B.Sc (Hons) Microbiology (CBCS Structure)

C-7: Molecular Biology

Unit 2: DNA Replication

Models of DNA Replication

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Reference – Molecular Biology of the Gene (6th Edition) by Watson et. al. Pearson education, Inc
Principles of Genetics (8th Edition) by D. Peter Snustad, D. Snustad, Eldon Gardner, and Michael J. Simmons, Wiley publications

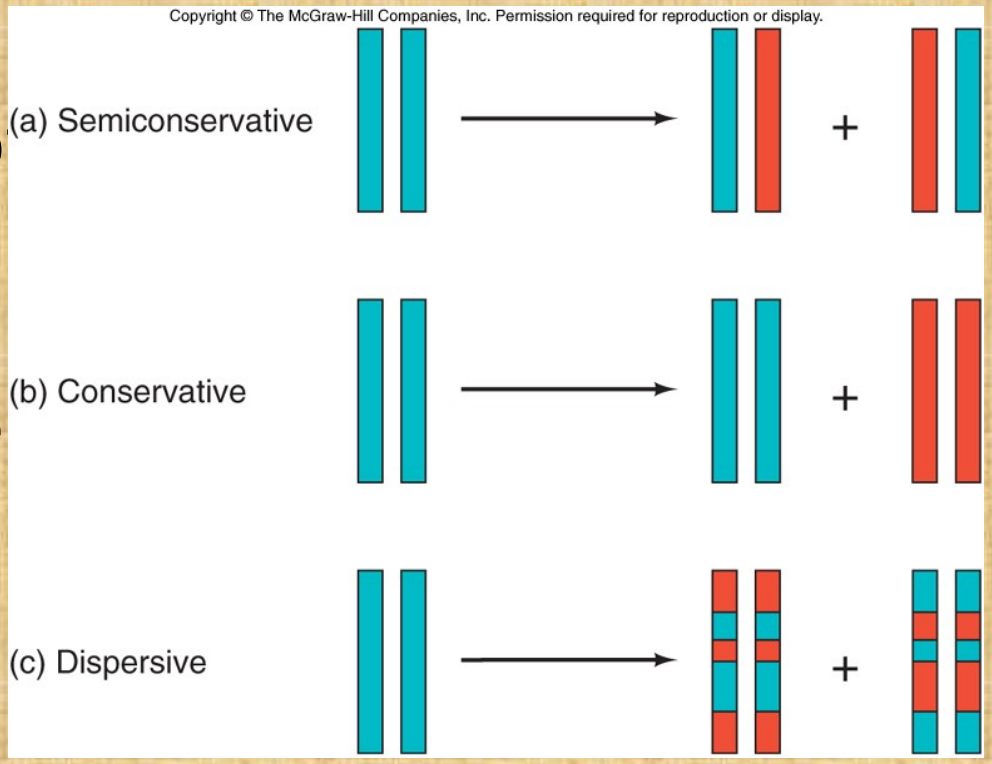
General Features of DNA Replication

- Double helical model for DNA includes the concept that 2 strands are complementary
- Each strand can serve as template for making its own partner
  - Semiconservative model for DNA replication is correct
  - Half-discontinuous (short pieces later stitched together)
  - Requires DNA primers
  - Usually bidirectional

Three Hypotheses of Replication

The three methods o
DNA replication
considered were:

1. Semiconservative
2. Conservative
3. Dispersive



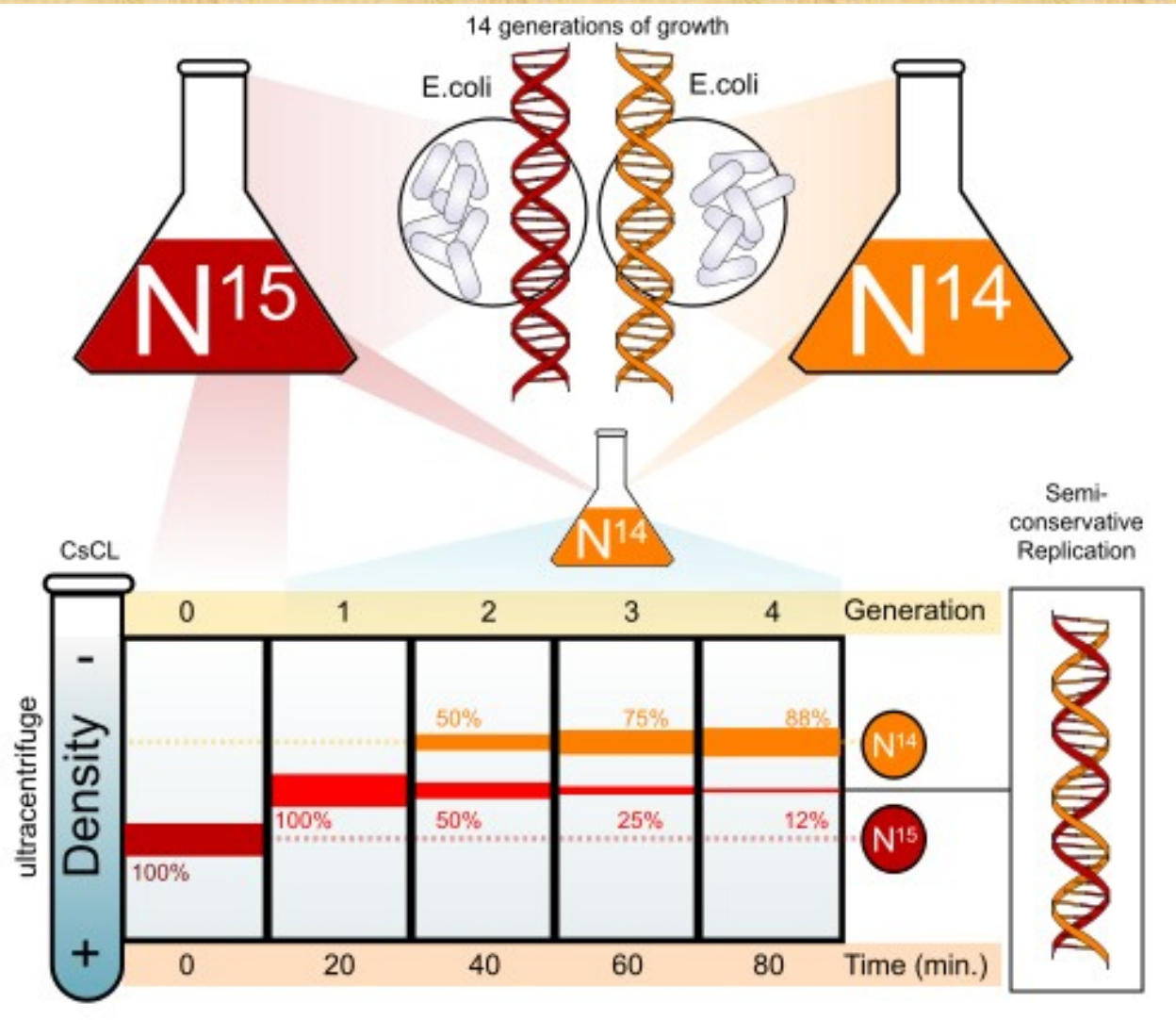
Semiconservative Process of DNA Replicatio

tson and Crick DNA Model - Suggested Semiconservative m

selson and Stahl Experiments

lbruck hypothesis in favor of dispersive mode of replication

Semi-conservative mode of replication – Experiment by Matthew Meselson and Frank W Stahl at California Institute of Technology in 1958



Meselson and Stahl Experiment

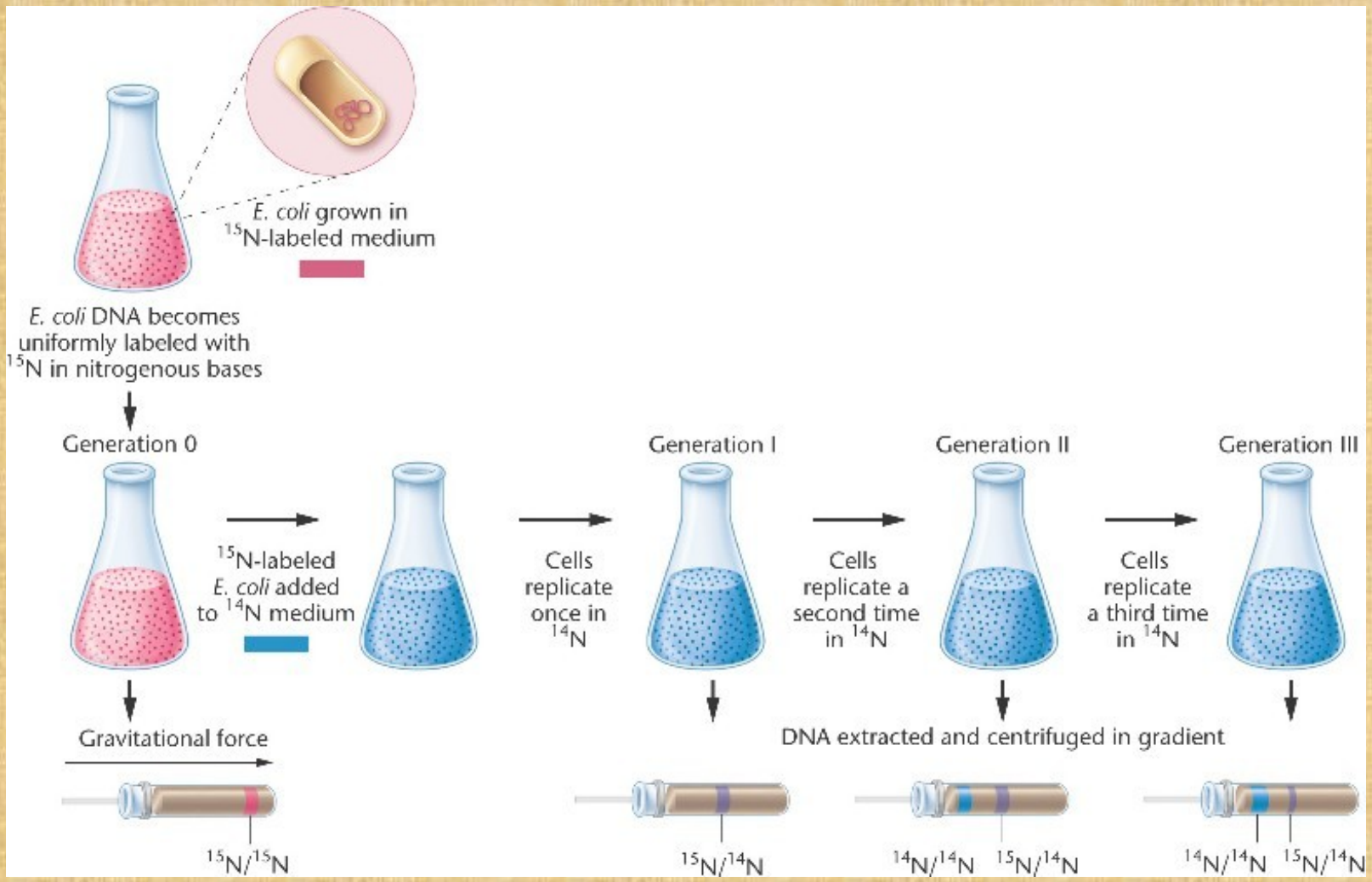
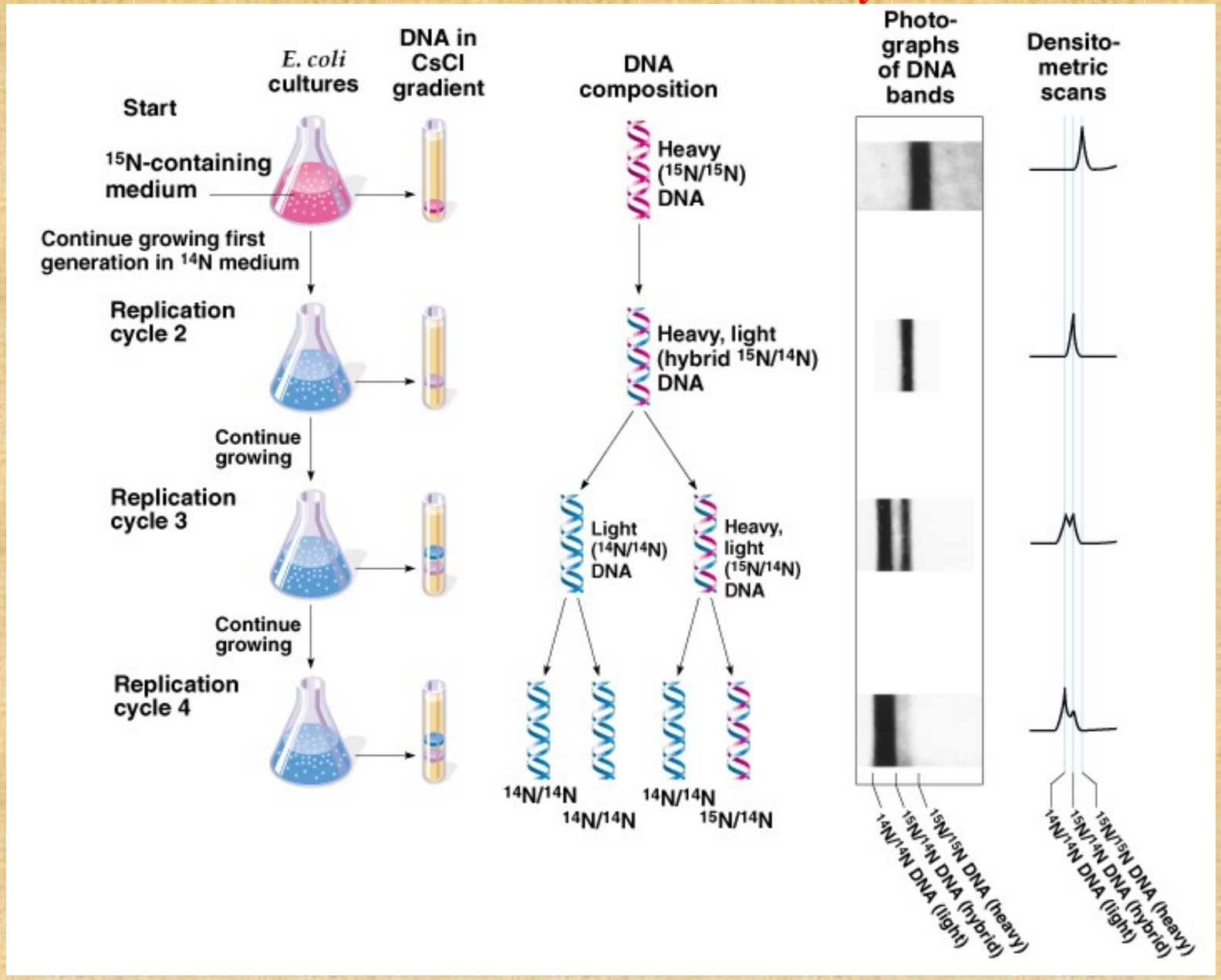
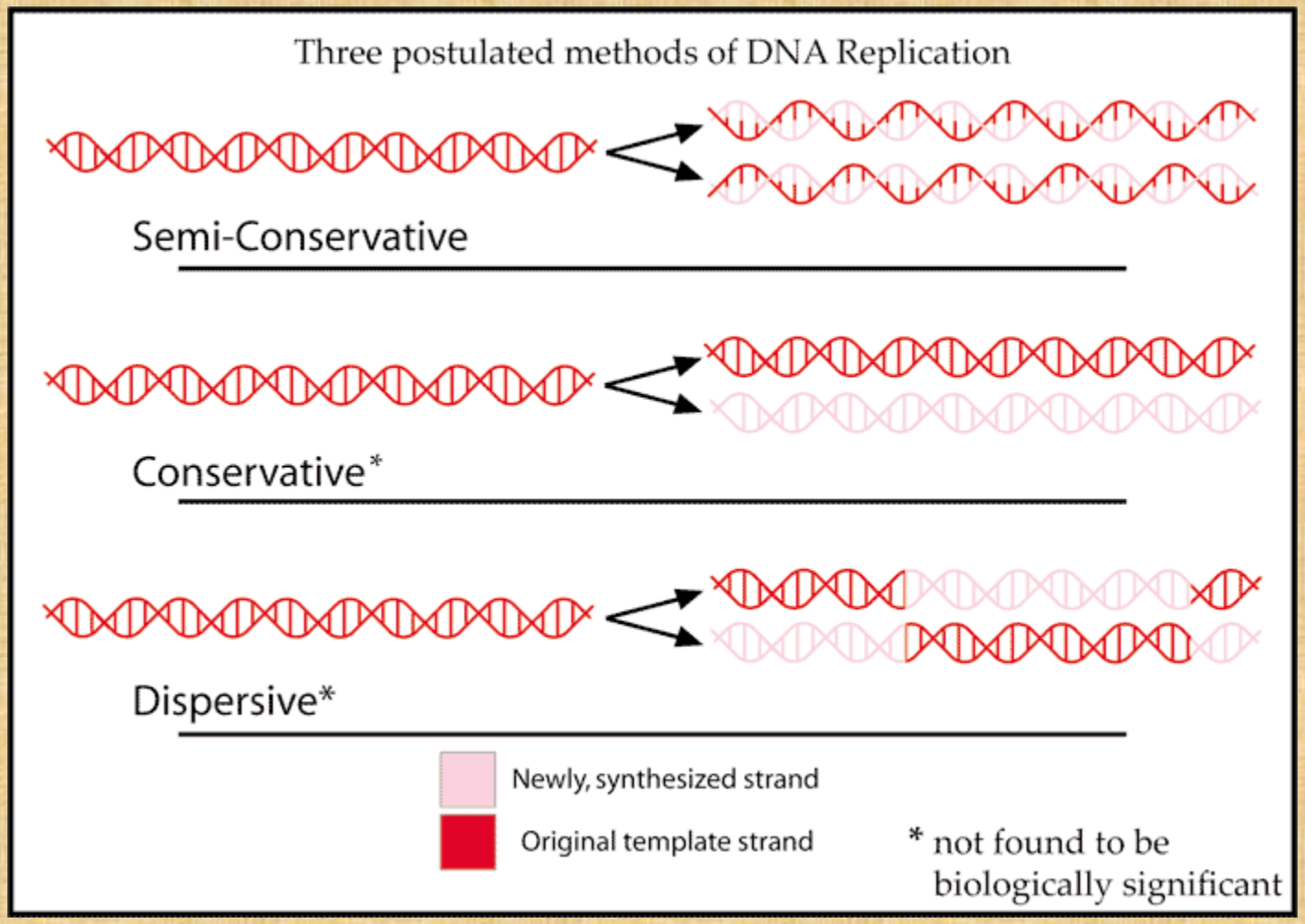


Fig. 3.2 The Meselson-Stahl experiment, which showed that DNA replicates semi-conservatively



Three possible mechanisms for DNA replication

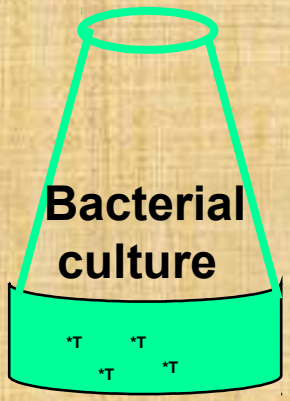


MODELS OF DNA REPLICATION

Replication Model	DNA Template	Breakage of Nucleotide Strand	Number of Replicons	Unidirectional or Bidirectional	Products
Theta	Circular	No	1	Unidirectional or bidirectional	Two circular molecules
Rolling circle	Circular	Yes	1	Unidirectional	One circular molecule and one linear molecule that may circularize
Linear eukaryotic	Linear	No	Many	Bidirectional	Two linear molecules

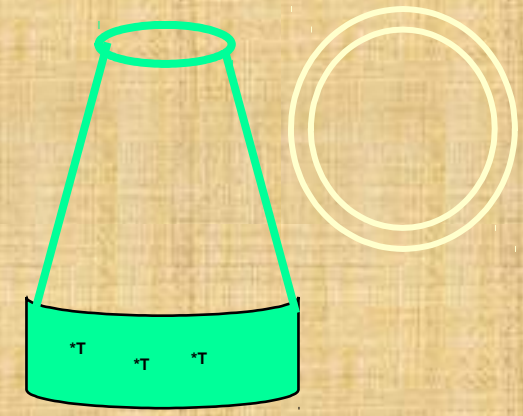
These models may differ with respect to the initiation and progression of replication, but all produce new DNA molecules by semi-conservative replication.

John Cairns



in media with low concentration of ^{3}H- thymidine

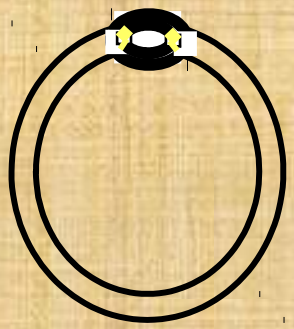
Grow cells for several generations
Small amounts of ^{3}H thymidine are incorporated into new DNA

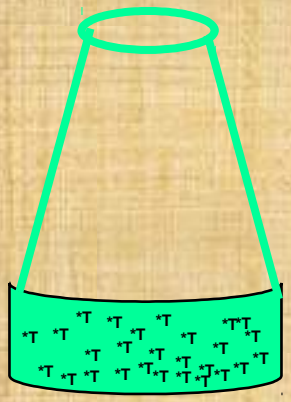


All DNA is lightly labeled with radioactivity

Add a high concentration of ^{3}H- thymidine

Grow for brief period of time





Dense label at the replication fork where new DNA is being made

Cairns then isolated the chromosomes by lysing the cells very very gently and placed them on an electron micrograph (EM) grid which he exposed to X-ray film for two months.

Theta Replication of Circular DNA Molecules

* The first physical evidence that E. coli DNA replicates as a circle came from an autoradio-graphic experiment.
* Cells were grown in a medium contain-ing ^{3}H-dT so that all DNA synthesized would be radioactive

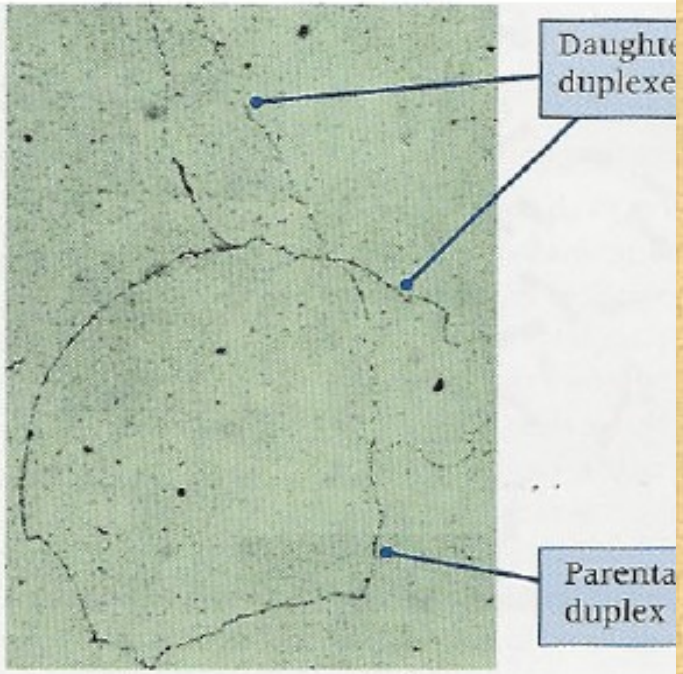
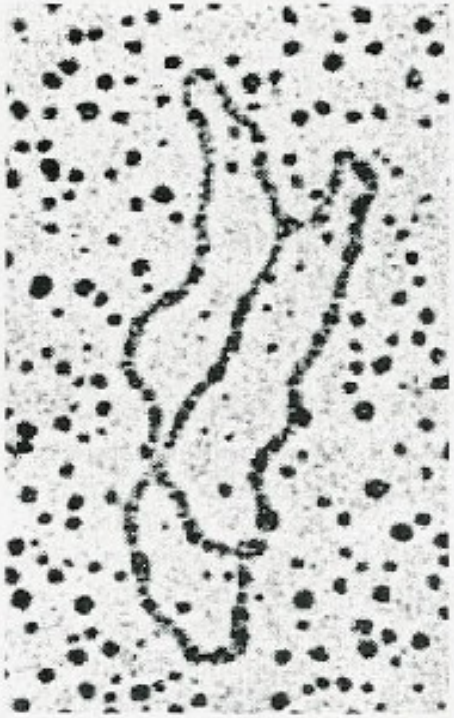


Figure 6.6 Autoradiogram of the intact replicating chromosome of an *E. coli* cell that has grown in a medium containing ^{3}H-thymine for slightly less than two generations. The continuous lines of dark grains were produced by electrons emitted by decaying ^{3}H atoms in the DNA molecu The image is observed with a light microscope. [From J. Cairns. *Cold Spri*

Theta Replication of Circular DNA Molecules

* ***The θ replication structure***

* ***Replication fork:*** ***the regions in which parental strands are separating and new strands are being synthesized***



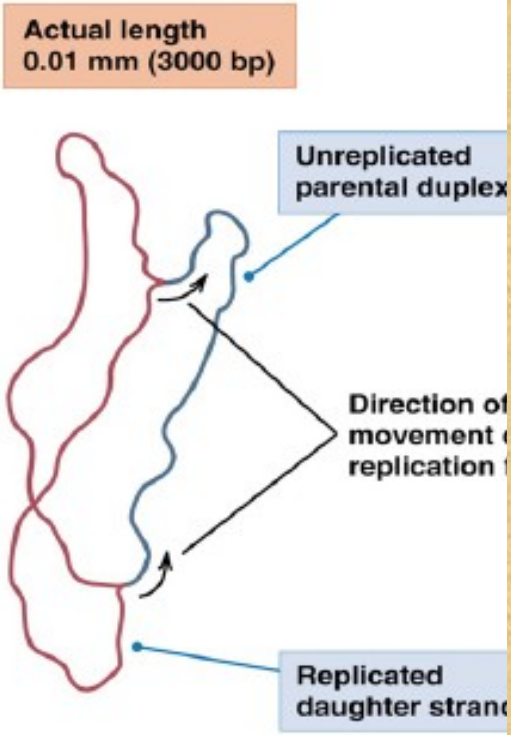
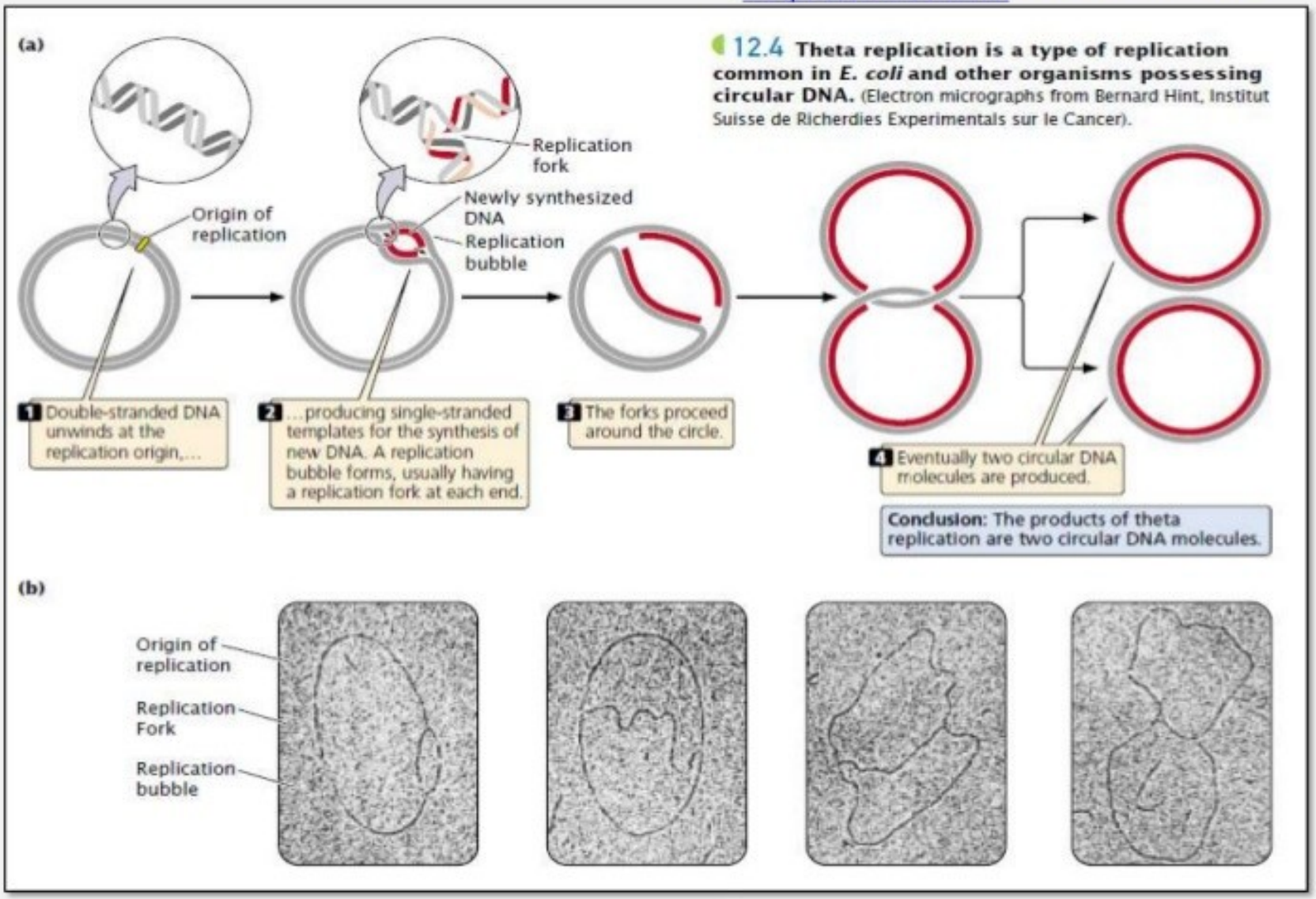


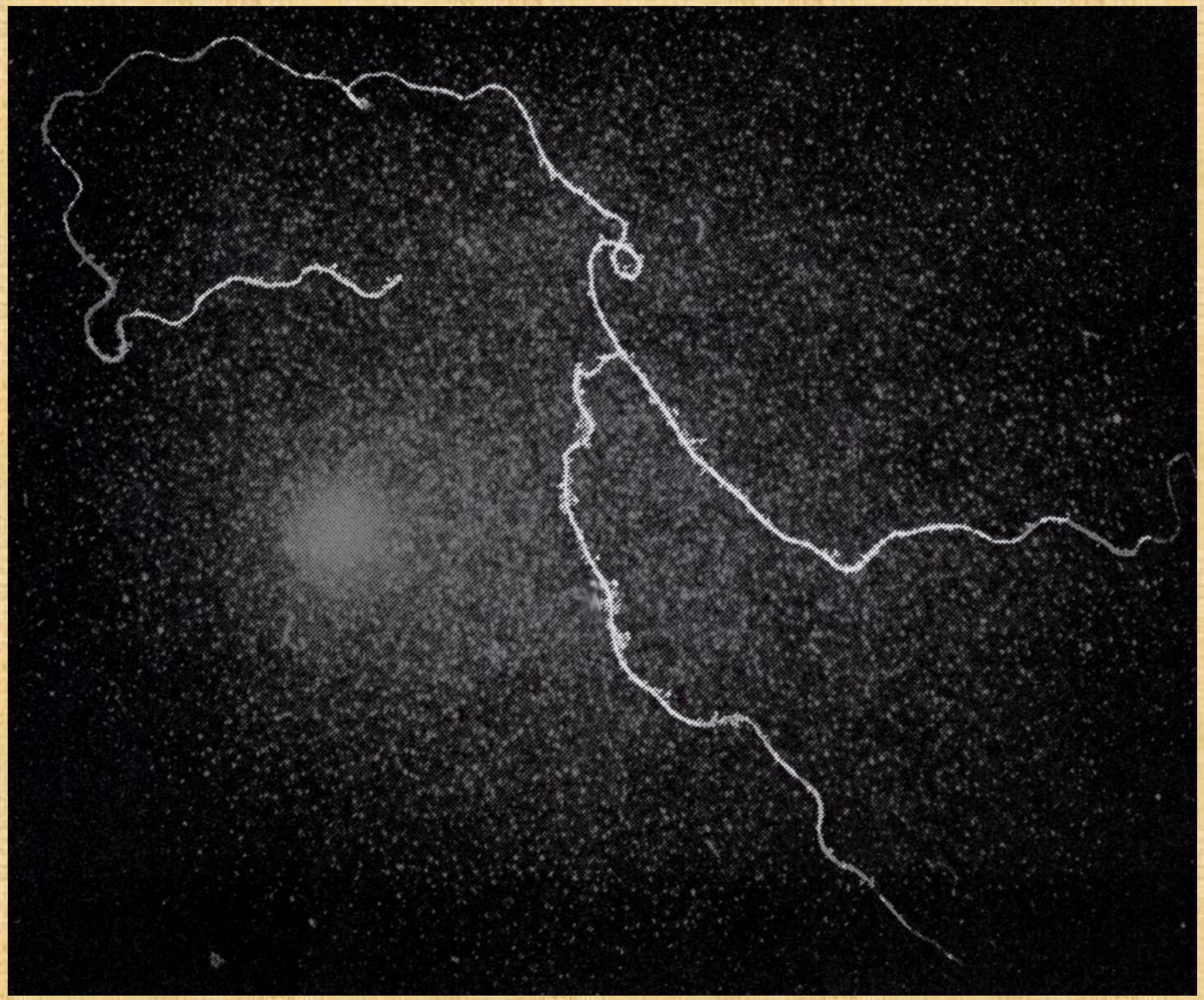
Figure 6.7 Electron micrograph of a small, circular DNA molecule replic
ing by the θ mode. The parental and daughter segments are shown in t
drawing. [Electron micrograph courtesy of Donald Helinski.]

THETA REPLICATION: *E. coli*

http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120073/micro03.swf::Bidirectional%20Replication%20of%20DNA



Replicating bubble in DNA from bacteriophage T7
Two replication forks heading towards opposite ends of the DNA



Replicating DNA from bacteriophage T7. One fork has reached the ends (A and A'). Second fork (arrow) is heading towards the opposite end of the DNA (B)

ROLLING CIRCLE REPLICATION

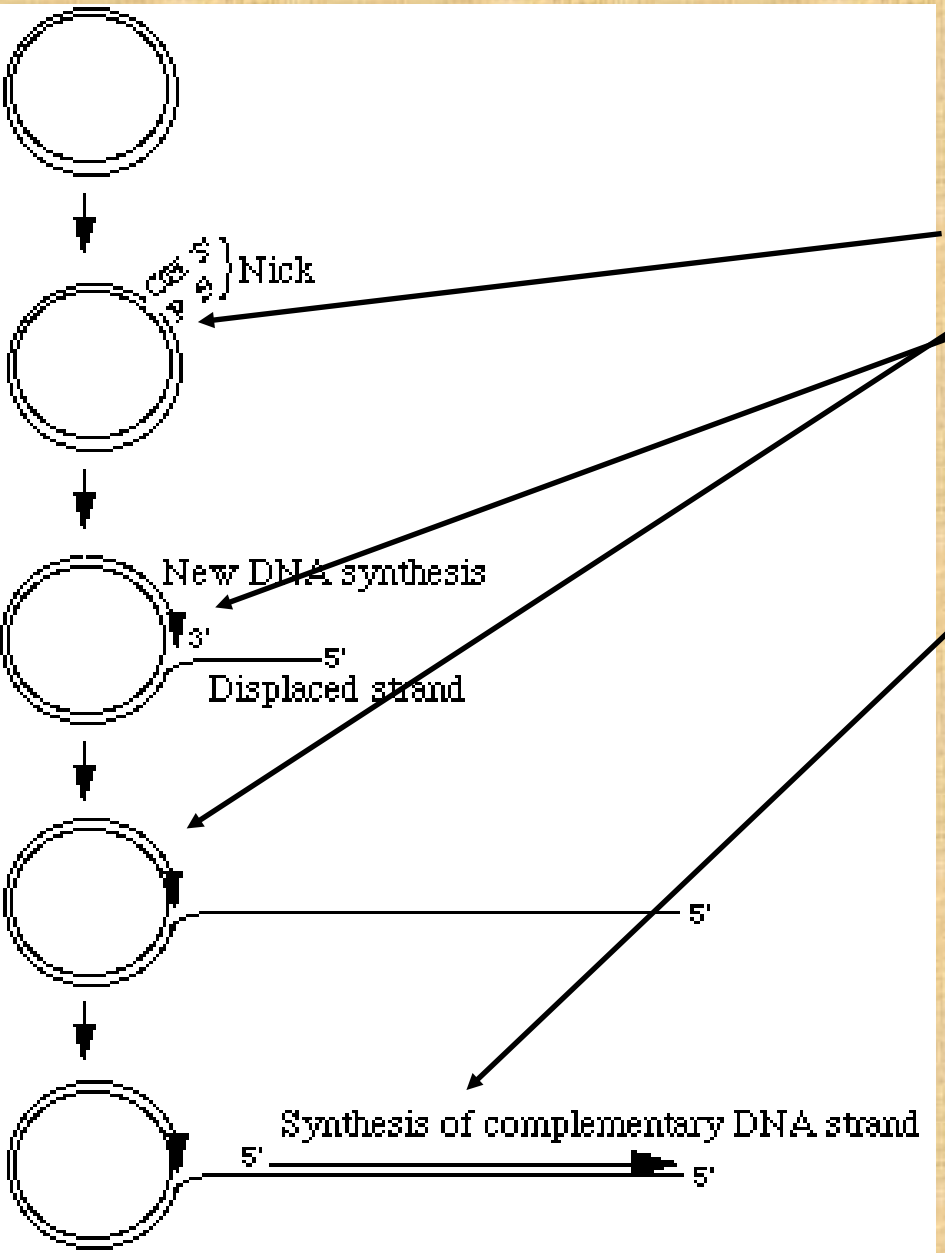
After initial replication of the circular P22 DNA by theta-replication, the DNA is replicated by rolling circle replication.

Rolling circle replication generates a long concatemer of linear, double-stranded DNA that can be packaged into phage heads

Rolling Circle”Common among bacteriophages

Circular DNA serves as template for

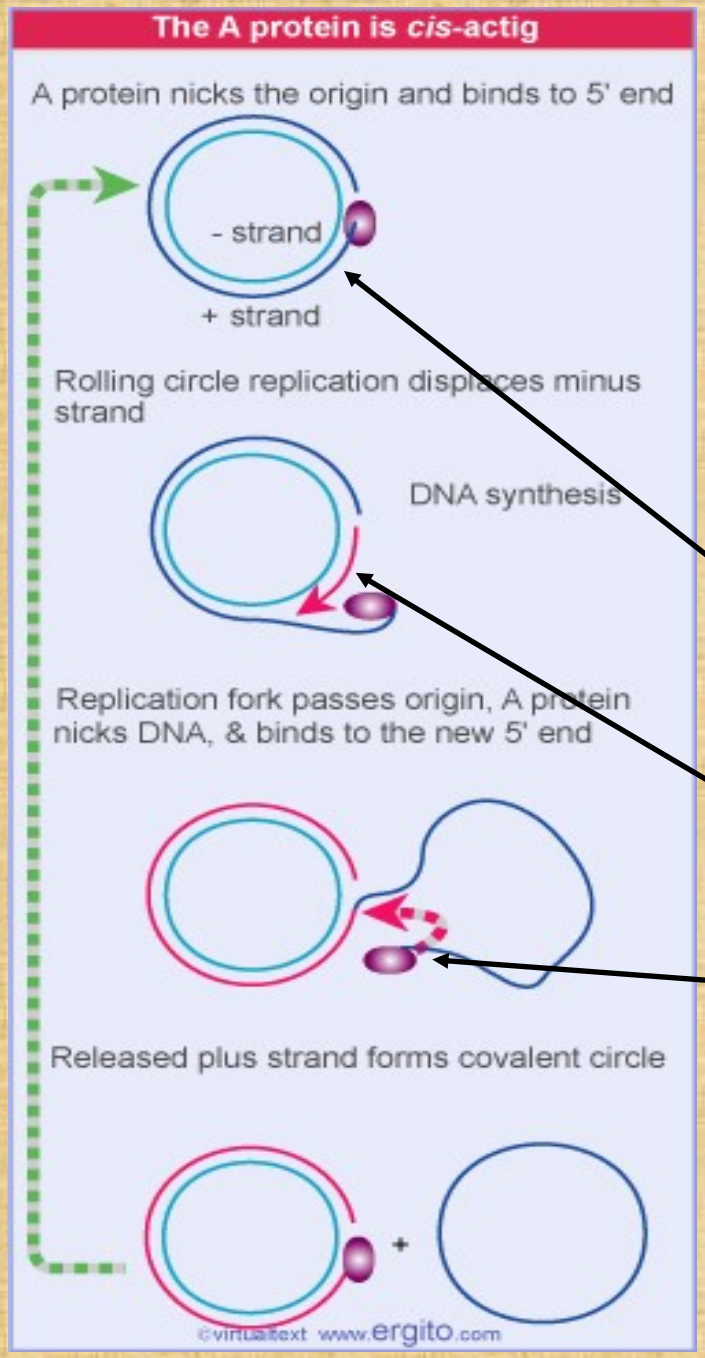
Rolling Circle I



- Few rounds of theta-replication
- Nick outer strand
- Extend 3’ end of outer strand, displacing original
- Synthesis of complementary strand using displaced strand as template
- Concatamers cut by RE’s, sealed
- Result several copies of circular

Rolling Circle I

- “Template “rolls”, extrudes leading strand
- Okazaki frags made on leading strand as it emerges.

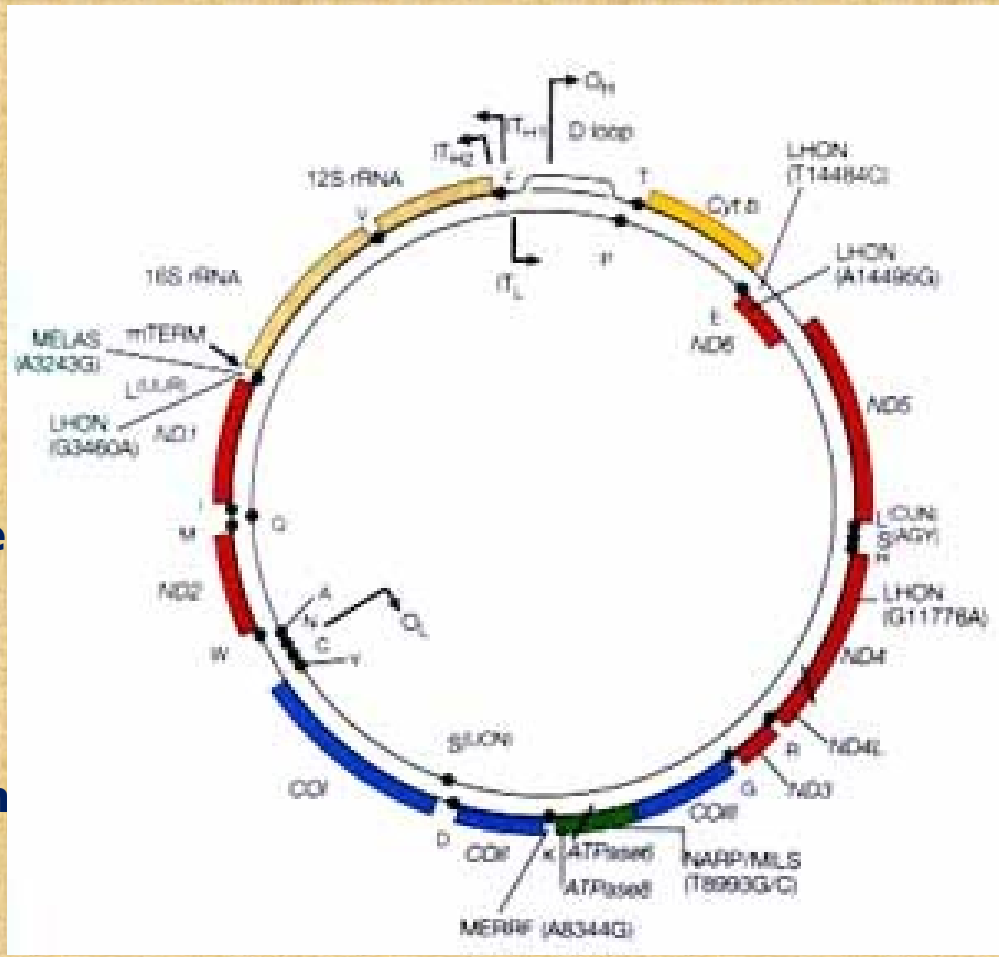


Rolling Circle II

- EX ΦX174
- Circular ssDNA chromosome
- Copy + strand using *E. coli* replication proteins to make ds circle (theta replication)
- Protein A (phage) cuts + strand
- Rolling circle replication
- Protein A cuts at unit length and circularizes (ligates) released ss chromosome

Mitochondrial Genome Structure

- **The mitochondrial genome is a circle, 16.6 kb of DNA. A typical bacterial genome is 2-4 Mbp.**
- **The two strands are notably different in base composition, leading to one strand being "heavy" (the H strand) and the other light (the L strand).**
- **Both strands encode genes, although more are on the H strand.**
- **A short region (1121 bp), the D loop (D = "displacement"), is a DNA triple helix: there are 2 overlapping copies of the H strand there.**
- **The D loop is also the site where most of replication and transcription is controlled.**
- **Genes are tightly packed, with almost no non-coding DNA outside of the D loop. In one case, two genes overlap: they share 43 bp, using different reading frames. Human mitochondrial genes contain no introns, although introns**



Mitochondrial Genome Replication

- **Replication starts with the H strand.**
  - **The origin of replication for the H strand is in the D loop, and it is initiated by an RNA primer generated from the L strand transcript.**
  - **After the new H strand is about 2/3 complete, the L strand origin of replication is uncovered. The L strand origin is on the old H strand; it is “uncovered” when the old H strand is displaced by the DNA polymerase synthesizing the new H strand.**
  - **The L strand origin folds into a stem-loop structure, which acts as a primer, and replication of the L strand begins.**
  - **Replication can be said to**