ELEC1210
DNA Replication

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Learning Outcomes

By the end of this session you should be able to:

• Explain the semi-conservative model of DNA replication and the experimental evidence to support this model

• Describe the different centrifugation techniques utilised to isolate molecules

• Explain how DNA is replicated

• Describe the properties of the replication fork

• Describe DNA sequencing and PCR
DNA replication

How is DNA copied?

We could imagine three alternative models:

- **Conservative**
- **Semi-conservative**
- **Dispersive**
Evidence
Meselson & Stahl experiment

Grew bacteria on heavy (radioactive) isotope of nitrogen $^{15}$N. Cells containing labelled DNA were then transferred to a medium containing the normal isotope of nitrogen $^{14}$N. The DNA was isolated after 1, 2, 3 ... generations.

Old DNA is $^{15}$N
New DNA will be $^{14}$N

Separate the DNA by density gradient centrifugation

DNA that contains $^{15}$N is denser than that containing $^{14}$N

At zero all DNA has $^{15}$N
After 1 generation - all DNA has density between $^{15}$N and $^{14}$N
After 2 generations - 50% has density of $^{14}$N, 50% is intermediate
After 3 generations – 75% has density of $^{14}$N, 25% is intermediate
Aside on centrifugation

Heavier particles sediment the fastest
Not an equilibrium method – everything goes to the bottom of the tube

Sucrose density centrifugation

Samples applied to the top of a tube that already contains a gradient of sucrose concentration.
(more concentrated at the bottom).
Also not an equilibrium method – eventually everything goes to the bottom of the tube. But gives better separation of the fractions if stopped at the right time.

Cesium chloride density gradient centrifugation

Samples are dispersed in a concentrated solution of cesium chloride.
An equilibrium method. Under the high centrifugal force the cesium forms a concentration gradient. The samples “float” at the position corresponding to their buoyant density. Separates samples according to **density**, not mass or shape.
Density Gradient Centrifugation
Separates molecules according to their buoyant density

Spin in a centrifuge in concentrated solution of caesium chloride. The high centrifugal forces generate a gradient in the concentration of the ions (denser at the bottom of the tube). The DNA “floats” at the position that corresponds to its buoyant density.
Conservative

\[ = ^{15}\text{N} \]

\[ = ^{14}\text{N} \]

15N or 14N but no intermediate forms

Denature 15N or 14N

Semi-conservative

Mixed 15N / 14N

Denature 15N or 14N

Dispersive

Mixed 15N / 14N

Denature mixed 15N / 14N
Semi-conservative

100% $^{15}\text{N}$ → 100% $^{15}\text{N}/^{14}\text{N}$ → 50% $^{15}\text{N}/^{14}\text{N}$ → 25% $^{15}\text{N}/^{14}\text{N}$

50% $^{14}\text{N}$
**Question** Which model of DNA replication – conservative, dispersive, or semiconservative – applies to *E. coli*?

**Experiment**

1. **15N medium**
2. Spin
3. Transfer to 14N medium and replicate
4. Spin
5. Replication in 14N medium
6. Spin
7. Replication in 14N medium
8. Spin

**Results**

- **Light (14N)**
- **Heavy (15N)**

1. DNA from bacteria that had been grown in medium containing 15N appeared as a single band.
2. After one round of replication, the DNA appeared as a single band intermediate between that expected for DNA with 15N and that expected for DNA with 14N.
3. After a second round of replication, DNA appeared as two bands, one in the position of hybrid DNA (half 15N and half 14N) and the other in the position of DNA that contained only 14N.
4. Samples taken after additional rounds of replication appeared as two bands, as in step 3.

**Conclusion** DNA replication in *E. coli* is semi-conservative.
Replication starts at a fixed point and is bi-directional

*E. coli* – circular double stranded genome

In eukaryotes there are **multiple** replication forks – each progressing in a **bidirectional** fashion
Remember that replication is bidirectional

- Origin of replication
- Bidirectional replicative forks
- Direction of replication
- Partially replicated chromosome

*E. coli* chromosome
DNA polymerase and the replication fork

Polymerase synthesizes new strand in 5’ to 3’ direction. Remember this is on the NEW strand being made.
The duplex is opened up and new bases are added at the 3’-end. i.e. *synthesis is from 5’→3’*

5’-ACGTGGTAGCTTAGCTAC-3’

Parent/template strand 3’-TGCACCATCGAATCGATG-5’

ALL DNA polymerases synthesize new DNA in the \textbf{5’→3’} direction

ALL require a single-stranded DNA template.

ALL extend from an existing strand (primer). This primer is made of RNA and is made by another enzyme (primase)
Chemistry of DNA replication

Deoxynucleotide triphosphate

Pyrophosphate
Overall new synthesis in 3'-5' direction

Leading strand

Lagging strand – made in short fragments in 5'-3' direction, which are then joined together

Okazaki fragments

The short fragments are then joined together by DNA ligase
The full process is complex and requires many different enzymes!
Two important uses of DNA polymerase reactions

1. DNA sequencing
2. DNA amplification - PCR
**DNA sequencing** (dideoxy, Sanger method)
Uses DNA polymerase to extend a primer which is bound to the single-stranded fragment to be sequenced

![Diagram of DNA sequencing](image)

The reactions contain all the usual dNTPs, plus a small amount of a modified nucleotide triphosphate – ddNTP – dideoxynucleotide triphosphate.

Can be added to the 3’-end, but then causes chain termination
If the reaction contains dATP, dCTP, dGTP, dTTP and ddATP synthesis will stop every time it incorporates a ddATP.

Denature and separate the products by polyacrylamide gel electrophoresis (short fragments run faster)
• DNA fragments move through a gel matrix from –ve to +ve
• **Short fragments move faster**

**Agarose** – physical gel.
Most often used for separating double stranded DNA fragments (typically 100-10,000 base pairs).
Horizontal submerged gel
DNA is visualised by adding a dye (ethidium) which fluoresces (orange) when bound to DNA.

**Polyacrylamide** – chemical gel.
Vertical apparatus run between glass plates
Most often used in denaturing gels, containing 8M urea, to separate single stranded DNA fragments (as in sequencing).
DNA is visualised by **autoradiography** ($^{32}$P) or by covalent attachment of a fluorescent group.
Do similar reactions with ddCTP, ddGTP, ddTTP

5’- TCAAGTTACCGTCGACGGCTAATGAACTGCTA

ddATP
ddTTP
ddCTP
ddGTP
Can also use ddNTPs that contain different coloured fluorescent dyes covalently attached.

ddATP  ddTTP  ddCTP  ddGTP

Combine into a single gel lane.
Next generation sequencing

- Short fragments of DNA as input, annealed to a slide using oligonucleotide adaptors
- PCR amplified to create multiple copies
- Use of fluorescent nucleotides for sequencing
- Image is taken after addition of each new nucleotide
- **use of Reversible terminator**

https://www.ebi.ac.uk/training/online/course/ebi-next-generation-sequencing-practical-course/what-next-generation-dna-sequencing/illumina-
Next generation sequencing

Sanger method sequencing

Sanger fluorescent dideoxynucleotide (ddNTP)

Next generation sequencing method

3’-O-blocked reversible terminator

Fluorescent dye and blocking group can be cleaved off to allow continuation of PCR and addition of next dNTP

adapted from Chen, C-Y, Front. Microbiol., 2014
Next generation sequencing

- Clusters of PCR-generated DNA on slide
- each cluster sequenced at the same time (in parallel)
Next generation sequencing

Computer algorithms are used to detect the signals and construct a sequence
Amplifying DNA
Polymerase Chain Reaction (PCR)

How to make millions of copies of a piece of DNA

1) **Denature** to produce single strands (heat)

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5'--->-3' =DNA sequence of interest-
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2) Add short primers (~18 mers) that are complementary to the ends of the sequence of interest. Lower temperature → anneal
Amplifying DNA
Polymerase Chain Reaction (PCR)

3) Thermostable DNA polymerase
   • *Taq* – originally isolated from *Thermus aquaticus*
   • able to withstand high temperatures required for DNA melting during PCR
   • required for DNA extension

4) Repeat steps of denaturation, annealing, extension
Summary

• What is the semi-conservative model of DNA replication and explain the experimental evidence to support this?

• How is DNA replicated and what are the properties of the replication fork?

• What is density gradient centrifugation and how does it differ from other centrifugation methods?

• Explain PCR and DNA sequencing methods.

• How is DNA separated on a gel?