

DNA REPLICATION

2/14/94, 2/13/95, 2/13/96, 2/10/97, 9 Feb 98, 7 Feb 00, 11 Feb 02, 14 Feb 03, 11 Feb 04, 14Feb05, 13 Feb 06, 18 Feb08, 17Feb09, 17Feb10, 11Feb11, 17Feb12

gmslg, P. 320-, Becker and Deamer: 399-, Griffiths et al, 7th: p 249-257, 9th: 279-292

HOW IS DNA REPLICATED?

Semiconservative (p 276)

Meselson and Stahl (1958)(p. 276)

grow *E coli* in ^{15}N , transferred to ^{14}N for one or two divisions,
CsCl gradient in ultracentrifuge (50,000 rpm for ON.) (P. 277)

[Also, **harlequin chromosomes**: two rounds of replication in bromodeoxyuridine in metaphase chromosome. Double labeling with 5 BU prevents staining]

Cairns (1963) **autoradiography** of replicating fork, Bidirectional, multiple origins in eukaryotic, (p. 278)

Kornberg (1956) isolated **DNA polymerase I (pol I)**, worked only with nucleotide triphosphates

displayed **three enzymatic activities**:

1. polymerizes 5' to 3' direction (p 254)
2. 3' to 5' **exonuclease** on mismatched bases
3. 5' to 3' exonuclease on 2x stranded (can remove RNA primer)

Pol I is the most abundant, edits and repairs

Two other DNA polymerases isolated later, **pol II**, & major replication enzyme: **pol III**

Okasaki: **Pulse and chase expt**: grow in cold, then in hot, then cold, then follow fate of newly synthesized labeled strand.
First light pieces, converted with time to heavy.
Ligase ties DNA pieces together in discontinuous strand.

PROCESS: (p. 282), 280:

VIDEO: simpler:

<http://www.youtube.com/watch?v=teV62zrm2P0>

more accurate:

<http://www.youtube.com/watch?v=4jtmOZaIvS0>

lagging strand, 282: replisome,

pol III holoenzyme contains at least 20 subunits, requires RNA primer
is major replication polymerase

Helicase disrupts H bonds, helps unwind DNA.

Single Strand Binding (SSB) protein stabilizes unwound DNA

gyrase (283) removes the resulting twists (class of enzyme: **topoisomerase**)

act either by causing single strand breaks, or double strand breaks
synthesizes 30 base long RNA, required as **primer**, with primasome

primase

leading strand synthesis uses 3'-5' **continuously** in direction of **replicating fork**

lagging strand discontinuous synthesis in opposite direction of fork, until meets previously synthesized fragment

pol I 5'-3' exonuclease removes primer, and replaced with newly synthesized DNA

ligase (281) ties these Okasaki fragments (length: 1000-2000 bases in viruses, 100-200 in eukaryotes)
Must be activated by ATP, then attaches to 5' end before it can bridge to 3' end

Telomeres: (289)

synthesis of lagging strand a problem at end of chromosomes. An enzyme **telomerase** (a reverse transcriptase) carries RNA template (AAUCCCAAU in Tetrahymena) which serves as template for DNA synthesis of adjacent repeats on the leading strand, allowing extension of the lagging strand. Not in *most* cells. Aging?

Exonuclease editing:

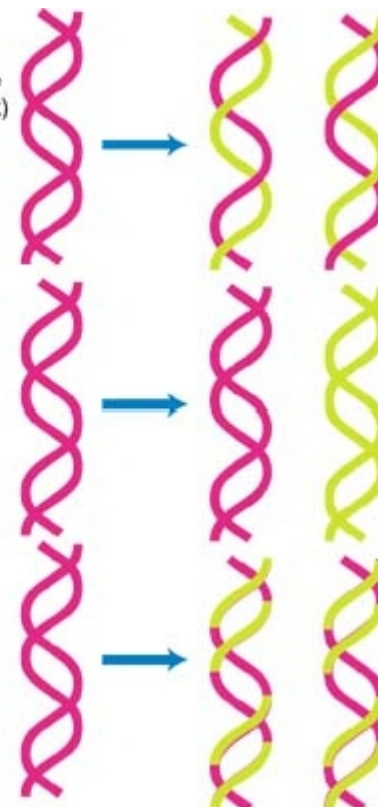
proofreading is done by pol I and pol III through **3'-5' exonuclease** (backs up to correct error)

Eukaryotic replication occurs at multiple sites, but only after passage through G-1 restriction point

Semiconservative
(Watson and Crick)

Conservative

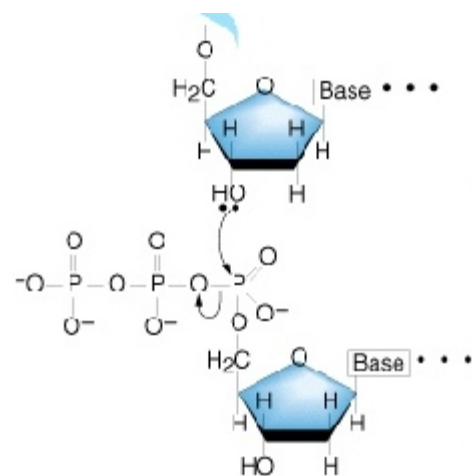
Dispersive

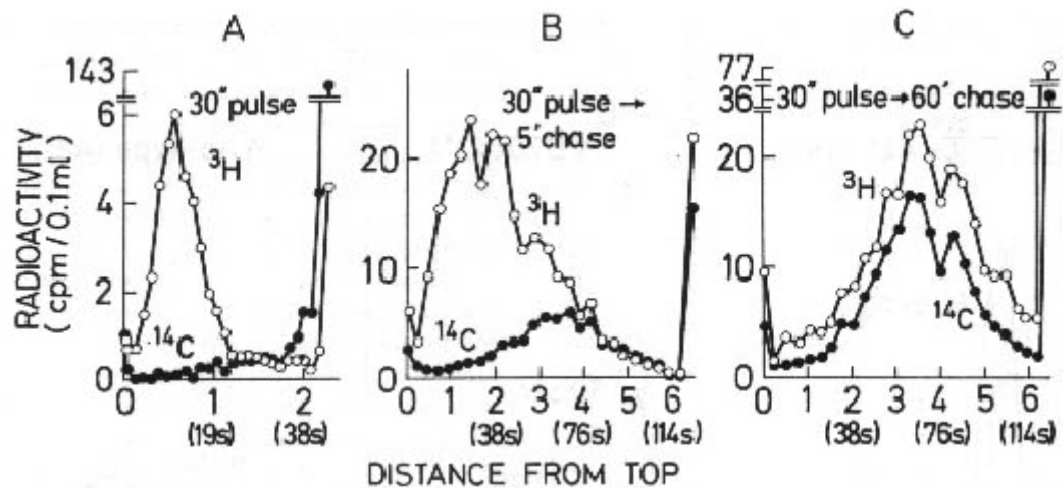


Autoradiograph



Interpretation





GENERATIONS

