DNA replication

Copying the genetic material

Need for replication

Since DNA is carrying the information on how each cell works then, when a cell divides, it is essential to duplicate the information, so both daughter cells get a complete copy.

The Watson-Crick model

The two strands separate and then each is used as a template to build a new complementary strand. No details about the molecular mechanism are suggested.
Modes of replication

• Others immediately suggested alternate approaches. The question was, how can these be tested?

In 1957 Matt Meselson and Frank Stahl come up with an idea that can distinguish among the three possible methods of replication. It uses two relatively new techniques: isotope density labeling and ultracentrifugation.

Most nitrogen has 7 protons and 7 neutrons in the nucleus (\(^{14}N\)), but there is a stable isotope that contains 8 neutrons (\(^{15}N\)) and so is heavier.
How to measure the density?

It is not practical to purify enough DNA to measure its density directly. Luckily, there is a method that will work with small quantities, and can distinguish between very small differences in density.

Isopycnic measurement

Isopycnic simply means ‘same density’.

Density gradient
DNA banding

DNA has a density of about 1.7 g/ml. Meselson and Stahl knew they could create a density gradient where the density of the liquid ranged from 1.6-1.8 g/ml.

Testing the idea

The experiment

Bacteria are grown for many generations in 15N, so all their DNA is heavy. At the start of the experiment they are transferred to fresh medium that contains 14N.
The experiment

- So, from now on all the new DNA being made will be light but any original strands will be heavy. Each of the three models make specific predictions about the result.

Predictions

- At start:  
  - Conservative
  - Semi-conservative
  - Dispersive

- After one cycle:  
  - Conservative
  - Semi-conservative
  - Dispersive

- After two cycles:  
  - Conservative
  - Semi-conservative
  - Dispersive

The result
How is it done?

Knowing that DNA replication is semi-conservative still tells us very little about the molecular mechanism.

DNA polymerase

It is a DNA polymerase; using DNA nucleotide subunits to create a larger molecule

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It is a DNA polymerase; it uses DNA nucleotide subunits to create a larger molecule

It is a DNA-dependent DNA polymerase. It only makes a DNA strand when there is one to copy
Polymerase action

The nucleotide triphosphate attacks the 3'OH of the last base in the strand and attaches via a phosphodiester bond. In the process two inorganic PO4 are lost while the $\alpha$PO4 becomes part of the DNA backbone.

The search for DNA polymerase

Arthur Kornberg and Severo Ochoa undertook a search for an enzyme that had the expected characteristics.

How to identify polymerase

Tube contains: labeled nucleotides and suspected polymerase

- Not the polymerase
  - Nothing happens
  - DNA Synthesized?
- In the polymerase
  - Most of Radioactivity on filter
### Results

<table>
<thead>
<tr>
<th>Incubate labeled NTP with</th>
<th>DNA present</th>
<th>Radioactivity on filter?</th>
</tr>
</thead>
<tbody>
<tr>
<td>No enzyme</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Random polymerase</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Real polymerase</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

### Pol I

Eventually, in 1955, Kornberg succeeded in purifying an enzyme with the right behavior, and called it DNA polymerase I. This is often termed pol I, or Kornberg’s enzyme.

### Problems with pol I

- Very quickly, people started to question whether this really was the enzyme used by the cell to replicate its DNA.
- Several problems arose, all of which were cause for concern.
Speed

It was far too slow

Direction

Pol I can only add a nucleotide triphosphate to the 3'OH group end of a strand.
Exonuclease activity

Pol I shows both 3’ and 5’ exonuclease activity.

Cannot initiate

Pol I can only add a base to an existing DNA strand.

A better polymerase

Eventually, in 1969, John Cairns isolated a mutant of *E. coli* that was almost totally lacking pol I, but which replicated its DNA normally. When he looked for polymerase activity in this mutant he found a new DNA polymerase, pol III.
**Pol III**

- The best thing about pol III was that it was very fast, quite capable of synthesizing DNA at 100,000 bases/min.

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**Problems to be solved**

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  - how new strands can be started

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  - how replication can proceed in both directions
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So, we need to understand:

- how new strands can be started
- how replication can proceed in both directions
- why the polymerases have exonuclease activity.

The answers to all of these came from the work of one person.

Reiji & Tsuneko Okazaki

In the mid 60's the Okazakis started to look at what happened to new DNA molecules as they grew.
Okazaki fragments

What he discovered was there were two distinct processes.

Continuous vs discontinuous

The two types DNA synthesis are termed continuous and discontinuous. The first occurs on the ‘leading’ strand where the enzyme can always add to a 3’OH group. The second takes place in bursts, as the two strands separate from each other.

Replication on the two strands
Reiji Okazaki was able to show that the individual fragments need an enzyme called **ligase** which links them together, to form a continuous piece of DNA.
Making Okazaki fragments

His wife continued the work. Her work suggested that, somehow, RNA is involved in the creation of new DNA strands.

RNA is in every fragment

If Okazaki’s experiment is repeated with uracil (only found in RNA), the same results are seen as with thymine.

RNA in the fragments

Every fragment starts with a small RNA primer.
Why have the primer? Because the RNA polymerase (primase) can do something the DNA polymerase can’t. It can initiate a strand without needing a free 3’OH group.

Once the primer has produced a free 3’OH end, pol III can add DNA very rapidly and accurately.

The accuracy comes from the ability of pol III to ‘proofread’.
If it senses an error then pol III uses its 5’ exonuclease to step back and remove the last base. It then inserts a new base, usually the right one. This double-checking means that pol III has an error rate of less than 1 in 1,000,000.

But we know that DNA doesn’t contain RNA; therefore the RNA primers must be removed, and replaced with DNA. So, we need an enzyme that can do both: Kornberg’s enzyme!
Kornberg’s enzyme in action

Its 5’ exonuclease removes the RNA: its polymerase adds DNA: its 3’ exonuclease acts in proofreading.

Starting replication

So far we have seen how the process of replication takes place once the DNA has separated and the polymerases are in place.

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But how does it all start? There must a time and place where the entire sequence is initiated.
In bacteria there is a specific site on the chromosome where replication always begins. It is termed the origin of replication.

In *E. coli*, this site is a 9 base-pair (bp) sequence that is repeated four times within the region. It is AT rich, allowing easier separation of the strands.

In eukaryotes each chromosome may have many origins of replication.

A protein (dnaA) binds to the origin of replication and starts to unwind the helix. It attracts other proteins to form an initiation complex.
Initiation of replication

One of these (helicase) unwinds DNA strands using ATP energy and moves processively (i.e., does not leave the DNA until replication is finished; it encircles the DNA strand) in the 5'-to-3' direction along DNA.

Other key proteins

SSB (single-strand binding protein) does not itself unwind DNA, but binds to and stabilizes unwound single-stranded DNA.

Other key proteins

As the helicase unwinds the DNA it creates a strain which must be relieved. This is the role of an enzyme termed gyrase.
Gyrase

DNA is HELICAL

* each molecule with helix wraps about the other once every 10 bp
* *E. coli* chromosome = 4,600,000 bp
* hence, 460 000 twists
  - each & every twist must be removed!

Proteins involved in replication

![Proteins involved in replication](image)
Proteins involved in replication

- **Promerase** synthesizes the RNA primer
- **DNA polymerase III** extends the leading strand
- **Sliding clamp** holds DNA polymerase in place during strand synthesis

**Table 14.1: Proteins Involved in DNA Synthesis**

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leading strand</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primase</td>
<td>Synthesizes RNA primer</td>
<td>Taps DNA polymerase III for synthesis of RNA primer</td>
</tr>
<tr>
<td>DNA polymerase III</td>
<td>Extends DNA strand on nascent DNA polymerase III</td>
<td></td>
</tr>
<tr>
<td>Sliding clamp</td>
<td>Holds DNA polymerase III in place during strand synthesis</td>
<td></td>
</tr>
<tr>
<td>DNA polymerase I</td>
<td>Removes the RNA primer and replaces it with DNA</td>
<td></td>
</tr>
<tr>
<td>DNA ligase</td>
<td>Catalyzes the joining of Okazaki fragments for a continuous strand</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 14.1**

1. DNA is opened, unwound, and primed
2. RNA primer synthesizes RNA primer
3. RNA primer synthesis is under way
4. Helicase opens double helix
5. Single-stranded DNA (SSD) invades single strands
6. Sliding clamp holds DNA polymerase in place
7. DNA polymerase III moves in a 3' → 5' direction, synthesizing leading strand
For eukaryotes, who have linear chromosomes, there is a big problem. The polymerase cannot replicate the very end of each chromosome, since it will fall off before completion.
CHROMOSOME SHORTENING DURING NORMAL DNA REPLICATION

3. DNA polymerase synthesizes the last Okazaki fragment in lagging strand.

4. No DNA synthesis occurs after primer is removed (no free 3' end for DNA polymerase); chromosome is shortened.

DNA polymerase

Last Okazaki fragment

TELOMERE REPLICATION

1. When the RNA primer is removed from the 5' end of the lagging strand (see Figure 14.15), a strand of parent DNA remains unreplicated.

2. Telomerase binds to the "overhanging" section of single-stranded DNA. Telomerase adds deoxyribonucleotides to the end of the parent DNA, extending it.

3. Telomerase moves down the DNA strand and adds additional repeats.

4. Primase, DNA polymerase, and ligase then synthesize the lagging strand in the 5'→3' direction, restoring the original length of the chromosome.

5. Missing DNA on the lagging strand

6. Telomerase with its own RNA template

Figure 14-14-2

Figure 14-15
TELOMERE REPLICATION

3. Telomerase moves down the DNA strand and adds additional repeats.

4. Primase, DNA polymerase, and ligase then synthesize the lagging strand in the 5′ → 3′ direction, restoring the original length of the chromosome.

WHAT IT LOOKS LIKE

This computer animation is running about 50x slower than the actual process.

LAB USES FOR REPLICATION

Our understanding of the molecular process has given rise to two great tools:

- Polymerase Chain Reaction (PCR)
- DNA sequencing
PCR

- Invented by Kary Mullis in 1983.
- It allows us to make many copies of a piece of DNA in a short time.

So, if you have a very small sample of DNA (i.e., from a crime scene) you often find yourself having to destroy it all in a single experiment. PCR allows you to make literally billions of copies that you can then use in a variety of experiments.

How PCR works

- It uses the ability of pol III to copy an existing molecule. Then you use it to copy both molecules, then to copy all four etc.
- Every cycle doubles the number of DNA molecules so, after 50 cycles, you have approximately 1,000,000,000 copies.

The mechanism

- The starting point is a DNA sequence of interest. A short (15-20 bases) sequence on either side of the target is synthesized in huge numbers. These will be the primers for the polymerase.
- The DNA is heated to separate the strands and primers are added
- As it cools the primers will hybridize with the DNA
- Pol III adds DNA to the primers and copies both strands.
- Reheat the DNA and repeat
The process

DNA sequencing

Originally it was almost impossible to determine the order of bases in a piece of DNA, at best a dozen or so might be identified. Since a typical gene is close to 1000 bases long, this wasn’t very useful.

A technique was developed that overcomes most of the problems.

Dideoxy bases
Normal addition of bases in DNA

Chain termination with dideoxy bases

Dideoxy reaction mix

Note that most of the nucleotides have the normal deoxyribose. A small percentage (~10%) of each nucleotide has dideoxyribose. These bases are labeled with a fluorescent dye. In this example, Guanine, Cytosine, Adenine, and Thymine. If a DNA molecule contains one of these dyes, it will glow the corresponding color.
Reactions within the tube

Each time the polymerase adds a complementary base to the growing chain, there is a chance that it will add a ddBase, and that chain stops growing.

Since there are millions of copies of the DNA molecule in the tube, most will proceed to the next base, where the two possible outcomes take place.

Over time the polymerase will leave a series of pieces, each terminating with a ddBase that glows with its specific color.

Reading the gel

Since the strands without a ddBase have no dye, they will not show up.

Human genome project

A single gel can only manage about 1000 bases. To get the entire 3,000,000,000 bases in the human genome it was randomly cut into 1000 base pieces and each sequenced. Then overlapping areas were found and linked together until the entire sequence was established.