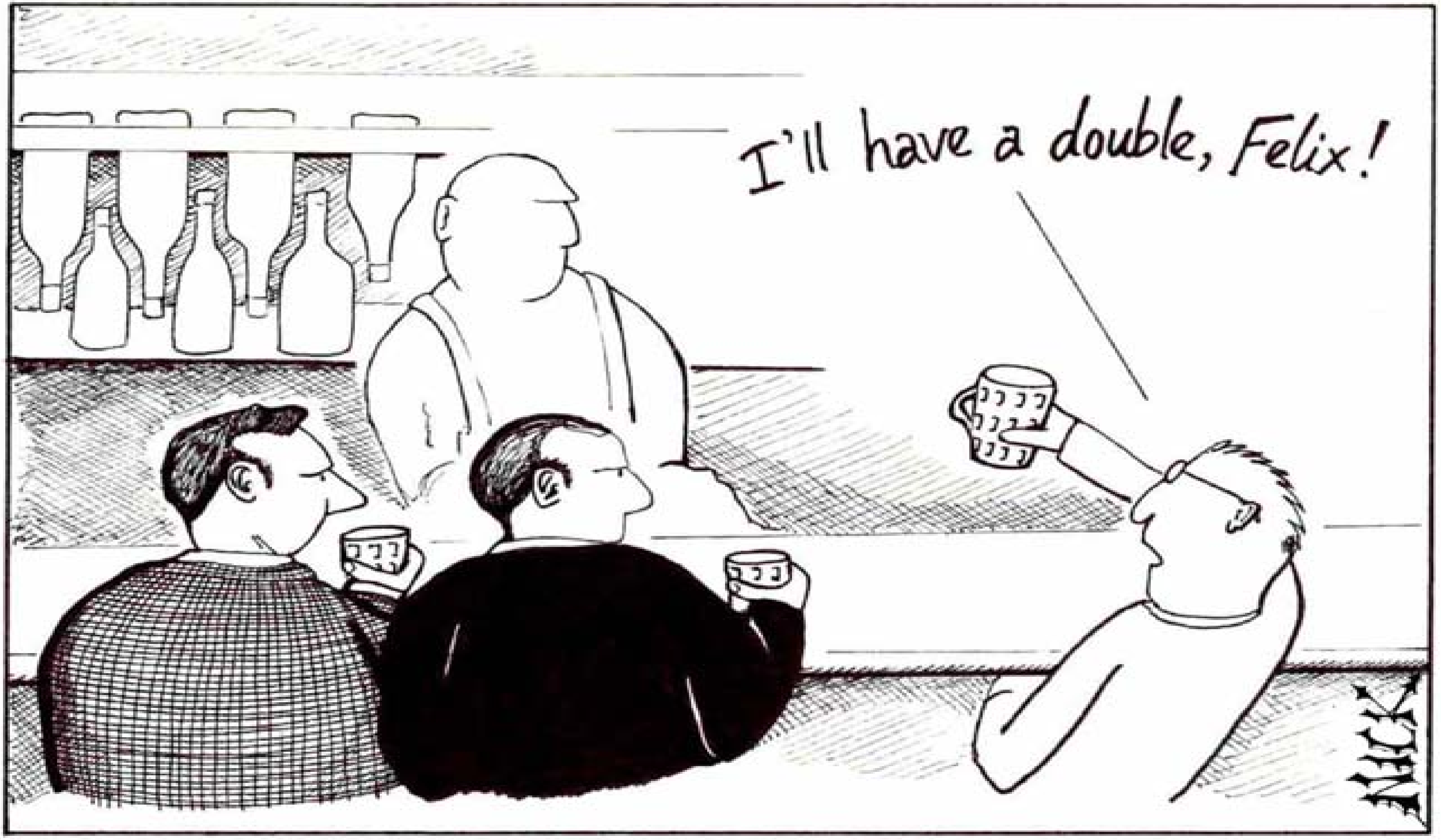
The Exception: RNA Viruses

Some viruses (including the AIDS virus) use RNA as their genetic material.

When these viruses infect a host cell, they typically make a DNA copy of their genome that then is inserted into the host genome (latent cycle) or is used to direct the lytic cycle.

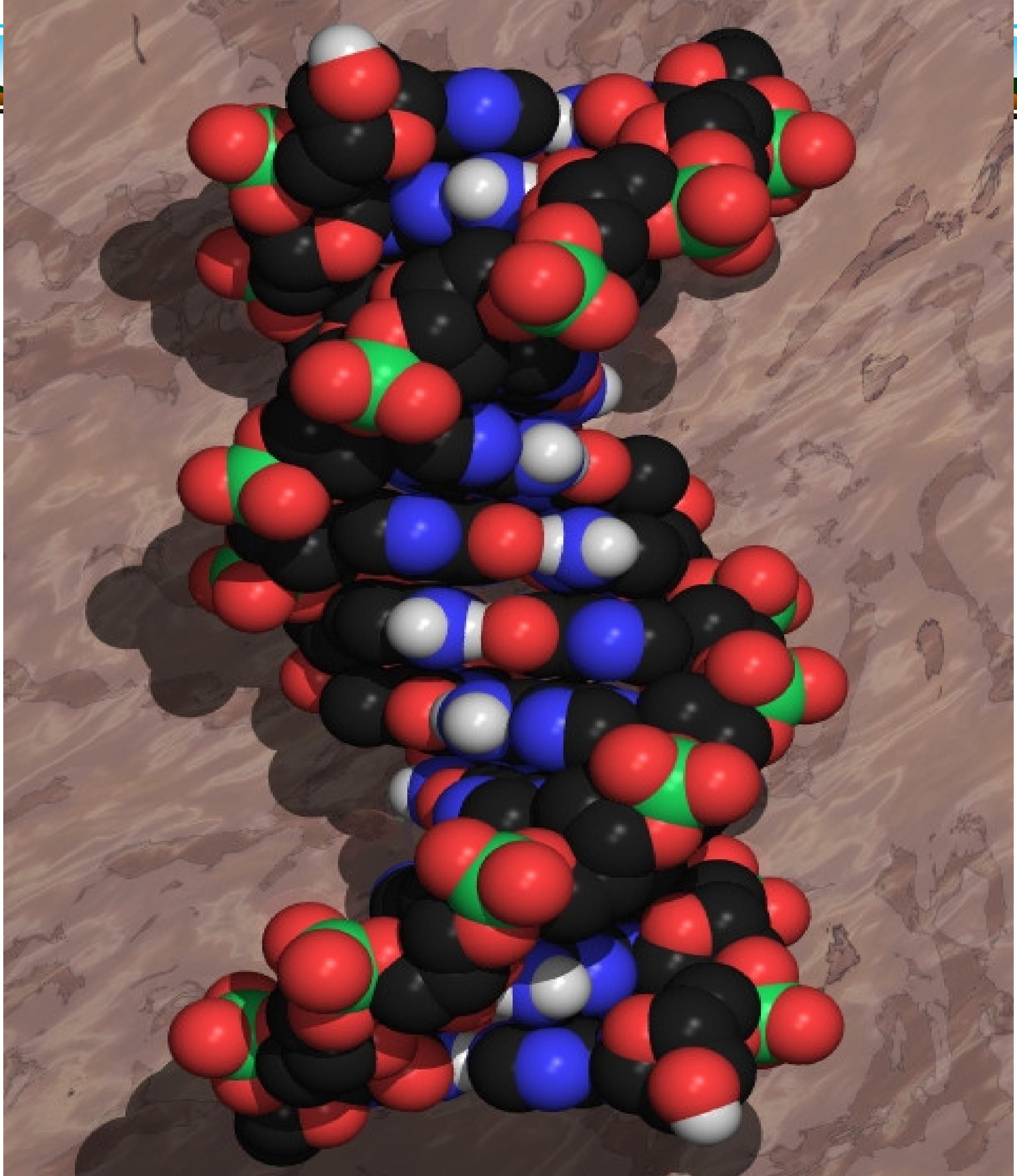
The viral enzyme is called **reverse transcriptase** because it makes a DNA copy from an RNA template.

Next Step: What does DNA look like?



Cambridge, 1953. Shortly before discovering the structure of DNA, Watson and Crick, depressed by their lack of progress, visit the local pub.

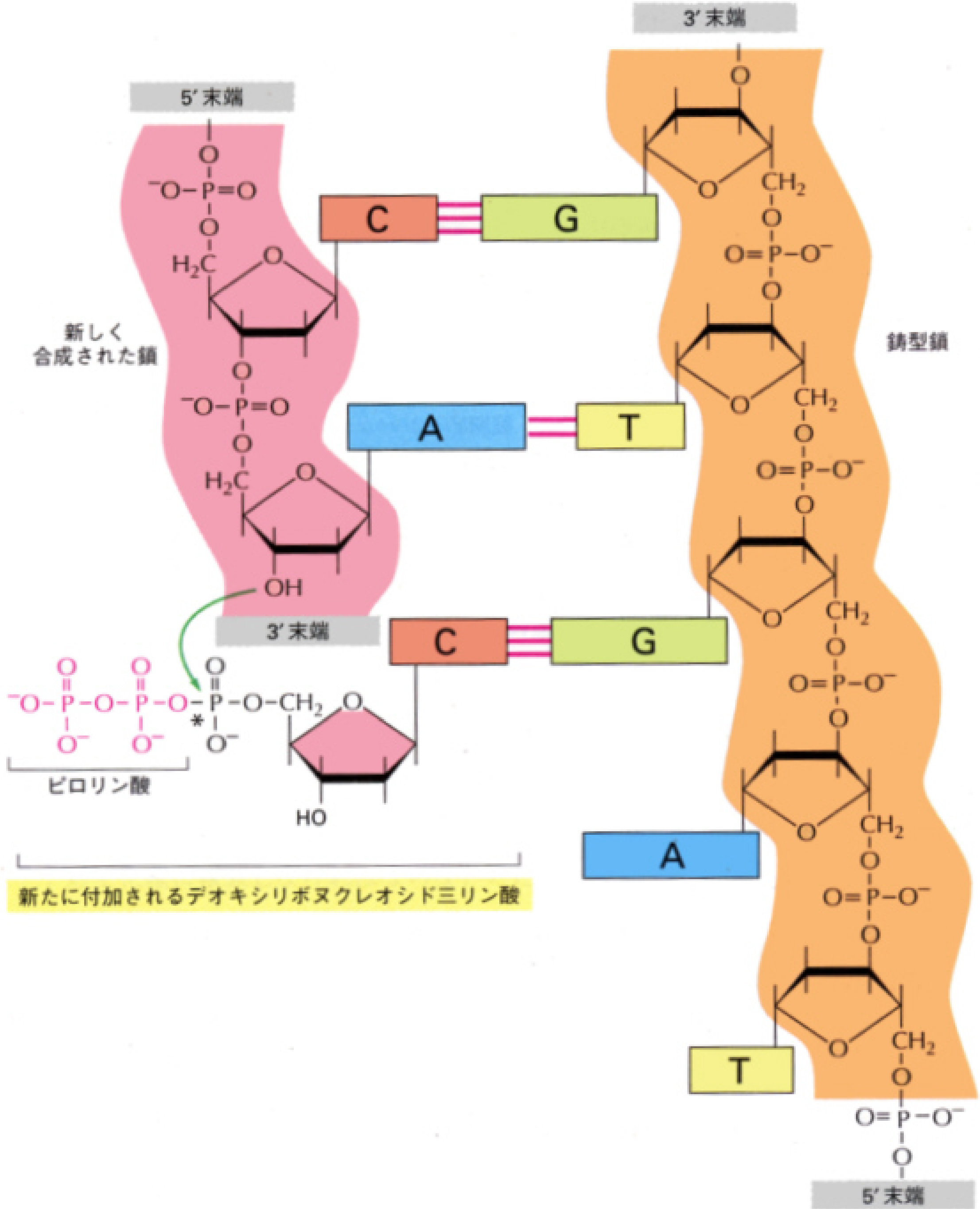
1953: Watson and Crick propose DNA is arranged in a double helix.



Features of DNA

DNA is *double-stranded*

- Two strands that are NOT identical, but in fact are complementary.



5′末端
3′末端
新しく
合成された鎖
鋳型鎖
C
G
A
T
C
G
A
T
OH
3′末端
ピロリン酸
HO
新たに付加されるデオキシリボヌクレオシド三リン酸
5′末端

Features of DNA

DNA is composed of nucleotides:

Sugar—deoxyribose

Phosphate

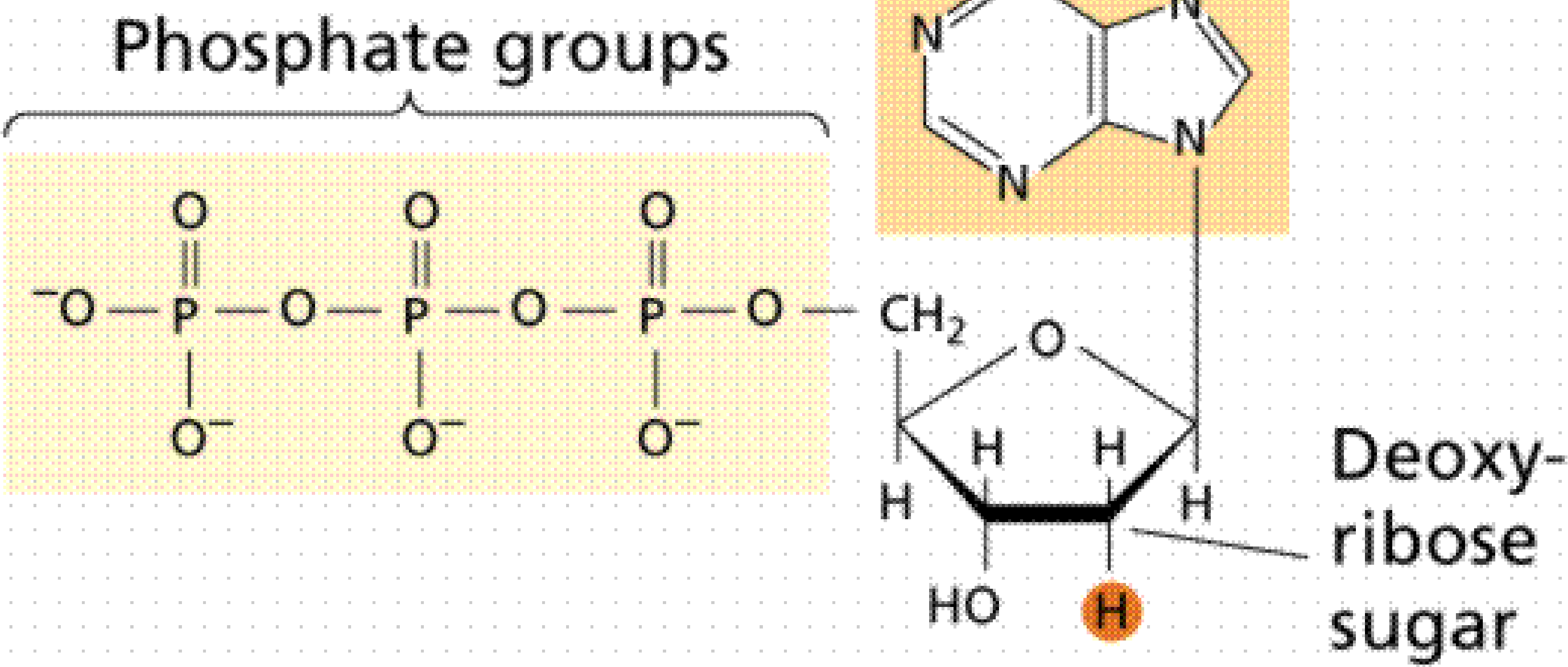
Base—Adenine and Guanine (purines)

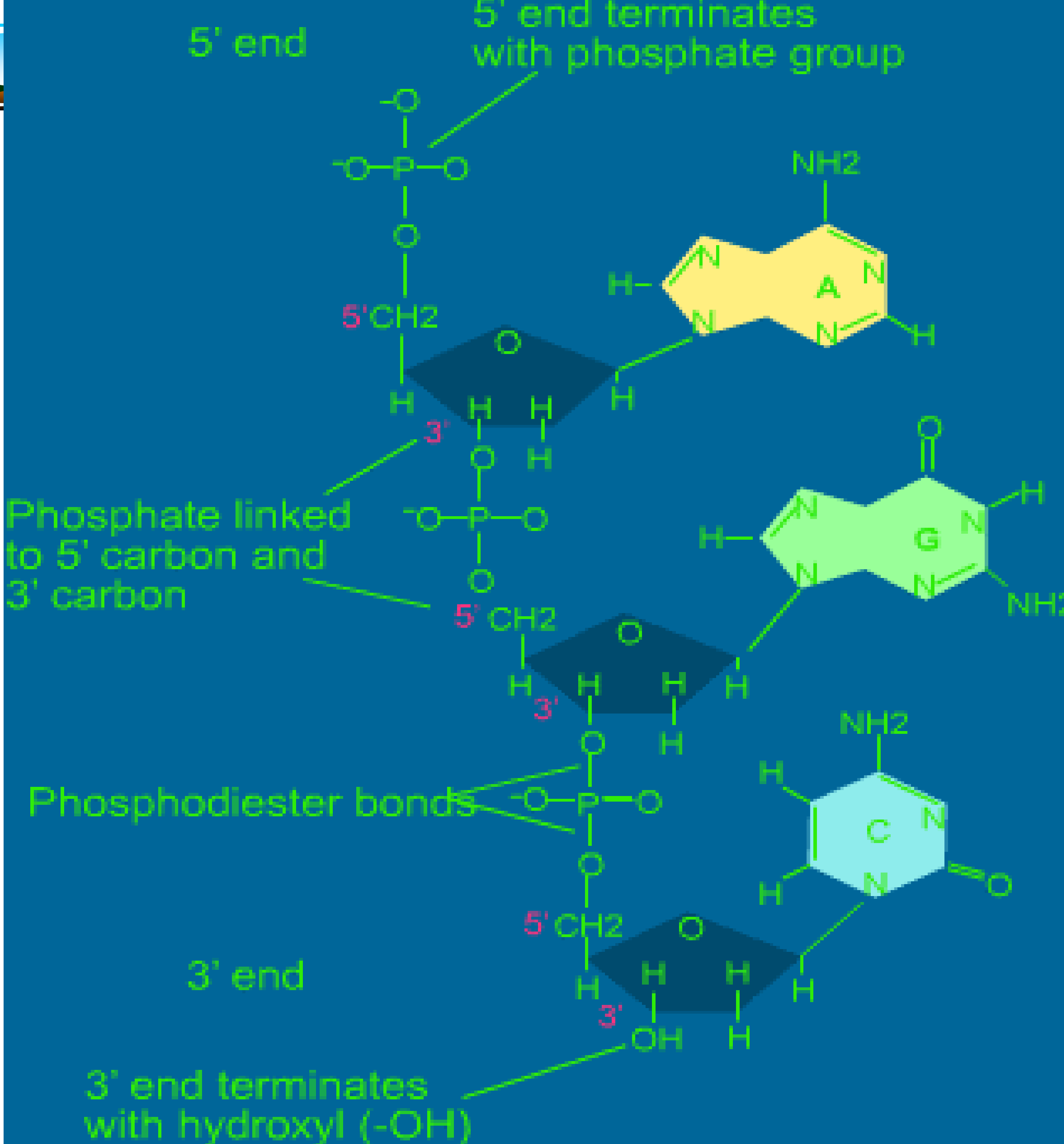
Thymine and Cytosine (pyrimidines)

Numbering convention: Cs in bases 1-XX

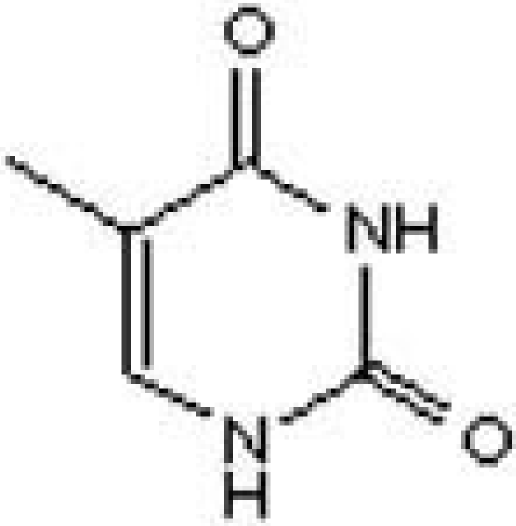
Cs in sugar 1’-5’

Deoxy-ATP (deoxyadenosine triphosphate)

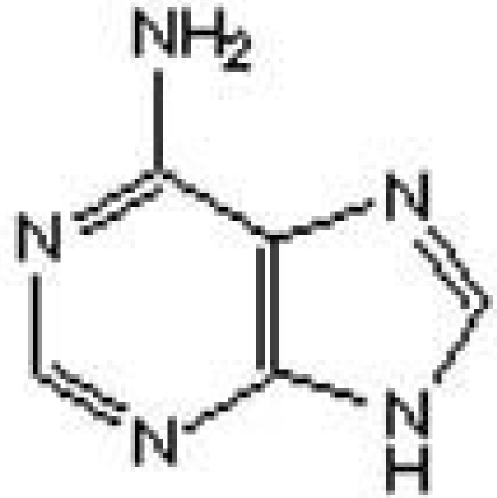




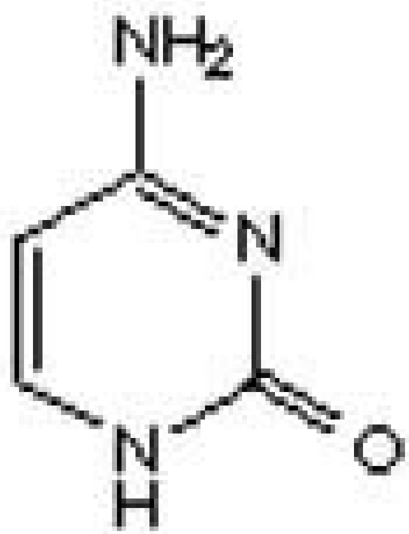
5' end terminates
with phosphate group
5' end
NH2
A
5'CH2
3'
Phosphate linked
to 5' carbon and
3' carbon
G
NH2
5' CH2
3'
NH2
C
Phosphodiester bonds
5' CH2
3' end
3'
OH
3' end terminates
with hydroxyl (-OH)



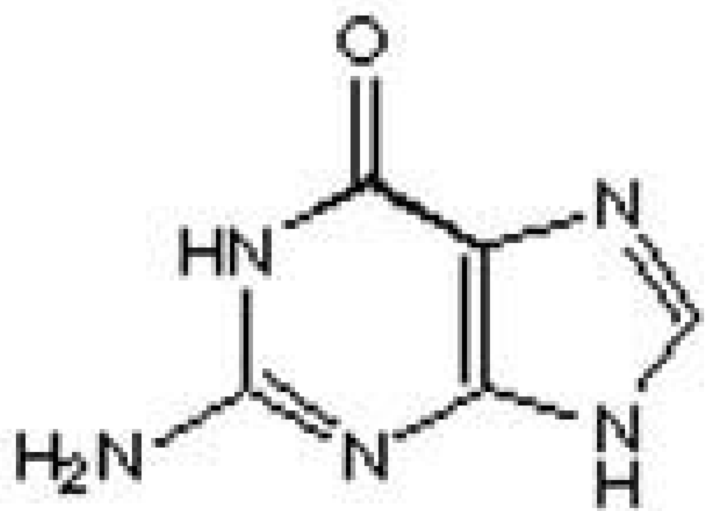
Thymine (T)



Adenine (A)



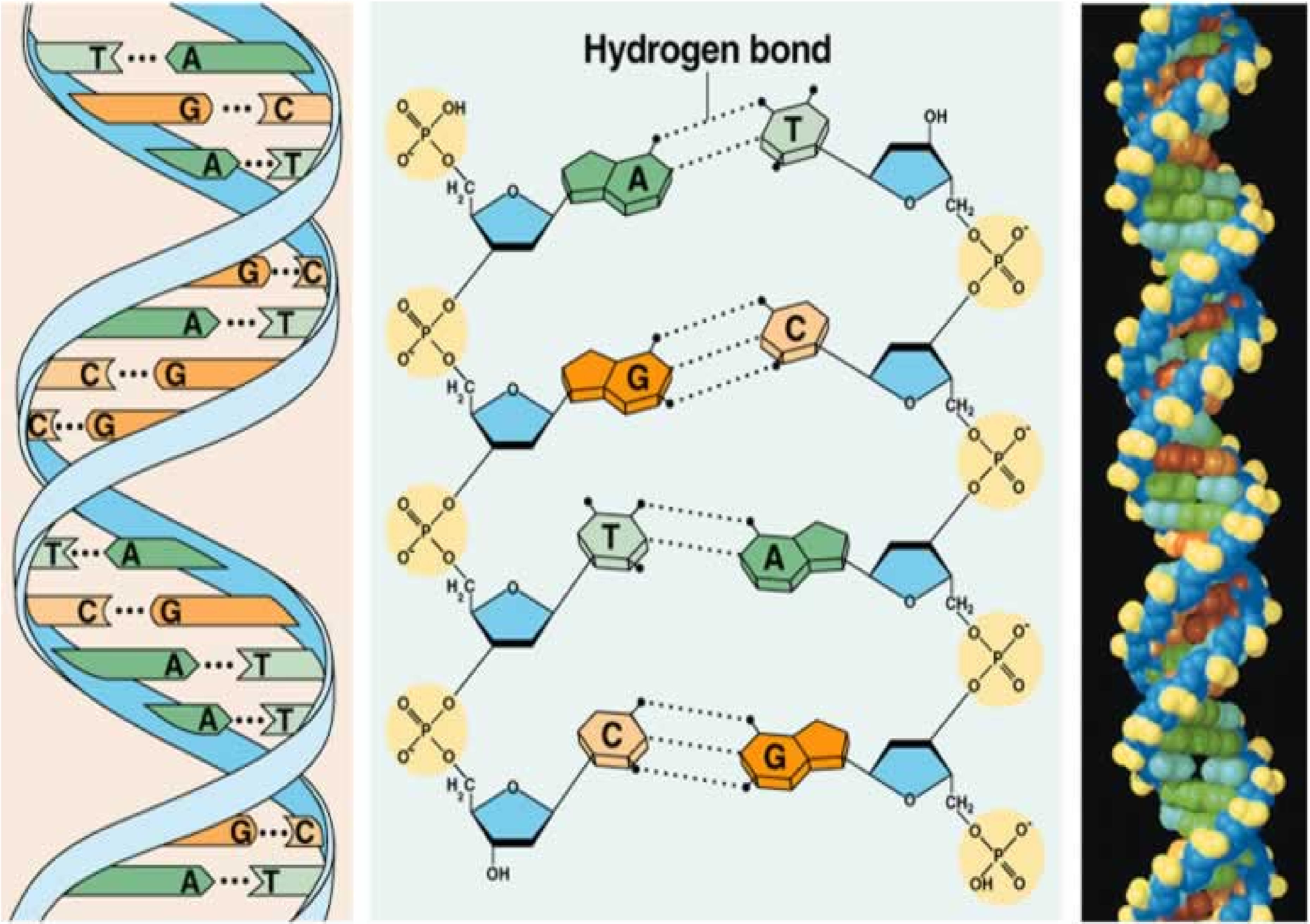
Cytosine (C)



Guanine (G)

So, how does complementarity work?

Base Pairing!


Hydrogen bond
T
A
G
C
OH
H_2C
CH_2
P
O

Features of DNA

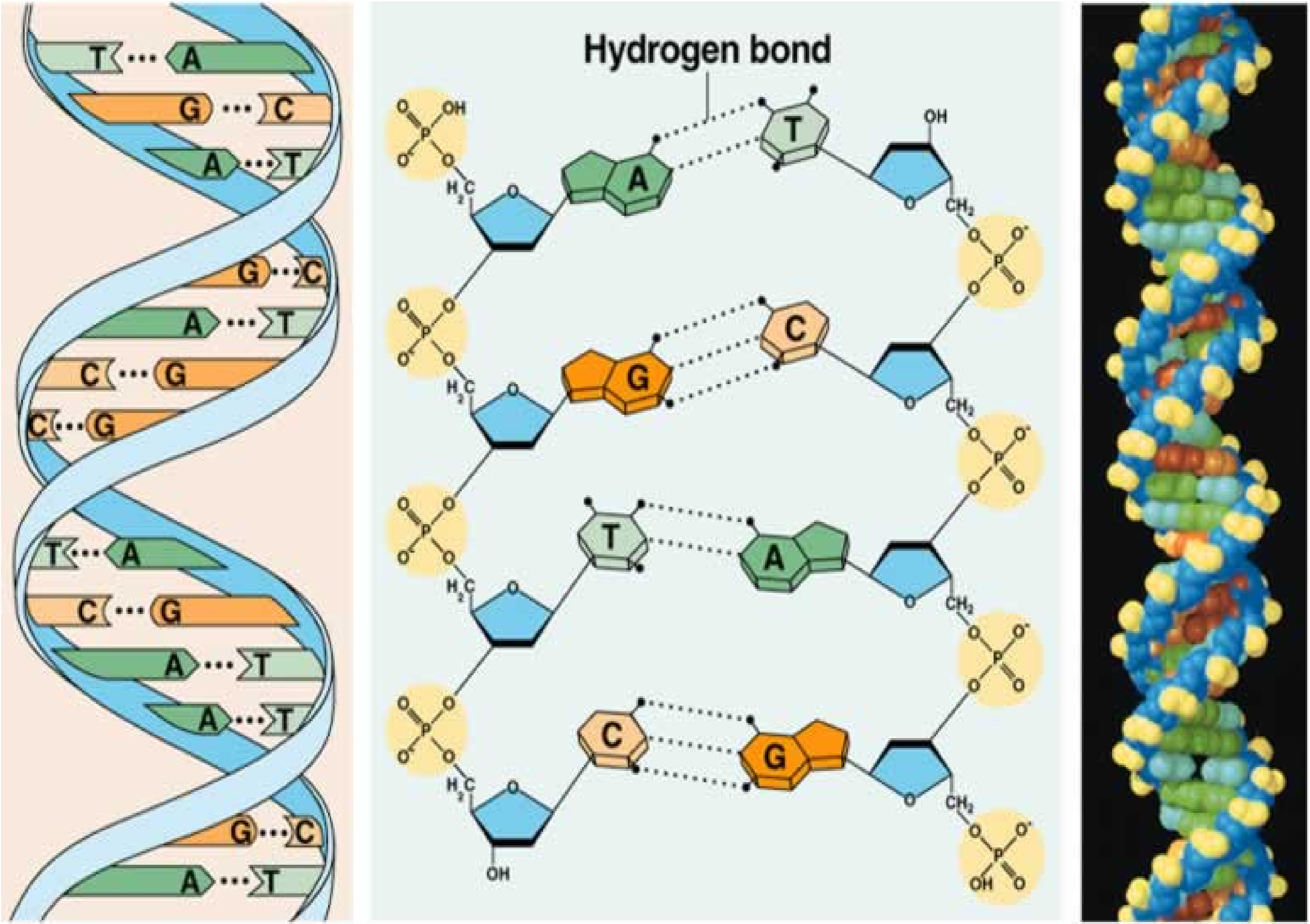
The DNA structure is such that the bases (adenine, guanine, cytosine and thymine) of opposing strands face each other.

Hydrogen bonds form between the bases, holding the strands together.

Hydrogen Bonds….remember your chemistry??

Hydrogen bond: a weak association between a covalently bonded hydrogen atom and an unshared electron pair from another covalently bonded atom (in this case oxygen and nitrogen)

Alone, they're pretty wimpy, but thousands in a row create a very stable force holding the two strands of DNA together.



Hydrogen bond

Base Pairing Rules

1a. Adenine always pairs with Thymine

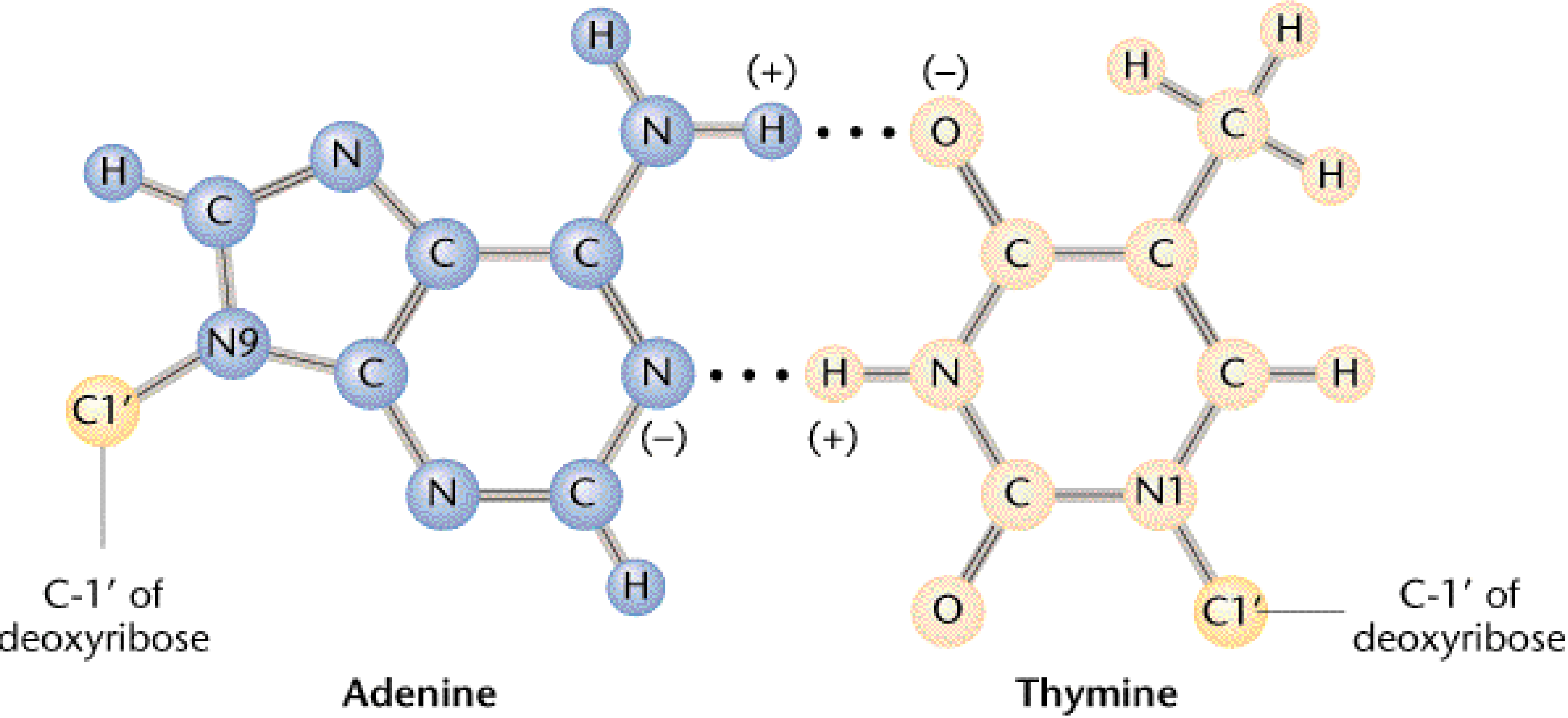
1b. Guanine always pairs with Cytosine

2a. A-T pairs have TWO hydrogen bonds

2b. G-C pairs have THREE hydrogen bonds

Adenine-thymine base pair

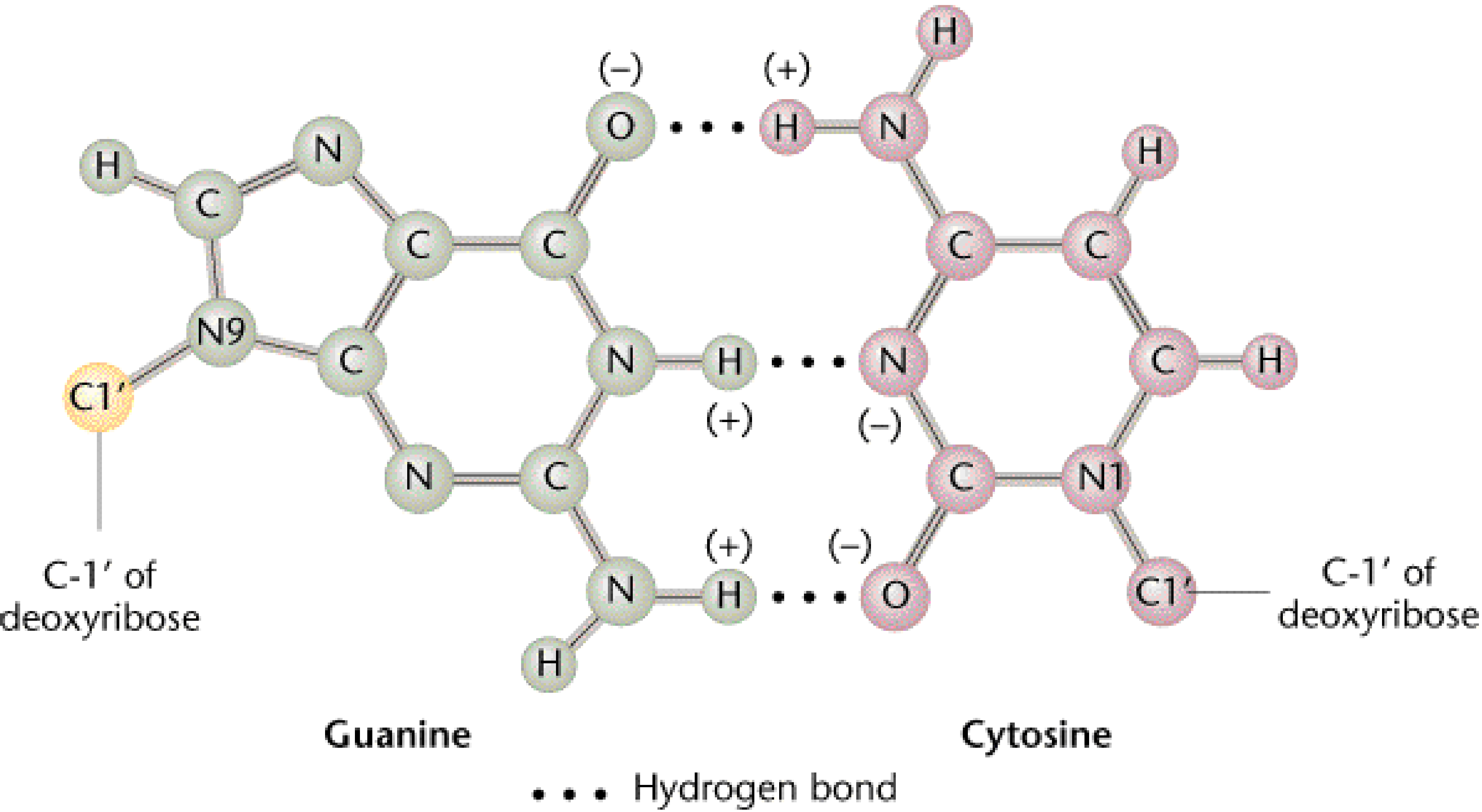
two hydrogen bonds



(Klug & Cummings 1997)

Guanine-cytosine base pair

three hydrogen bonds



(Klug & Cummings 1997)

DNA Replication

DNA Replication

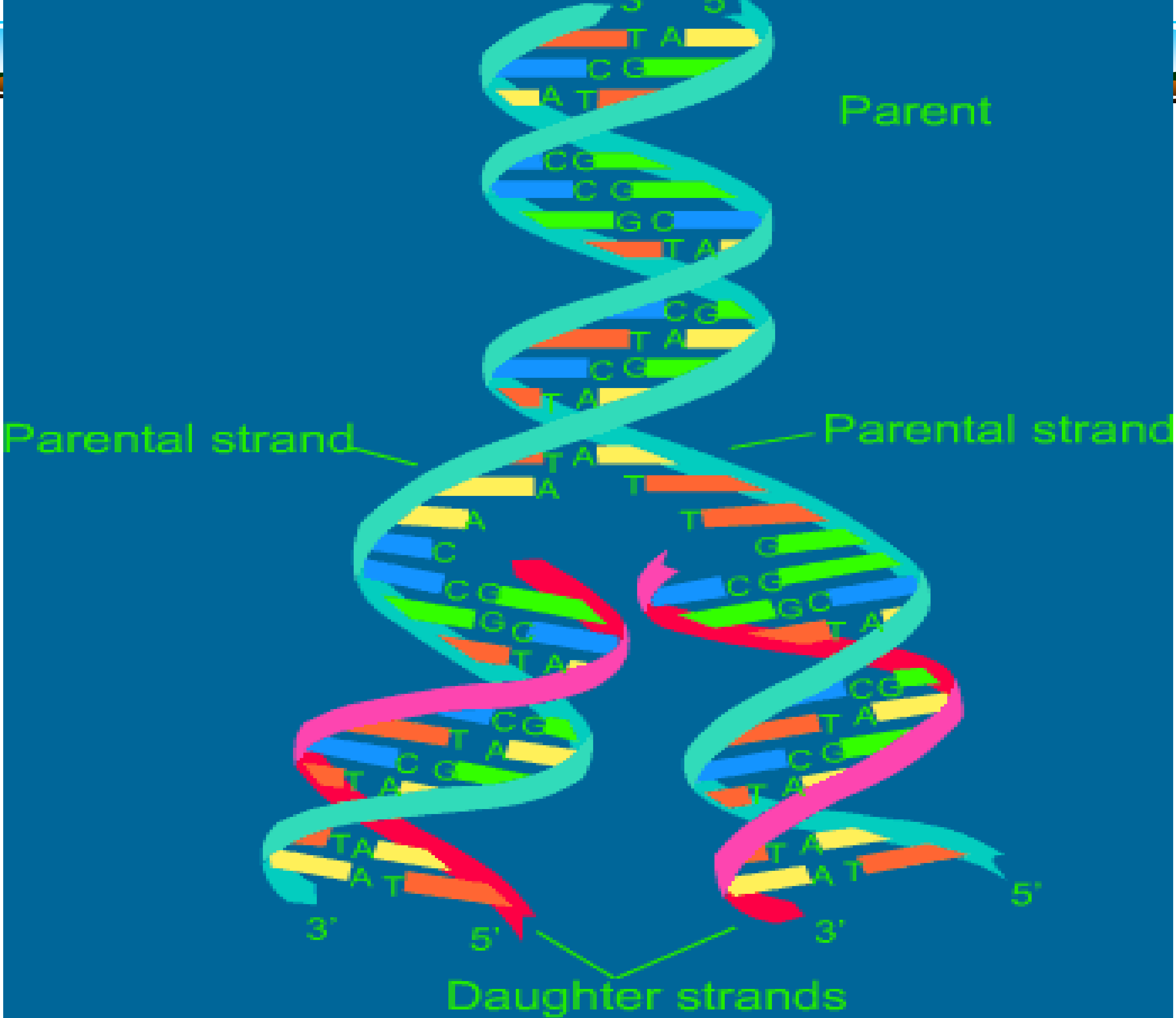
Four characteristics of genetic material:

1. Replication
2. Information storage
3. Information expression
4. Change (variation) by mutation

Replication

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

J.D. Watson and F.H.C. Crick, 1953


Parent
Parental strand
Parental strand
3'
5'
3'
5'
Daughter strands

How might DNA Replicate?

The complementary nature of DNA lends clues to how DNA might copy itself:

1. Conservative: “old” double strand goes to one daughter strand intact, other daughter cell gets a new copy.
2. Dispersive: Parental strands are dispersed into two new double helices following replication. Both daughter cells would receive “old” and “new,” but would involve cleavage of the parental strands. Most complicated and least likely

How might DNA Replicate?

3. Semi-conservative: Each daughter cell receives one new strand, one old strand.

Each strand serves as a template to synthesize the complementary strand.

Meselson-Stahl Experiment (1958)

Strongly supported semiconservative hypothesis….

Grew *E. coli* in medium that contained only $^{15}NH_4Cl$ as the nitrogen source for many generations such that all the N within the bacteria was radioactive.

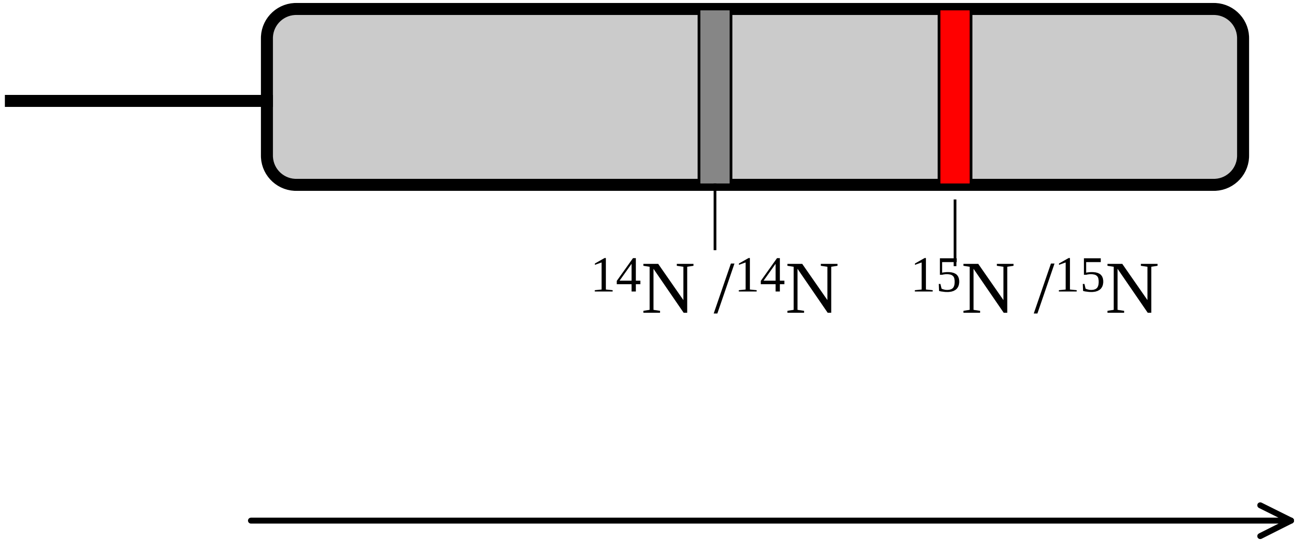
Meselson-Stahl Experiment (1958)

The natural form of N is ^{14}N, which is lighter than ^{15}N.

DNA can be separated by weight using centrifugation

DNA made with ^{15}N is heavier than DNA made with ^{14}N and will form a discrete band from ^{14}N-DNA

Sedimentation Equilibrium Centrifugation



Gravity generated by centrifugal force

Meselson-Stahl Experiment (1958)

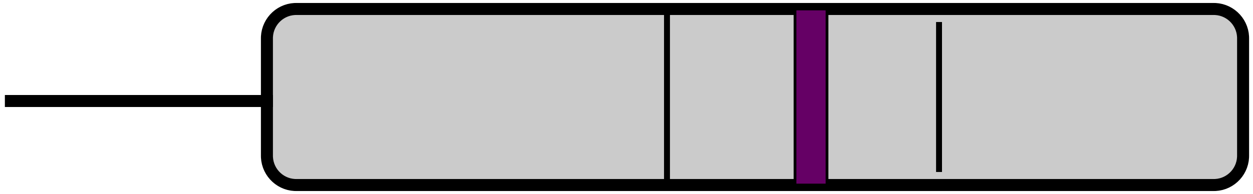
When the E.coli were switched to non-radioactive N-source (^{14}N), all “new” DNA would be made with ^{14}N.

After one generation, there was only one band of intermediate density (1:1 ^{15}N :^{14}N)

(If replication were conservative, there would be two bands)

Meselson-Stahl Experiment (1958)

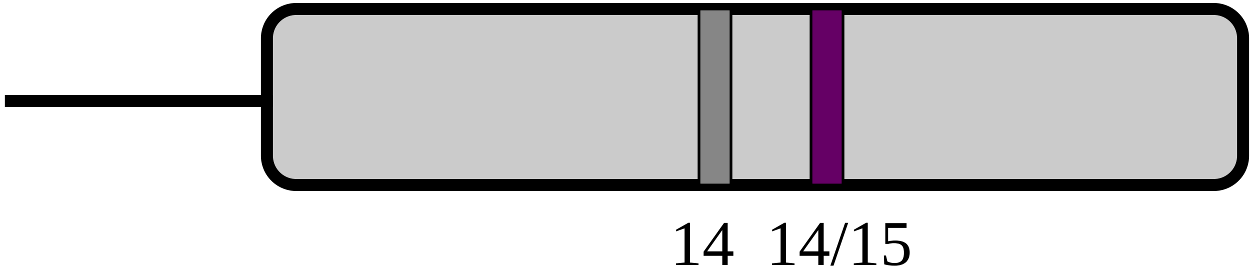
Generation One:



$^{14}N/^{15}N$

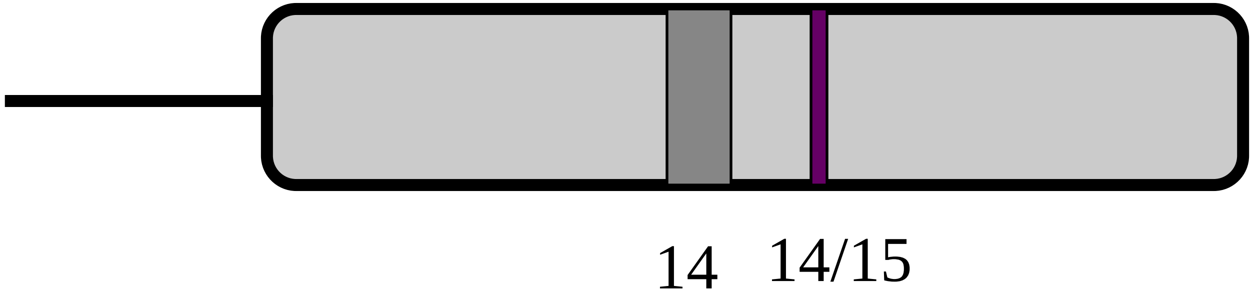
Meselson-Stahl Experiment (1958)

Generation Two



Meselson-Stahl Experiment (1958)

Generation Three



Over time, the proportion of ^{14}N-DNA increased, while the proportion of $^{14/15}N$-DNA decreased

Meselson-Stahl Experiment (1958)

Conclusion: Replication is semi-conservative

Why not dispersive or conservative?

- -When denatured, only discrete ^{14}N and ^{15}N bands were observed (not dispersive)
- -The proportion of $^{14/15}N$-DNA decreased (not dispersive)

Meselson-Stahl Experiment (1958)

-^{15}N-DNA/ ^{15}N-DNA band was not observed again (purely radioactive molecule was not preserved)

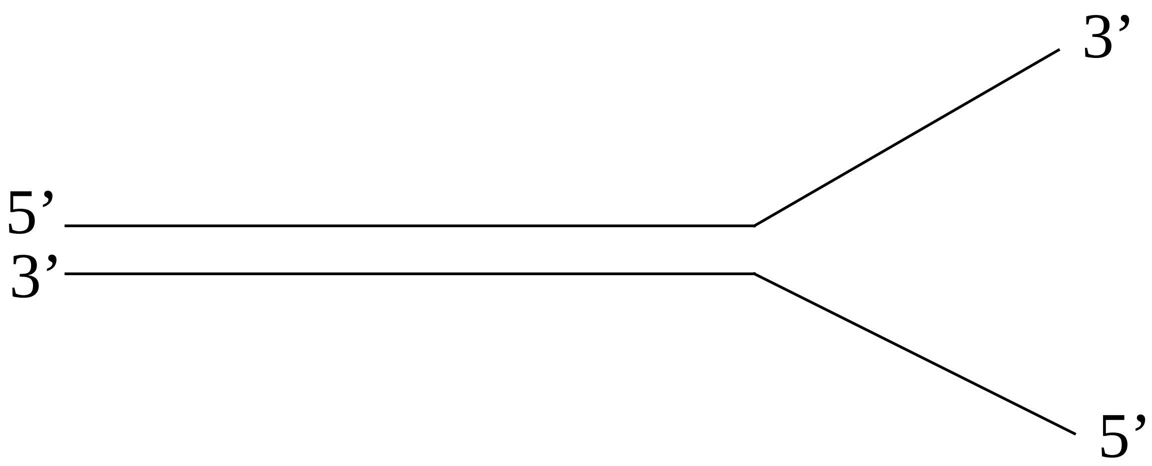
Replication in eukaryotes was later proved to occur by the same means.

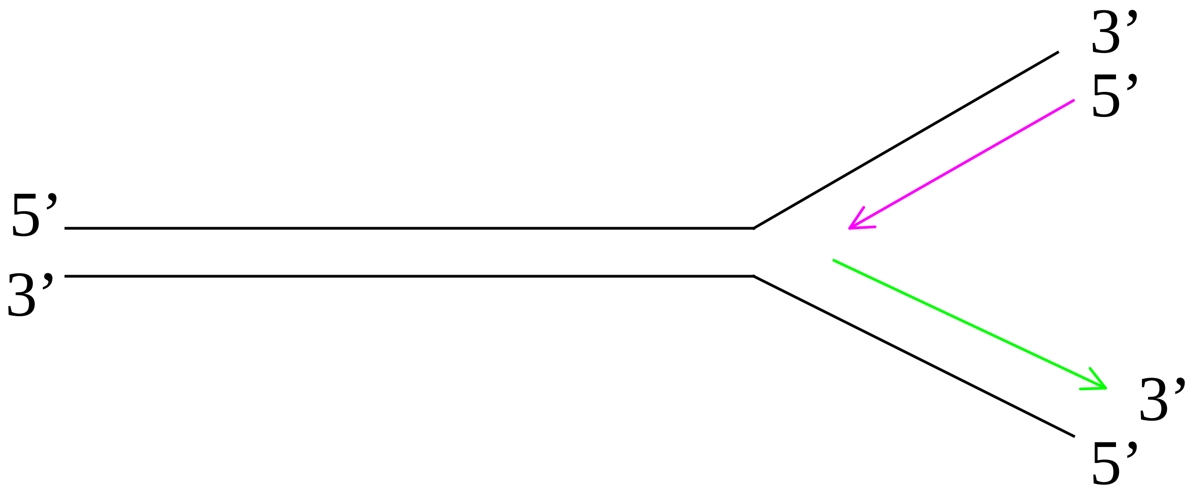

New strand

Replication… where does it start?

Origin of replication is a replication fork

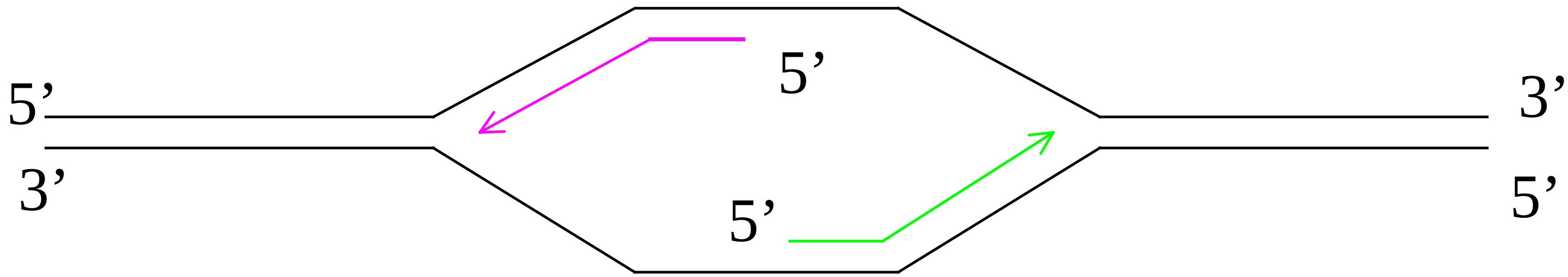
DNA strands separate from each other, each strand is used as a template to synthesize the complement





3’
5’
5’
3’
3’
5’

Replication is Bidirectional

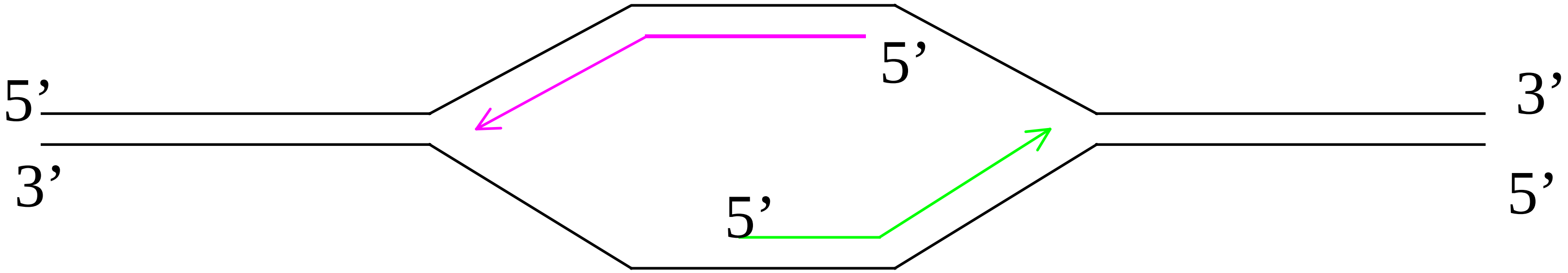


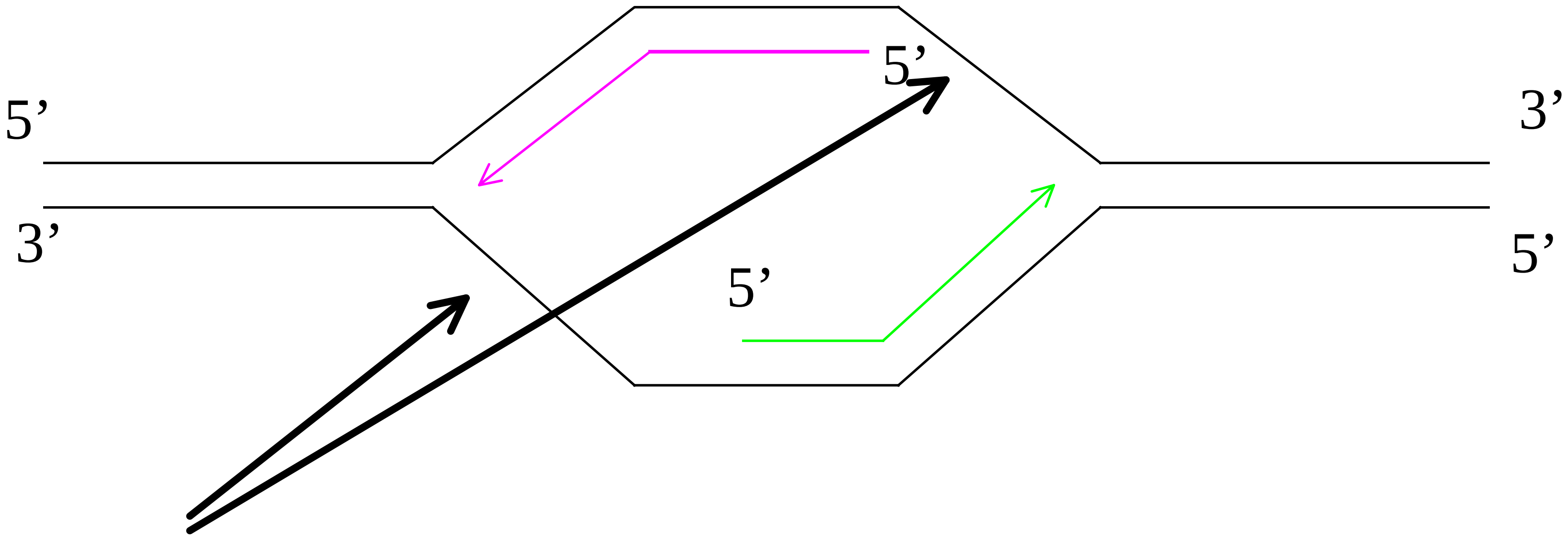
The bubble gradually opens up as the DNA unzips and is replicated

Can you see a problem?

DNA is always synthesized in the direction 5’ to 3’ (new nucleotide has its 5’ end stuck to the 3’ end hanging off the strand.)

Look at the picture again:



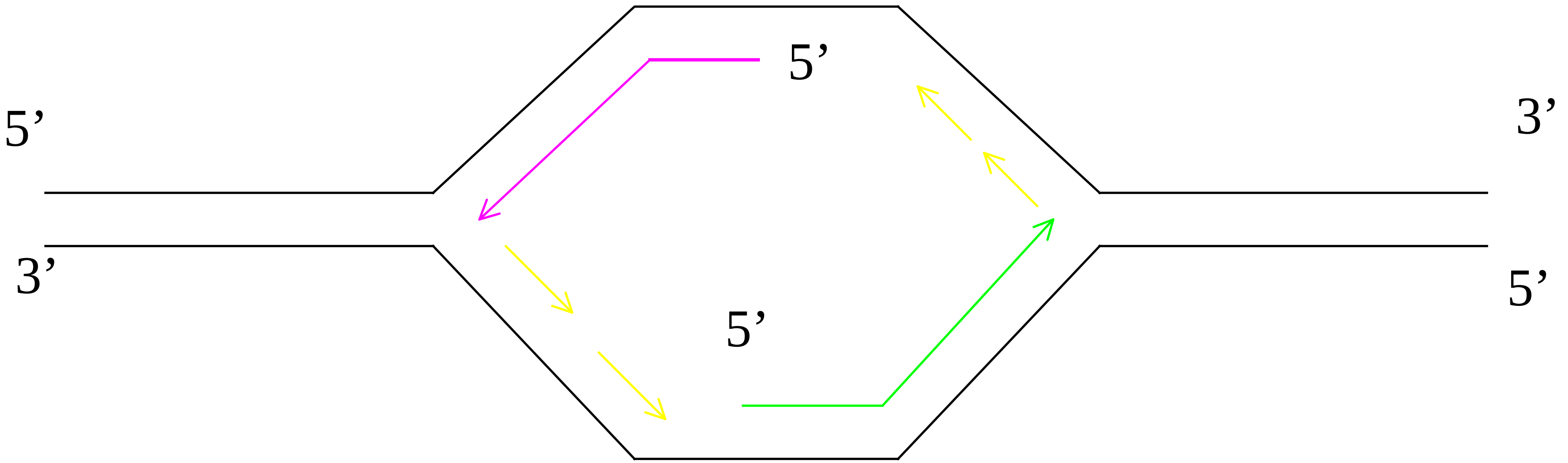


What about these two strands?? How are they replicated if the direction must be 5' to 3'?

The solution?

Okazaki fragments!

This Japanese researcher figured out that the other strand of DNA is synthesized in short 5’ to 3’ fragments that are later ligated (fused) together


5’
3’
5’
3’
5’
5’

So, remember that replication is bidirectional and semidiscontinuous.

Prokaryotes (bacteria and most viruses)

Bidirectional

One chromosome,

One origin of replication

Two forks

Eukaryotes

Bidirectional

Multiple origins along chromosomes

Replicating forks merge

Enzymes Drive Replication

- DNA Polymerases synthesize DNA
- Helicases unwind DNA
- Single-stranded binding proteins (SSBP) hold DNA in its unwound state
- Exonucleases (may be part of polymerases) remove nucleotides to fix errors

RNA Structure

Ribose instead of deoxyribose

Uracil replaces Thymine

Single-stranded (except in some viruses)

RNA

Three classes of RNA in animals:

1. mRNA: messenger RNA
2. rRNA: ribosomal RNA
3. tRNA: transfer RNA

DNA Recombination
(Crossing Over)

Recombination

Genetic exchange between two homologous, double-stranded DNA molecules.

Occurs at equivalent positions along two chromosomes with substantial DNA sequence homology.

Models for Recombination

Based on proposals put forth by Robin Holliday and Harold Whitehouse in 1964.

Depend on complementarity of DNA strands

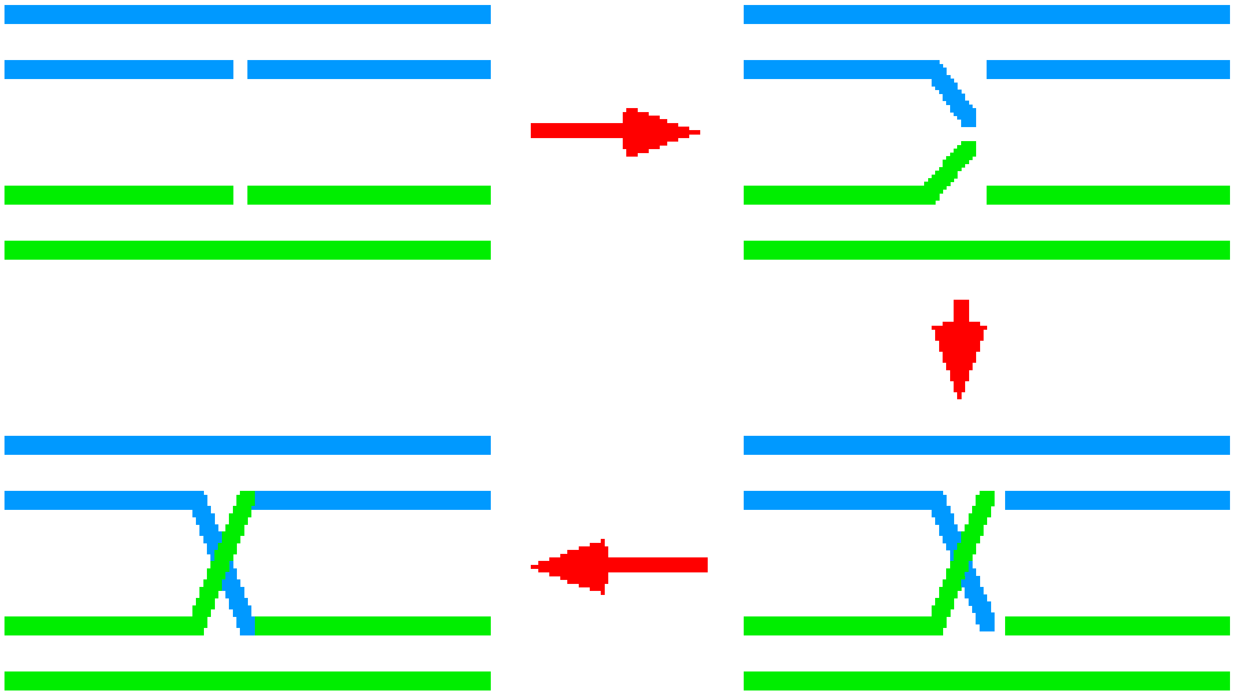
Rely on enzymatic processes

Basic Model

- Two pair DNA duplexes
- An endonuclease nicks one strand of each (breaks the phosphodiester bond in the backbone)
- The ends of the strands are displaced
- The homologous regions of the displaced strands pair up
- Ligase seals the nicks

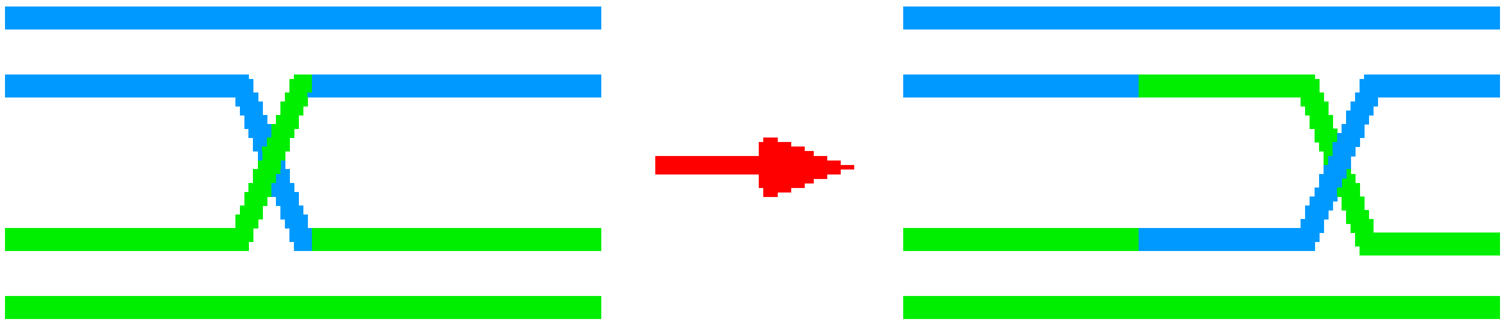
Basic Model

The hybrid duplex formed is a **heteroduplex**:



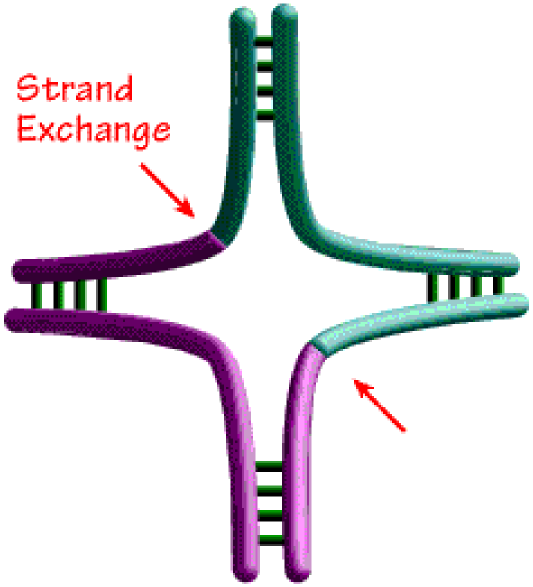
Basic Model

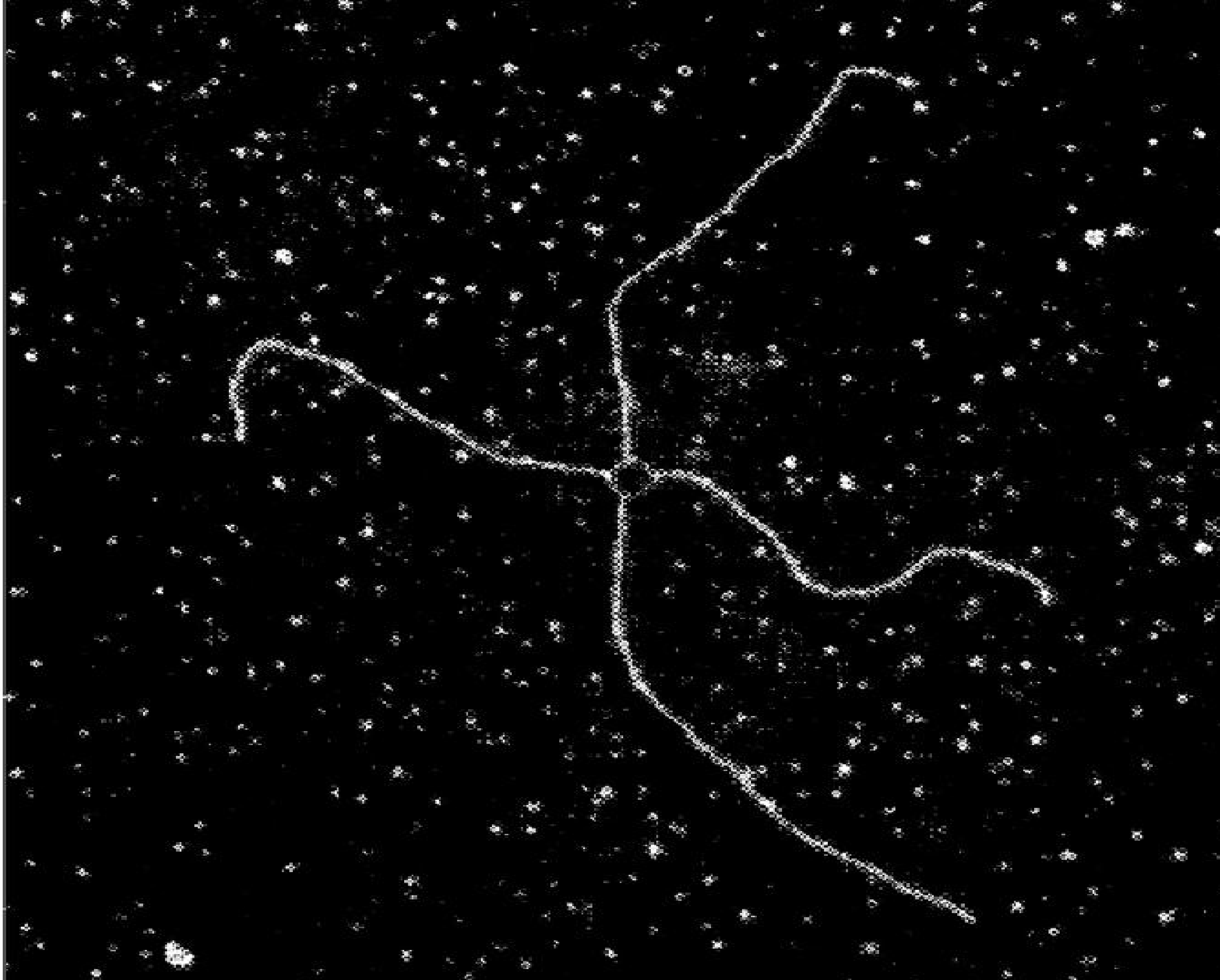
The cross bridge then migrates down the strand in a process called **branch migration** as hydrogen bonds are broken, then reformed.



Basic Model

If the duplexes separate and the structure rotates 180º, an intermediate structure is formed called a **Holliday Structure**.





Evidence for these models

1. Visualization of the intermediate planar Holliday structure.
2. Discovery of Rec A protein in E. coli that promotes exchange of reciprocal single-stranded DNA molecules.
3. Discovery of other enzymes essential to nicking, unwinding and ligation of DNA.