# Lecture 1

Sunday, 4 March 2018
10:24 pm

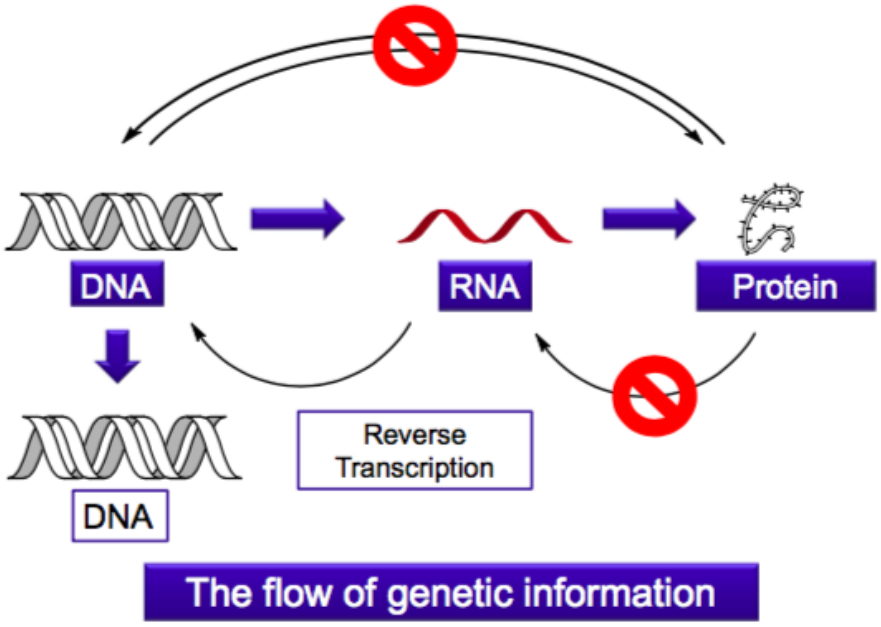
## Assessments Summary

| Assessment task | Due Date and Time | Mark Contribution | Submission |
|---|---|---|---|
| ELN | Each fortnight, to be completed before the next lab session | 25% | Complete on Labarchives |
| Data Analysis and presentation/Poster | Friday, week 11, 25th May | 5% | Present |
| PeerWise authoring and evaluation tasks | Each fortnight, to be completed before the next tutorial session | 10% | Submit Online to PeerWise site |
| ELMA design and interpretation | Weeks 12 and 13 | 10% | Submit at the end of the lab session |
| Final Exam | Exam period | 50% | Attend Final Exam |

Amino acid side chains can be

- Hydrophobic, hydrophilic
- Positive, negatively charged

Movement of information




OH removed from 2' carbon to make the end more stable

- New nucleotides join to the 3' carbon

Backbone is hydrophilic and negatively charged
Nucleotides attach to 3' end. Release Ppi (pyrophosphate)
Bases have donors and acceptors

- Donors have hydrogen
- Acceptors do NOT have hydrogen

Read DNA 5' -> 3'

Purines: 2 rings
Pyrimidines: 1 ring

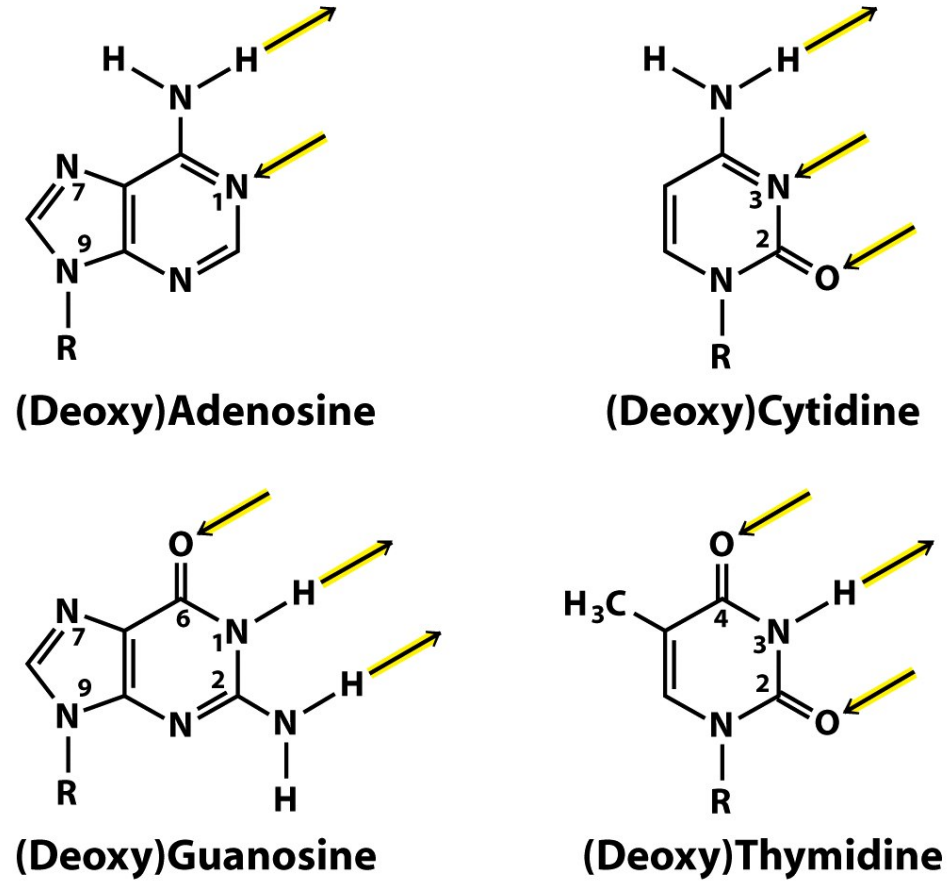



Figure 19-6 Principles of Biochemistry, 4/e


pKa: the pH where 50% molecules are protonated, 50% not protonated

All 4 bases absorb roughly the same

- Does not change the UV absorbance
- Double stranded DNA absorbs less than single stranded DNA

Experiments are about

- Putting strands together
- Pulling strands apart

Polymerase is known by their product

- DNA polymerase makes DNA
- Add things to the 3' end

# Lecture 2

Tuesday, 6 March 2018
3:07 PM

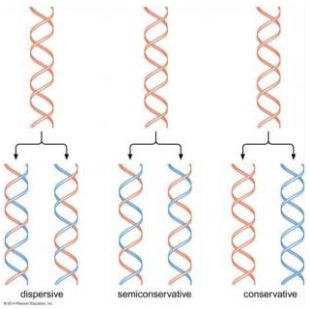
*You don't need to know protein names.*
*You need to know the **enzymatic functions** of the proteins.*

DNA poly needs a primer

- The primer provides the OH end to attach dNTP molecules to
- Synthesis from 5' to 3' (the template stand copied 3' to 5')
- dNTP has 3 phosphates (tri-phsophate)
- Joins to OH with phosphodiester bond
- Releases pyrophosphate which is quickly hydrolised to phosphate

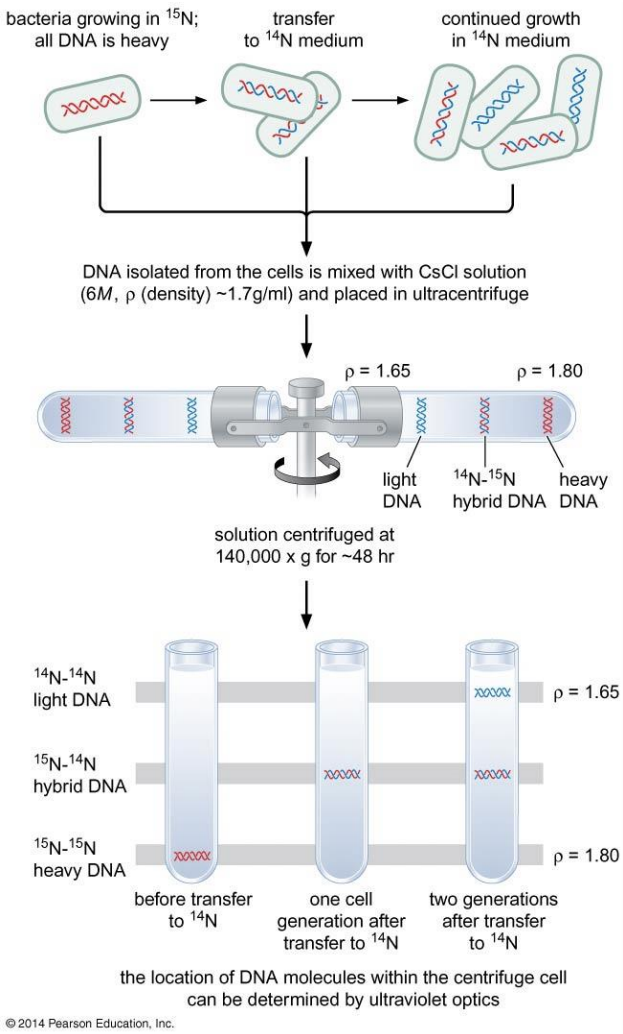
Three possible models of replication

1. Conservative
2. Semi-conservative (we now know this is correct)
    - Meselson-Stahl proved this
3. Distributed




Meselson-Stahl experiment

- Grow E-coli in N-15 (heavy isotope)
- Move cells to N-14 (light) and continue to grow




Bacterial DNA replication

- Have circular genome (plasmid)
- Replication starts at oriC (origin of replication)
- 9-mer motif -- 9 nucleotides long, repeated along the OriC
    - Origin-recognition protein, DnaA, binds to this
- 13-mer motif -- 13 nucleotides long, repeated
    - AT rich. AT has 2 h-bonds, whereas GC has 3 h-bonds
    - AT is easier to split apart
    - Change the structure of DNA to allow separation
- **Helicase** unzips genes
- Single stranded binding protein (**SSBP**) holds the strands apart
- **Primase** inserts RNA primer
- **DNA pol3** can start copying
    - Copying is bidirectional

DNA POL3

- Alpha enzyme does the polymerase
- Beta enzyme (sliding clamp) holds the DNAPOL3 to DNA

Processivity

- Ability to catalase many consecutive reactions which releasing substrate
- DNAPOL3 has high processivity

Leadings and lagging

- Leading strand synthesized continually
- Lagging strand synthesized in Okazaki fragments
    - The looped part is called the "Trombone slide"
    - Primers needed for each fragment
    - After fragments are made
        - Primer is removed using Rnase H
        - 5' exonuclease removes the 5' nucleotide (DNAPOL1)
        - DNAPOL1 fills the gap
        - Fragments sealed using DNA ligase

**FROM MBLG:**

Difference between DNA pol 1 and pol 3?

- Both have 5' to 3' polymerase (new strand)
- Both have 3' to 5' exonuclease (proof reading)
- ONLY pol 1 has 5' to 3' exonuclease

# Lecture 3 Prokaryotic Replication

Friday, 9 March 2018
11:02 am

| | |
|---|---|
| Exonuclease | Cut from the end of the DNA strand |
| Endonuclease | Cut from the middle of the DNA strand |

If DNA POL1 and POL3 do not fix a mismatch, there is mismatch machinery to fix this

- Mismatch repair can happen anywhere along the DNA

Plasmids are not perfectly circular

- DNA is negatively supercoiled
  - Negative just refers to the direction (left-handed coiling)
  - In opening the DNA, Helicase adds supercoiling
- Topoisomerase add or remove supercoiling
  - By cleaving either one or two of the strands
  - Type 1 cut one strand and remove supercoiling
  - Type 2 cuts 2 strands and add supercoiling

TYPE 1

- Cut one strand
- Energy from potential energy in stressed DNA

TYPE 2

- Cut both strands
- Introduce negative supercoiling
- Requires energy input

After plasmid replication

- Circular genome is catenated
- Type 2 topoisomerase can decatenate (separate the rings)

DNA POL1 can be used for

- PCR
- DNA sequencing
- Probe labelling

PCR

- You need template, dNTP, primers (Primase), polymerase
- Steps
  - Separate the double strand by heating the sample (95 degrees)
  - Lower temperate so primers can stick (anneal)
  - Lower temperate for polymerase to perform
- Each cycle will denature DNA POL1
  - Instead of DNA POL1, we now use Taq polymerase (thermus aquaticus)
  - Taq lives in hot springs, proteins are heat stable
- In PCR, the **primers used are made of DNA**
  - Primers do not need to be removed because they are made of DNA

DNA is stabilized with salt ions. More salt, higher melting temperature
More GC%, higher melting temperature
Longer length chain, higher melting temperature

PCR advantages

- Quickly synthesize many copies (doubles each cycle)
- Completely in-vitro (in glass)
- Can use very low amounts of source DNA

Limitations

- Start of target sequence must be known to make primer
- Sensitive to contamination

Why did we use POL1 in PCR and not POL3?

- Easier to make POL1 like enzyme
    - POL3 is more complicated and difficult to maintain in experimental conditions
- POL3 sliding clamp is much more complicated
    - The sliding clamp is used to keep POL 3 attached to the DNA

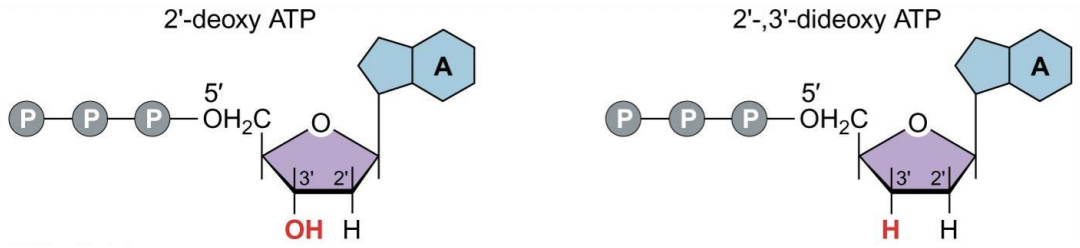
Replications occurs both ways along the circle (halves the replication time)

# Lecture 4

Tuesday, 13 March 2018
11:00 am

Sanger sequencing

- Uses ddNTP
  - no OH on 2' and 3' carbons
  - Terminates DNA polymer
- ddNTP labelled with radio isotope or fluorescent dye
  - denoted *ddNTP
  - Label on the alpha phosphate




- If ddAPT terminates a chain, we know the base at that position is a T
- Sequences are run through gel, and we can read the bands
  - Short sequences travel further
- We use a Taq variant which works better with ddNTP

Fluorescence-based sequencing

- Each ddNTP is labelled differently so we don't need 4 different sequencing runs

Next generation sequencing (NGS)

- Uses light emitted when a nucleotide in added to the chain
- See MBLG

Probe labelling

- When you need to incorporate radiolabel into DNA

Making the probe

- Nick translation
  - Start with double-stranded DNA
  - DNAse adds a nick to the DNA
  - POL1 removes DNA using 5' to 3' exonuclease
  - DNA polymerase fills the gap with labelled dNTP
- Random primed labelling
  - Start with single-stranded DNA
  - Random hexamers (6 bases long) will bind to regions of DNA and act as primers
  - DNA polymerase elongates from primer with labelled dNTP