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BCMB2001 Lecture notes (Part One)

Week 1 lecture 1: Introduction to the Central Dogma

- DNA and RNA are polymers made of nucleotides
- All nucleic acid copying occurs in the nucleus
- Most eukaryotic genes have non-coding regions made of small nuclear RNA (~98%) known as introns. Found as big sections in the middle of genes
- Micro RNA regulates the rate of translation
- Retroviruses largely carry out reverse transcription
- Uracil has no CH3. Adding CH3 turns into Thymine to prevent confusion with corrupted cytosine.

Because translation and transcription don't occur together there are lots of different options for gene regulation

The base sequence of DNA does not affect the overall absorbance because all four bases absorb approximately the same. If double stranded nucleic acid absorption is less it is because bases are stacked together so covered from photons.

Week 1 Lecture 2: Prokaryotic DNA Replication

Aims

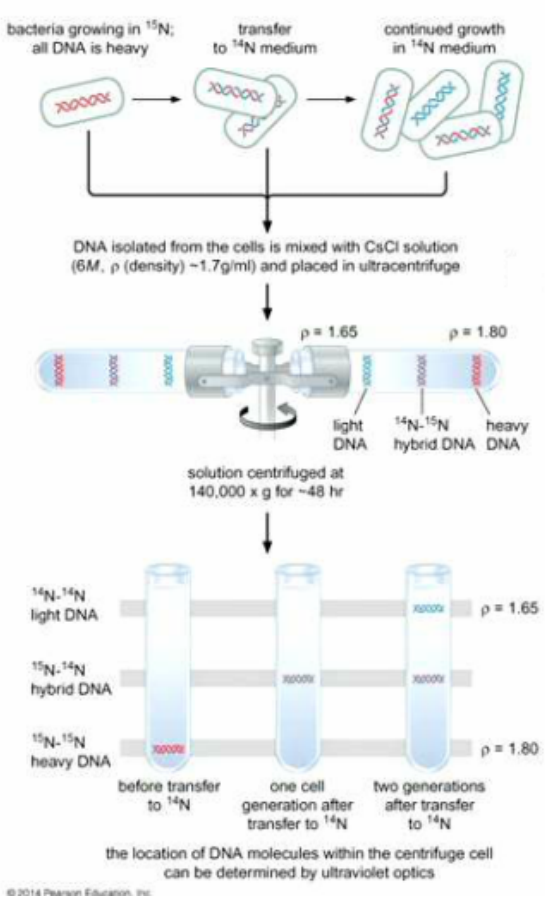
1. Review the concept of DNA replication as presented in BIOL1007
2. Outline the specific processes by which DNA is replicated in E.coli
3. Identify the enzymes involved in prokaryotic DNA replication and describe their activities

Semi Conservative replication: Meselson Stahl

How do we know that the semi-conservative model is correct?

Two other proposed models for replication were conservative (double helical structure produces a new one which does not incorporate any of the template) and dispersive (Parts of the DNA are copied and then pieced together ending up with a hybrid molecule with composites of the old one scattered through both new molecules.

Meselson and Stahl grew E.coli in media containing heavy ^{15}N isotope so all the DNA in the bacteria contained the heavy nitrogen



It was then switched to a medium containing the normal isotope, ^{14}N and allowed to grow.

DNA was isolated at different points, initial and after one and two generation.

The DNA was then run on a caesium chloride gradient. Spinning DNA in the caesium chloride results in a density gradient with heavy molecules in the bottom of the tube

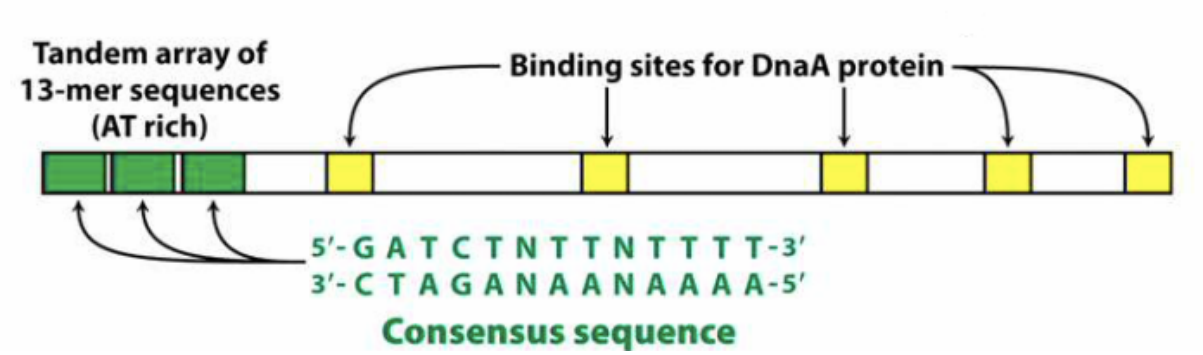
UV light absorption could be used to identify the DNA in the tube. Density centrifugation showed that the initial DNA was the heaviest but after one generation it became lighter, with the second generation made up of lighter and lighter still strands (two bands).

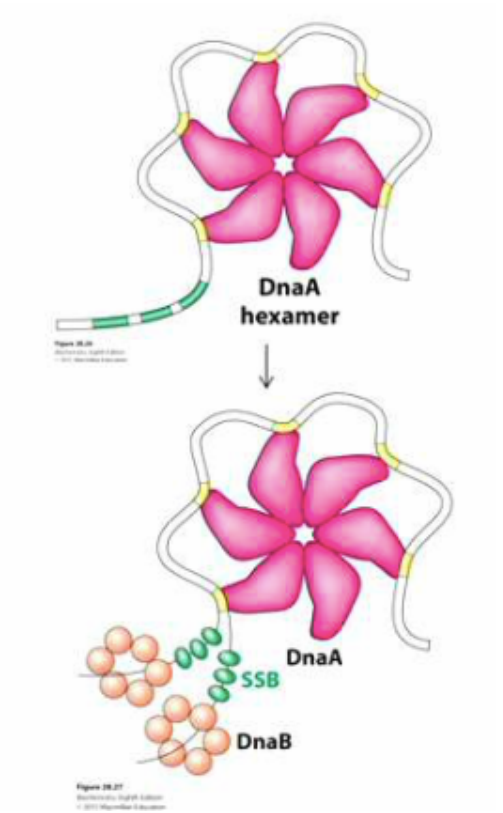
DNA Replication in E. coli

E. coli has a circular genome with 4.6 million base pairs. Replication occurs at a single point known as the origin of replication or OriC locus.

The first stage of replication is called initiation where the OriC is activated. The OriC consists of two repeated motifs

1. 9-mer motif: Sequence of 9 nucleotides present in several copies. In E. coli there are five repeated motifs for recognition by the *origin recognition protein* (*DnaA*) where it makes specific contact with those sequences
2. 13-mer motif: 13 nucleotides repeated 3 times. AT rich (lots of A and T base pairs) because they only have two hydrogen bonds so are easy to tear apart





In order for replication to happen, the origin recognition protein (DnaA) binds to the yellow sequences. It also then interacts with itself to form a complex. The formation of the complex and bending of the DNA in the position below facilitates the separation of the strands for DNA replication to proceed

Helicase (DnaB) is then recruited and wraps itself around each DNA strand, continually separating the strands allowing for replication to occur

Single stranded DNA binding proteins hold the DNA separate after it has been pulled apart by DNA by binding to DNA to prevent re-annealing

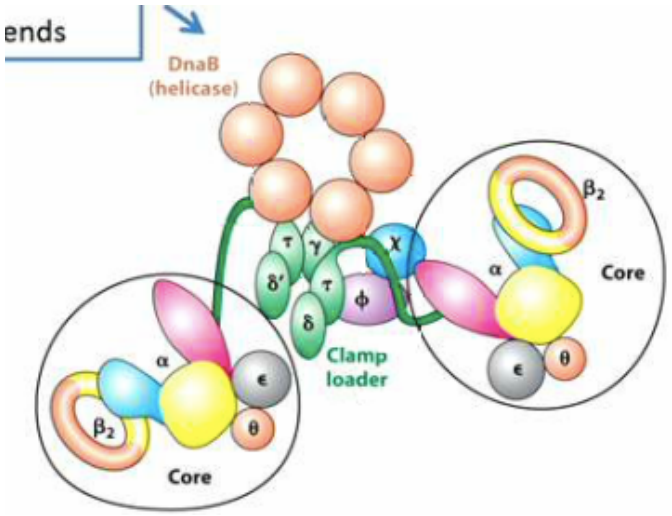
Primase then lays down an RNA primer.

Next, *DNA Polymerase III* assembles, and replication commences.

Replication is bi-directional and occurs with two replication forks moving away from the OriC.

Helicase is always associated with the DNA Pol III as it lays down new nucleotides

The alpha subunit of DNA Pol III adds the nucleotides. The holoenzyme (whole enzyme) however does other things as well as synthesis.



The beta subunit (sliding clamp) keeps the polymerase attached to the DNA. This is because DNA polymerase needs to be attached to the substrate (template strand) even after the first reaction is catalysed. This allows the enzyme to have a high processivity (ability of enzyme to catalyse many consecutive reactions without releasing substrate) because it can stay associated with DNA for thousands of base pairs

DNA polymerases can only synthesise the leading strand (5' to 3') and thus produces Okazaki fragments in the lagging strand as it cannot synthesise in 3' to 5' direction.

The RNA primer is then removed by *RNase H* (Ribonuclease). *DNA Polymerase 1* provides the 5' to 3' exonuclease activity that removes the last part of the RNA primer on the lagging strand.

DNA pol 1 also fills in the gap left by the primer. *DNA ligase* then fixes the new primer bit to the back bone because pol 1 cannot fix the new part to the existing backbone (it only joins to the existing 3')

Week 1 Lecture 3: Prokaryotic Replication cont.

Fidelity of DNA Replication

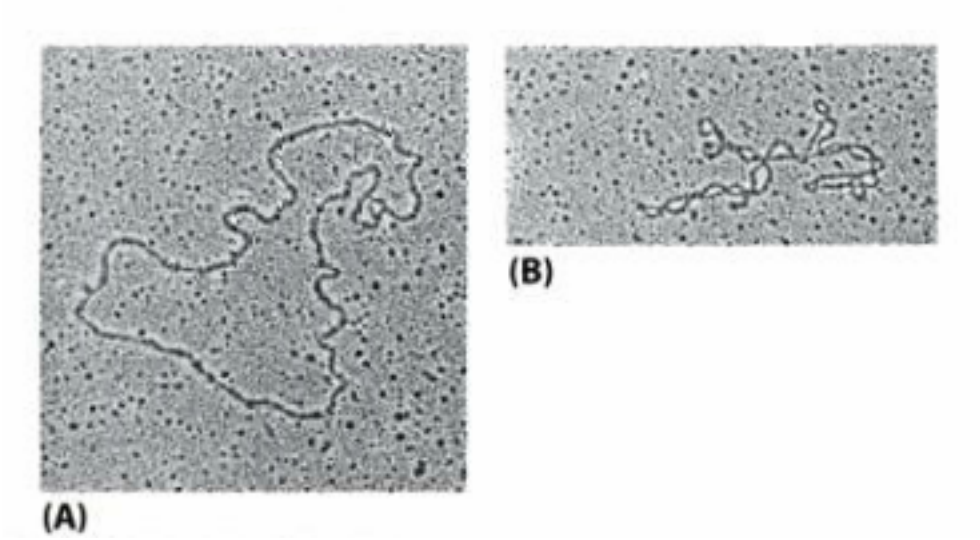
It is vital that DNA replication produces DNA duplexes that are identical in sequence to the parent DNA duplex, i.e. there are no mistakes. If mistakes are made this might have consequences to the functional level of the protein.

This fidelity is carried out in many ways

- DNA polymerases select the correct base for incorporation.
- DNA Pol I and III have proofreading activity so can remove misincorporated bases. (occurs in 3' to 5' direction: 3' to 5' exonuclease activity. "*nuclease*": any enzyme that can degrade nucleic acid, "*exonuclease*": cuts back from the end of the DNA strand "*endonuclease*": can cut within the DNA strand)
- Other DNA repair mechanisms (mismatch repair machinery) can remove the mismatched bases. Distinct from exonuclease activity because it can happen anywhere on the DNA strand instead of just the end

Challenge of Supercoiling

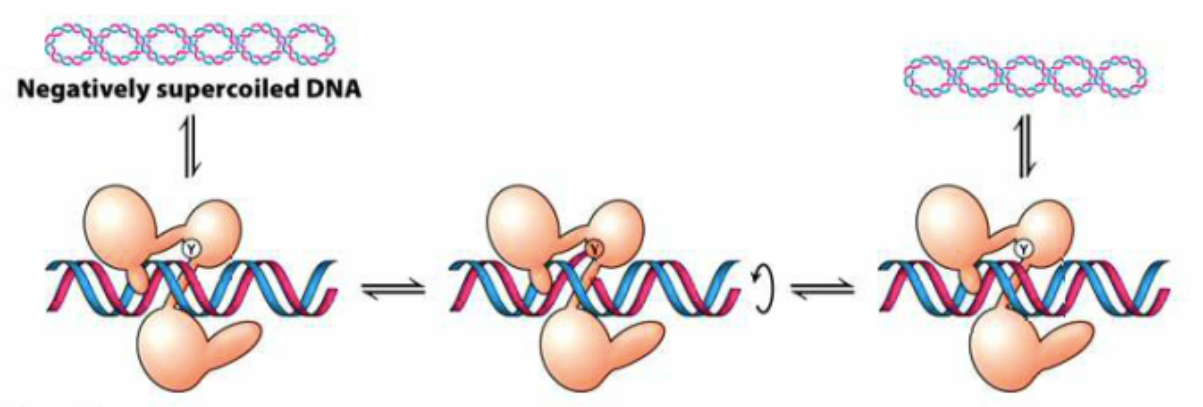
Bacterial DNA is negatively supercoiled (B)- twisting overlayed over the circular genome, allowing for compaction of genome to fit within the cell. Negative refers to direction of the twists.



Topoisomerases introduce or eliminate supercoils.

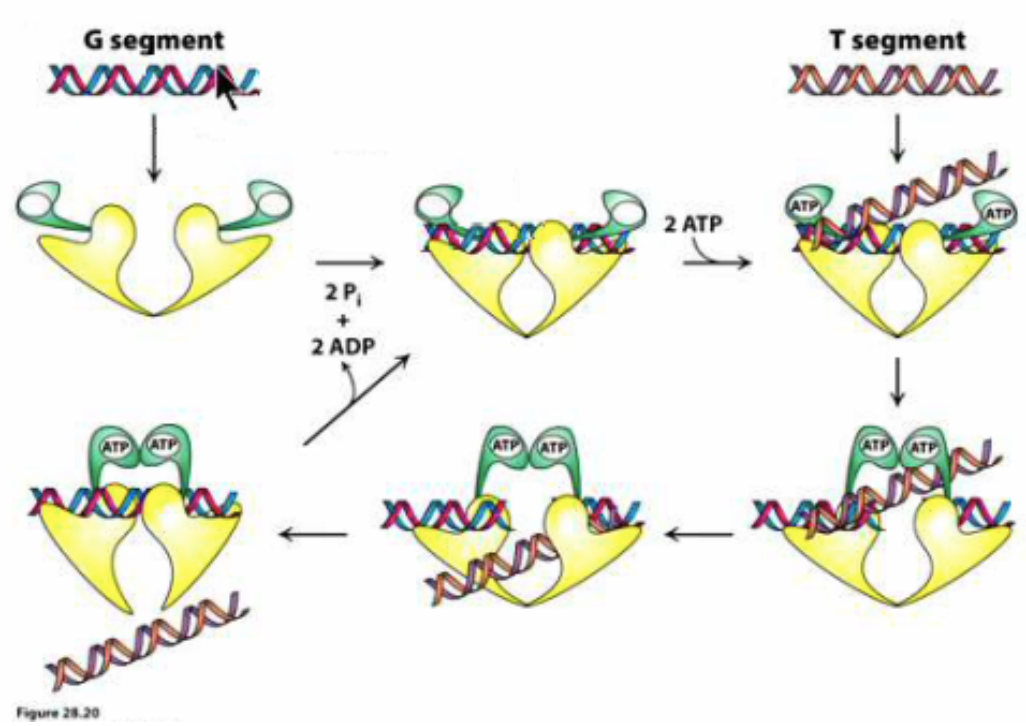
Type I topoisomerases relax supercoiled DNA by eliminating supercoils. They do this by cutting **one** of the strands, allowing some of the DNA to pass through the break, and then sealing the gap again.

Firstly, the topoisomerase attaches to the DNA and cleaves a certain site. One of the amino acids in the type I topoisomerase holds onto the fragmented end of the DNA and lets the rest of the strand swivel around, allowing for a change in the number of coils in the DNA structure.



Type II topoisomerase cleaves **both** of the DNA strands to add negative supercoils, contributing to the packaging of the genome for storage in the cell. (details of mechanism are not vital)

Basic mechanism is that both strands of part of the genome (G segment) are cut, and then a different part of the same genome (T segment) is allowed to pass through the gap. The gap is then resealed, forming a coil.



During replication opening DNA by helicase introduces local positive supercoils into the DNA. Introducing negative supercoils by the topoisomerase counteracts this supercoiling to keep the strand straight for replication.

Type II topoisomerase also allows for *decatenation* at the end of the replication process (separating the two circular genomes).

Properties of DNA Polymerases

Enzymatic activity	**DNA Pol I**	**DNA Pol III**
5' to 3' polymerase	✓	✓
5' to 3' exonuclease	✓	
3' to 5' exonuclease	✓	✓