6. Modern genetics- Cell control

After their formation in a multicellular organism, most new cells differentiate, becoming specialised to carry out a particular function. In doing so, the majority lose the ability to divide by mitosis.

The genetic code comprises 64 different sequences of three nucleotide bases, each of which encodes a specific amino acid. Polypeptides are made by ribosomes that assemble amino acids in a sequence that is prescribed by the base sequence of a gene. Protein synthesis involves transcription and translation. Whereas all the DNA of prokaryotes encodes polypeptides, much of the DNA of eukaryotes does not encode polypeptides.

The zygote of a multicellular animal or plant is a single diploid cell. It contains the genome, the complete set of chromosomes that are characteristic of the species and gives rise to all the somatic cells of that animal or plant. All the somatic cells produced by replication are then identical and contain the same chromosomes, raising the question of how cells can be produced for different functions in different tissues if they are all genetically the same.

Factors Affecting Gene Expression:

There are many ways in which the transcription or translation of genes can be regulated in eukaryotes, with the main five being,

- DNA regulatory sequences and transcription factors.
- Post-transcriptional modification of mRNA.
- Destruction of mRNA.
- DNA methylation.
- Histone acetylation.

The final three are said to be epigenetic modifications, meaning that they are inherited by daughter cells, but are not the result of changes to the DNA.

Regulatory sequences and transcription factors:
Only about 2% of the DNA in the nuclei of human cells form genes, the other 98% is non-coding, which only has the purpose of regulating the switching on and off of genes. Every gene is associated with a short base sequence of non-coding DNA that performs this regulatory function. There are two types of regulatory sequence.

- **Promoters-** Short base sequences that lie close to their target genes. They initiate transcription by enabling RNA polymerase to bind to the gene they regulate.
- **Enhancers-** Short base sequences that lie some distance from their target genes. They stimulate promoters causing an increase in the rate of transcription of the genes they regulate.

Both lie 'upstream' of their target genes. In order for a gene to be transcribed, RNA polymerase must be able to attach to it, and if it cannot, the gene will not be transcribed, and the polypeptide it codes for will not be produced. In eukaryotes, RNA polymerase cannot initiate transcription itself, but instead it is regulated by the gene's promoter. One or more specific proteins called transcription

example soaking the cells and plasmids together in ice-cold calcium chloride, followed by a brief heat shock at 42 degrees for two minutes. Bacteria that have taken up the plasmid are 'transformed,' and if the plasmid contained one or more entire genes, they are also 'transgenic.'

Identifying Transformed Bacteria:

As you cannot observe either the bacteria or the plasmids, it is unknown whether any plasmids contain recombinant DNA or if they joined back on themselves (self-ligated), any bacteria have taken up plasmids, or whether any bacterium that has taken up a plasmid has taken up a self-ligated plasmid or a recombinant plasmid. It is necessary to know which bacteria are transformed, so that only transformed bacteria can be grown in a culture medium.

There are several ways to identify transformed bacteria, with most involving marker genes on the plasmids. One way is to culture the mixture of bacteria onto agar plates. Each colony that grows is the clone of a single cell. We can use the example of a plasmid containing a gene for the resistance to an antibiotic 'A,' and having the point where it is cut, and the other gene is inserted being in the middle of a gene responsible for the resistance to antibiotic 'B.'

Once the plate has been formed, we can take an imprint by gently pressing down on it with sterile filter paper, and then we press the filter paper onto the surface of an agar plate containing antibiotic A. We can repeat this process, pressing the filter paper onto the surface of an agar plate in which the agar contains antibiotic B.

The bacteria that successfully took up the plasmid, and therefore had resistance to antibiotic A, would grow on the replica plate containing A. The bacteria that successfully took up the plasmid *and* contained a recombinant plasmid, would grow on the replica plate for A, but would not grow on the plate for B. If it did grow on replica plate B, the gene conferring this resistance would be intact, meaning it had self-ligated.

We can then transfer samples of the successful colonies from the original agar plate to sterile nutrient medium and allow them to increase in number.

Other Methods used to Insert DNA into Cells:

If scientists wish to transform any cells other than bacteria, they can use different vectors to transfer the target DNA, including the following four methods,

- **Viruses-** Affect the cells they infect by inserting their own nucleic acids into a host cell, and this can be exploited by using viruses to transfer recombinant DNA into host cells. The virus must be weakened to do this so that it does not cause the death of host cells.
- **Liposomes-** Small lipid droplets used to coat plasmids containing recombinant DNA. The lipid coating allows the droplet to cross cell-surface membranes.
- **Gene Guns-** Use air pressure to enable particles of heavy metals, such as gold or titanium, coated in recombinant DNA to be 'fired' through the surface of cells. The DNA can be fired directly into an organ or tissue culture.
- **Electroporation-** Uses electricity to create pores in cell surface membranes of the target organism. The affected cells can then able to take up naked recombinant DNA and incorporate it.