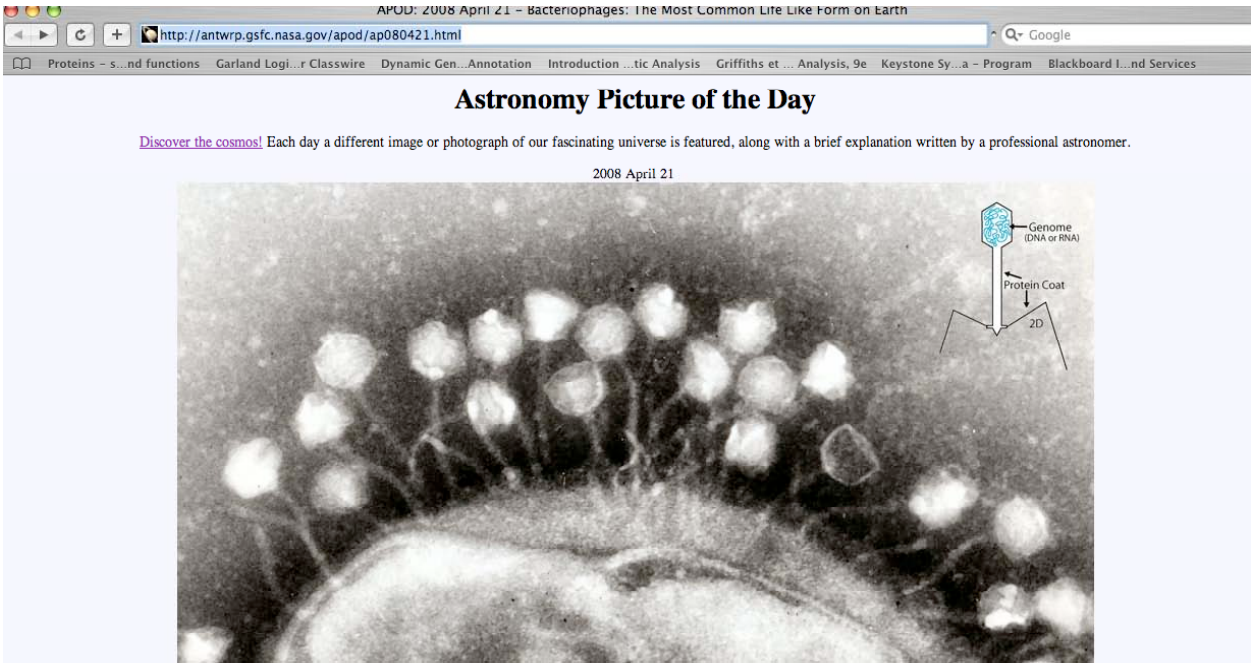
Biol 205 Spring 2008

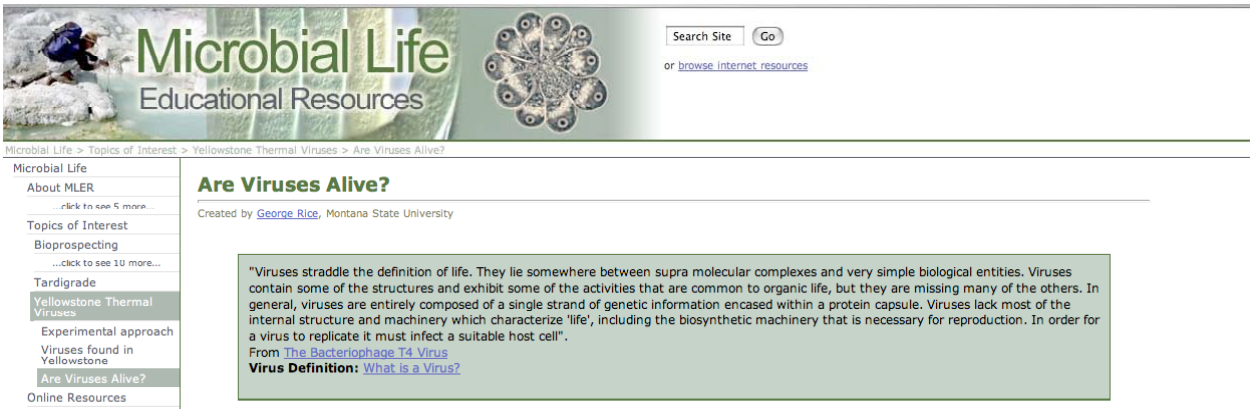
Astronomy picture of the day (4/21/08)

http://antwrp.gsfc.nasa.gov/apod/ap080421.html



Are viruses alive?

http://serc.carleton.edu/microbelife/yellowstone/viruslive.html



Week 3 Lecture 3 Replication of DNA

For exam 1 you are responsible for the structure of DNA – through pg 25 of this version of the lecture notes

Reading Assignments:
Chapter 5 DNA and Chromosomes
pg. 169-181 *(Browse through pgs 172-174 but you are not responsible for these experiments)*

Chapter 6 DNA Replication
pg. 195-197; 201-207

<u>Jargon Review:</u> Is there any terminology that you want to review, clarify or discuss futher?

<u>Loose ends from previous lectures:</u>

How does a rouge form (conformer) of a prion protein induce a protein in the normal form to follow its lead?

Mechanism not clear – see next page

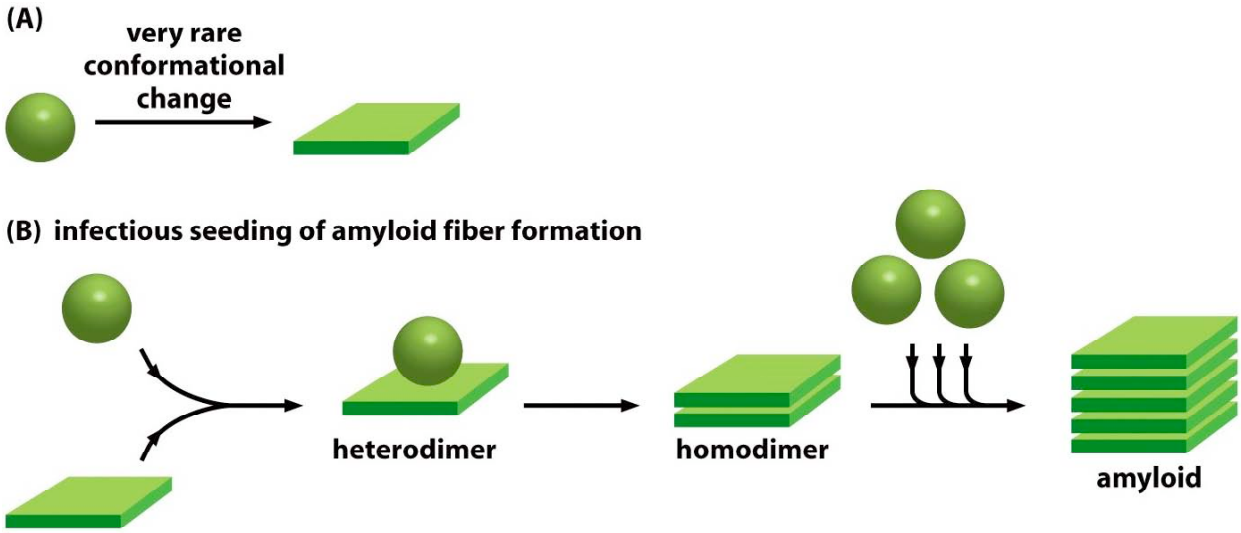
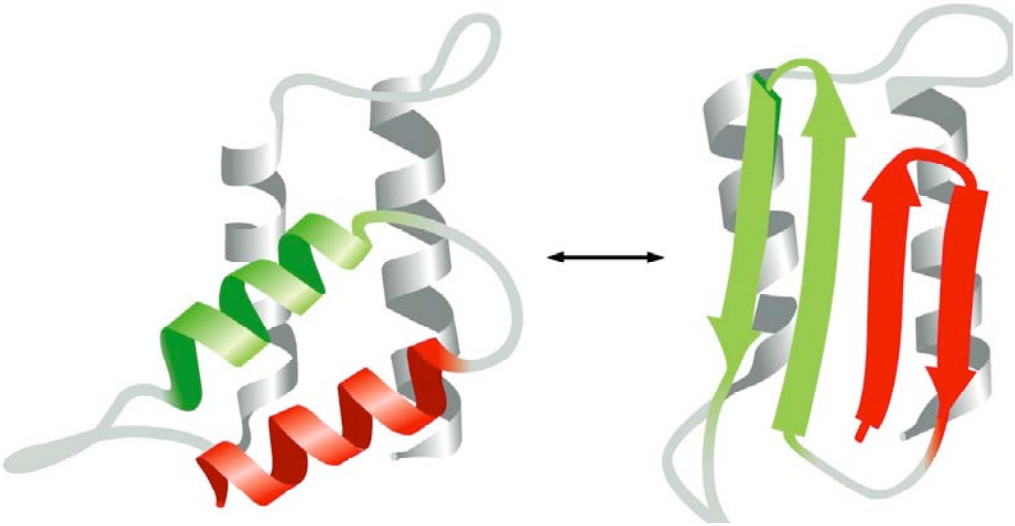


Figure 6-95ab Molecular Biology of the Cell 5/e (© Garland Science 2008)

A) Schematic illustration of the type of conformational change in a protein that produces material for a cross-beta filament. (B) Diagram illustrating the self-infectious nature of the protein aggregation that is central to prion diseases. PrP (prion protein) is highly unusual because the misfolded version of the protein, called PrP*, induces the normal PrP protein it contacts to change its conformation, as shown. Most of the human diseases caused by protein aggregation are caused by the overproduction of a variant protein that is especially prone to aggregation, but the protein aggregate cannot spread from one animal to another.



(D) One of several possible models for the conversion of PrP to PrP*, showing the likely change of two α helices into four β strands. Although the structure of the normal protein has been determined accurately, the structure of the infectious form is not yet known with certainty because the aggregation has prevented the use of standard structural techniques.

(C) Drawing of a cross-beta filament, a common type of protease-resistant protein aggregate found in many human neurological diseases. Because the hydrogen-bond interactions in a β sheet form between polypeptide backbone atoms, a number of different abnormally folded proteins can produce this structure.

THINKING ABOUT DNA REPLICATION:

- *What proteins and enzymes are involved?*
- *What is the specific chemistry of the chain elongation reaction?*
- *How does a cell efficiently and accurately duplicate extremely long polymers?*
- *How does a cell "decide" when to replicate its DNA?*
- *How does a cell replicate its DNA efficiently without making too many errors?*

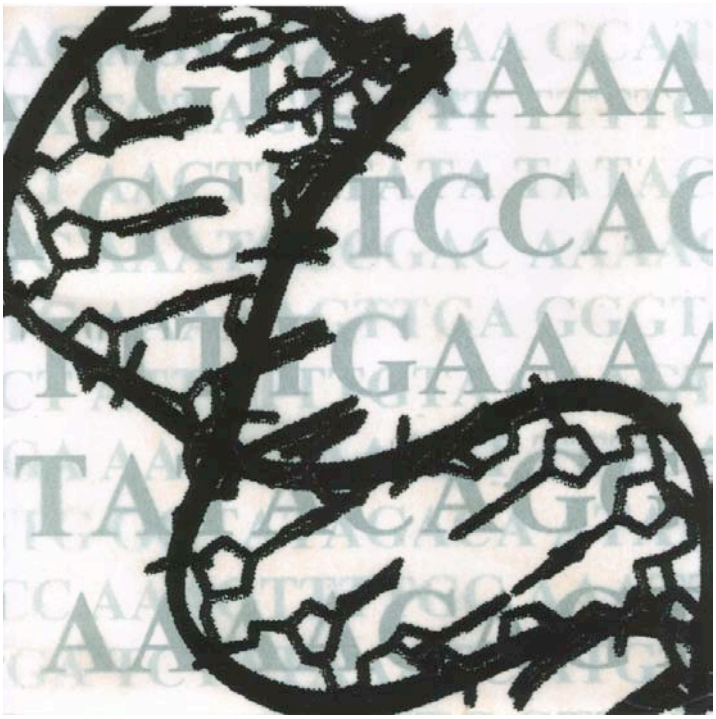
TTACCCATTCAGCCCATTCCCTGCAAACCAGTGGAGTATCCGCTGCAGCTGCTGCAC
AGCCCCCCTGCCCCAGTGGTGAAGAGGCCTGGGGCCATGGCCACCCACCACCCCCTGCAGGAGC
CCTCCCAGCCCCTGAACCTCACAGCCAAGCCCAAGGCCCCCGAGCTGCCCAACACCTCCAGCTC
CCCAAGCCTGAAGATGAGCAGCTGTGTGCCCCGCCCCCCCAGCCATGGAGGCCCCACGCGGGAC
CTGCAGTCCAGCCCCCCGAGCCTGCCTCTGGGCTTCCTTGGTGAAGGGGACGCTGTCACCAAAG
CCATCCAGGATGCTCGGCAGCTGC……..
…….. etc, etc, etc,

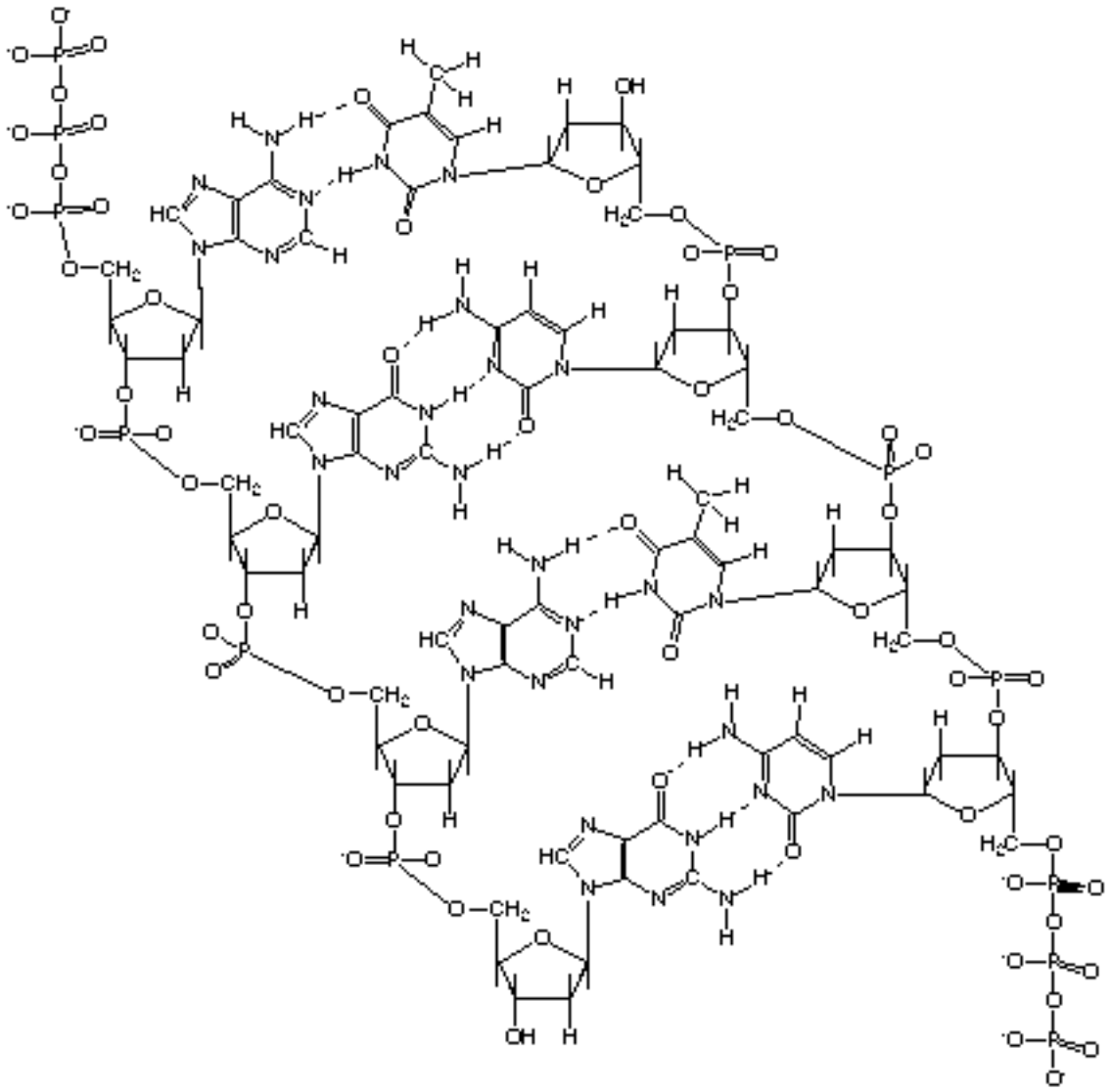
THis is a shorthand for

TTACCCATTCA GCCCATTCCCTGC AAACCAGTGGAGTATCCGCTGCAGCTG
AATGGGTAAGTCGGGTAAGGGACGTTTGGTCACCTCATAGGCGACGTCGAC

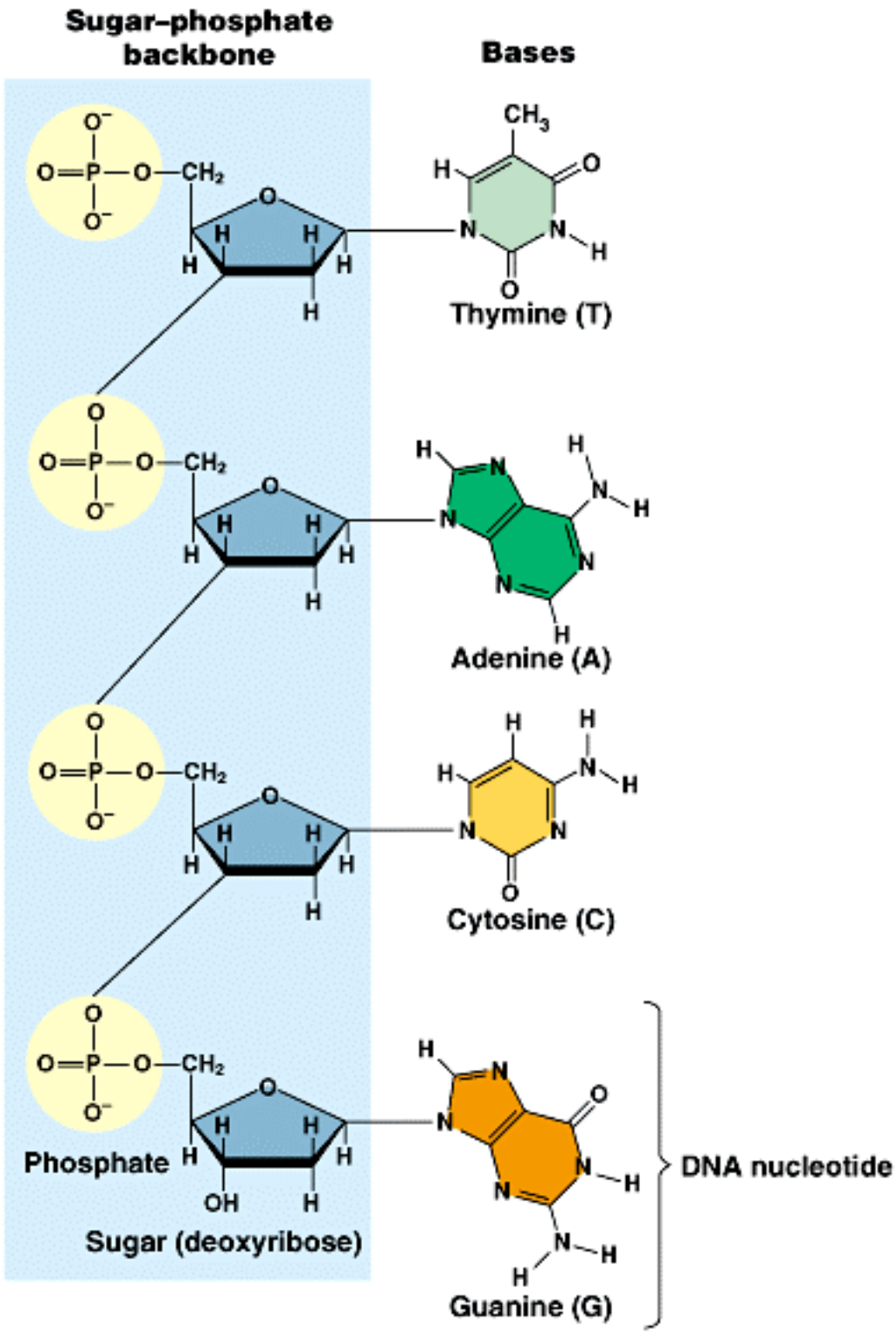
ETC. ETC. ETC.

What does this symbolism mean chemically?





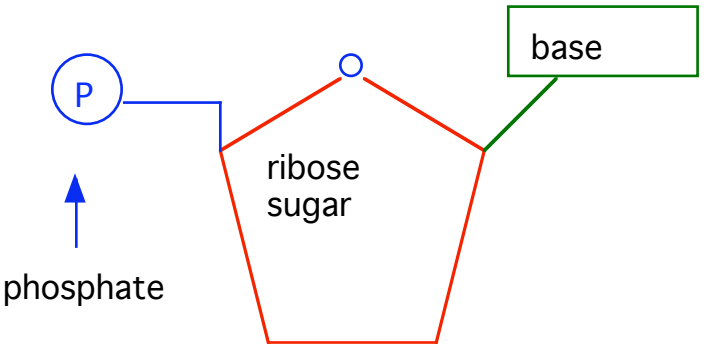
Lets take this molecule apart



DNA is a polymer of four different monomer units

Each monomer unit contains:
1. a carbohydrate: deoxyribose (in ring form)
2. a phosphate attached to the deoxyribose
3. one of four different nitrogenous bases

The nitrogenous bases are called
A = adenine G = guanine
T = thymine C = cytosine



Each monomer unit has this generic structure.
The monomer units differ in the structure of the base

Jargon review

nucleic acids = DNA and RNA polymers

G, C, A, T = purine and pyrimidine bases

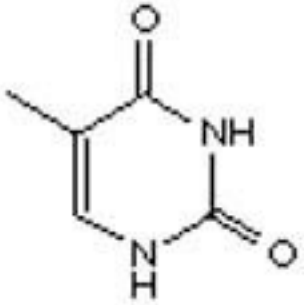
Purines:
G= guanine
A = adenine

Pyrimidines
C = cytosine
T= thymine

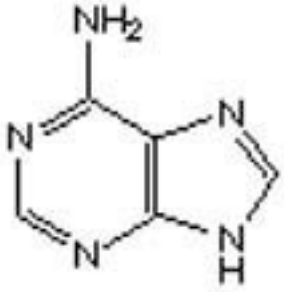
monomer units are called:
nucleotides or bases

When paired (via hydrogen bonds) in double-stranded DNA - ***base pairs or nucleotide pairs***

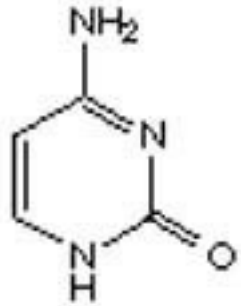
Add base



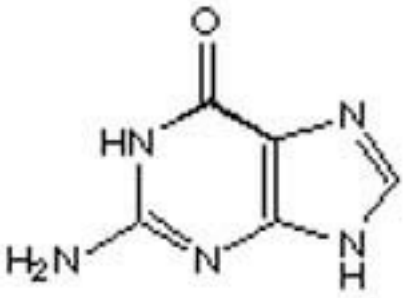
Thymine (T)



Adenine (A)



Cytosine (C)

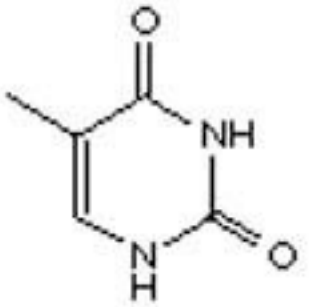


Guanine (G)

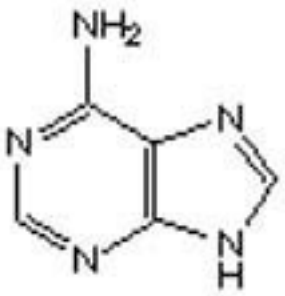
thymine and cytosine are pyrimidine bases

adenine and guanine are purine bases

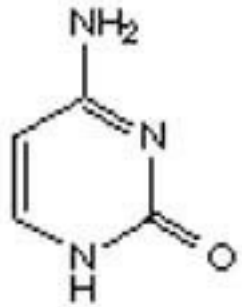
Understanding the hydrogen bond is the key to understanding the structure and information storage capacity of DNA



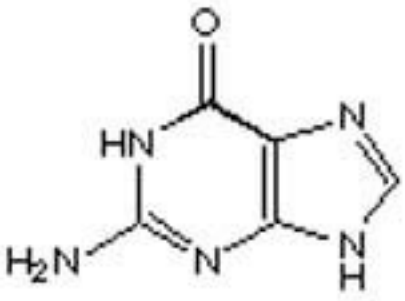
Thymine (T)



Adenine (A)



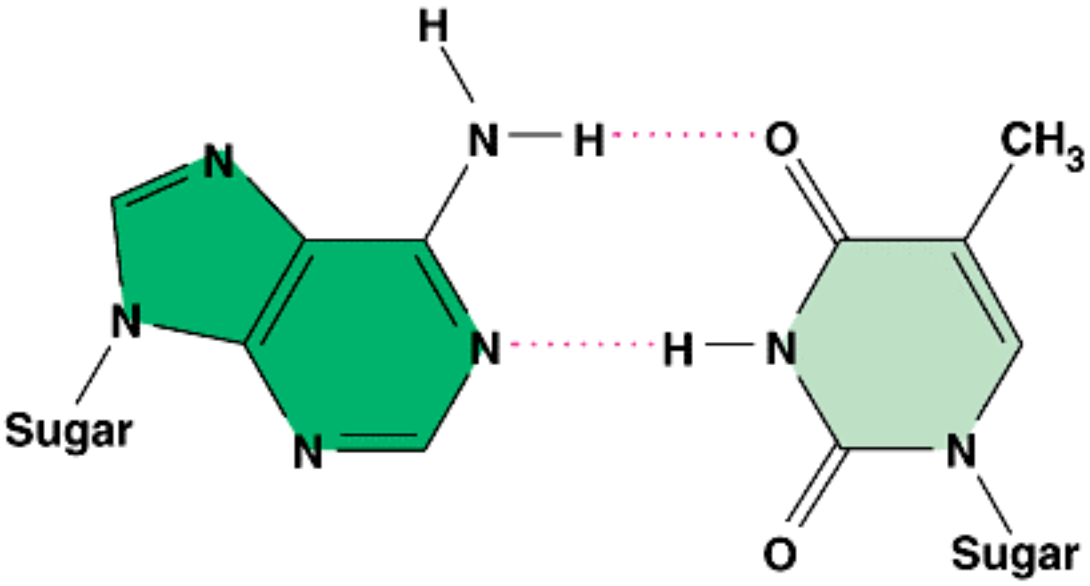
Cytosine (C)



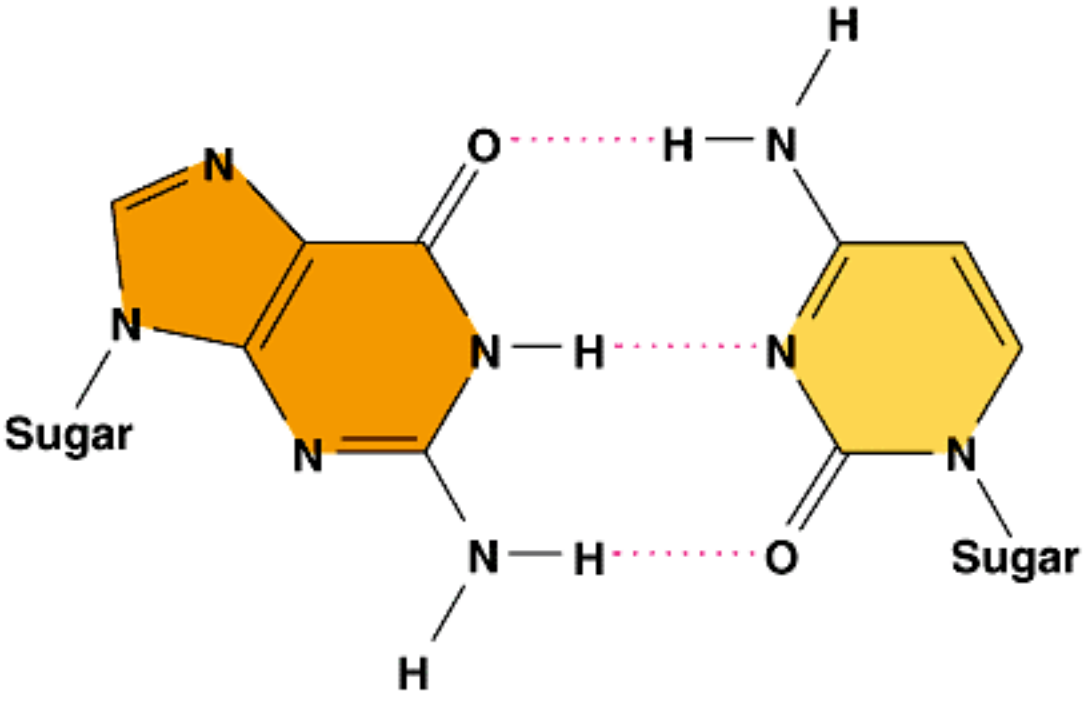
Guanine (G)

Examine these molecules for the presence of hydrogen bond donors and acceptors

C and G have complementary H-bonding surfaces
A and T have complementary H-Bonding surfaces



Adenine (A) Thymine (T)

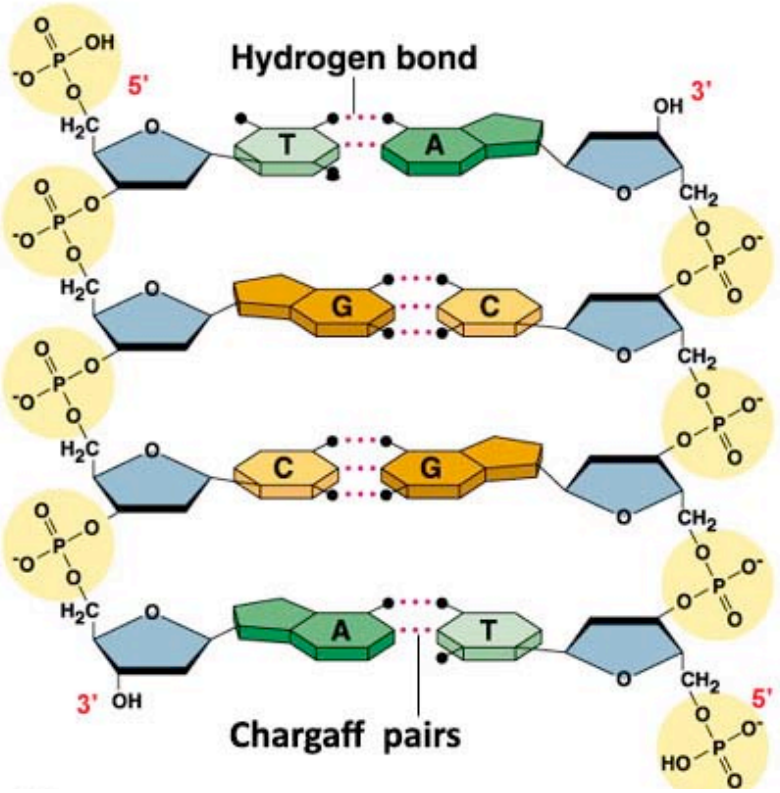


Guanine (G) Cytosine (C)

Hydrogen bonds & structural complementarity between purines and pyrimidines are the key to the

1) double helical (double polymer) structure of DNA
2) faithful replication of the DNA molecule
3) conversion of the digital information in DNA into the form of proteins during transcripton and translation

NOTE: the uniform diameter of the double helix



History of life on earth represents an unbroken chain of genetic continuity (that is, transmission of genetic information) lasting at least _____ years?

Transmission or propagation of a genetic program requires 2 basic steps common to all life on earth:

- **copying process -- replicating the DNA**
- **a distribution process -- getting the genetic information properly distributed to the progeny cells (*The nature of the distribution process affects the inheritance patterns of genetic traits*)**

THINKING ABOUT DNA REPLICATION:

- *What proteins and enzymes are involved?*
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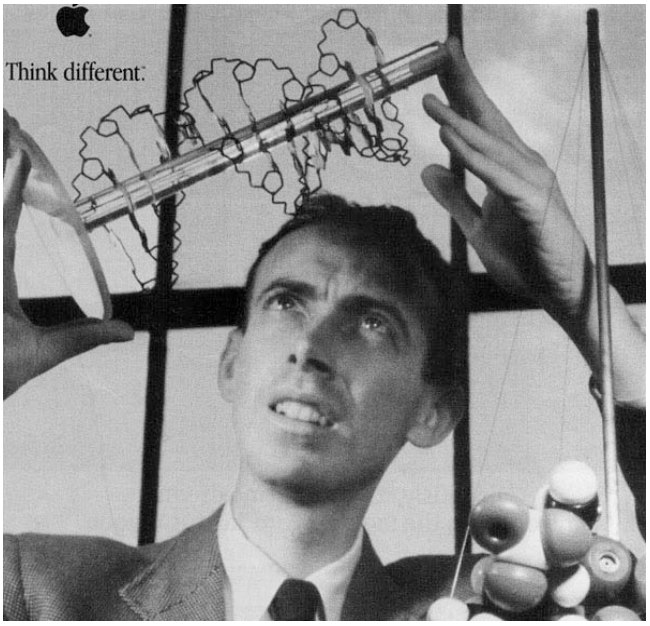


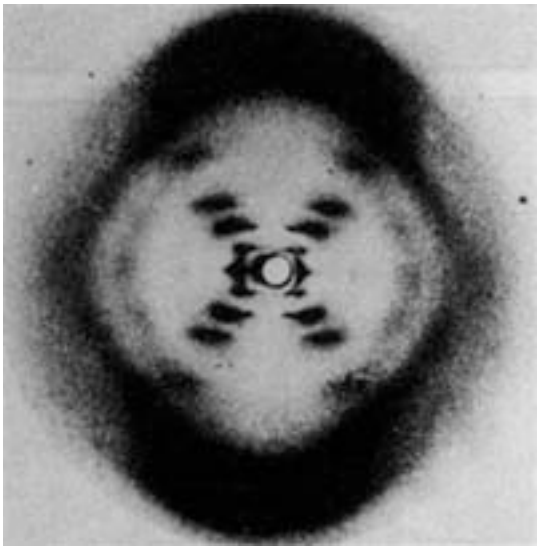
James Watson & Francis Crick were great model builders

http://www.physics.ucla.edu/~cwp/Phase2/Franklin,_Rosalind@841234567.html

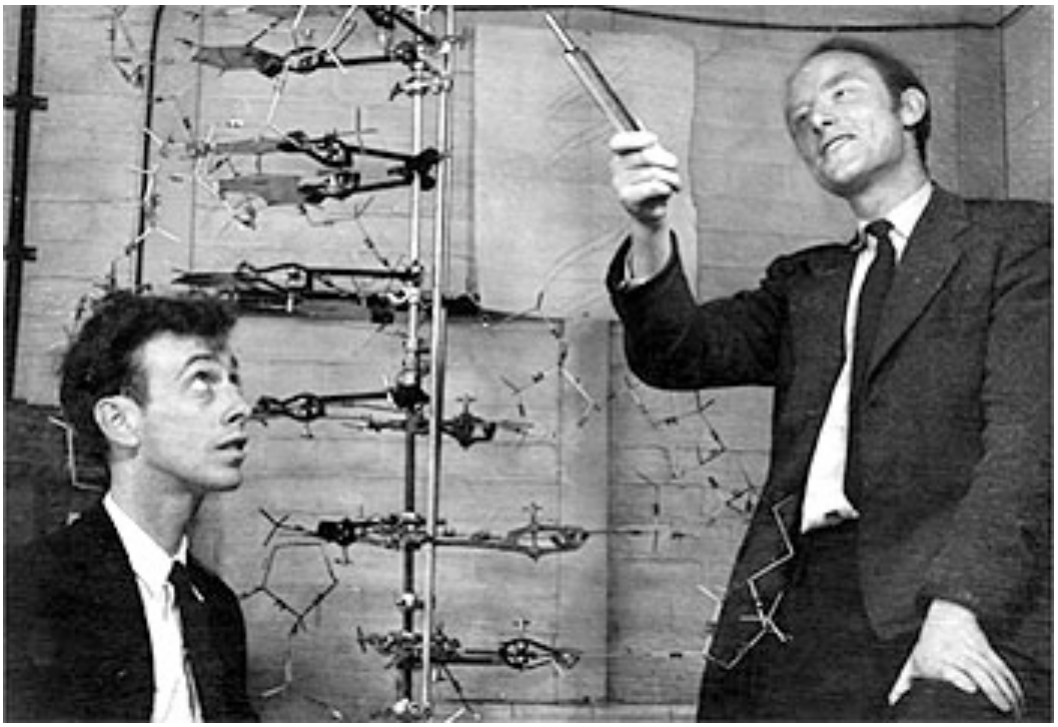
BUT, what did Rosalind Franklin do that Watson & Crick didn't do?

She actually collected data on the structure of DNA!

As a scientist Miss Franklin was distinguished by extreme clarity and perfection in everything she undertook. Her photographs are among the most beautiful X-ray photographs of any substance ever taken. Their excellence was the fruit of extreme care in preparation and mounting of the specimens as well as in the taking of the photographs.



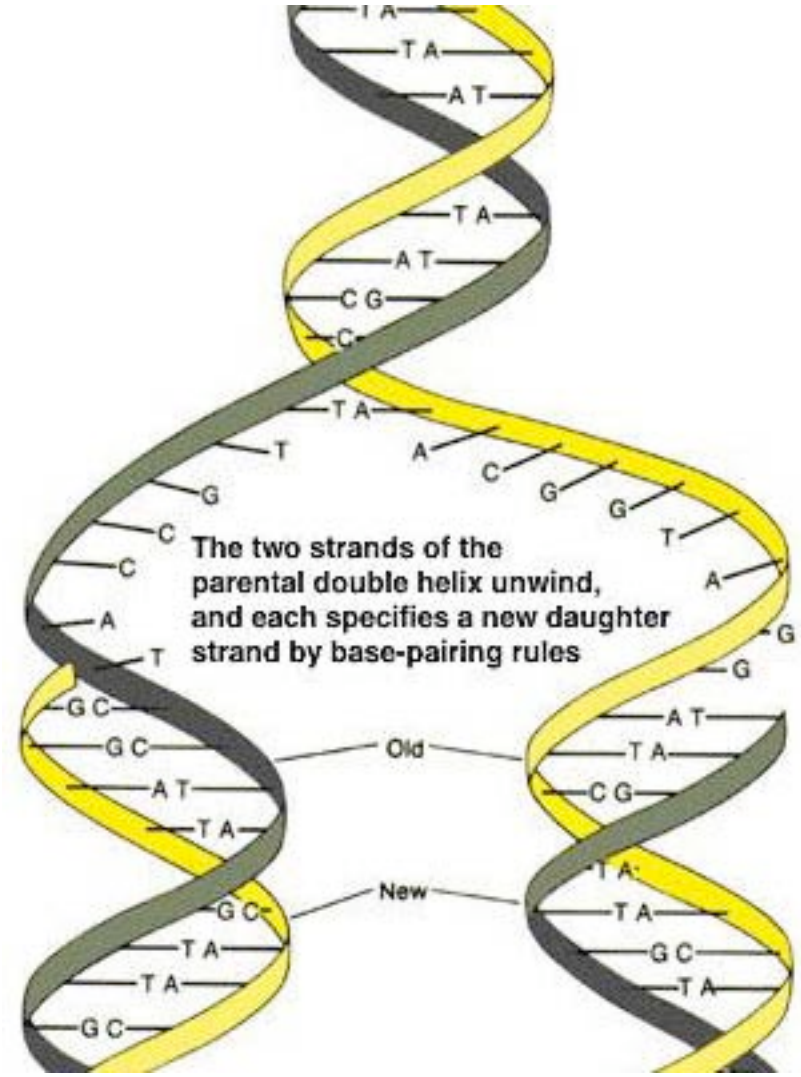
1953 Rosalind Franklin's X-ray diffraction image of DNA



http://www.nature.com/genomics/human/watson-crick/

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

The structure of DNA that Watson and Crick proposed was very appealing because it provided an simple, elegant and obvious mechanism for its duplication



Semi-conservative DNA replication:

1. **The parental strands of the DNA double helix separate**
2. **Each parental strand serves as template for the synthesis of a complementary copy**
3. **The nucleotide sequence of the newly synthesized daughter strand is determined by**
   - **the sequence of the parental template**
   - **the pairing (hydrogen-bonding) specificities of the purine and pyrimidine bases**

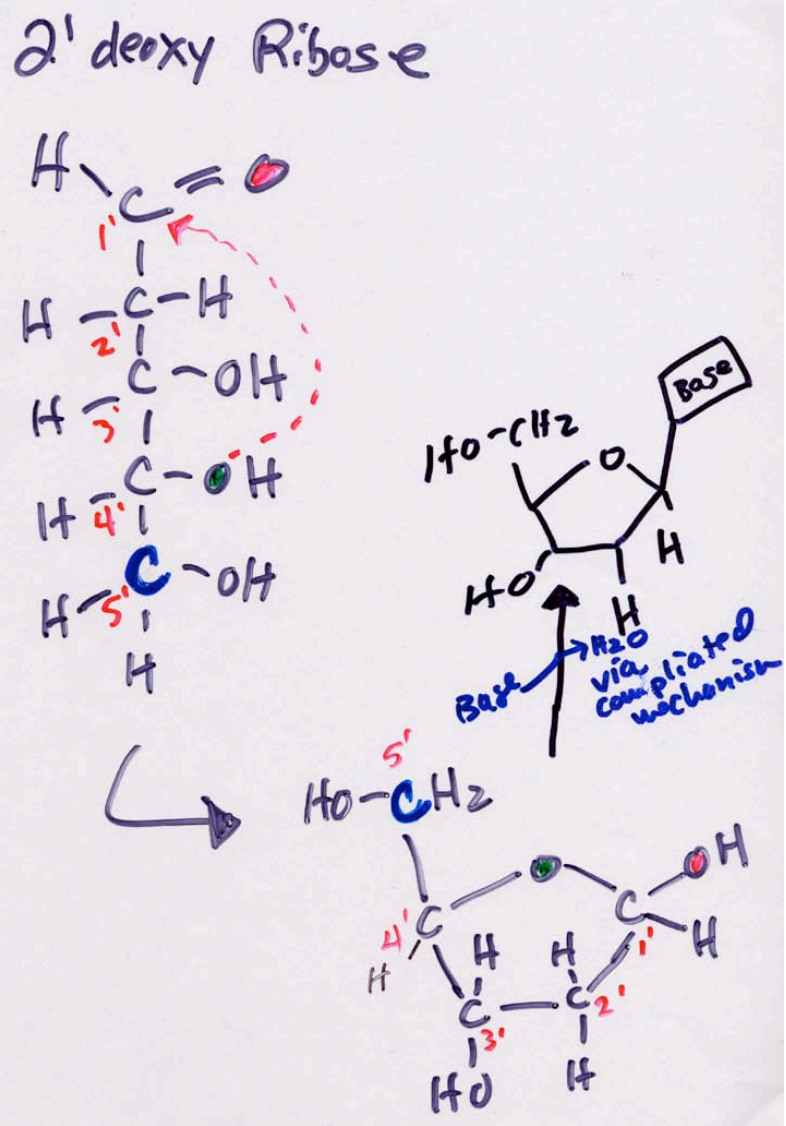
DNA REPLICATION IS BIOCHEMICAL VERY COMPLEX INVOLVING DOZENS OF DIFFERENT PROTEINS

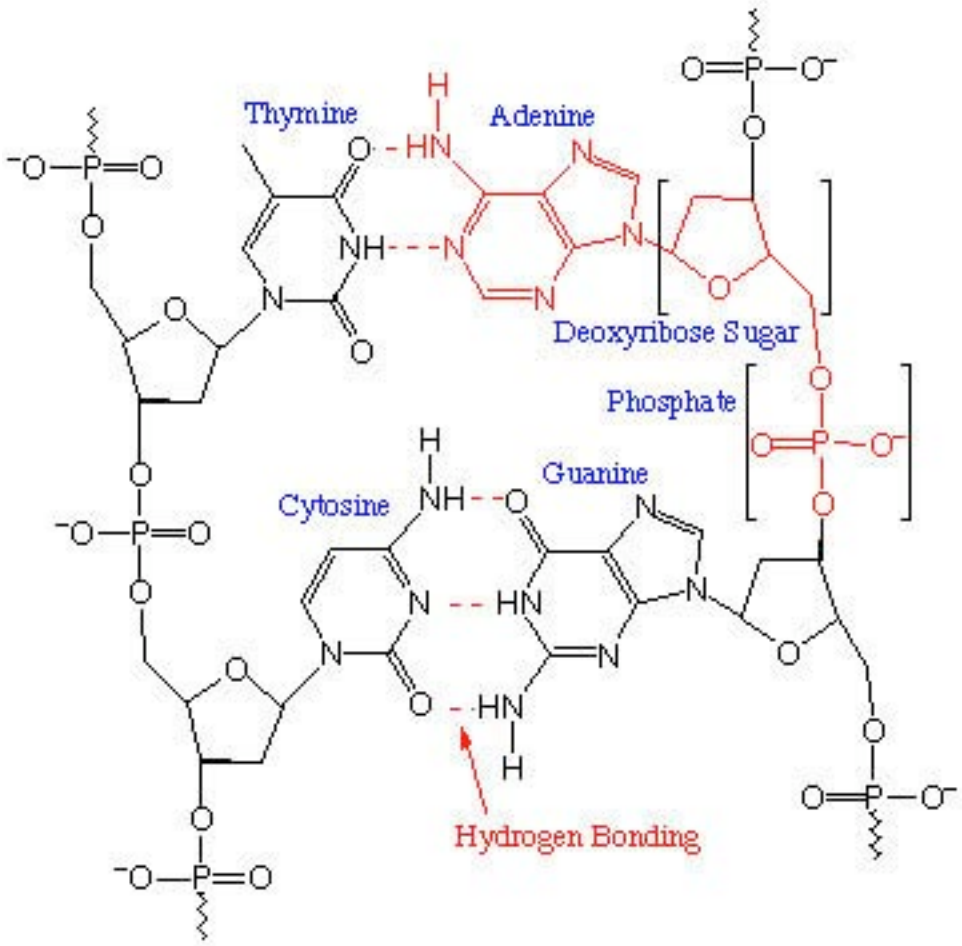
We will focus our attention on DNA polymerase
DNA polymerase ***catalyzes the synthesis of a DNA polymer -- that is, the formation of covalent bonds between monomer units***

DNA polymerase substrates are

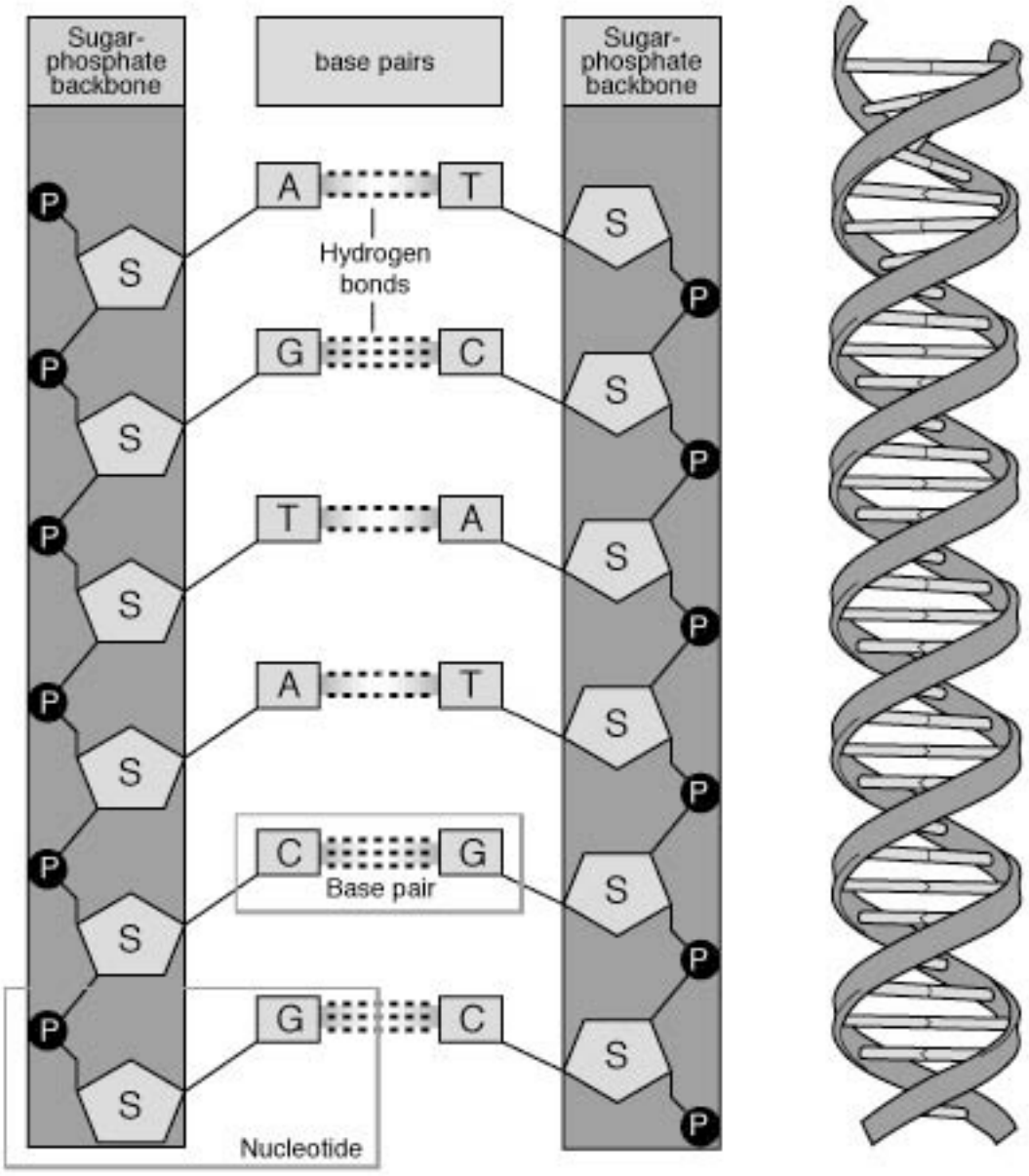
- ***primer-template complex***
- ***nucleotide subunits***

TRACKING 3' AND 5' ENDS:





MARK THE 3’ AND 5’ ENDS



3’ end 5’ end 3-D

2-D look at DNA: note strands are *antiparallel*

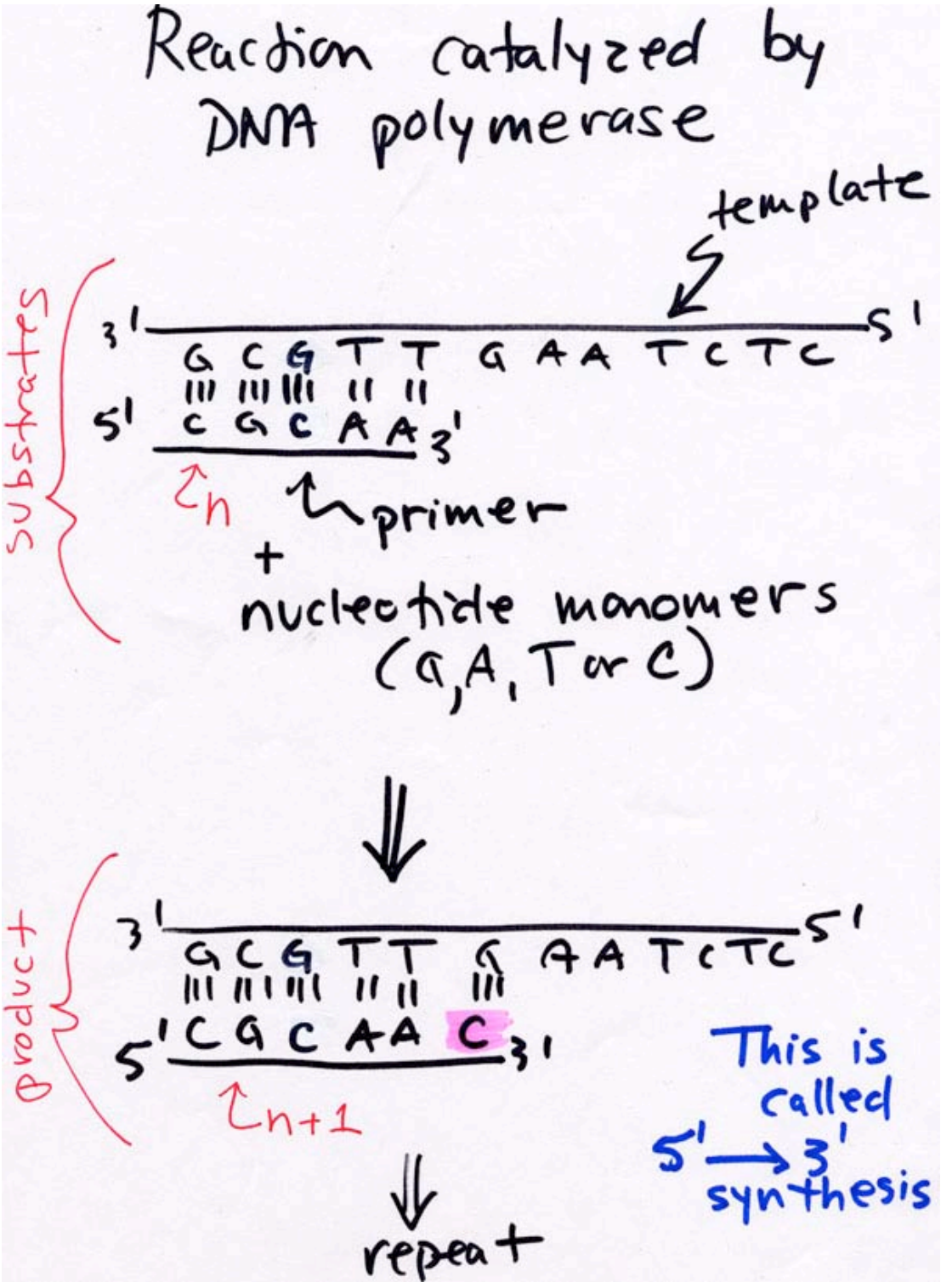
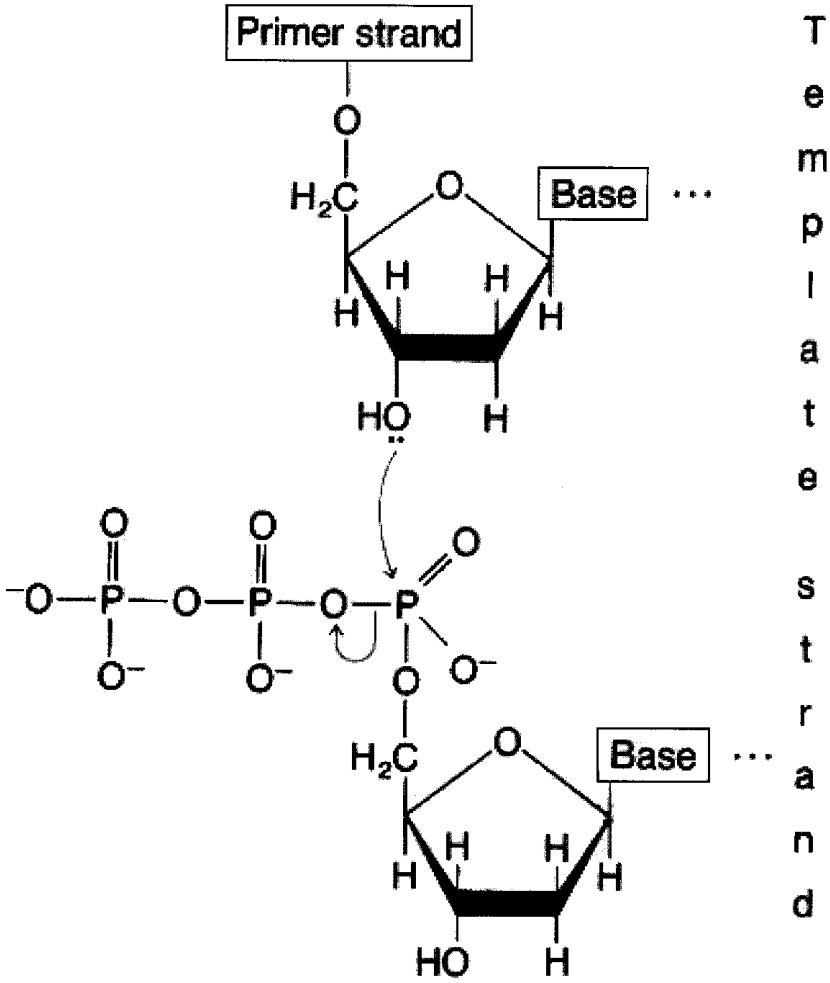

Reaction catalyzed by DNA polymerase
template
substrates
3'
G C G T T G A A T C T C
5'
5' C G C A A 3'
n
primer
+
nucleotide monomers (G, A, T or C)
product
3'
G C G T T G A A T C T C
5'
5' C G C A A C 3'
n+1
This is called 5' → 3' synthesis
repeat

Figure shows features common to all DNA polymerases:

1. **it takes "instructions" from a template -- the parental strand of DNA**

template: **a gauge, form or mold used as a guide to the form of a piece being made**

2. **it can only catalyze the addition of a nucleotide monomer to a 3' carbon of ribose**
4. **it cannot catalyze the addition of a nucleotide monomer to the 5' carbon of ribose**
5. **this is called 5' to 3' synthesis**



DNA SYNTHESIS OCCURS 5' TO 3'

The monomer substrates are in the form of a dNTP

d= 2' deoxy N = A, C, G or T TP = triphosphate

dNTP's are chemically reactive monomer units

Note again that it is the 3' end of the primer chain that forms the bond with the "incoming" monomer

[Also note that the primary energy currency in the cell is ATP]

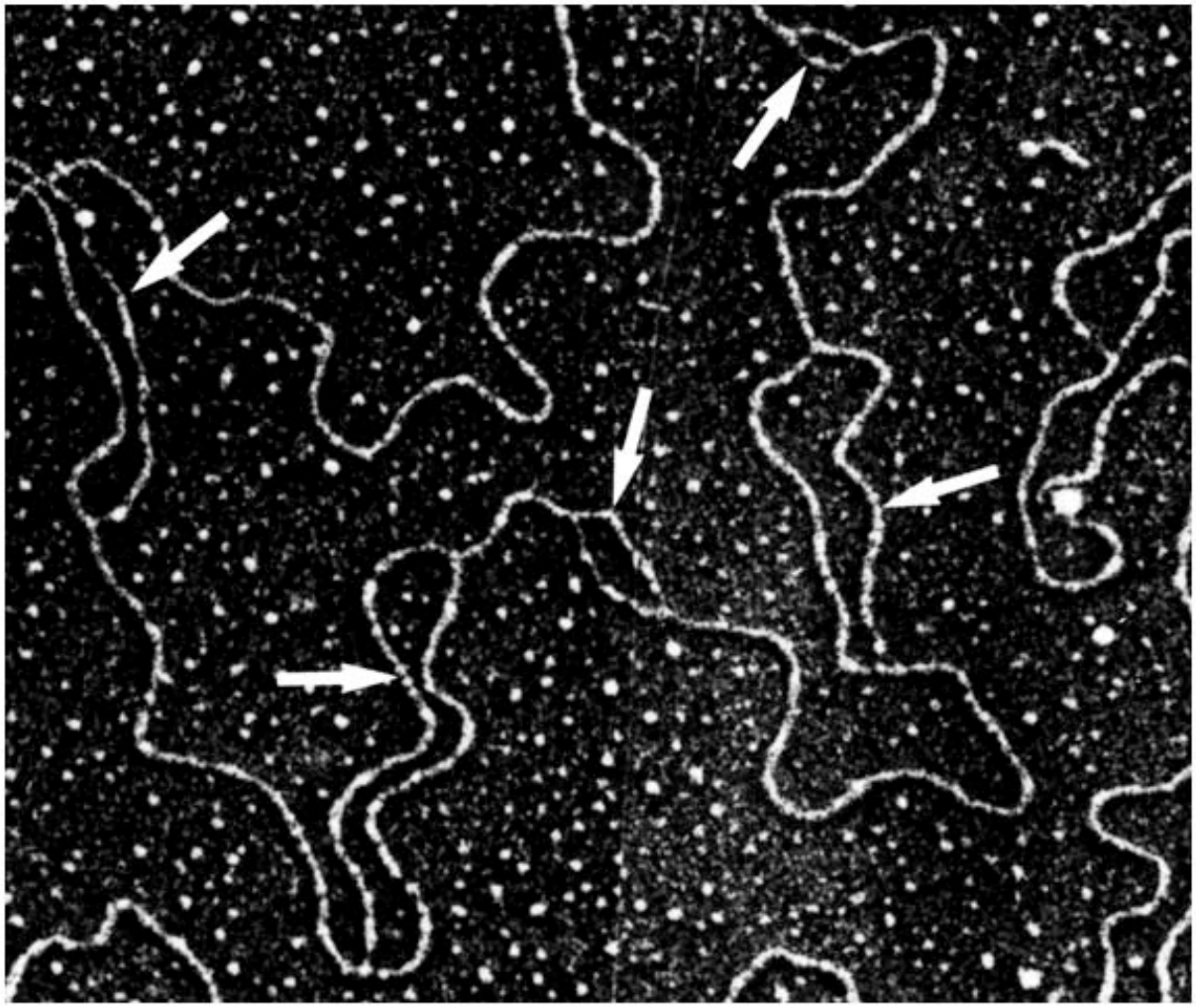


FIGURE 10-5 Electron micrograph of DNA extracted from rapidly dividing nuclei of early *D. melanogaster* embryos. The arrows mark replication bubbles; the diameters of the DNA chain in both arms of these bubbles indicate that they are double-stranded. [See A. B. Blumenthal, H. J. Kreigstein, and D. S. Hogness, 1973, *Cold Spring Harbor Symp. Quant. Biol.* **38:**205; courtesy of D. S. Hogness.]

Facing up to leading and lagging strands
ON BOARD

Another "problem" with DNA polymerase

- DNA polymerase cannot lay down the first nucleotide of a DNA strand
- It requires a "bit" of polymer to add onto
- This short segment of polymer is called a *primer*
- It provides a 3' hydroxyl for the DNA polymerase to add onto

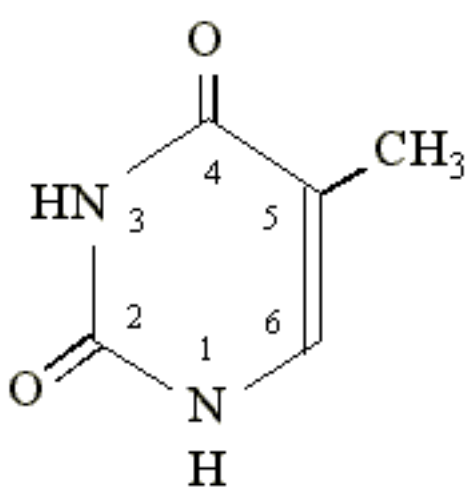
This "problem" adds a significant complication to the DNA replication process

Confronting the primer issue:
If DNA pol can't start a DNA polymer what does?

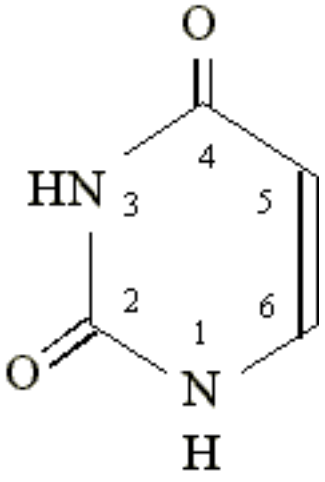
An enzyme called *primase* synthesizes a short polymer of RNA

What are the chemical differences between RNA and DNA?

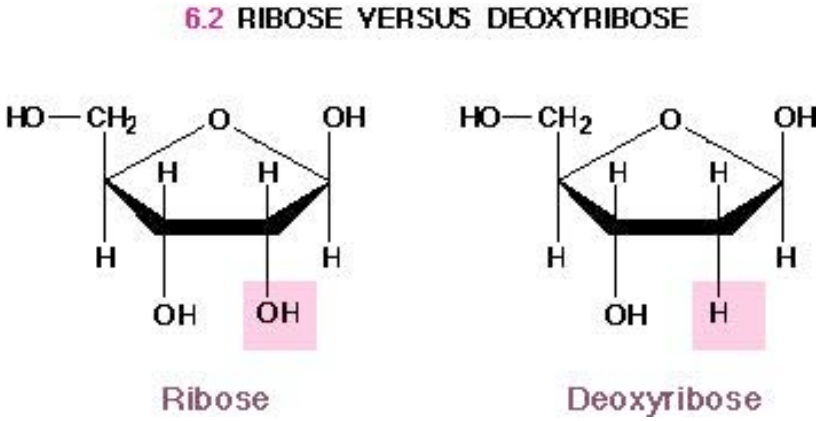
- **single-stranded not double-stranded**
- **2’ hydroxyl on ribose ring**
- **uracil replaces thymine in RNA**
- **uracil = thymine with respect to base-pairing (H-bonding) characteristics because the methyl group on thymine doesn’t influence H-bonding**



Thymine
(DNA)



Uracil
(RNA)



RNA DNA

The biochemistry of DNA replication is enormously complex

Many other proteins involved in the process of DNA replication (no need to memorize this list – just think about the complexity of the process)

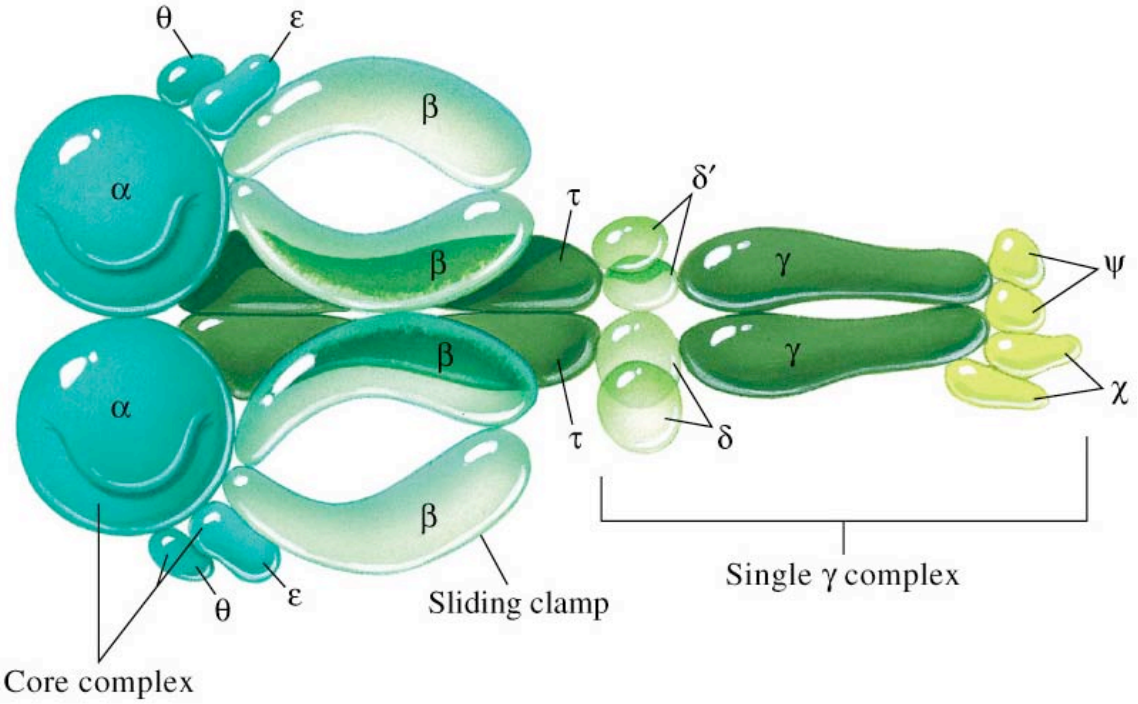
- ***helicase:*** separating the DNA strands
- ***single-stranding binding protein:*** protecting the separated strands and making them accessible to DNA polymerase
- ***topoisomerase:*** relieving torsional stress on the molecule (from the unwinding)
- ***primase, exonuclease & DNA polymerase:*** make and replace the RNA primer
- ***3'-5' exonuclease proofreader:*** ensures a high level of accuracy of the process
- ***sliding clamp:*** ensures that the polymerase remains bound to the DNA as the replication fork moves
- ***sliding clamp loader:*** transiently opens up clamp so it can to topologically linked to the DNA
- recognizing the origin of replication
- coordinating the activities of all of the proteins

Check out these animations:
http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter14/animations.html#

DNA polymerase need helpers

TABLE 20.1 Subunits of DNA polymerase III holoenzyme

Subunit	M_r		Gene		Activity
α	130 000	core	*pol*C/*dna*E		Polymerase
ε	27 000	core	*dna*Q/*mut*D		3′→ 5′ exonuclease
θ	8846	core	*hol*E		?
β	40 000		*dna*N		Forms sliding clamp
τ	71 000		*dna*X		Enhances dimerization of core; ATPase
γ	47 000	γ complex	*dna*X		Enhance processivity; assist in replisome assembly
δ	38 700	γ complex	*hol*A		
δ′	36 900	γ complex	*hol*B		
χ	16 600	γ complex	*hol*C		
ψ	15 174	γ complex	*hol*D		



The sliding clamp: evolution's solution to the holding-on-but-letting-go problem

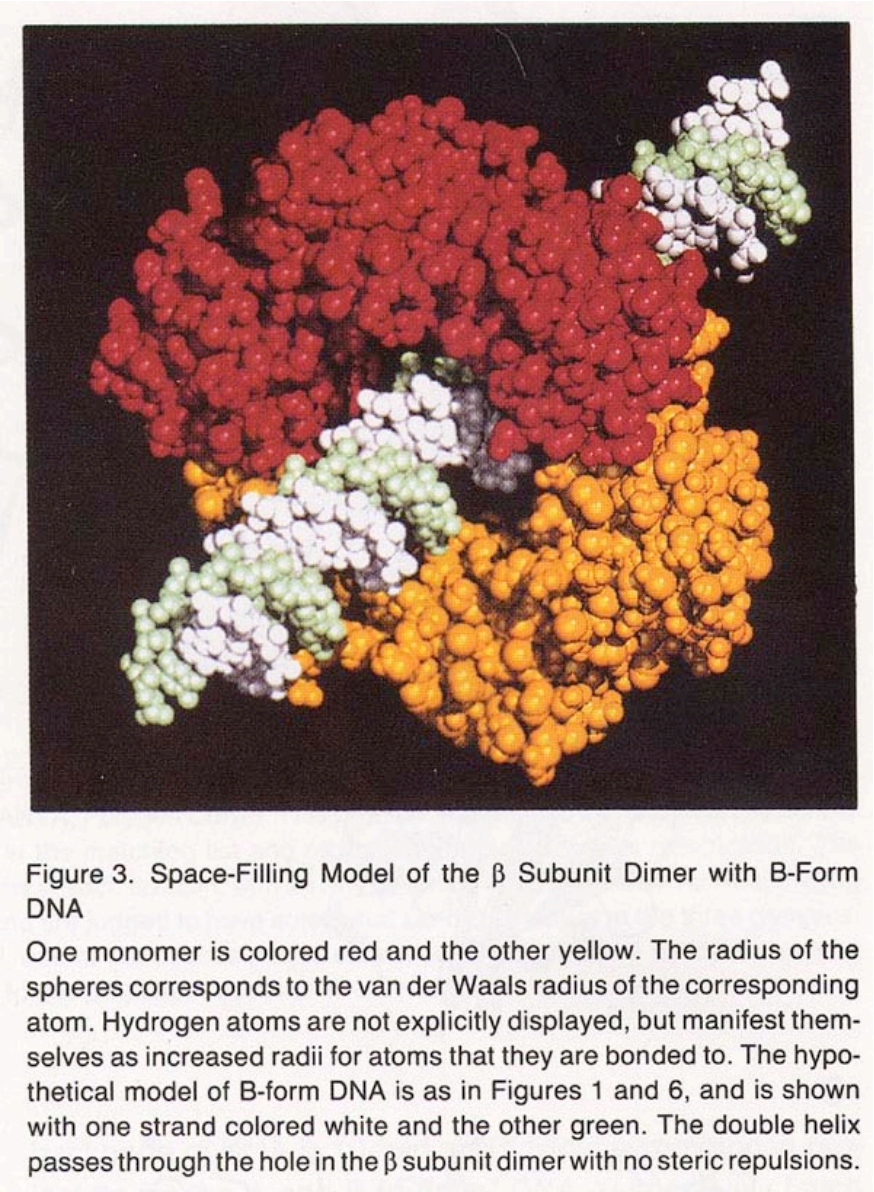


Figure 3. Space-Filling Model of the β Subunit Dimer with B-Form DNA

One monomer is colored red and the other yellow. The radius of the spheres corresponds to the van der Waals radius of the corresponding atom. Hydrogen atoms are not explicitly displayed, but manifest themselves as increased radii for atoms that they are bonded to. The hypothetical model of B-form DNA is as in Figures 1 and 6, and is shown with one strand colored white and the other green. The double helix passes through the hole in the β subunit dimer with no steric repulsions.

DNA replication in all organisms is highly accurate (viruses are the big exception)

OVERALL error rate (after two "proofreading" steps) is ***1 mistake in every 10^9-10^{10} nucleotides copied!***

Recall that a single copy of the human genome has 3 billion base-pairs.

When a thirty year old man breeds with a 30 year old woman:

- **his DNA (in his sperm cells) has been copied 430 times against her 33 cell divisions (in egg cells).**
- **with thirteen times as many errata in his DNA, about 185 of the 200 copying mistakes in each human conception may come from the sperm.**
- **however, a woman's eggs are more likely to carry serious errors in chromosome numbers, and these errors increase with maternal age.**

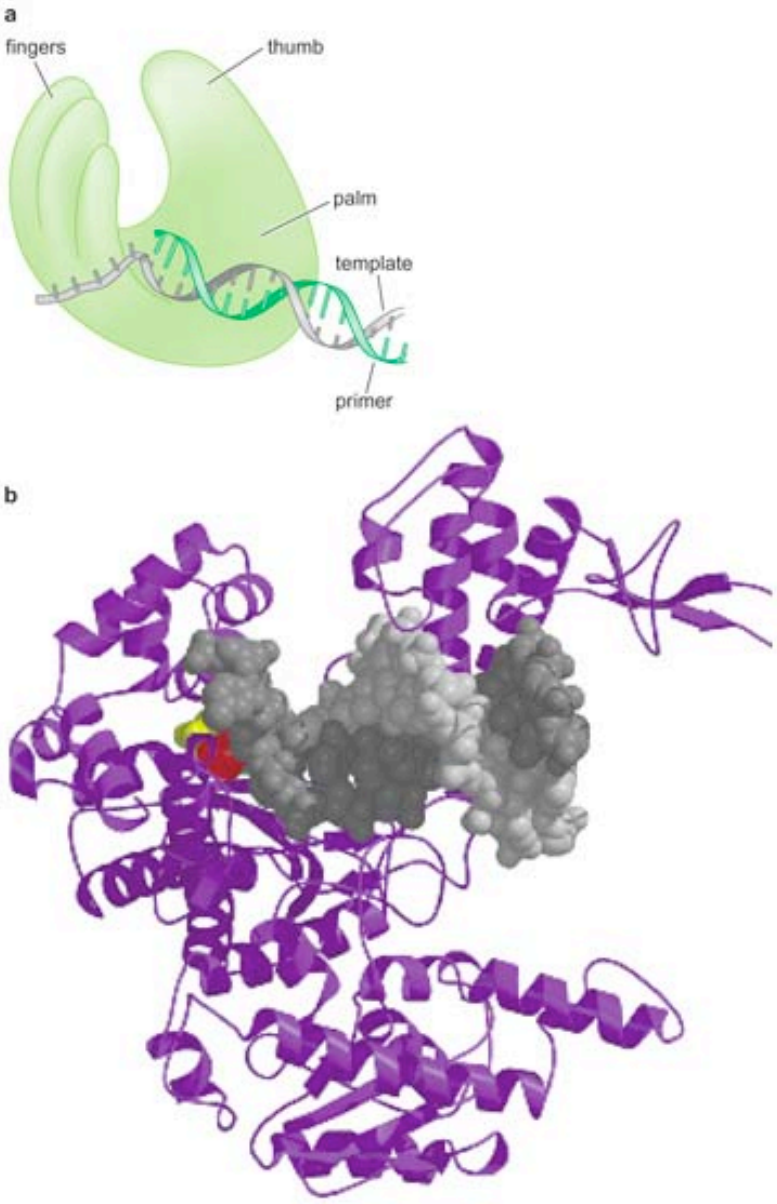
DNA replication in all organisms is highly efficient:
in *E. coli* rate of polymerization can be as high as ***1000 nucleotides per second***

Evolutionary Considerations:

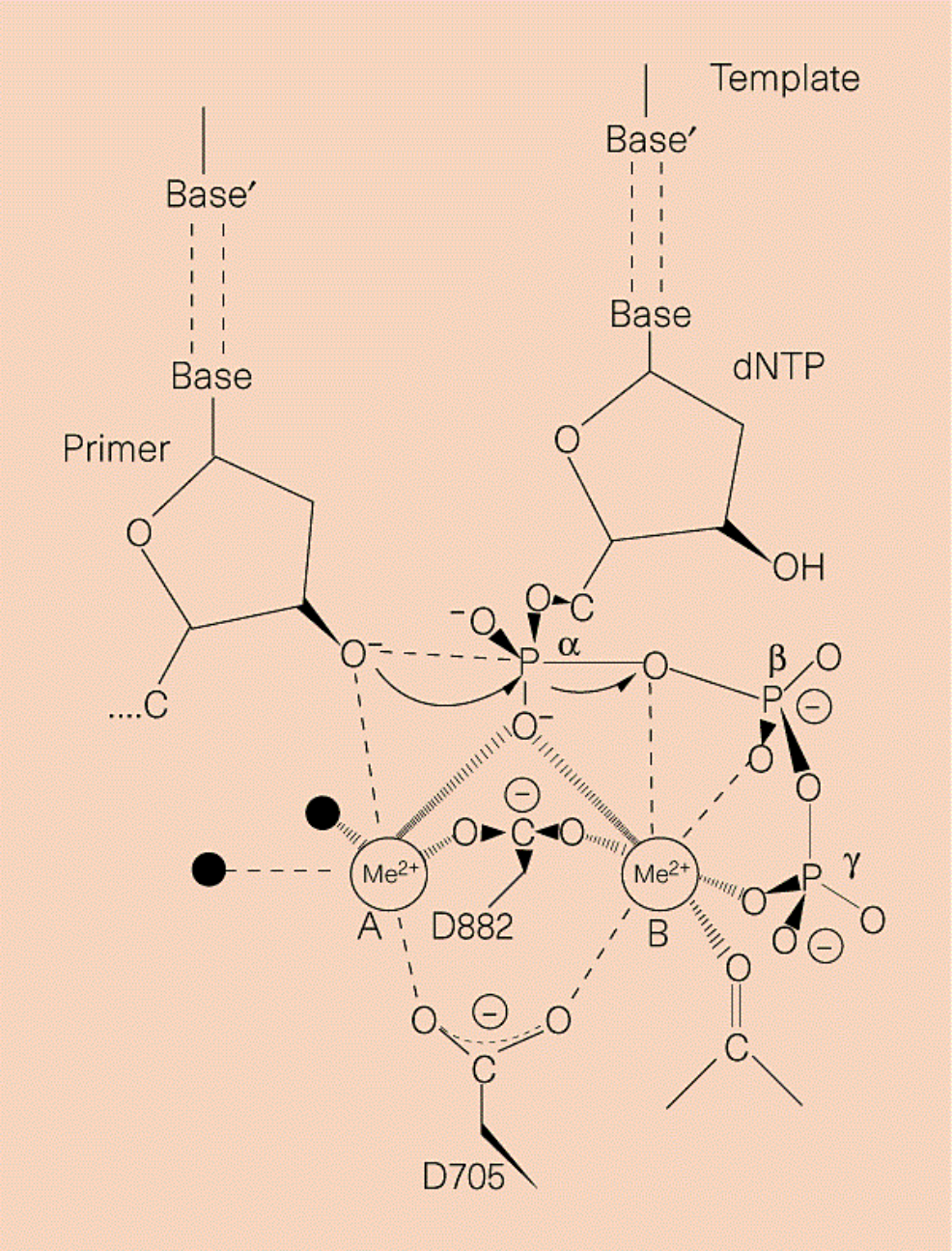
- although the biochemical details differ, all organisms use the same basic strategy for DNA replication
- in some ways DNA replication is beautiful example of a highly efficient, highly accurate cellular process
- in some ways the process seems overly complicated -- all this RNA primer business and leading and lagging strands
- if we were to design the system, we might do it in a different way

Two additional considerations:

- All cellular processes are products of evolutionary history (and not of an engineer)
- The biochemical limitations of DNA polymerase appear to relate directly to the very high fidelity (accuracy) of this enzyme



a. The 3-D structure of DNA polymerase resembles a right hand. Site of catalysis is located in the crevice between the fingers and thumb. b. The fingers and thumb are composed of alpha helices. The incoming dNTP is shown in red (base and ribose) and yellow (triphosphate moiety). The template strand is dark gray and the primer is shown in light gray.



active site of DNA polymerase showing mechanisms listed previously -- ***alignment of substrates***; ***charge stabilization (by the metal ions);*** bond "straining"

DNA polymerase: enzyme that catalyzes the formation of a DNA polymer

Note the exonuclease domain that performs the proofreading