DNA replication

How is DNA copied?

We could imagine three alternative models:

- Conservative
- Semi-conservative ✔
- Dispersive

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
**Evidence**

Meselson & Stahl experiment

Grew bacteria on heavy (radioactive) isotope of nitrogen $^{15}\text{N}$

Cells containing labelled DNA were then transferred to a medium containing the normal isotope of nitrogen $^{14}\text{N}$. The DNA was isolated after 1, 2, 3 ... generations.

Old DNA is $^{15}\text{N}$

New DNA will be $^{14}\text{N}$

Separate the DNA by density gradient centrifugation

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

At zero all DNA has $^{15}\text{N}$

After 1 generation - all DNA has density between $^{15}\text{N}$ and $^{14}\text{N}$

After 2 generations - 50% has density of $^{14}\text{N}$, 50% is intermediate

After 3 generations – 75% has density of $^{14}\text{N}$, 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.

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.
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.

**DNA polymerase and the replication fork**

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 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
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

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.

Overall new synthesis in 3’-5’ 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!

Direction of fork opening

Leading strand

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

O

BAS

E

O

P

O

O

O-

P

O

P

O-

O

O-

Missing OH
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

Can also use ddNTPs that contain different coloured fluorescent dyes covalently attached with 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 method

Sanger method sequencing

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

Sanger fluorescent dideoxynucleotide (ddNTP)

Reversible blocking group


Next generation sequencing

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

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)

2) Add short primers (~18 mers) that are complementary to the ends of the sequence of interest. Lower temperature → anneal

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?