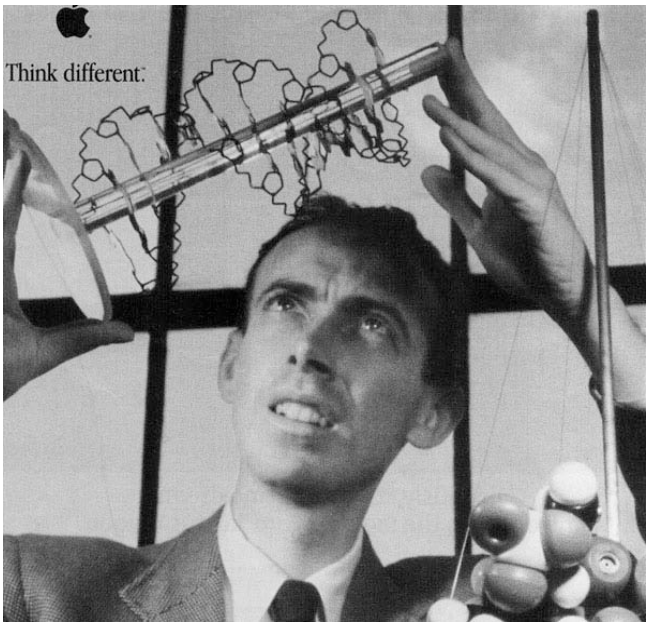
5/1/06 DNA replication

How does the transmission of genetic information occur?

Transmission of genetic information requires two steps:

1. copying step called DNA replication
2. "parcelling out" or distribution step



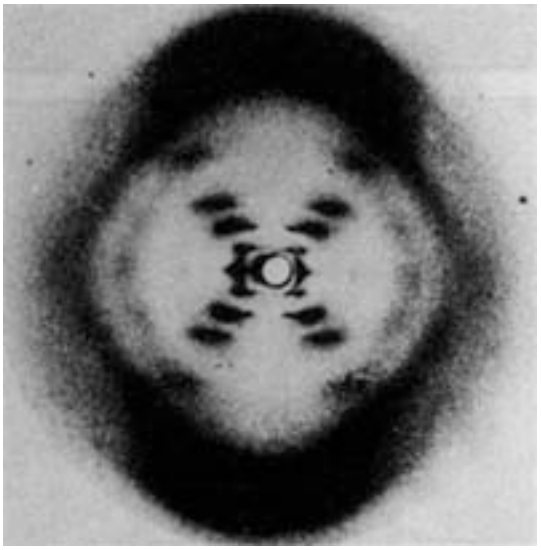
James Watson & Francis Crick were great model builders

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

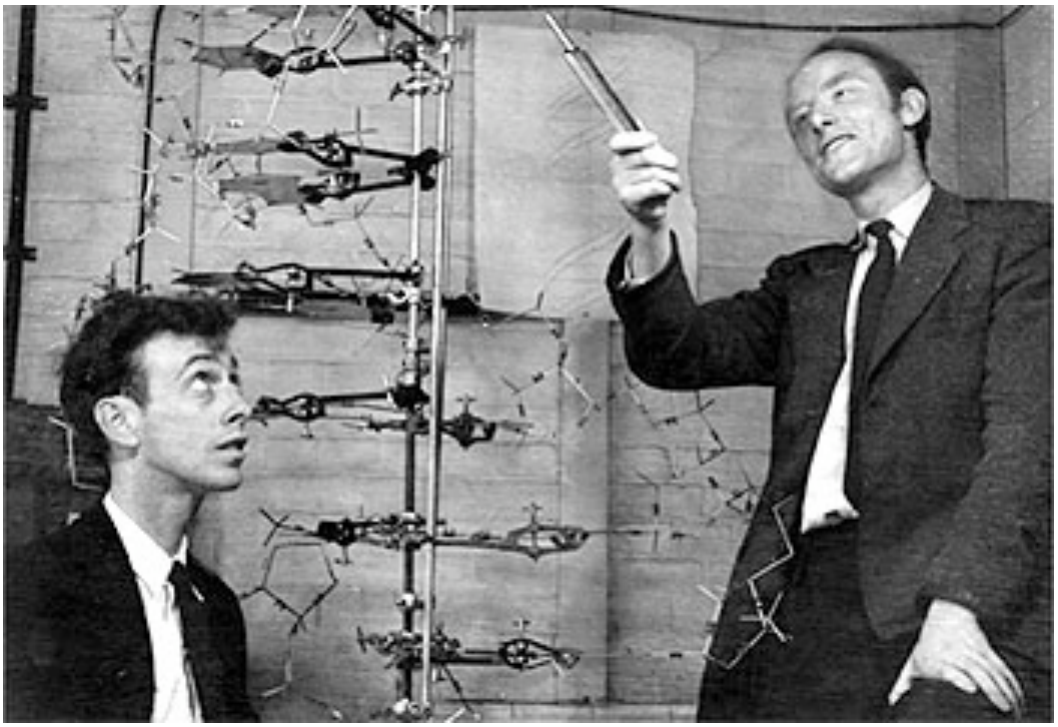
BUT, what did Rosalind Franklin do that Watson & Crick didn't do?

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.



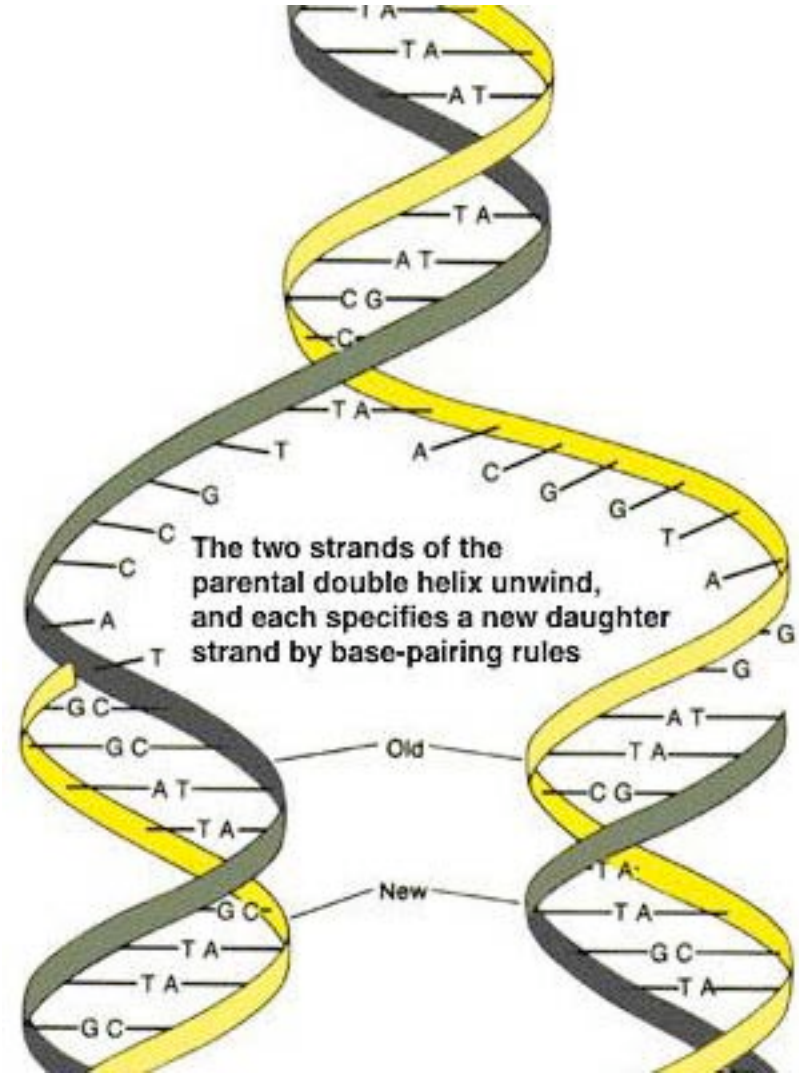
1953 Rosalind Franklin's X-ray diffraction image of DNA



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**

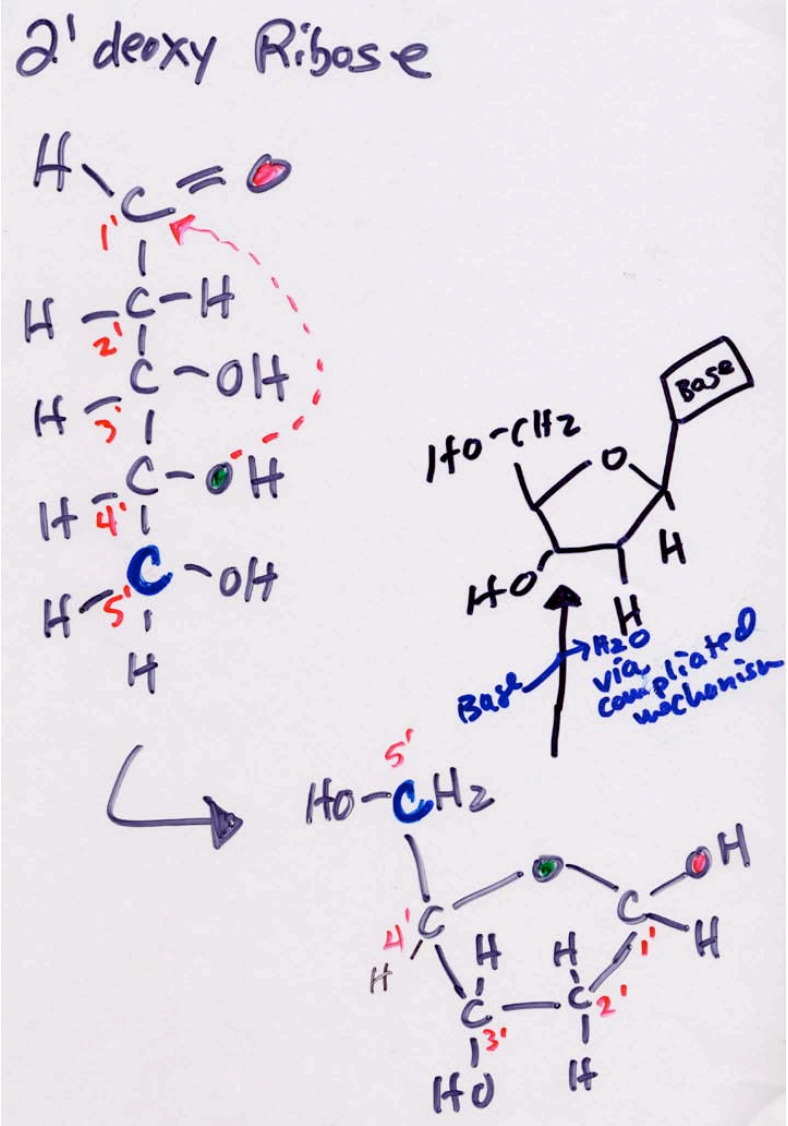
DNA REPLICATION IS BIOCHEMICAL VERY COMPLEX INVOLVING DOZENS OF DIFFERENT PROTEINS

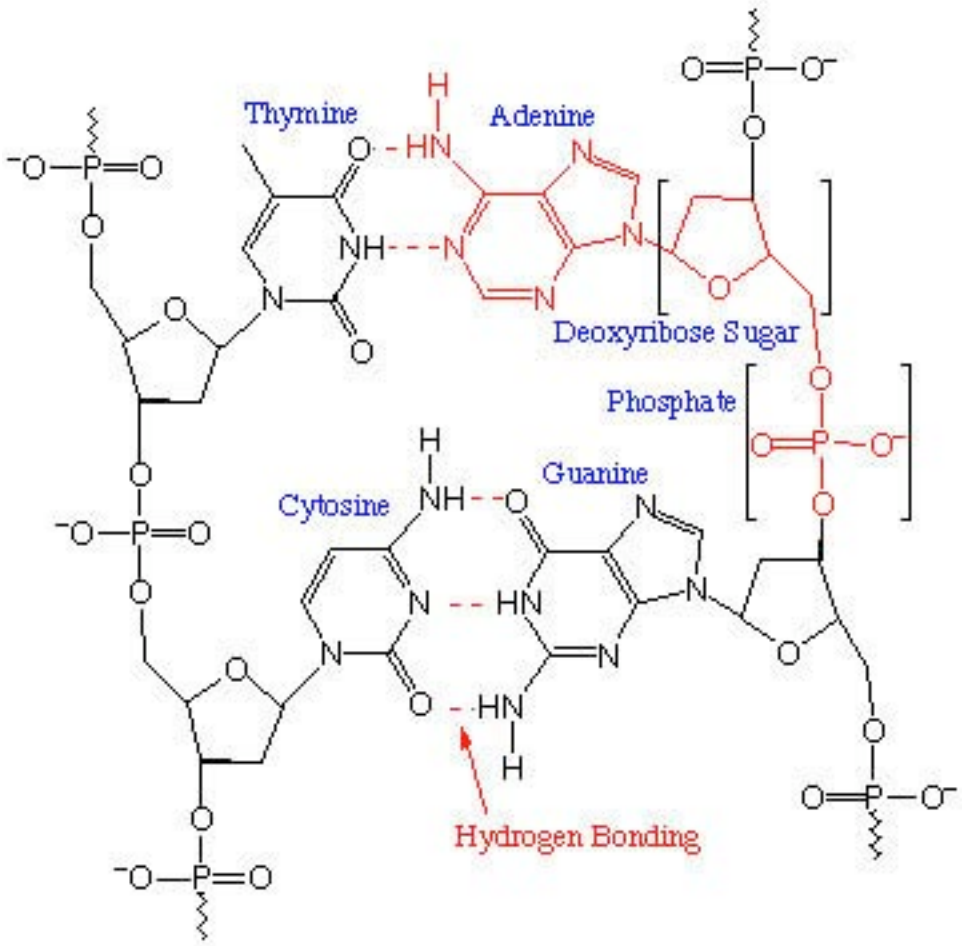
We will focus our attention on DNA polymerase
DNA polymerase ***catalyzes the synthesis of a DNA polymer -- that is, the formation of covalent bonds between monomer units***

DNA polymerase substrates are

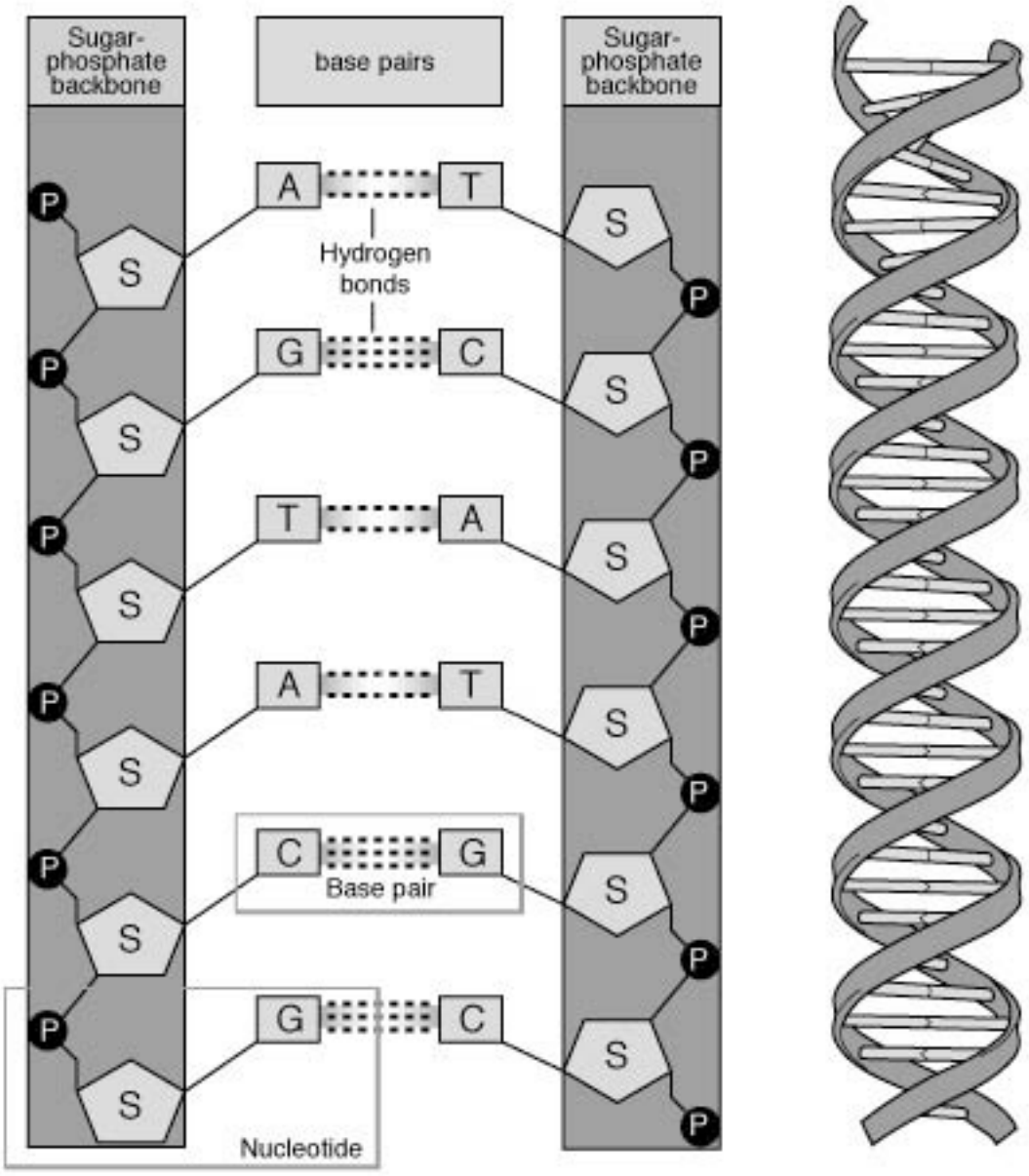
- ***primer-template complex***
- ***nucleotide subunits***

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*

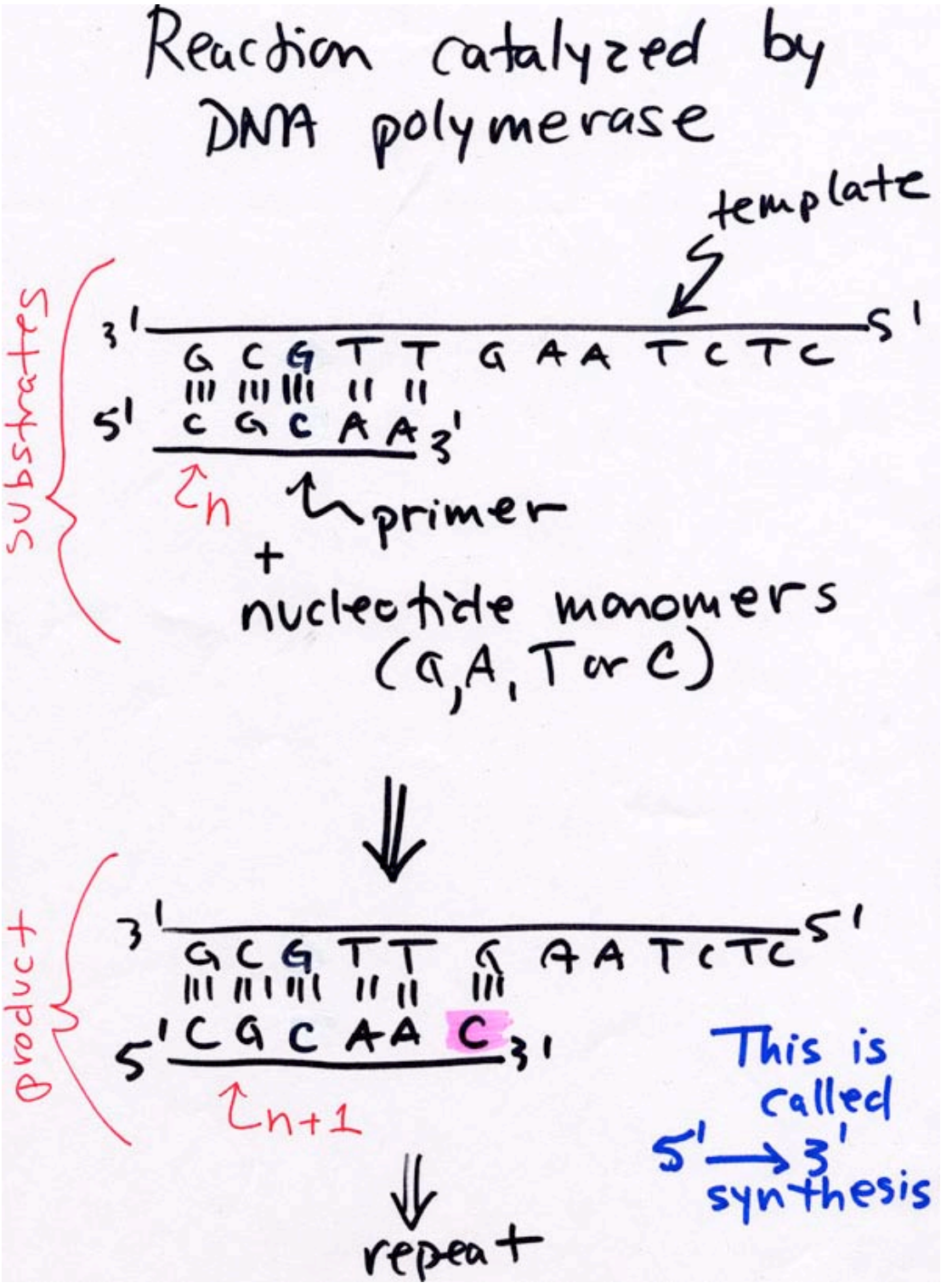

Reaction catalyzed by DNA polymerase
template
substrates
3'
G C G T T G A A T C T C
5'
5' C G C A A 3'
n
primer
+
nucleotide monomers
(G, A, T or C)
product
3'
G C G T T G A A T C T C
5'
5' C G C A A C 3'
n+1
This is called
5' → 3'
synthesis
repeat

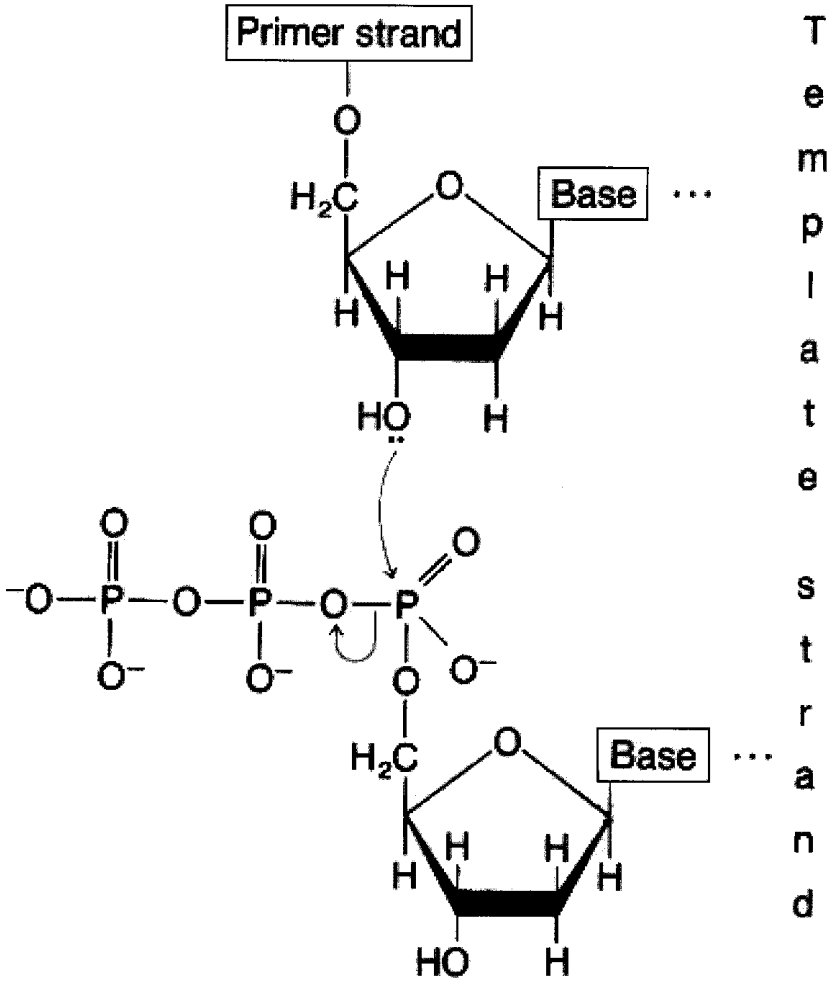
Figure shows features common to all DNA polymerases:

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

- **it cannot catalyze the addition of a nucleotide monomer to the 5' carbon of ribose**
- **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]

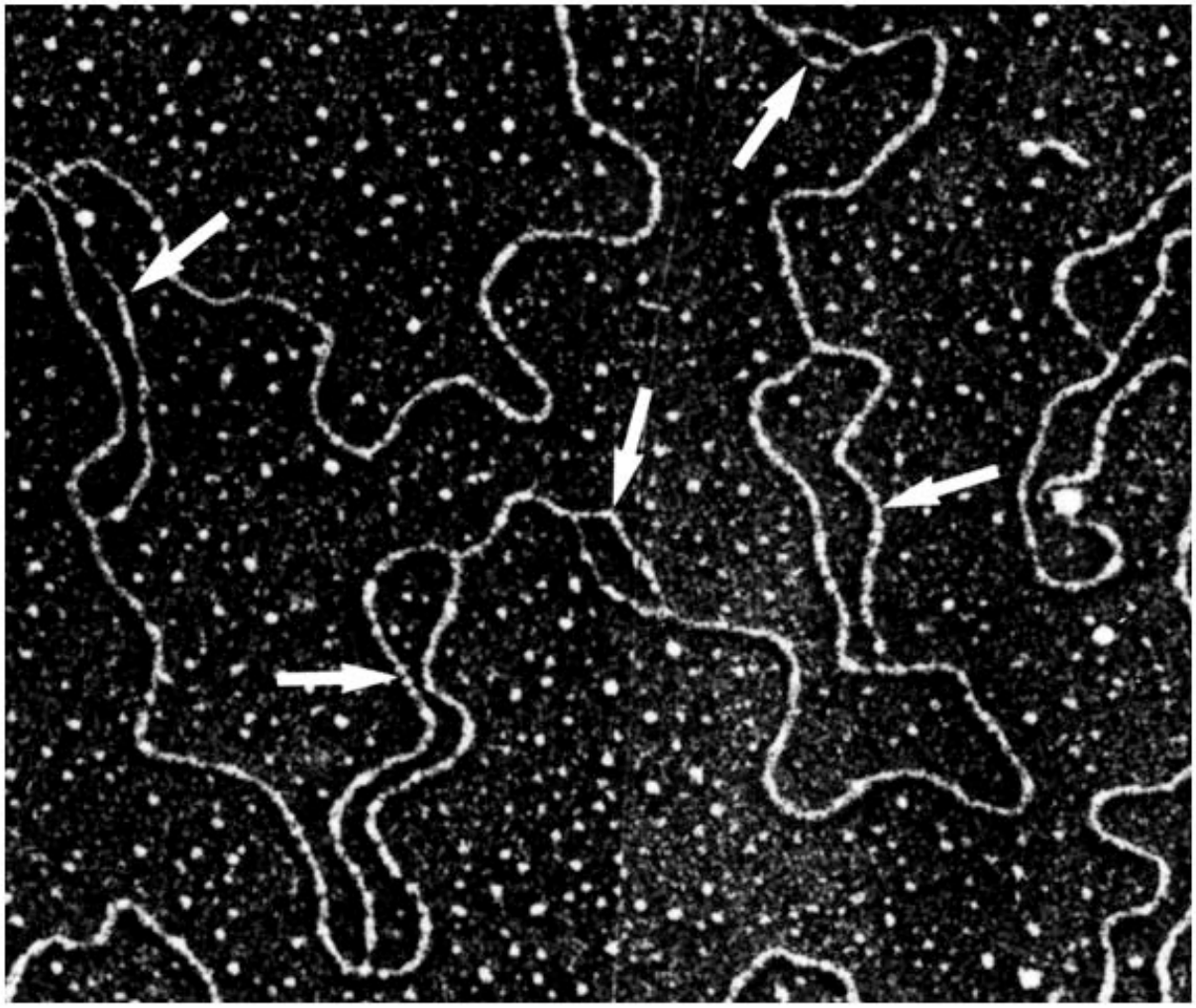


FIGURE 10-5 Electron micrograph of DNA extracted from rapidly dividing nuclei of early *D. melanogaster* embryos. The arrows mark replication bubbles; the diameters of the DNA chain in both arms of these bubbles indicate that they are double-stranded. [See A. B. Blumenthal, H. J. Kreigstein, and D. S. 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

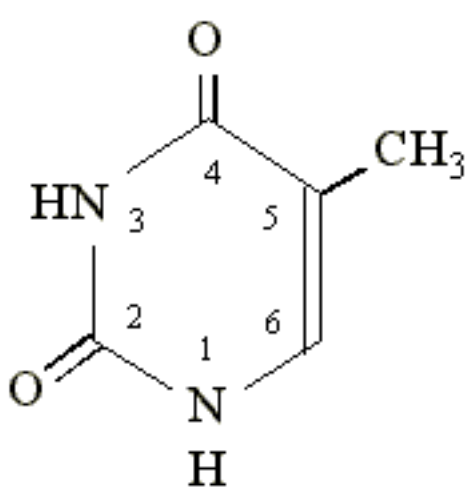
This "problem" adds a significant complication to the DNA replication process

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

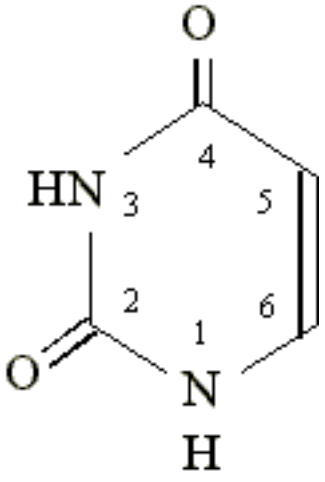
An enzyme called *primase* synthesizes a short polymer of RNA

What are the chemical differences between RNA and DNA?

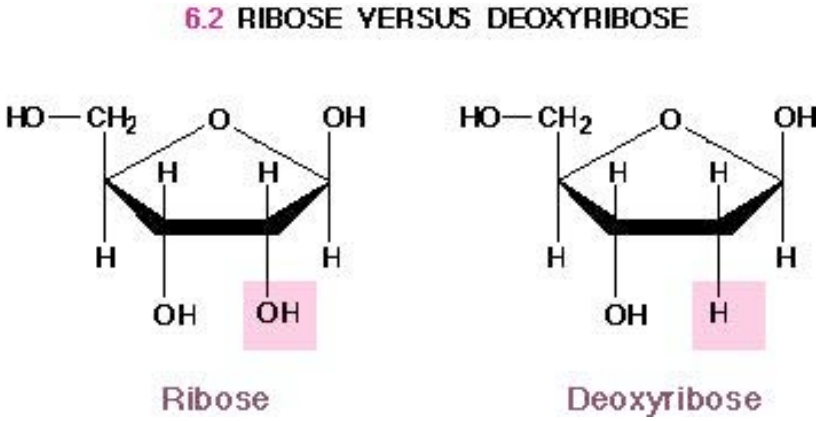
- **single-stranded not double-stranded**
- **2’ hydroxyl on ribose ring**
- **uracil replaces thymine in RNA**
- **uracil = thymine with respect to base-pairing (H-bonding) characteristics because the methyl group on thymine doesn’t influence H-bonding**



Thymine
(DNA)



Uracil
(RNA)



RNA DNA

Many other proteins involved in the process of DNA replication

- separating the DNA strands
- protecting the separated strands and making them accessible to DNA polymerase
- relieving torsional stress on the molecule (from the unwinding)
- making and replacing the RNA primer
- ensuring a high level of accuracy of the process
- recognizing the origin of replication
- coordinating the activities of all of the proteins

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 division (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.**

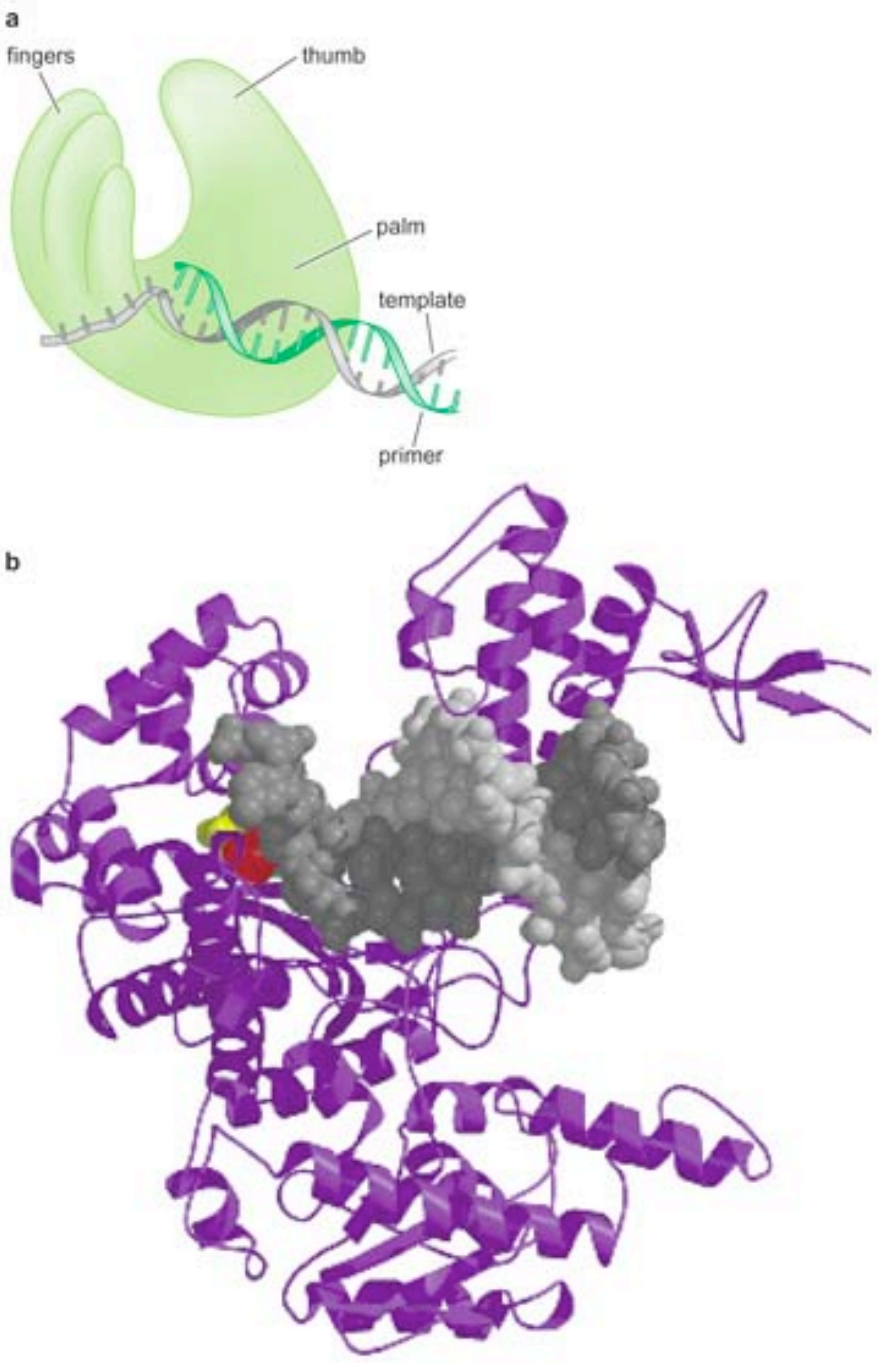
DNA replication in all organisms is highly efficient:
in *E. coli* rate of polymerization can be as high as ***1000 nucleotides per second***

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 the 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.