HIGHER ORDER GENETICS

9. ORGANISATION OF DNA IN MICROBIAL GENOMES

What is the diversity of genomic structure observed in different microbes
Size of genomes:
Virus > Bacteria > Archaea > Eukaryotes

Viral genomes are very complex and your lecturer seems to be infinitely fascinated by it. Viruses are not considered life forms, because they cannot replicate on their own. Instead, they need to hijack their host's cellular machinery. In fact, viruses are considered inactive whilst outside a host.

But that doesn't mean they don't have genes! Viruses are simple structures. They have protein coats that house their genetic material, which can either be DNA or RNA. Once they attach themselves onto a host, they inject their genetic material into the host, ready for replication.

There are two different types of viruses:
1. RNA viruses: dsRNA, ssRNA (e.g Influenza, HIV, poliovirus)
2. DNA viruses: dsDNA, ssDNA (e.g Smallpox, giant viruses)

Bacterial genomes have single circular chromosomes (E. coli, Haemophilus influenzae), two circular chromosomes (Vibrio cholerae etc…), or linear chromosomes (Borrelia burgdorferi).
In lots of cases, bacteria may have multiple types of chromosomes (e.g Agrobacterium tumefaciens have circular and linear chromosomes).

Bacteria often have a *main chromosome* (one or two; circular or linear), which contains its essential genetic material needed for growth and division, and *extrachromosomal plasmids*, which do not contain essential genes but may give them a selective advantage to grow in certain conditions.

In some cases, bacteria may actually have viral DNA that was integrated into its genome when it was infected by a bacteriophage (transduction).

Bacteria also has *transposable elements* which will be covered in subsequent lectures.

Bacterial plasmids replicate autonomously, and independently of the main chromosome. Thus, a bacteria can have multiple copies of the same plasmid. The number of times a plasmid replicates is genetically coded by the plasmid itself, - so the origin of replication "knows".

How can DNA be transferred between microbial organisms

Vertical transfer: DNA on the chromosomes including mobile DNA, plasmids, and some bacteriophages can be passed on from the parent to the daughter cells.

1. Transformation: Bacteria take up free DNA fragments (plasmids) in the environment. They must first be made competent as very few bacterial species are naturally competent. This is horizontal gene transfer.

   Making cells competent
   There are two methods to make cells competent as most species are not naturally competent.
   1. Chilling cells, and the treating them with Mg and Ca ions to increase permeability.
   2. Electroporation Discharge electric current to temporarily make the membrane permeable.

2. Transduction: Bacteriophage (Virus) inserts DNA through infection, which uses the host's cellular machinery to replicate. During this process, it may incorporate the hosts DNA into its own genome. Once the bacteria lyses, the bacteriophage will continue to infect more bacteria, thus injecting the original bacteria's DNA into other bacteria.
3. Conjugation: Donor cell projects a sex pilus to the recipient cell. One strand of the F plasmid from the donor cell is nicked and transferred to the recipient cell. Each bacteria will then produce a complementary strand, hence each having a copy of the F plasmid.
4. Transfection: Foreign DNA can be introduced into a cell by housing it in liposomes and the action of membrane fusion. Alternatively, a gene gun can be used to fire DNA coupled with an inert solid directly into the nucleus.

How can we exploit these systems in the context of our own experimental approaches

In order to see whether a cell has taken up a gene of interest, we can also make them take up a gene that allows them to be differentiated from untransformed cells (e.g antibiotic resistant genes).

10. CHROMOSOME STRUCTURE AND FUNCTION

How DNA is organised in both prokaryotes and eukaryotes
First, what I find so remarkable is that each chromosome is a single piece of DNA. It's a lot of genetic information wound up in such a compressed form. Eukaryotes are diploid, meaning they contain two sets of chromosomes, whilst prokaryotes are haploid, meaning they contain only one set of chromosomes.

DNA usually sits loose in the nuclease. It is only when the cell needs to divide that the DNA wounds itself up into chromosomes for ease of segregation.

So, here's the problem: Each cell has a nuclease about 6 micrometers in diameter, and it needs to house about 2m worth of DNA into it. The DNA must fit in a nucleus whose diameter is 367000 times smaller than its length. It's the same as trying to cram in 15 km of string inside a gold ball. So the level of compression is truly amazing.

What's more is that DNA has a negatively charged phosphate group, and as we know, like charges repel each other. If they repel, they hinder compression.