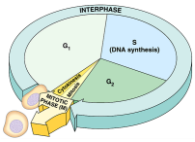
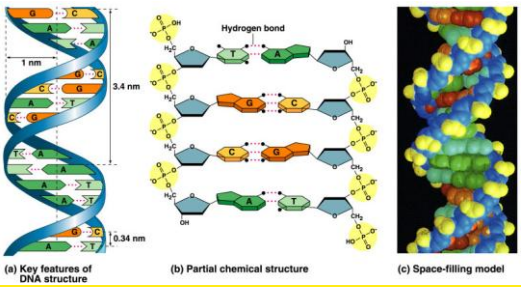


DNA Replication

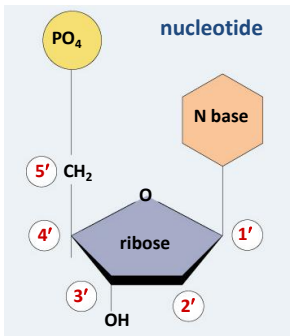
Double helix structure of DNA



"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."
Watson & Crick

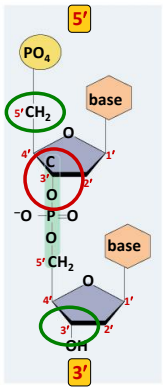
Directionality of DNA

- You need to number the carbons!
  - it matters!



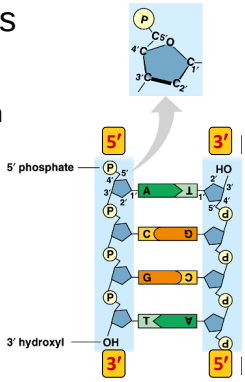
The DNA backbo

- Putting the DNA backbone together
  - refer to the 3′ and 5′ ends of the DNA
    - the last trailing carbon

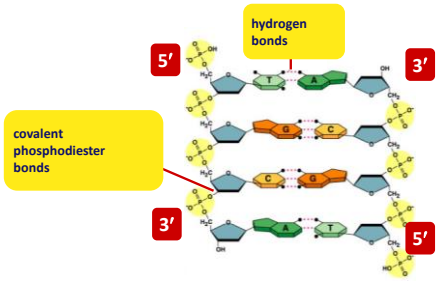


Anti-parallel strands

- Nucleotides in DNA backbone are bonded from phosphate to sugar between 3′ & 5′ carbons
  - DNA molecule has “direction”
  - complementary strand runs in opposite direction



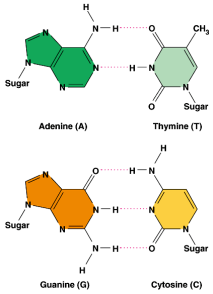
Bonding in DNA



….strong or weak bonds?
How do the bonds fit the mechanism for copying DNA?

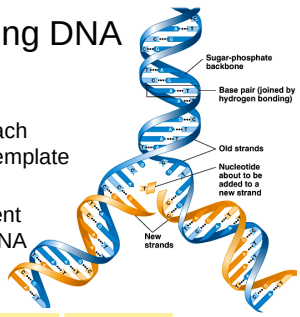
Base pairing in DNA

- Purines
  - adenine (A)
  - guanine (G)
- Pyrimidines
  - thymine (T)
  - cytosine (C)
- Pairing
  - A : T
    - 2 bonds
  - C : G
    - 3 bonds



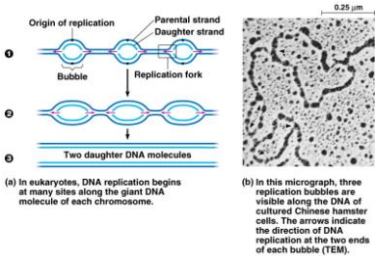
Copying DNA

- Replication of DNA
  - base pairing allows each strand to serve as a template for a new strand
  - new strand is 1/2 parent template & 1/2 new DNA
    - semi-conservative copy process



DNA Replication

- Large team of enzymes coordinates replication

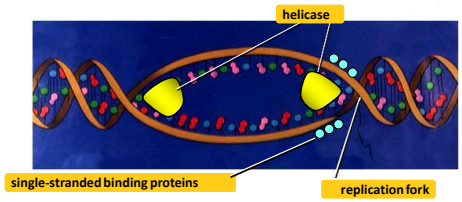


(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

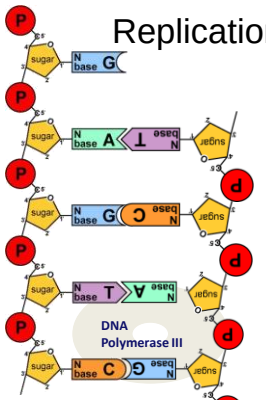
(b) In this micrograph, three replication bubbles are visible along the DNA of cultured Chinese hamster cells. The arrows indicate the direction of DNA replication at the two ends of each bubble (TEM).

Replication: 1st step

- Unwind DNA
  - helicase enzyme
    - unwinds part of DNA helix
    - stabilized by single-stranded binding proteins



Replication: 2nd step

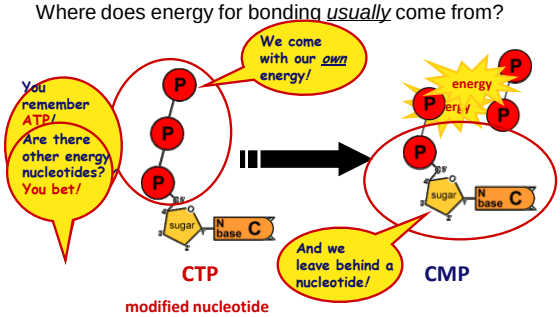


- Build daughter DNA strand
  - add new complementary bases
  - DNA polymerase III

Where's the *ENERGY* for the bonding!

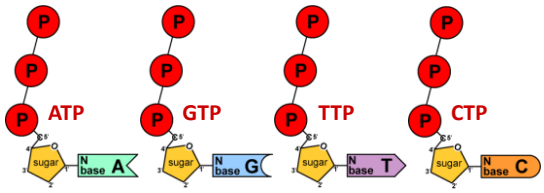
Energy of Replication

Where does energy for bonding *usually* come from?



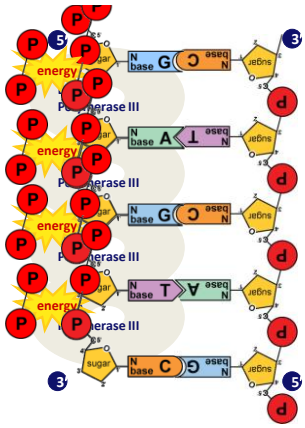
Energy of Replication

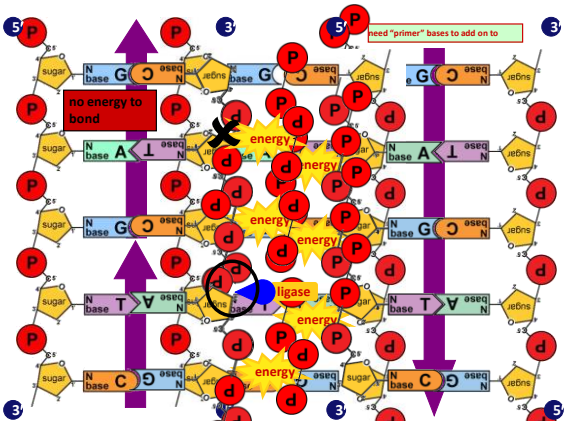
- The nucleotides arrive as nucleosides
  - DNA bases with P–P–P
    - P-P-P = energy for bonding
  - DNA bases arrive with their own energy source for bonding
  - bonded by enzyme: DNA polymerase III



Replication

- Adding bases
  - can only add nucleotides to 3′ end of a growing DNA strand
    - need a "starter" nucleotide to bond to
  - strand only grows 5′→3′



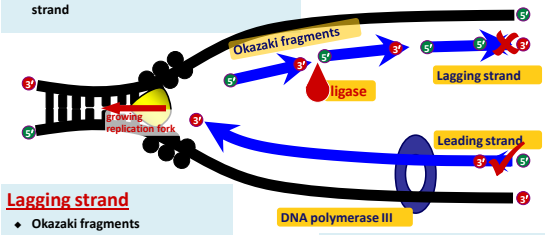


Okazaki

Leading & Lagging strands

Limits of DNA polymerase III

- can only build onto 3′ end of an existing DNA strand



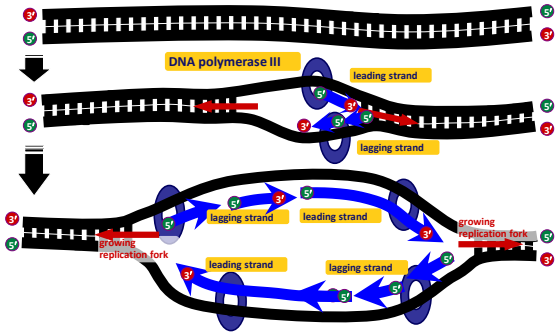
Lagging strand

- Okazaki fragments
- joined by ligase
  - "spot welder" enzyme

Leading strand

- continuous synthesis

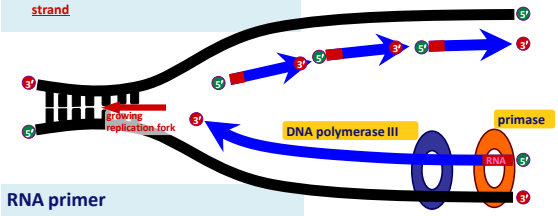
Replication fork / Replication bubble



Starting DNA synthesis: RNA primers

Limits of DNA polymerase III

- can only build onto 3′ end of an existing DNA strand



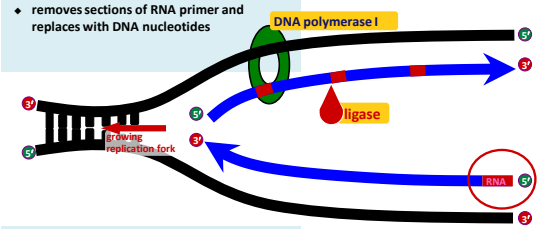
RNA primer

- built by primase
- serves as starter sequence for DNA polymerase III

Replacing RNA primers with DNA

DNA polymerase I

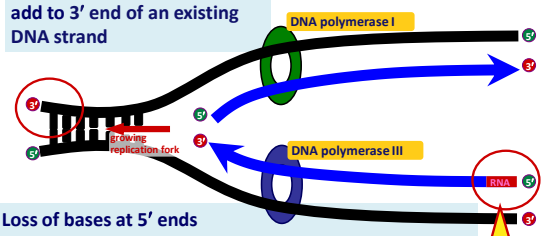
- removes sections of RNA primer and replaces with DNA nucleotides



But DNA polymerase I still can only build onto 3′ end of an existing DNA strand

Chromosome erosion

All DNA polymerases can only add to 3′ end of an existing DNA strand



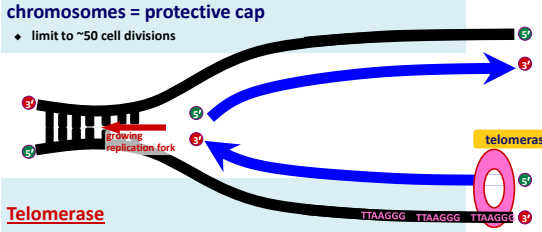
Loss of bases at 5′ ends in every replication

- chromosomes get shorter with each replication
- limit to number of cell divisions?

Telomeres

Repeating, non-coding sequences at the end of chromosomes = protective cap

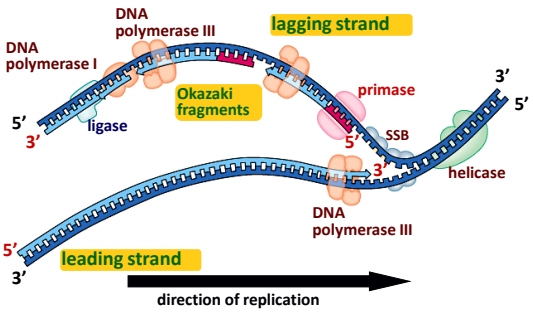
- limit to ~50 cell divisions



Telomerase

- enzyme extends telomeres
- can add DNA bases at 5′ end
- different level of activity in different cells
  - high in stem cells & cancers -- Why?

Replication fork



SSB = single-stranded binding proteins

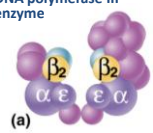
DNA polymerases

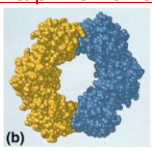
- DNA polymerase III
  - 1000 bases/second*!*
  - main DNA builder
- DNA polymerase I
  - 20 bases/second
  - editing, repair & primer removal

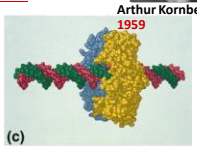
Thomas Kornberg
??

Arthur Kornberg
1959

DNA polymerase III enzyme

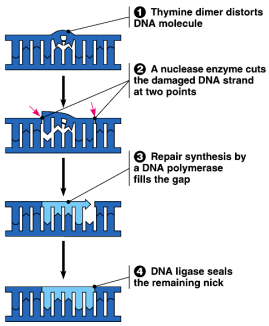






Editing & proofreading DNA

- 1000 bases/second = lots of typos!
- DNA polymerase I
  - proofreads & corrects typos
  - repairs mismatched bases
  - removes abnormal bases
    - repairs damage throughout life
  - reduces error rate from 1 in 10,000 to 1 in 100 million bases



Fast & accurate!

- It takes *E. coli* <1 hour to copy 5 million base pairs in its single chromosome
  - divide to form 2 identical daughter cells
- Human cell copies its 6 billion bases
  - remarkably accurate
  - only ~1 error per 100 million bases
  - ~30 errors per cell cycle

What does it really look like?