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A2 Biology Unit 5

DNA & Gene Expression

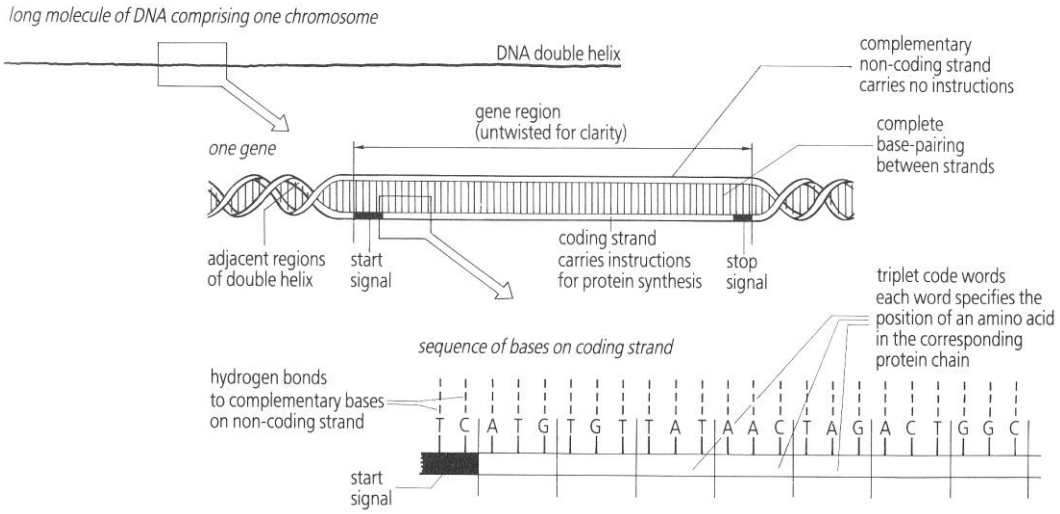
The genetic code

- is the base sequence of DNA which determines the amino acid sequence (primary structure) of a each protein. The code is …

- Degenerate
- Composed of codons
- Non-overlapping
- Universal

Protein synthesis

1. Transcription



Summarise the steps below

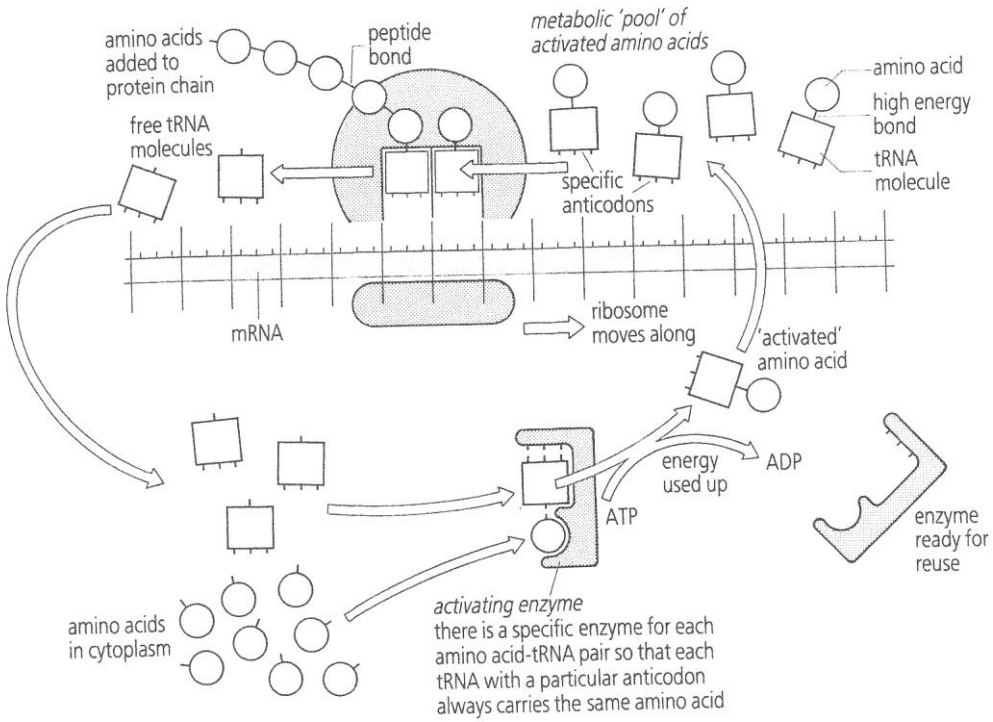
Introns and exons

EXON section of DNA which codes for amino acids in polypeptide chain
INTRON section of DNA with no coding function (junk DNA)

During transcription, the introns are removed and the exons are **spliced** together to form strands of mRNA. One length of DNA can make a number of different strands of RNA by splicing the exons in different orders.

A **gene** may be defined as a strand of mRNA which codes for a single protein.

Translation



Summarise the process of translation:

Gene mutation

A mutation is a change in DNA

Gene mutation	an alteration in the base sequence of the genetic code. It results in the formation of a new allele. e.g. cystic fibrosis
Chromosome mutation	a change in the number of chromosomes i.e. the amount of DNA. e.g. Down's syndrome

Substitutions

A nucleotide in the DNA chain is replaced by another with a different base. This can result in the following possible consequences:

- **nonsense mutation**
- **mis-sense mutation**
- **silent mutation**

Deletions

The loss of a nucleotide from the DNA base sequence

This completely messes up the code. Why?

Causes of mutations

- **spontaneous** random one or two per 100,000 genes per generation.
- **Mutagens** e.g. radiation and chemicals which alter DNA molecule

Oncogenes and cancer

The rate of cell division is controlled by **proto-oncogenes** which speed it up and **tumour suppressor genes** which slow it down.

Proto-oncogenes are switched on by external growth factors which bind to receptors on the cell membrane. This triggers DNA to replicate. The activity of the proto-oncogenes is normally suppressed, however, by tumour suppressor genes.

Uncontrolled cell division (cancerous growths) may result if

- Tumour suppressor genes mutate and fail to inhibit the proto-oncogenes
- Proto-oncogenes mutate to **oncogenes** and cause the receptor protein in the membrane to be permanently activated

Control of gene expression

Differentiation

occurs at the 16 cell stage. It is caused by switching genes off. This can happen in 2 ways:

1. Preventing transcription

Transcription depends on **transcriptional factors** which move from the cytoplasm into the nucleus and bind to a specific region of the DNA allowing RNA polymerase to attach and start the transcription process. Normally these binding sites are blocked by **inhibitors** so the gene is switched off.

Question: Oestrogen and other fat soluble steroid type hormones work by unbinding specific sites of transcription. (How does this compare with the action of glucagon, a protein type hormone?

2. Small interfering RNA (siRNA)

Short pieces of double stranded RNA bind to complementary regions of mRNA causing it to break apart. siRNA is of particular interest to medical research. Imagine being able to switch off a gene e.g. oncogene with a drug.

Totipotent stem cells

Undifferentiated embryonic cells retaining the ability to develop in any way. In animals only pre-16 cell stage blastula cells can do this. In plants **meristematic** cells are totipotent

Pluripotent stem cells

After the 16 cell stage, a **blastocyst** is formed. Part of this forms the placenta and an **inner cell mass** consists of pluripotent cells which develop into all the different body tissues. These cells individually have a limited scope for differentiation.

Adult stem cells

From from pluripotent cells of the blastocyst and retain some stem cell ability. e.g bone marrow stem cells may differentiate into red blood cells, white blood cells and platelets.

DNA technology

Steps involved in the manufacture by gene technology of human insulin

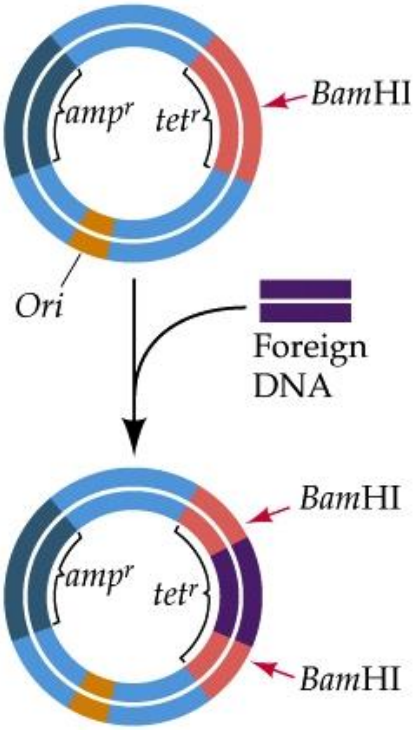
1. **isolation** of DNA fragment
2. **insertion** into a vector
3. **transformation** into a host cell
4. **identification** of transformed host cells using gene markers
5. **cloning** host cells to produce insulin

Isolation of gene using reverse transcriptase

- Extract mRNA from beta cells of Islets of Langerhans
- Use reverse transcriptase to convert to DNA

Insertion into vector

- **Plasmid** vector with two antibiotic resistance genes: one is resistant to ampicillin (*amp*), the other to tetracylcine (*tet*)
- Both the plasmid and the gene for insulin are split open by the same restriction enzyme (*Bam*HI). This creates **palindromic sticky ends**.
- The enzyme **ligase** joins the insulin gene to a site in the middle of the *tet* gene.



Transformation into a host cell

- Plasmid is reintroduced to a bacterial host cell
- This is achieved with alterations of temperature and the addition of Ca^{2+} ions which make the bacterial membrane more permeable.
- Only a few bacteria will take up the plasmid and become transformed

Identification

- Bacterial cells are grown in a medium containing the antibiotic ampicillin. This destroys all the bacterial cells without the plasmid but does not reveal which ones have been transformed with the insulin gene.
- The remaining cells are spread thinly on a new culture plate where they grow into separate, genetically identical colonies.
- A replica is made of this plate and treated with tetracycline. (**replica plating**)
- The transformed cells die and their position can be compared to the colonies of the other plate.

Cloning

- Bacteria transformed with recombinant DNA are grown in a fermenter
- Human insulin produced by the bacteria is extracted and purified.

***In vitro* gene cloning**

The production of insulin (above) is an example of ***in vivo* cloning** i.e. the gene is multiplied within living cells. *In vitro* cloning means gene multiplication in laboratory glassware.

Polymerase Chain Reaction (PCR)

- Mix DNA sample, free DNA nucleotides, DNA primers (one for each strand) and DNA Polymerase (Taq polymerase)
- Heat components to **95ºC** (1 minute)
  - Breaks the hydrogen bonds between complementary bases on each strand
  - Makes the DNA single stranded
- Cool to **55ºC** (45 seconds)
  - Primers anneal to the 3' end
  - Taq Polymerase can now bind to primers
- Heat to **72ºC** (2 minutes)
  - Optimum temperature for Taq Polynmerase

- A new strand of DNA is synthesised from each template strand.

Use of recombinant DNA technology

Benefits of DNA technology

- Industrial / medical products

- Genetically modified plants

- Genetically modified animals

Risks of DNA technology

- **Ecological risks**

- **Economic risks**

- **Antibiotic resistance**

- **New pathogens**

Gene therapy

2 examples should be understood in some detail

1. **Cystic Fibrosis**

Caused by a mutation creating a recessive allele (about 1 in every 20,000 has the disease (recessive homozygous condition)

Effect: A channel protein controlling exit of Ca^{2+} ions called **CFTR** (cystic fibrosis transmembrane-conductance regulator) fails to

functions so water is retained in epithelial cells of lung and gut. This results in thick mucus deposits and consequent infections.

Treatment: two possible approaches:

- **Germ line treatment**

- **Somatic cell line treatment**

Somatic cell Gene Therapy	Germline cell Gene Therapy
Delivery into the recipient cell is **tricky** as cell needs to be removed, treated and replaced = Ex-vivo Therapy	Straight forward delivery as techniques are not cell specific
Treatment is short lived and needs to repeated regularly	Modifications are found in future generations
Difficulty getting allele into the genome, viruses trigger immune response, Liposomes are not efficient	Unethical to engineer embryos
Modifications are limited to the patient	All cells have modification and any mitosis results in daughter cells having the trait as well

2. SCID (Severe Combined Immunodeficiency)

Caused by: a defect in the gene which codes for the enzyme **ADA** (adenosine deaminase)which normally destroys toxins which kill white blood cells.

Effect: sufferers cannot make antibodies and have no cell-mediated immune response.

Treatment:

- Insert isolated ADA gene into a retrovirus
- Clone retrovirus in host cells *in vitro*
- Mix retroviruses with patient's T cells
- Reintroduce recombinant T cells into patient

Problems and risks of gene therapy

- Effect is short lived
- It can induce immune response
- Viruses can have other effects
- Genes are not always expressed
- Long term effects of changing the human genome

Locating and sequencing genes

DNA probes and hybridisation

DNA probe is a short manufactured length of single stranded DNA, labelled with radioactive isotope or fluorescence.

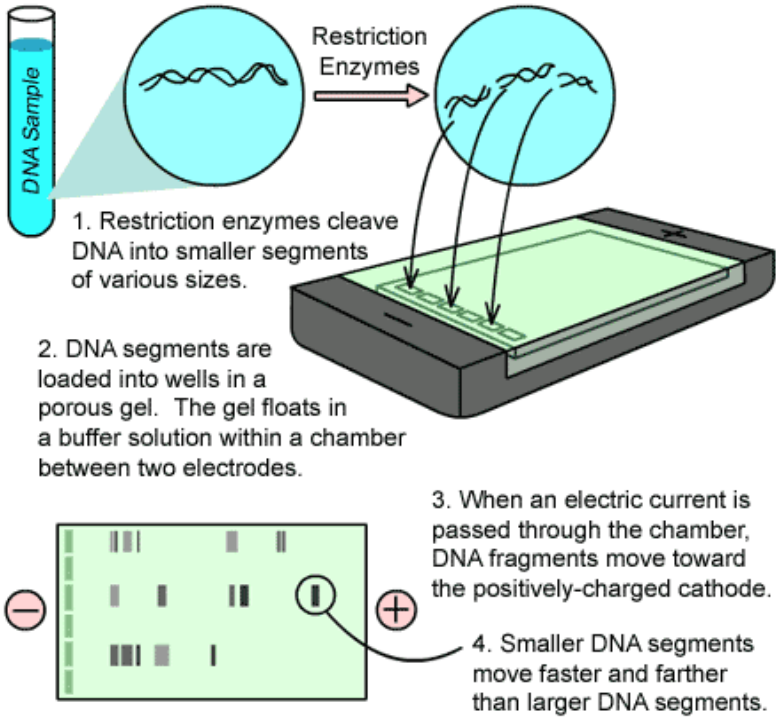
The DNA under investigation is split into single strands and the probes added. Hybridisation happens and the site of the specific sequence can be identified.

DNA sequencing – the Sanger method

- Cut the DNA under investigation into many different size lengths using terminators i.e nucleotides which are unable to bond with the next nucleotide in the chain. There are four different terminators one for each base (A,C,G,T)
- Add a few of these terminators along with plenty of normal nucleotides to a cloned sample of the DNA in a PCR machine
- Random binding occurs and lots of different length fragments are formed with the As, Cs, Ts and Gs, all cut a various points along the length of the DNA strand
- Separate the fragments using gel electrophoresis
- The smallest fragment will be a single nucleotide at one end of the chain, th largest, the opposite end.

- The process can be run automatically using 4 different fluorescent markers allowing the gel to continue up a single narrow capillary gel, using lasers

Figure S-2: Gel Electrophoresis



Alternative methods

Restriction mapping

- Use restriction enzymes in pairs to cut DNA at specific recognition sites
- Separate fragments with gel electrophoresis and compare lengths between the cuts.

Genetic screening

How is it done?

It involves using **DNA probes** which hybridise and mark specific recessive alleles which would otherwise not be known about e.g. cystic fibrosis, sickle cell disease, oncogene alleles.

Many can be done at once by fixing hundreds of different DNA probes to a glass plate.

An example: Sickle cell anaemia

Cause: a single base substitution (A for T) coding for one of the polypeptide chains of Hb.. It produces CAC (valine) instead of CTC (glutamic acid)

.

Effect: a recessive allele Hb^s producing a molecule of Hb with a sticky patch**.** At low O^2 levels, the Hb molecules tend to stick together forming long threads, causing the red cell itself to deform into a banana (sickle) shape. This causes anaemia as they are unable to carry O^2. It also increases the risk of clotting.

Genetics: The alleles are co-dominant. The heterozygous form is generally symptomless but in severe exercise or other O^2 deficiency they may exert an effect.
Sickle cell trait gives a selective advantage against **malaria**

Implications of genetic screening

- **Who decides?**

- **Who has access to the results?**

- **Responsibility for carrier**

- **Defect or disease?**

Genetic Fingerprinting

Introns (non-coding bits of DNA) contain many repetitive sequences called core sequences. For each individual they form a distinct and unique pattern.

- Extraction and multiplication

- Digestion

- Separation by gel electrophoresis and Southern blotting

- Hybridisation

- Development

- Interpretation

Uses of genetic fingerprinting

- Forensics

- Medical diagnosis

- Plant and animal breeding