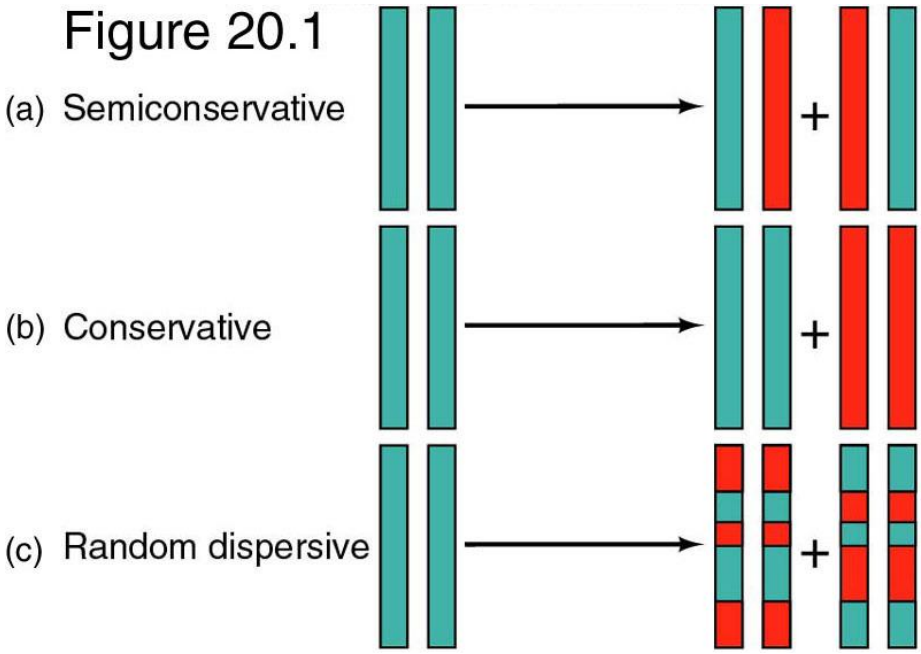
BIOL3090 Final Exam Summary, Pete's Lectures

Lecture 1: DNA Replication

- The five rules of DNA replication:
  1. DNA is made of two complementary strands (always)
  2. DNA replication is semiconservative (always)
  3. DNA replication is semidiscontinuous (always)
  4. DNA replication requires RNA (always)
  5. DNA replication is bidirectional (usually)

(2.) DNA Replication is Semiconservative

- Meselson and Stahl proved this in 1958
- At the time they had to distinguish between three possible options: semiconservative, conservative and random dispersive replication

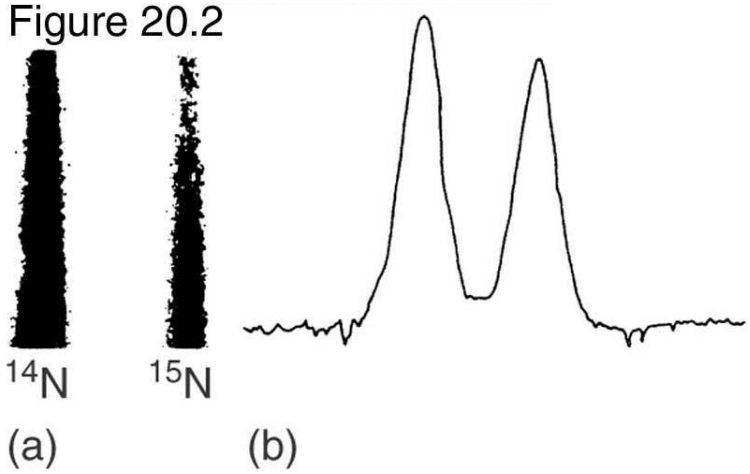


a) Semiconservative: Two strands separate. One strand in each new pair is conserved and the other daughter strand is synthesised. **b)** Conservative: Each parental strands lead to duplication, but remain paired together. **c)** Random dispersive: Random mixing of parent and daughter strands in each new pair.

- They labelled *E. coli* DNA with heavy nitrogen (^{15}N) by growing cells in a medium enriched in it
- They then switched the cells to normal medium containing ^{14}N
- They then separated the DNA on a CsCl gradient (ultracentrifugation)

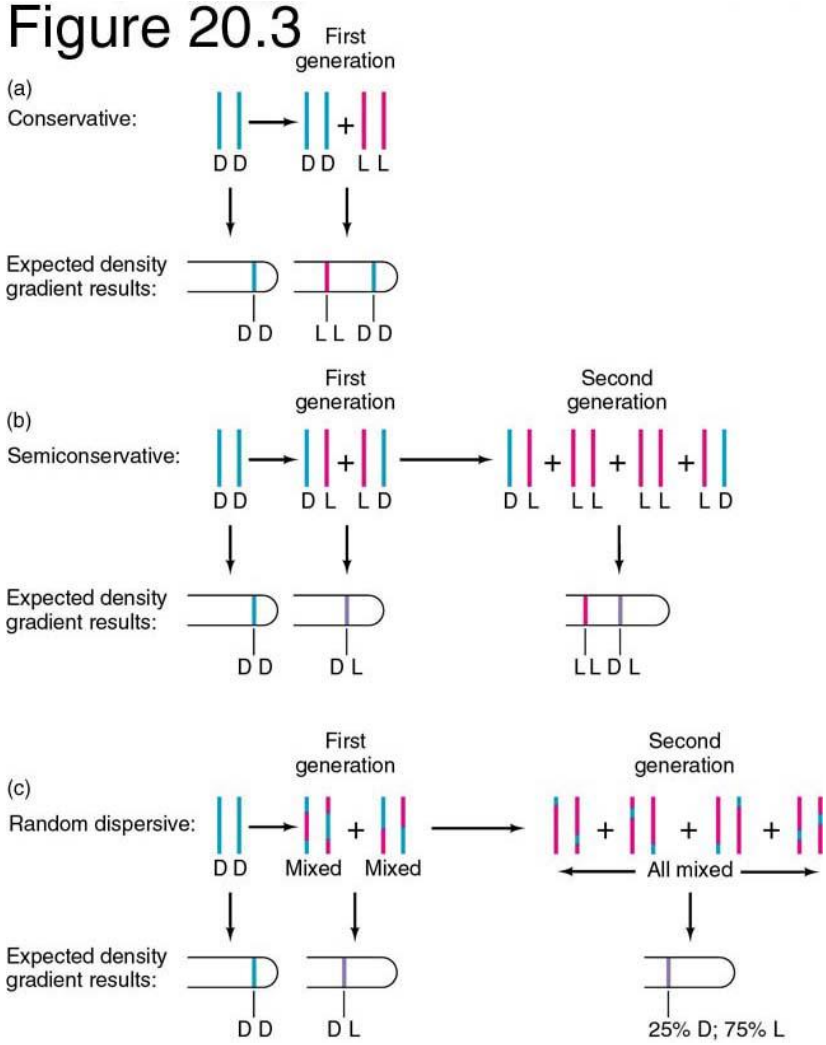
- ^{14}N and ^{15}N DNA are separated by this method

Figure 20.2

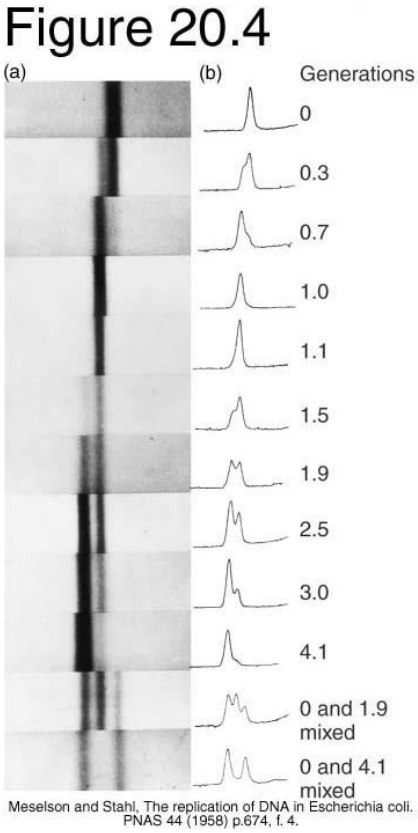


- Considering the three possible methods of replication, they expected outcomes after one round of replication of ^{15}N labelled DNA in a ^{14}N medium would be:

Figure 20.3



- After one round of replication, they saw the D/L hybrid band, which ruled out conservative replication but it was still possible either semiconservative or random dispersive replication
- Further rounds of replication showed an increasingly darker L/L band and a fading D/L band, showing that DNA replication is semiconservative

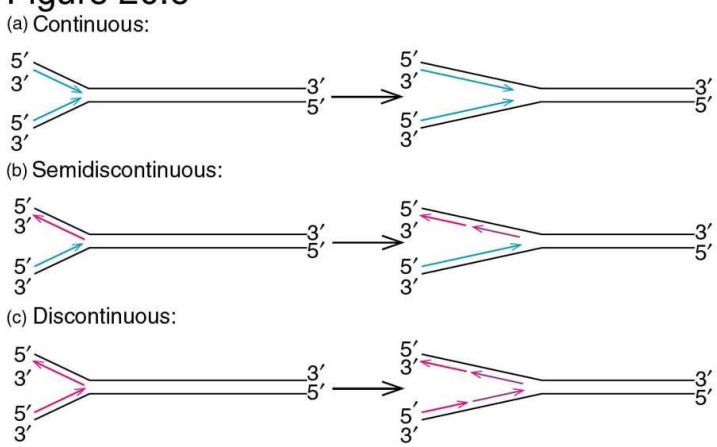


(3.) DNA Replication is Semidiscontinuous

- For semiconservative DNA replication, DNA strands must separate to serve as templates
- The issue with this is replication occurs in the 5' → 3' direction ONLY (DNA Pol can only add new nucleotides to the 3' –OH group of a growing daughter strand)
- Okazaki figured that this must mean DNA replication is semidiscontinuous, i.e. one (leading) strand is made continuously in the 5' to 3' direction, but the other (lagging) strand is synthesised discontinuously
- From this he made 2 predictions: 1. Since half the newly synthesised DNA is made in short fragments, you should be able to label and catch these before they are stitched together by allowing very short pulses of labelling with a radioactive DNA precursor. **2.** Elimination of DNA ligase should allow

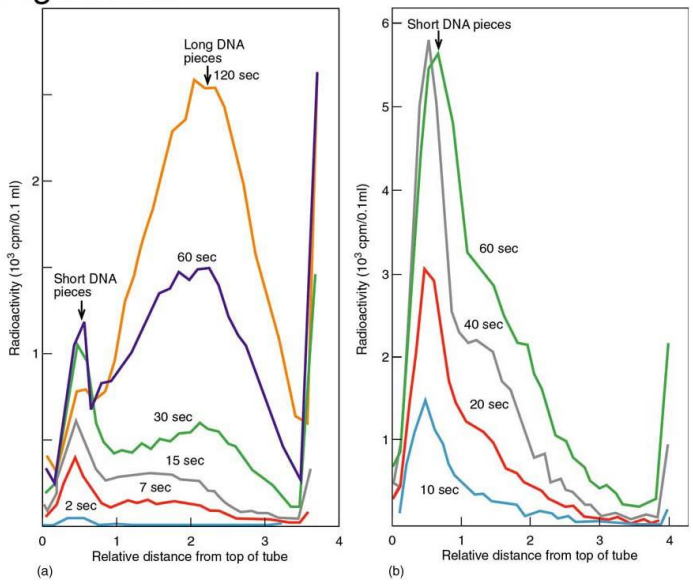
detection of the short DNA fragments even with long pulses of radioactive DNA precursor

Figure 20.6



- Okazaki pulse labelled *E.coli* replicating T4 DNA
- Pulses as short as 2 seconds caught the small DNA fragments before they were ligated
- They used ultracentrifugation to separate the fragments based on size (distance from top of tube)
    - When they increased their pulse intervals they saw longer fragments of DNA as this gave DNA ligase enough time to join the fragments together
- When they used a DNA ligase-deficient mutant, they saw accumulation of short DNA fragments even when they increased their pulse intervals

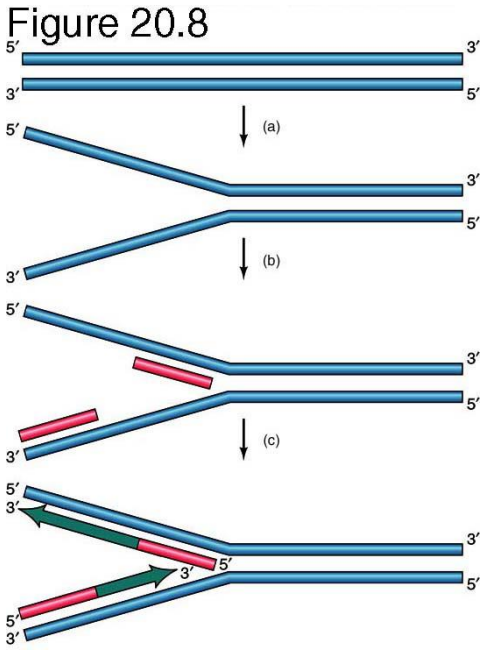
Figure 20.7



- These fragments are call Okazaki fragments are about 1-2kb in size

(4.) DNA Replication Requires RNA

- RNA is needed for the priming of DNA synthesis
- RNA Pol can take a NTP and make RNA from is, but DNA Pol can't just take a dNTP and make DNA
- This is because DNA Pol requires a 3' OH to add the dNTP to
- This is supplied by an RNA primer
- The enzyme primase is a type of RNA Pol
- It synthesises 8-12 nucleotides of RNA (which is ~1 turn of a DNA helix



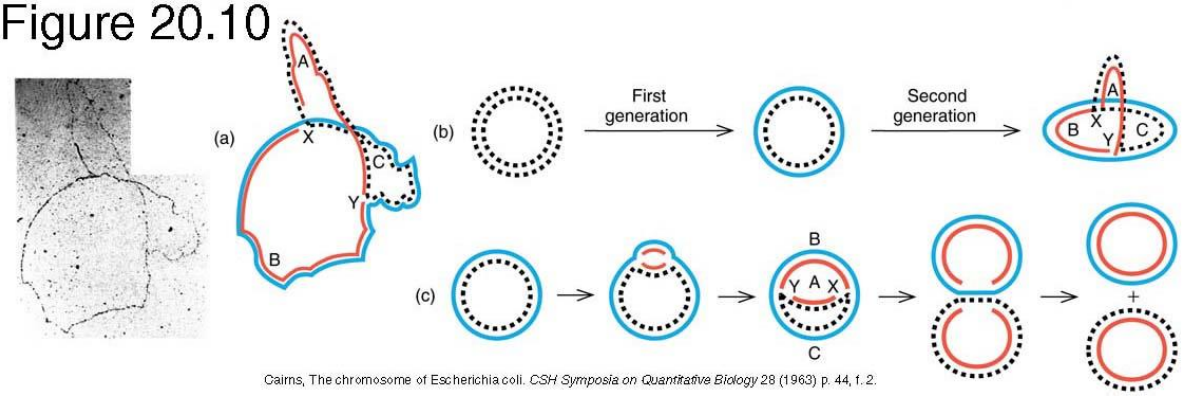
- Okazaki's wife conducted work where she labelled intact RNA primers (still attached to Okazaki fragments) with [^{32}P]GTP and a capping enzyme
- They used DNase to destroy the DNA part then used electrophoresis to determine the size of the primers
- They had to create mutant *E. coli* deficient in the nucleases that degrade RNA primers because otherwise intact primers were almost impossible to isolate

(5.) DNA Replication is Bidirectional (usually)

- Cairns, 1960: Labelled replicating *E. coli* DNA with H^3-T (the only base unique to DNA)
- He let the DNA undergo one round of replication and part of the second
- All the DNA should have at least one labelled strand
- After one round of replication, there is a labelled (blue) and unlabelled (dotted) strand

- After another round of replication there is one plasmid with two labelled strands and one with a labelled and unlabelled (c)
- Labelling extent determine darkness level when imaged by an autoradiograph

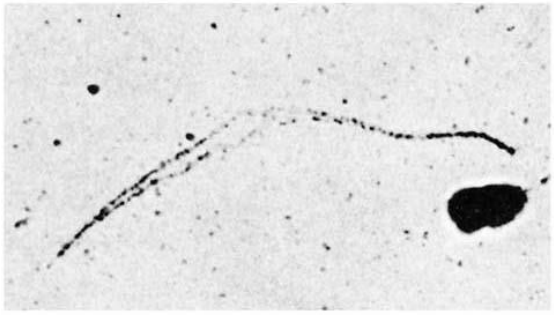
Figure 20.10



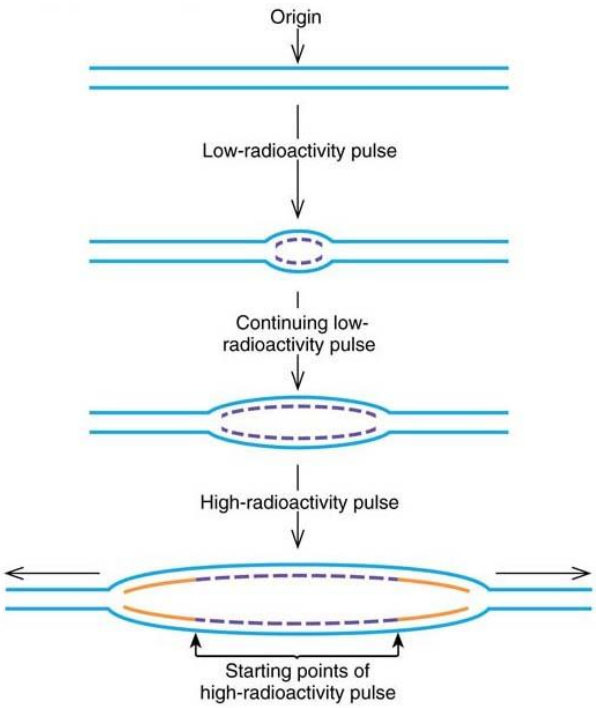
Cairns, The chromosome of Escherichia coli. *CSH Symposia on Quantitative Biology* 28 (1963) p. 44, f. 2.

- Although this showed two points of origin of DNA replication, this experiment did not show if replication was uni- or bidirectional
- This was shown later by Gyurasits and Wake in *Bacillus subtilis*
- They allowed *B. subtilis* to grow in the presence of a weakly labelled DNA precursor and then for a short time with a more strongly labelled precursor (both times ^{3}H-T)

Figure 20.11



(a)

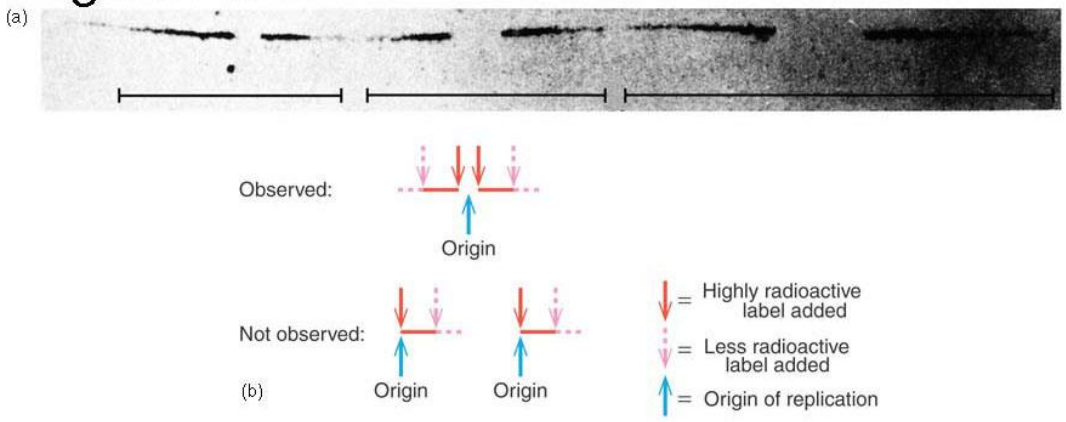


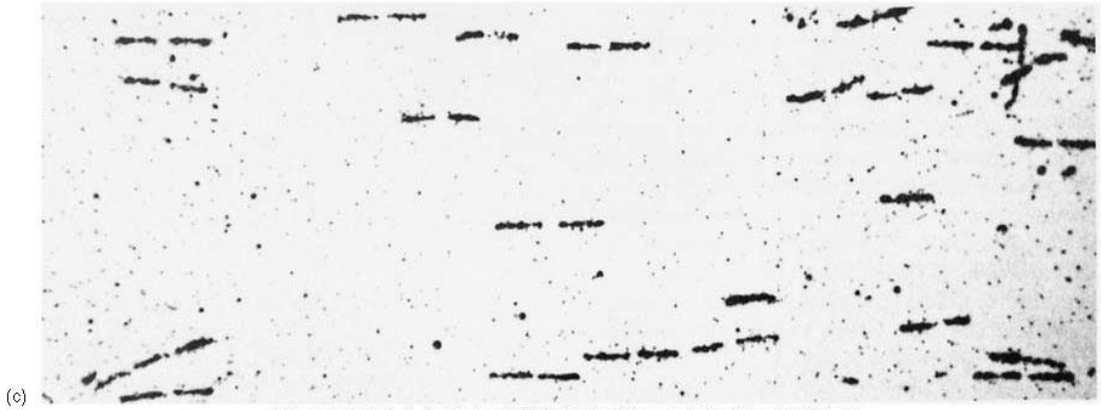
(b)

Gyurasits and Wake, *J. Molec. Biol.* 73 (1973) p. 58, by permission of Academic press.

- The incorporation of the strong label in both replication forks indicate they were both active at the time of exposure, indicating bidirectional replication was taking place

Figure 20.12





Huberman and Tsai, *J. of Molec. Biol.* 75 (1973) p. 8, by permission of Academic Press.