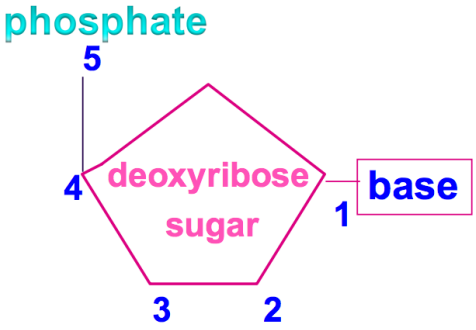
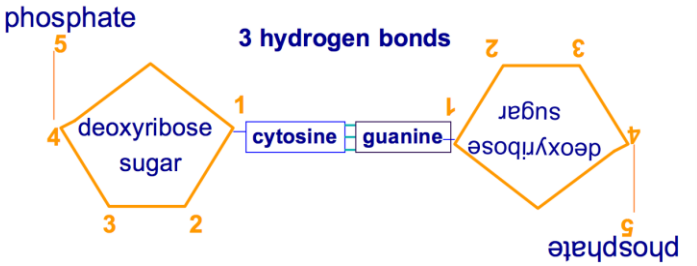
STRUCTURE OF A NUCLEOTIDE

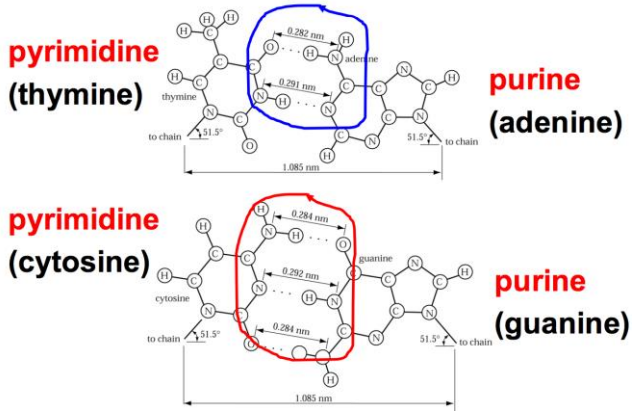


- Consists of three parts: Deoxyribose sugar, a phosphate group and a nitrogenous base.
- Adenine (**purine),** Cytosine, Guanine (**purine),** Thymine
- **Purine:** 2 carbon rings of nitrogen-containing
- **Pyrimidines:** 1 carbon ring
- **Phosphate** is attached to the 5-carbon end and the **base** is attached to the 1-carbon end

- The two strands are antiparallel but complementary
- Adenine pairs with Thymine
- Guanine pairs with Cytosine
- The bases are joined by hydrogen bonds
- 5-carbon base is attached to the 1-carbon
- the 5 or 3 determines whether the phosphate is up?
- 5-prime end on one strand is opposite to the 2-prime end on the other → **antiparallel**
- on one strand, the 5-carbon is up and the other strand is down (that's the antiparallel nature)



- **triple bond** between GC and **double bond** between AT
- the bond between the phosphate and sugar is called a **phosphodiester bond** which is really hard to break



Where are genes found?

- Mostly located in **chromosomes**, but also in mitochondria, chloroplasts and plasmids
- The position of a gene on a chromosome is called a **locus.**

Where are chromosomes found?

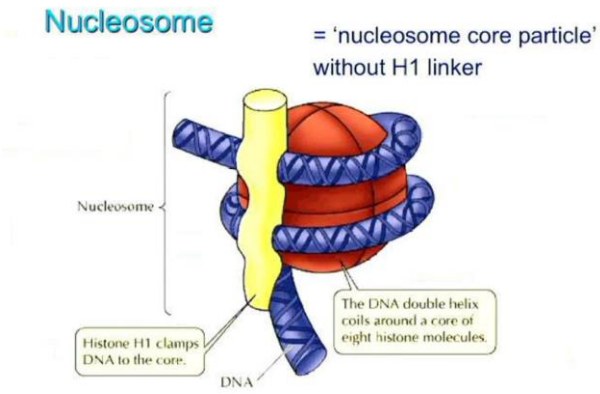
- Prokaryotes: in the cytosol
- Eukaryotes: in the nucleus

What is a chromosome?

- Consists of DNA and protein
- Can be single stranded (one molecule of DNA)
- Or double stranded (Two molecules of DNA) → sister chromatids
- DNA + protein = chromatin (chromosome but not visible)
- Chromosomes are only visible during cell division
- Chromosomes become visible during mitosis
- During S-phase that the replication occurs
- DNA is found in association with histones in chromosomes – nucleosome

Nucleosome:

- H1 clamps DNA
- Wound around the protein to form the 'beads on a string' look



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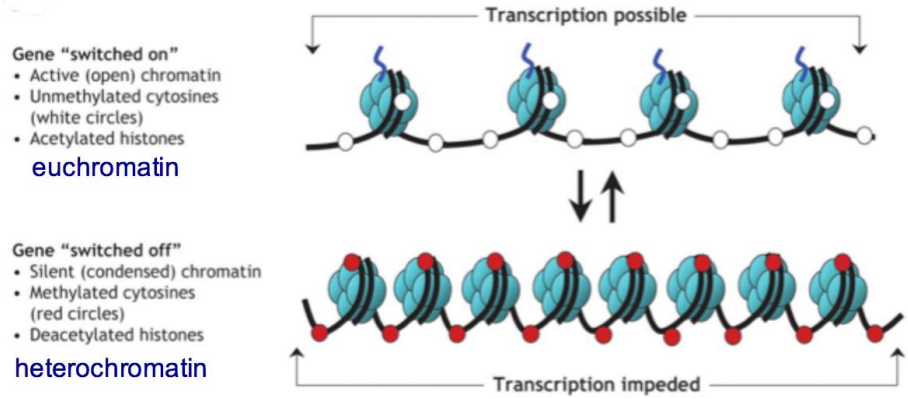
Histone Modification – A way of regulation gene expression

- Genes are switched on and off so they are not active in all cells at all times



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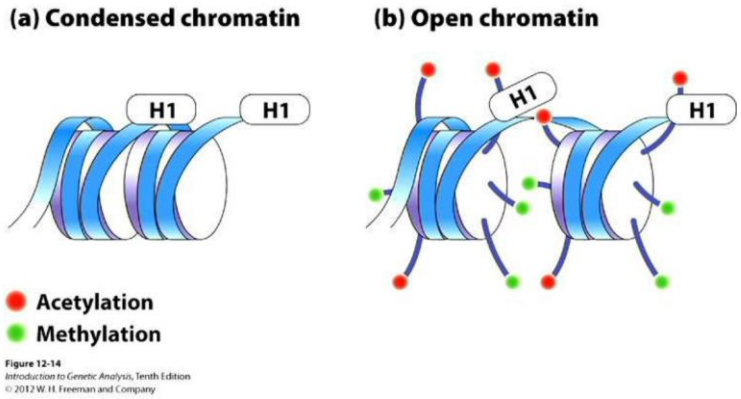
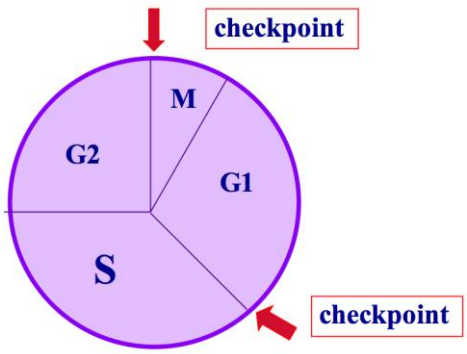
- chromatin can be modified by acetylation or methylation
- when the chromatin is strung out, then it allows the DNA between the nucleosomes to be expressed → euchromatin
- when tightly packed, the enzymes cant get to the DNA to express the gene, so we 'silence' the gene
- Chromatin can be modified by acetylation or methylation

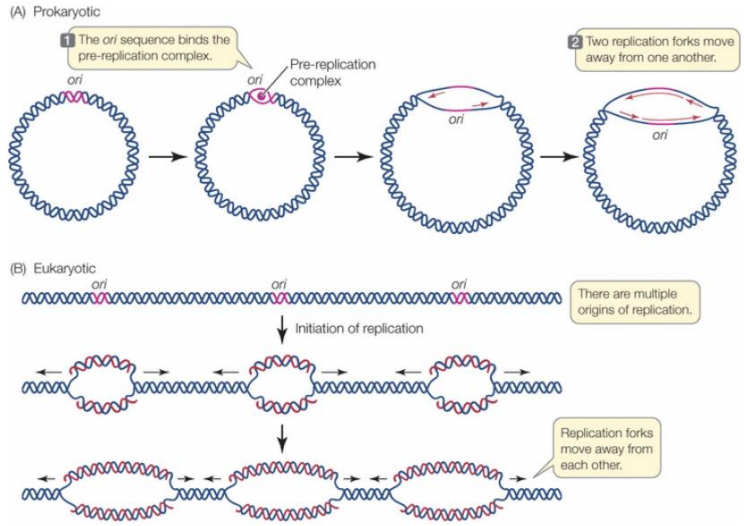


DNA REPLICATION

- DNA replication occurs during the S (synthesis) stage of the cell cycle
- Chromosomes go in double stranded
- At the end of mitosis, the chromosomes are single stranded
- Come out of the S phase as double stranded as the DNA has replicated
- Semi-conservative (half-conserved) → half of molecule is saved and used to create new molecule
- Each strand is a chromatid at the end of S-stage and the end of G2



- **Prokaryote**: circular chromosome
- In a **Eukaryote:**



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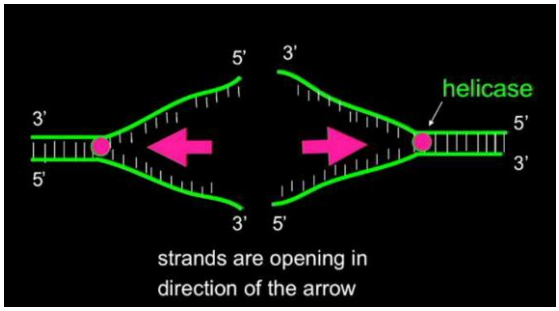
- along the chromosome, there are many regions that replication starts

DNA REPLICATION IN E.COLI

- Helicase separates strands of DNA so that we are exposed to the bases
- DNA hates being double stranded, so it will snap shut to be double stranded again
- So in come a battery of proteins that link to the DNA and keep it single-stranded and keep it open for the replication
- As we open it up, it causes tension down the molecule. So to relieve the tension, another protein comes in called DNA Topoisomerase (gyrase)
- It makes little nicks in the DNA to relieve the tension and joins it back up (prevents the DNA strand becoming twisted during unwinding)
- The clamp loader loads the sliding clamp at intervals along the lagging strand, DNA polymerase I fills in any gaps after removal of the RNA primers and DNA ligase ligates the Okazaki fragments on the lagging strand
- Two enzymes: gyrase and helicase
- Then we have specialised proteins
- DNA polymerase III which doesn't start replicating unless we have a short double-stranded region to start replication
- We have primer RNA to start a double-stranded section
- Enzyme can only work in one direction
- Can only read the DNA template 5' to 3'.
- Can only add bases to the 3' end
- This is called the leading strand as it can only be replicated on the end of the 3'
- We have to wait for the molecule to open up, replicate, open up more, replicate out
- We need another primer to replicate
- The lagging strand is replicated in fits of starts
- DNA polymerase III makes short sequences called okazaki fragments
- Lots of primers added by primase to start replication
- Beta-clamp keeps the DNA polymerase on the strand
- We end up with all this RNA that needs to be removed by DNA polymerase I
- **Leading strand:** 5' to 3'
- **Lagging strand:** 3' to 5'

LECTURE 3



- The clamp is a complex of proteins in the shape of a donut which keeps the DNA polymerase in place and increase efficiency of replication
- Primers are removed
- Gaps are filled with complementary bases
- Okazaki fragments are joined
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DIFFERENCE BETWEEN PROKARYOTIC AND EUKARYOTIC DNA REPLICATION

- **Speed:** its faster in prokaryotes than eukaryotes
- Humans: 3000 bases per min
- Bacteria : 30, 000 bases per min
- Okazaki fragments are longer in prokaryotes than eukaryotes
- **Enzymes:**
  → Prokaryotes: DNA Polymerase III
  → Eukaryotes DNA Polymerase: Alpha (initates DNA replication in association with primase, Delta (replication of lagging strand), Epsilon (replication of the leading strand) and Gamma (replication of the mitochondrial DNA)

THE LAGGING STRAND

- Need DNA exposed
- In comes primase and adds the RNA primer
- In comes DNA polymerase III
- We need to get rid of the RNA and tahts the DNA polymerase I

ERRORS IN DNA

- Errors during replication can be repaired on the spot; the DNA polymerase can go backwards 3' → 5' and cut out an error and replace the base
- When it goes forward it polymerases (adds bases) when it goes backwards it excises and edits DNA
- 1. During DNA replication, an incorrect nucleotide may be added to the growing chain
- 2. The proteins of the replication complex immediately excuses the incorrect nucleotide
- 3. DNA polymerase adds the correct nucleotide and replication proceeds
- mistakes can be mended on the spot

Two types of DNA repair:

a) Mismatch repair

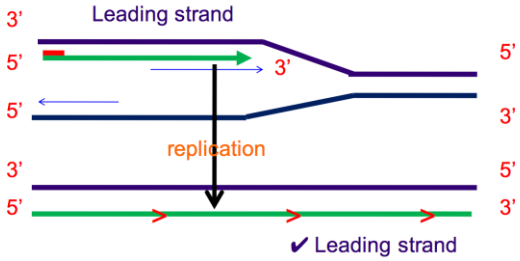
1. During DNA replication, a nucleotide was mispaired and missed on proofreading
2. The mismatch repair proteins excise the mismatched nucleotide and some adjacent nucleotides
3. DNA polymerase I adds the correct nucleotides

b) Excision repair

1. A nucleotide in DNA is damaged
2. The excision repair proteins excise the damaged nucleotide and some adjacent nucleotides
3. DNA polymerase I adds the correct nucleotides by 5' to 3' replication of the short strand

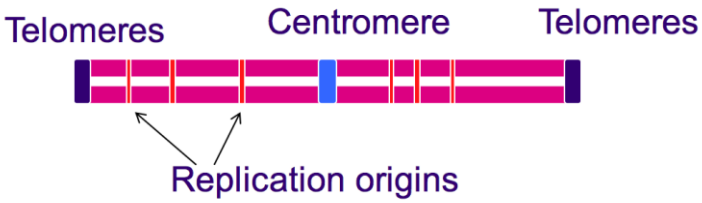
Chromosomes:

- Require 3 elements to function
- **Telomeres: structural stability**
  What happens to telomeres during DNA replication?
  → remember DNA polymerase adds nucleotides to the 3' end
  → DNA polymerase requires a double stranded molecule to start – so primers added
  → one strand has discontinuous replication and each piece (okazaki fragment) requires a primer
  → a problem occurs on the lagging strand ends of chromosomes
  → repeated sequences at telomere eg. Human TTAGGG, repeated about 2500 times, Arabidopsis (plant) it is TTTAGGG
  → protect ends of chromosomes by looping or recruiting protective proteins
  → problem however with replication of lagging strand
  → over time the ends degrade and genes are lost and the cell dies
  → **Telomere replication:** replication at the ends of chromosomes



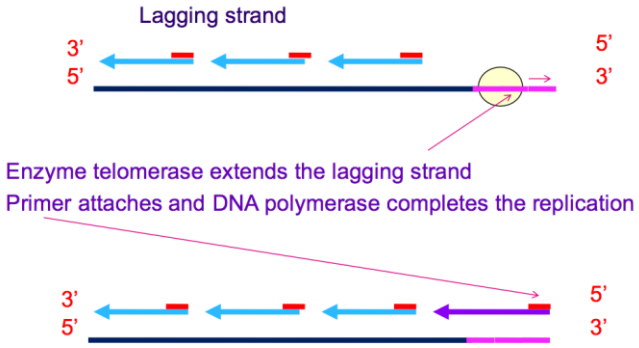
→ replication at the ends of chromosomes: lagging strand a problem not enough DNA template for primer.

- Centromeres: essential for segregation at cell division (acentric fragments gt lost on the spindle)
- Origins of replication: DNA replication start point



Telomerase and Aging

- Telomerase is absent from most cell types
- Unreplicated DNA is degraded so telomeres shorten with age
- Lose around 50-200 bases each round of replication
- Genes on the ends of the chromosomes are lost so the cell dies



- In some cells, **telomerase enzyme** is available
- Telomerase extends the lagging strand
  → primer attaches and DNA polymerase completes the replication
- Telomerase has an inbuilt RNA part which is complementary to the telomere sequence
- The RNA part is the template for the extension.