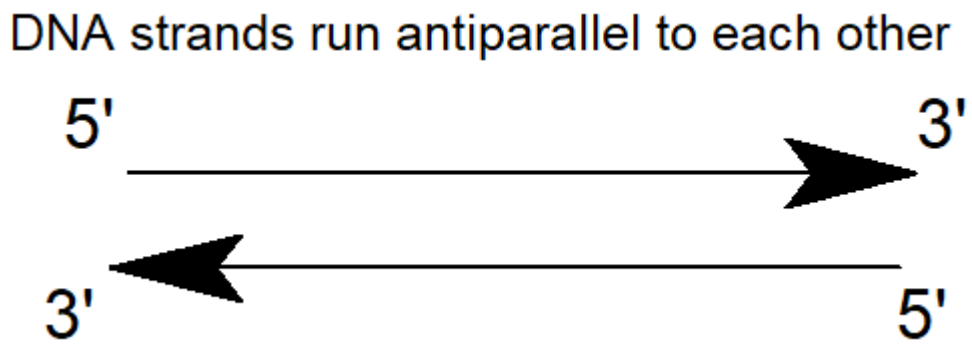
# LECTURE 1

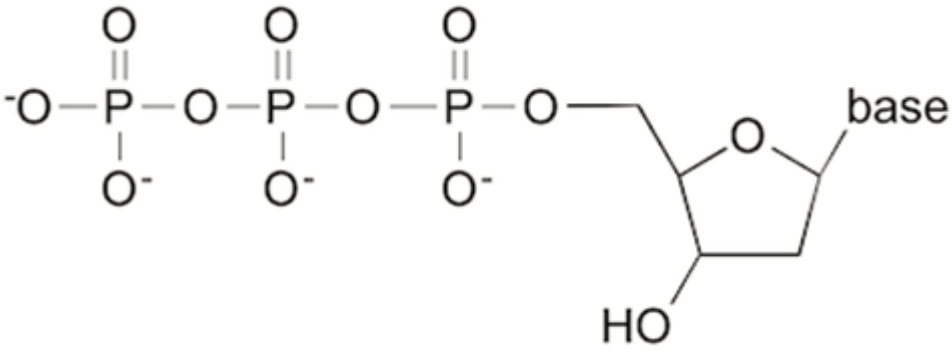
## DNA Structure Recap

- DNA runs antiparallel with base pairs stacked on the inside.

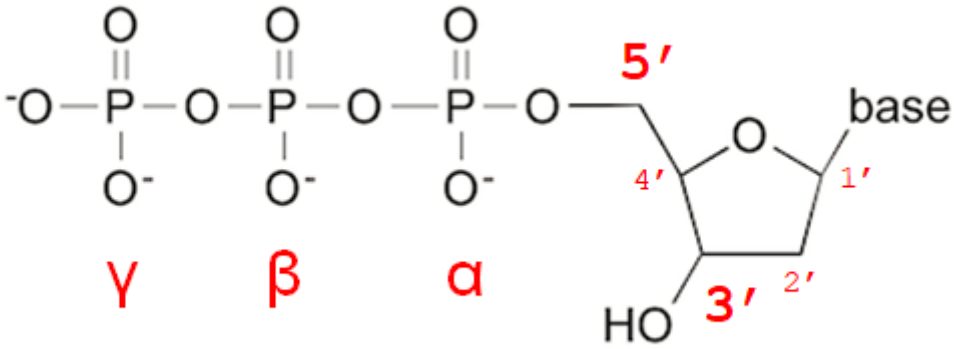



### Deoxynucleotide triphosphates

- (dNTP - dCTP, dGTP, dATP, dTTP)




▼ Label the above diagram with 5', 3' alpha, beta, and gamma.




▼ How is DNA made from the deoxynucleotide triphosphates, and what is the driving force?

- The hydrolysis of pyrophosphate is the driving force for DNA synthesis, the DNA is cut off from the last to phosphate groups and added to 3'.

# The Problem

- **Every time a cell divides its entire DNA content must be exactly replicated.**
  - e.g. For E.coli DNA replication  takes ~40 mins (quite fast)
  - Humans have 6×10^9 base pairs, E.coli have 4.6×10^6

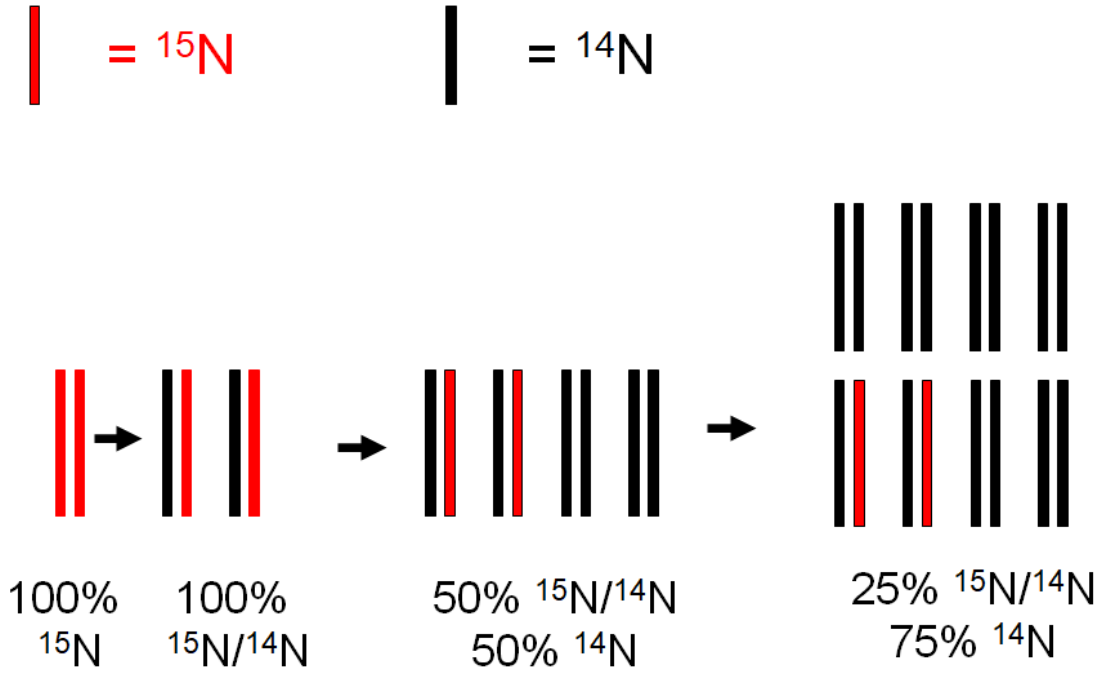
# Replication

## Semi-Conservative Replication - how was it discovered?

▼ Explain the experiment that was conducted in order to find out that DNA replicated via semi-conservative replication.

1. Bacteria was grown on heavy isotope of Nitrogen 15.
2. Then this heavy bacterial DNA was transferred to a medium containing Nitrogen 14.
3. The DNA was isolated and separated (via density gradient centrifugation as Nitrogen 15 is heavier than Nitrogen 14) after each generation.
4. 1st Generation - All DNA density is between 14N and 15N

5. 2nd Generation - 50% of DNA is at 14N Density and 50% is intermediate between 14N and 15N
6. 3rd Generation - 75% of DNA is at 14N and 25% is intermediate.



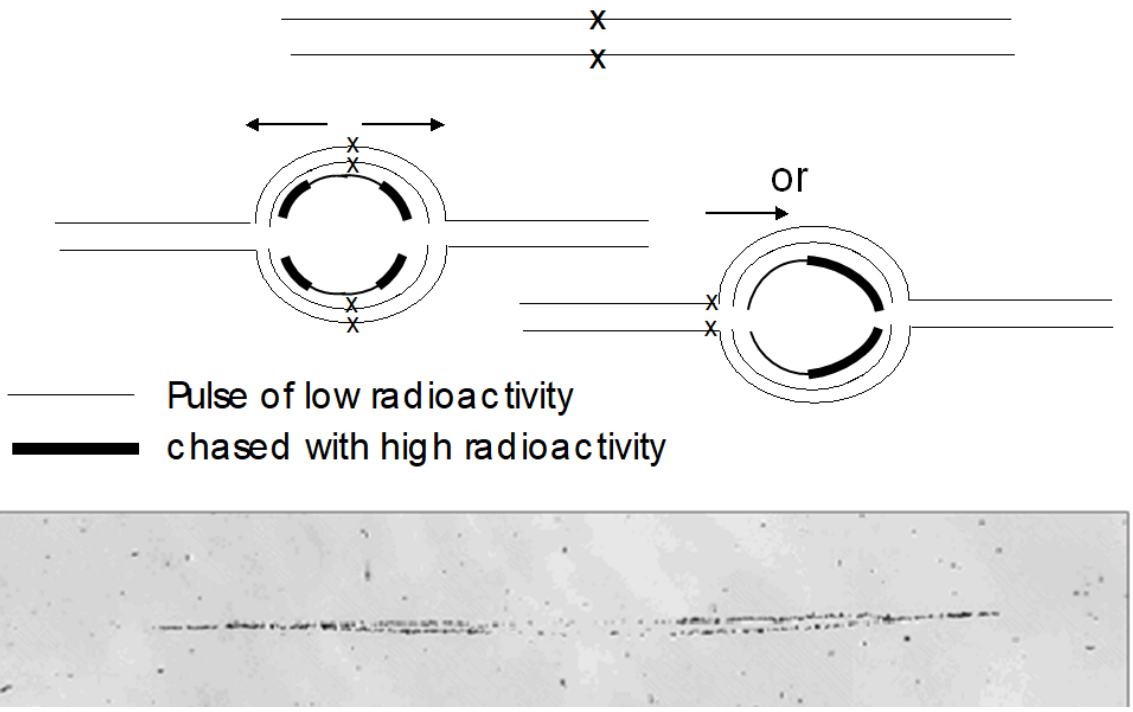

## Bidirectional replication

▼ Why is bidirectional replication complicated?

1. Antiparallel nature of DNA
2. Coiling of strands
3. Circular nature of bacterial genomes
4. Stacking of bases within helix

▼ How did we know that replication was bidirectional?

An experiment was conducted wherein DNA was given a pulse of low radioactivity and then high radioactivity. This would create thin strands and thick strands. This way we would be able to see the direction from the origin of replication. It was found to be bidirectional.




# Enzymology - DNA Polymerase I

- First enzyme to be isolated (By Kornberg 1958)
- Single polypeptide chain (mw, 109000) ~400 Molecules per cell
- Requires all 4 dNTPs, a template strand and primers.
- DNA Polymerase I is NOT self priming and can only add onto existing 3'-OH terminuses
- Polymerization is processive (10-100 nucleotides per binding event) ~10 nucleotides/s
- Chain growth is always 5'-3'
- Pol I binds to nicked or gapped DNA (Can occur due to errors such at U being used in place of T), it will chew out DNA ahead and make new DNA. Does not bind to intact single stranded DNA (ssDNA) or double stranded DNA (dsDNA)

## DNA Polymerase I has multiple actions:

▼ What are the 3 actions of DNA Pol I?

1. 5'-3' polymerase (making new DNA)