1 Chromatin

1.1 Heterochromatin

- Highly condensed chromatin
  - Meiotic and mitotic chromosomes (no tangles, no breaks)
  - Centromeres (central portion of a chromosome) and telomeres (the ends of linear chromosomes)
  - One X chromosome in human females (Barr body, which is not transcribed)
- Heterochromatic regions of interphase chromosomes are areas where gene expression is suppressed. Since the DN is very condensed, the information cannot be accessed. Some regions in during the interphase are thus compact, some are not so much.

1.2 Euchromatin

- Relatively non-condensed chromatin
- Euchromatic regions of intrephase chromosomes regions where genes tend to be expressed.
- The reversible switching from euchromatic to heterochromatic regions is modulated by covalent modification of histones, the presence of chromatin remodelling complexes and RNA polymerase (transcription) complexes.

There are several implications of existence of condensed chromatin for gene expression:

- the interphase chromosomes are in discrete regions of the nucleus
- The expressing gene within the chromatin can be re-oriented. If there is a need to express a gene, transcription can happen by decondensing particular regions of a chromosome. If the chromatin should not be expressed, everything stays condensed.

  For example, chromatin can be remodelled to form loops in order to access to DNA.

1.3 Transcription Factories

Transcription factories are regions of the nucleus that have lots of RNA polymerase, lots of substrates, lots of materials for transcription.

Question. What are the advantages to the cell of being able to package DNA into a heterochromatics state?

1.4 DNA Replication

DNA replication is important.

Question. Is DNA repllication conservative or semiconservative?

Before the cell division, a cell is a mother cell (or parental cell). Afterwards, they are daughter cells.

In the conservative model, when the parental cell divides, one of the cells have the same strands, the other has all the new strands.

In the semiconservative model, one of the strands in each daughter cell is old, the other one is new.

Only the semiconservative method has been observed.

Question. What is the direction of the DNA replication?

Note the following:

- DNA is antiparallel.
- New DNA is synthesised from five' to three'
- The template is read 3' to 5'.

This implies that three modes of behaviour are possible:

- Unidirectional growth of single strands from two starting points (for example, linear viruses use this technique)
- Unidirectional growth of two strands from one starting point. The leading strand is in the same direction as the separation. The other strand is called a lagging strang. (for example some plasmids use predominantly this method).

  The fragments produced are called **Okazaki fragments**.
- Bidirectional growth from one starting point.

  There are two replication forks, each leading a leading and a lagging strand. (for example, eubacteria and bacteria use this method.

Question. Where does DNA replication start?

There are two possibilities:

1. Always starts from the same location on DNA.

   What are some of the characteristics of the sequences at replication origins?

   - Easy to open, A-T rich
   - Recognized by and bound by indicator proteins
2. Random

There is a single origin of replication in bacteria, while multiple in eukaryotes.

Question. How does DNA replication proceed in bacteria?

The style of replication only apples to circular genomes

Question. What happens at the DNA replication forks?

With time there are more DNA separation, and also Okazaki fragments occuring which stitch to the leading strand. The replication fork is assymetrical, and the leading strand is replicated continously, while the lagging strand is replicated discontinuosly.

1.5 Overview of DNA replication

1. Separate the DNA strands
2. Synthesise the DNA
3. Proofread newly synthesized DNA

Ingredients for synthesis: origin, primers, dNTPs, ATP (as an energy source), DNA polymerase, accessory proteins

1.6 DNA Synthesis

This reaction is catalyzed by DN polymerase.

This reaction relies on accurate base pairing to make the DNA.

First, take a primer. A primer is the first part of the growing polypeptide chain.

The appropriate base pairing will induce the dNTP to catalyze the reaction which will cut off the pyrophosphate group that, in turn, is gradually degraded.

Steps in the bacterial DNA replication are as follows:

1. origin of replication

   Initiator proteins for replication in E.coli bind to origin and help the helicase to bind, for which ATP is required.

2. binding of initiator proteins

   First, primase binds to helicase, forms a primosome, to which the rest of the replication machinery binds.

3. unwinding by helicase, brought by a helicase-loading protein

   Helicases unwind and separate strands. The predominant helicase goes 5'-3' along the lagging strand template.

   There are 5 subunits in each helicase.

4. binding of single-strand binding proteins

   Following the action of helicase, single strand binding proteins keep DNA strands separated.

   A helicase protein separates the strands by binding ssDNA and prevents strands from H-bonding.

5. RNA primers made by primase

   In order to beign, DNA polymerase requires a bound primer.

   The purpose of the primase in replication, which proceeds in the direction 5'-3', is to synthesise an RNA primer.

   Note that primase, DNA primase and RNA primase are all the same.

6. DNA polymerase

   Close to fingers and in the palm of the DNA polymerase is the synthesis centre.

   If there is a mismatch of pairs, 3'-5' exonuclease site in the wrist area will chop it off.

7. sliding clamp (also known as a β clamp) holds polymerase onto DNA

   Polymerase does not hold to the strand, so sliding clamps keep the polymerase catalyzing the reactions.

8. nick sealing by DNA ligase

   A special DNA repair system is responsible for removal of the RNA primer and replacing it with a correctly matched DNA sequence.