

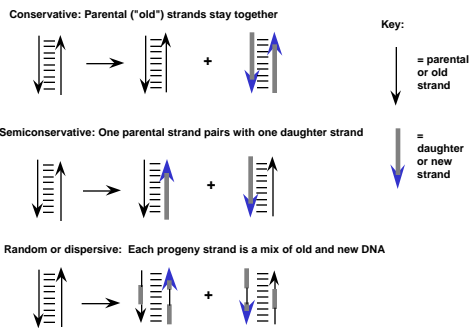
## DNA replication

Semiconservative replication  
Replicative Structures  
Replication Fork

## Watson & Crick's prediction

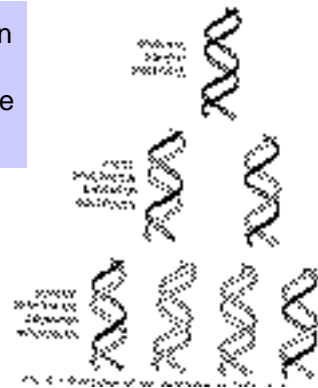
- "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."
- Nature, April 25, 1953: "Molecular Structure of Nucleic Acids: A structure for deoxyribose nucleic acid"

## Possible modes of replication of DNA



## Predicted pattern for semiconservative replication

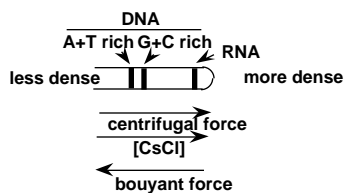
Meselson and Stahl, PNAS



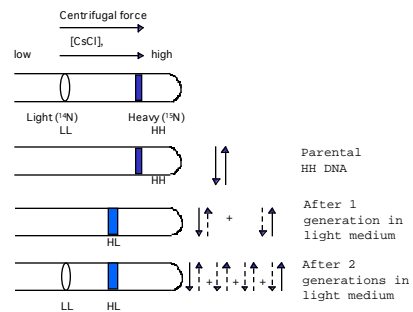
## Sedimentation equilibrium to measure DENSITY

Use a gradient of CsCl so that the molecules will band at the [CsCl] corresponding to their density.

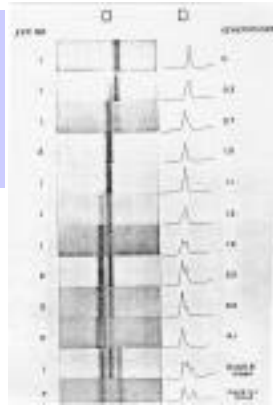
The position at which the molecule bands is independent of its size.



## Meselson & Stahl: Test for semiconservative replication



Density gradient results:  
Semiconservative replication



Meselson and Stahl, PNAS

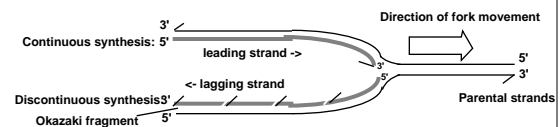
Conclusions from Meselson & Stahl

- Production of HL and disappearance of HH shows replication is NOT conservative.
- Production of LL shows replication is NOT random.
- Data support a semiconservative mode.
- The parental DNA strands are used as templates for the synthesis of new strands, directed by **base complementarity**.

Problems with polymerizing 2 strands

- All DNA polymerases synthesize new DNA in a 5' to 3' direction
- All DNA polymerases need a primer, i.e. a 3'-OH to which new nucleotides can be added.
- Synthesis of the new strand oriented away from direction of fork movement:
  - Discontinuous
  - Okazaki fragments: short DNA fragments initially made on the lagging strand and then ligated together.

Semidiscontinuous Replication at the Fork



What enzymatic activities are needed?

- |                |                |
|----------------|----------------|
| DNA polymerase | Helicase       |
| Make primers   | Topoisomerases |
| Ligase         |                |

DNA polymerases

How to identify proteins needed for replication

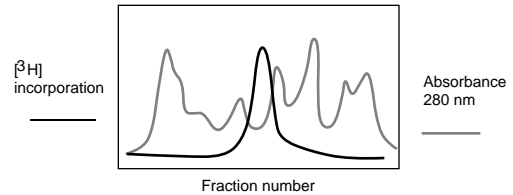
Methods to find proteins involved in replication

- Biochemical
- Genetic
- Combine them: *in vitro* complementation

### Biochemical approach: Assays for polymerase activity

- Incorporation of radiolabeled thymidine or dTTP into higher molecular weight polymers
  - DNA precipitates in trichloroacetic acid, nucleotides do not
- Gel electrophoresis can resolve short primers from the extended product

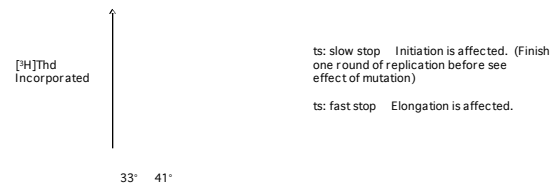
### Fractionate a cell extract and isolate the polymerizing activity



### *dna* mutants

- Conditional lethal mutants
  - E.g. mutants grow at low temperature (33° C) but not at high temperature (41° C)
  - Temperature sensitive (*ts*) mutants
  - Screen *ts* lethals for inability to make DNA at the restrictive temperature
- Complementation analysis: How many gene products are needed for DNA synthesis?

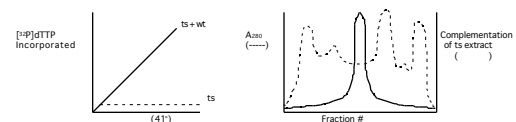
### Stage of replication affected by *dna* mutants



### *In vitro* complementation

- Combines biochemistry and genetics
- Allows isolation of any enzyme whose function is needed for the process under study (e.g. replication) *without knowledge of its enzymatic activity.*
  - Fractionate an extract from a wild-type strain
  - Assay each fraction for the ability to complement the defect (i.e. restore DNA synthesis) in an extract from a *ts* strain.

### Example of *in vitro* complementation



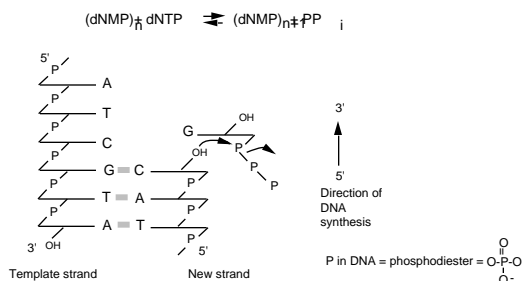
### Isolation of DNA polymerase I

- A. Kornberg (1956) isolated a DNA polymerizing activity from *E. coli*.
- Required a template
- Required a primer
- Synthesized the complement of the template
- Size: 928 amino acids, 103 kDa
- Encoded by *polA* gene

### Reaction catalyzed by DNA Pol I

- Add dNMP (from dNTP) to 3' end of a growing chain
- Release pyrophosphate (PP<sub>i</sub>)
- Reversible: pyrophosphorolysis
- Requires template, primer, Mg<sup>++</sup>, and all 4 dNTPs

### DNA Pol I adds a nucleotide and releases PP<sub>i</sub>



### Subsequent hydrolysis of pyrophosphate



Catalyzed by a separate enzyme:  
pyrophosphatase  
Helps drive the reaction forward  
(toward synthesis)

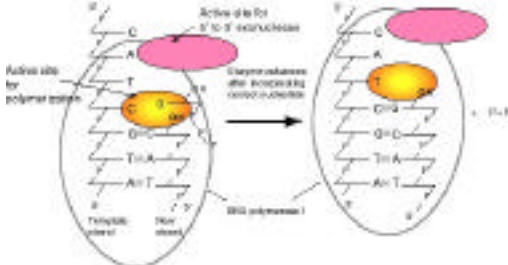
### Exo- vs. Endonucleases

- Exonucleases remove nucleotides from the ends of DNA (and/or RNA) molecules.
  - Catalyze hydrolysis of phosphodiester bonds
  - Will NOT work on circular DNA
- Endonucleases cleave in the middle of DNA (and/or RNA) molecules.
  - Catalyze hydrolysis of phosphodiester bonds
  - DO work on circular DNA

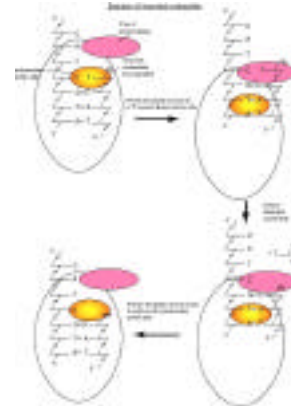
### DNA Pol I is a 3' to 5' exonuclease

- Provides a proofreading function
- If an incorrect nucleotide is added, the enzyme recognizes the mismatch and removes the incorrect nucleotide.
- The incorrect nucleotide is removed by hydrolysis.
- Proofreading is common to many but not all DNA polymerases.

### Chain elongation by DNA polymerase I



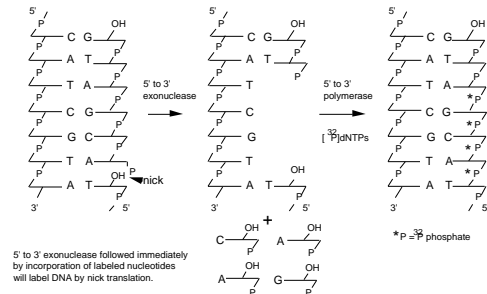
### Excision of incorrect nucleotide by 3'-5' exonuclease activity of DNA polymerase I



### DNA Pol I is a 5' to 3' exonuclease

- Removes nucleotides in base-paired regions
- Can remove DNA or RNA
- Physiological function appears to be repair of damaged DNA and removal of RNA primers from Okazaki fragments
- Can be used to label DNA *in vitro* by nick translation
- Not a common activity of other DNA polymerases

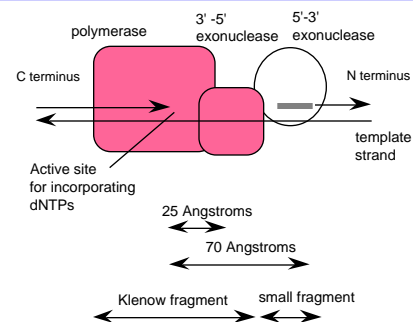
### 5' to 3' exonuclease activity



### DNA Pol I is a multi-domain protein

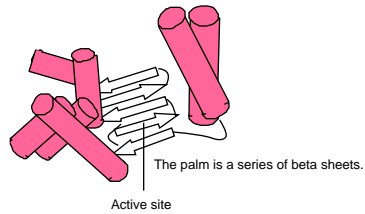
- Cleavage by the protease subtilisin can separate the 5' to 3' exo from the polymerase and proofreading 3' to 5' exo.
- Polymerase + 3' to 5' exonuclease are in the remaining Klenow fragment
- Polymerase active site is in the palm of the cupped right hand
- 3' to 5' exonuclease is about 25 Angstroms away
- Klenow fragment has two functional domains

### 3 functional domains in 1 polypeptide



### Polymerase domain resembles a cupped right hand

The "cupped" fingers are a series of alpha helices.



### Many DNA polymerases have a similar structure

- DNA polymerase from *Thermus aquaticus*
  - Polymerase
  - 3' to 5' exo domain is present but inactive
  - 5' to 3' exo is also present
- Cupped right hand structure also seen in
  - T7 RNA polymerase
  - HIV reverse transcriptase (RNA dependent DNA polymerase)
  - Others
- DNA Pol I: founding member of ONE class of polymerases

Null mutants at *polA* (encoding DNA Pol I) are viable!