

A. Replication in Prokaryotes:

Meselson-Stahl Experiment (1958): Replication is Semi-Conservative

Experiment:

*Grow E. coli on $^{15}\text{NH}_4\text{Cl}$; Transfer cells to $^{14}\text{NH}_4\text{Cl}$
Isolate DNA after 1 or 2 replications
Analyze DNA: CsCl density gradients*

After 1 round of DNA replication:

*Single band of DNA in tube
Density intermediate between ^{15}N and ^{14}N
Conclude: Conservative model incorrect*

After 2 rounds of DNA replication:

*Two bands of DNA in tube
One intermediate band as above; One band equal to ^{14}N standard
Conclude: Dispersive model incorrect*

B. Replication in Eukaryotes

Taylor *et al.* (1957); DNA Synthesis in Root Tips

*^3H -Thymidine; Visualize with Autoradiography
 ^3H causes precipitation of silver grains on film
Conclude: Replication is semi-conservative*

C. Origin of Replication in Chromosomes

*Single origin in circular bacterial chromosomes
Multiple origins in linear eukaryotic chromosomes
Replication proceeds bi-directionally*

*Unit of replication = Replicon
Bacterial: Entire chromosome
Eukaryotic: 500 - 1000 replicons / chromosome
Not all active at every mitosis*

D. Replication Fork = Site of DNA Synthesis

*New strand synthesized 5' to 3' direction
5' end of new strand synthesized first; NTPs added to free 3' end of new strand
One strand (leading) synthesized continuously
One strand (lagging) synthesized discontinuously
Discontinuous pieces (Okazaki fragments) later joined*

E. Requirements for Replication in *E. coli*

1. *dNTPs (A,T,G,C)*

2. *ss DNA template strands*

3. *Proteins associated with replication fork*

*Unwinding proteins, helicases, gyrases
Stabilize DNA at fork; Relax supercoiling*

4. *Short RNA primer to initiate replication; Synthesized by special RNA polymerase*

5. *DNA polymerases: Several types known*

*DNA polymerase III: Primary replication enzyme (large, multimeric)
Adds dNTPs to new strand*

DNA polymerase I: Proofreader

*More abundant and stable than polymerase III;
Exonuclease activity – removes incorrect bases*

Identity revealed by studying polA mutants

*Still replicate DNA; Deficient in DNA repair
High rate of spontaneous mutation
Normal Function: Remove primer, correct errors*

6. *DNA Ligase*

*Seals gaps in sugar-phosphate backbone
Connects Okazaki fragments*

F. Analysis of Replication

Biochemical: Isolate proteins involved; Analyze enzyme activities; Characterize functions

Genetic: Isolate mutants; Characterize defect; Identify gene product

Replication is Essential Process

*How do you maintain mutants defective in replication?
Use conditional mutants (e.g. Temperature sensitive)*

*Maintain mutants at low T
Mutant protein functional at low T
Study defect in replication at high T
Altered protein: Non-functional at high T
Normal 3D shape not maintained at high T*

Variety of genes required for replication identified:

<i>polA</i>	<i>DNA polymerase I</i>
<i>polB</i>	<i>DNA polymerase II</i>
<i>dnaE,N,Q,X,Z</i>	<i>DNA polymerase III subunits</i>
<i>dnaG</i>	<i>Primase</i>
<i>dnaA,I,P</i>	<i>Initiation</i>
<i>dnaB,C</i>	<i>Helicase at oriC</i>
<i>oriC</i>	<i>Origin of replication</i>
<i>gyrA,B</i>	<i>Gyrase subunits</i>
<i>lig</i>	<i>DNA ligase</i>
<i>rep</i>	<i>Helicase</i>
<i>ssb</i>	<i>Single-stranded binding protein</i>
<i>rpoB</i>	<i>RNA polymerase subunit</i>

Ends of Eukaryotic Chromosomes

Lagging strand problem:

*Primer not synthesized at extreme end of chromosome
Primer not replaced by DNA strand at chromosome end*

Solution: Chromosome ends - Repeated DNA sequences

*Synthesized by telomerase enzyme complex
Added to chromosome using RNA template
Repeated sequences form dsDNA hairpin
Provides strand for DNA polymerase to extend*

Telomerase: Helps to prevent chromosome shortening

*Activity in human cells reduced at maturity
May protect against unwanted cell proliferation
Reduced activity may contribute to aging*

