MEGA LECTURE

AS Biology – Revision Notes
Unit 2 – Genes And Genetic Engineering

The Genetic Code

1. DNA (deoxyribonucleic acid) consists of a sequence of nucleotide, each of which has:
    a. A phosphate group.
    b. A sugar group (deoxyribose sugar).
    c. A base – adenine (A), thymine (T), cytosine (C) or guanine (G).
2. The sugar and phosphate groups form a sugar-phosphate backbone to DNA. The bases in the two complementary DNA strands bond together by hydrogen bonding – A bonds to T and C bonds to G. These strands then twist around to form a double helix structure.
3. DNA is arranged into chromosomes:
    a. There are 46 (23 pairs) in humans.
    b. The chromosomes are arranged in homologous pairs – i.e. two with the same types of gene along them.
    c. Alleles for a gene will occupy the same locus on each chromosome.
4. There is only 0.1% difference in DNA between humans. SNPs (single nucleotide polymorphisms) are responsible for individual characteristics.
5. DNA is a successful molecule:
    a. It is a huge, coiled, molecule, containing masses of information in a small space.
    b. Characteristics are accounted for by small variations in the structure.
    c. It is very stable, and can take physical stress.
    d. It can reproduce very accurately.
6. DNA replication occurs during the interphase, and is semi-conservative replication:
    a. Helicase enzymes break hydrogen bonds between bases.
    b. DNA polymerase enzymes join free bases in the cytoplasm to ach strand.
    c. This produces two identical strands, with ½ old and ½ new DNA (i.e. semi-conservative).
7. Meselson and Stahl's experiment gave evidence for semi-conservative replication:
    a. Bacterial DNA was radioactively labelled with heavy nitrogen.
    b. The bacteria were allowed to replicate, and a sample was taken from each generation.
    c. In generation 0, the entire DNA was ^{15}N.
    d. In generation 1, the entire DNA was a mixture of half ^{15}N and half ^{14}N.
    e. In generation 2, half of the DNA was all ^{14}N, and half was a mixture.
8. Each triplet of bases in DNA is called a codon, and as there are four different bases, this gives 64 combinations. Each codon codes for a specific amino acid, so the sequence of codons in a gene relates to the sequence of amino acids in a polypeptide. Some codons are stop codons, which signify the end of the gene.
9. RNA (ribonucleic acid) is different from DNA:
    a. Thymine is replaced with uracil (U).
    b. The sugar will be ribose rather than deoxyribose.
    c. RNA forms short single-strand lengths as messenger RNA (mRNA), and small molecules joined to an amino acid as transfer RNA (tRNA).
10. In the nucleus, DNA is copied into mRNA by transcription:
    a. The DNA is 'unzipped'.
    b. RNA polymerase builds up an mRNA strand (up to the stop codon), complementary to the DNA strand.
    c. The strand of mRNA then passes out of the nucleus through nuclear pores, to the ribosomes either free in the cytoplasm or bound to the rough ER.
11. At the ribosomes, mRNA is used to build a polypeptide by translation:
    a. The mRNA is attached to the ribosome.
    b. A tRNA molecule with an anticodon complementary to the mRNA codon attaches to the mRNA.
    c. The RNA then shifts along and the next tRNA molecule can attach to the mRNA.
    d. The two amino acids form a peptide bond, and the first tRNA molecule breaks off.
    e. This continues until the polypeptide coded for by the gene has been produced.
12. A gene mutation occurs when there is a change in the sequence of bases such as when a gene is incorrectly copied. This can result in a change in the structure of the polypeptide that is coded for. A mutation can be caused by mutagens, such as UV light, tobacco, x-rays etc.
13. There are four main types of mutation:
    a. Addition – one extra base in the sequence is added, causing a frame shift.

   b. Deletion – one base in the sequence is removed, causing a frame shift.
   c. Substitution – one base is substituted for another, causing one different codon in the gene.
   d. Inversion – one codon is inverted, causing one different codon in the gene.
14. Mutations can affect the structure of a protein:
   a. They can be lethal if the tertiary structure is altered, so that the enzyme will not work, causing a metabolic block. This is very likely to occur with a frame shift.
   b. There can be no effect – If the changed codon still codes for the same amino acid then the protein produced will be the same. If a different amino acid doesn't affect the tertiary structure, then it will still function.
   c. It can be beneficial, if it makes the protein work better – this will be evolution.

The Cell Cycle And Reproduction

1. Normal cells (i.e. not gamete producing cells) replicate by mitosis. The two daughter cells will contain the full number of chromosomes (diploid), and will be identical to the parent cell.
2. The cell cycle has five main stages:
   a. Interphase – The cell grows to full size, and carries out its specialised functions. Towards the end of the interphase, DNA replication occurs.
   b. Prophase – The chromosomes coil together and shorten, forming two chromatids joined at the centromere. The two chromatids are identical. The nuclear membrane begins to disappear, and the centrioles go to opposite ends of the cell, beginning to produce spindle fibres.
   c. Metaphase – The spindle fibres reach across the whole cell, and the nuclear membrane has disappeared. The chromatid pairs line up on the equator of the spindle.
   d. Anaphase – The chromatids are pulled apart by the spindle fibres to the poles of the cell.
   e. Telophase – A nuclear membrane is formed around each set of chromosomes, and they form chromatin threads. The centrioles divide. Cytokinesis occurs, so that the cytoplasm divides in half to form two separate cells. The interphase will now begin in each cell.
3. Vegetative propagation is the natural asexual reproduction of plants:
   a. Runners, stolons, rhizomes and tubers are all used to produce new plants asexually.
   b. These are meristematic regions, whereby rapid growth takes place.
   c. Plants produced in this way will be clones of the original plant – so any good properties of the parent will be passed on, but so will any vulnerability to diseases etc.
   d. This can be carried out artificially by using cuttings containing a meristematic region.
4. Tissue culture is used to commercially grow plants:
   a. The principle of totipotency states that any part of a plant can be made from any other part (in theory).
   b. The meristem is isolated, and cultured to form a callous (mass of undifferentiated cells).
   c. This is then sub-divided, and plantlets are grown and cultured.
5. Micropropagation is also used commercially to grow plants, but leaf axil cuttings are taken as the meristem, grown into a shoot, and then further divided. This is repeated to give as many shoots as needed, and then the shoots are placed in a growth medium to grow into plantlets.
6. In order to produce more of a species of mammal per generation, two methods can be used:
   a. Superovulation – FSH (follicle stimulating hormone) is administered to the parent to cause the release of several mature eggs at once. This will result in non-identical twins.
   b. Cloning – The embryo is separated at an early stage into multiple embryos, each of which will grow into the offspring. This will result in identical twins.
7. To clone an embryo:
   a. A donor cow is treated with FSH so superovulation takes place – the multiple egg cells are removed from the cow.
   b. The eggs are fertilised in vitro with the sperm from a bull (with desired characteristics).
   c. The fertilised eggs are cultured until 16 cells big, and then the cells are separated.
   d. The nuclei of the cells are transferred to other egg cells, and these are grown and transferred into the uterus of another cow (a surrogate mother).
8. To clone an adult (e.g. Dolly):
   a. A cell is taken from the udder of the sheep to clone, and the nucleus is removed.
   b. An unfertilised egg is taken from another sheep – the nucleus is removed and discarded.
   c. The nucleus from the first sheep is inserted into the enucleated cell using a micropipette.
   d. This is cultured to the 16-cell stage, and then placed into the uterus of a surrogate mother.
9. Meiosis is the basis of sexual reproduction, and gives rise to variation:
   a. The DNA replicates into chromatid pairs.

   b. The homologous chromatid pairs then cross over at chiasmata to result in a recombination of the alleles (they are in effect swapped around).
   c. The cell then divides in the first meiotic division, but the homologous pairs of chromosomes are separated – one goes to each new cell – and so the two daughter cells will be haploid.
   d. The cells divide again in the second meiotic division, similar to mitosis. This therefore produces four haploid cells.
10. The production of sperm is called spermatogenesis – diploid spermatogonia develop into primary spermatocytes, which divide by meiosis to each produce four spermatids that develop into sperm.
11. The production of ova is called oogenesis – oogonia are produced by mitosis, but only one will continue to develop into a primary oocyte. In the first meiotic division this produces a secondary oocyte and the first polar body. In the second meiotic division the secondary oocyte divides to form the ovum and the second polar body. The polar bodies will degenerate.
12. Ova have the following adaptations:
   a. They have a large cytoplasm containing yolk droplets (proteins and lipids) for growth after fertilisation.
   b. They have a clear jelly-like coating that the sperm must break through in order to fertilise.
   c. They are about 100µm in diameter.
13. Sperm have the following adaptations:
   a. They have a head containing the nucleus and very little cytoplasm.
   b. At the tip of the head is the acrosome – enzymes to digest the coating of the ovum.
   c. The middle piece contains a large number of mitochondria to provide ATP for swimming.
   d. The tail contains protein filaments that can contract to produce a swimming motion.
   e. They are about 20µm long.
14. In the life cycle of a species, meiosis will halve the chromosome number, mitosis will keep it the same, and fertilisation will double the chromosome number.

Genetic Engineering

1. Genetic engineering is the removal of genes from one organism and insertion into another.
2. The genome is the entire DNA sequence of an organism, i.e. all of the genes and the entire non-coding DNA in between.
3. A transgenic organism is one that has had its DNA altered by the insertion of genes from another organism.
4. Recombinant DNA is DNA that has been mixed with the DNA of another species. Usually the entire genome will be intact – only one or two specific genes are added.
5. Restriction endonuclease enzymes will cut DNA at a specific base sequence. They will cut in a staggered manner to make 'sticky ends' on the DNA cut. Sticky ends can be joined together using ligase enzymes.
6. DNA can be isolated from cells for use in genetic engineering:
   a. For plant cells, the cellulose cell walls must be physically broken up by grinding in sand.
   b. This is mixed in a buffer solution, along with SDS detergent to emulsify lipids and break down membranes within the cells. It is incubated at 65°C to disrupt the proteins.
   c. This mixture is then centrifuged to remove the cell debris and leave the DNA in the supernatant.
   d. Adding ice-cold ethanol will cause the DNA to form a precipitate, which can be removed.
7. In order to locate a specific gene:
   a. The polypeptide for which the gene codes is analysed and converted into an mRNA sequence.
   b. The mRNA is converted into DNA using the enzyme reverse transcriptase, in the presence of radioactively labelled cytosine.
   c. This is then used as a radioactive gene probe, so when it is added to the DNA it will bind to the gene, pinpointing its location.
8. Plasmids are small circles of DNA found in bacteria that can be used as a vector – i.e. as the carrier of another gene.
9. To incorporate a gene into a bacterium:
   a. Use a restriction endonuclease enzyme to cut out the gene that is needed.
   b. Isolate a bacterial plasmid, and use the same restriction endonuclease to cut it, so as to produce complementary sticky ends.
   c. Splice the DNA into the plasmid with ligase enzymes.

   d. Place the vector DNA (the plasmid) into a bacterium, so that it becomes incorporated into it. The bacterium will then be able to produce the correct product.
10. Genetic markers can be used to show whether or not a bacteria has taken up the gene:
   a. A gene for antibiotic resistance is placed into the plasmid alongside the required gene.
   b. The transgenic bacteria are cultured.
   c. A replica plate (a circular sterile pad) is placed onto the transgenic bacteria, and then onto a medium containing the antibiotic for which the marker gene provided resistance.
   d. Only those bacteria that have incorporated the genes will continue to culture, and these colonies can be collected and cultured from.
11. Fermentation can be used to collect the gene product on a large scale in industry. The bacteria colony is placed in a medium in the fermenter containing all the nutrients needed. This is rotated with a stirrer, and sterile oxygen is bubbled through. The medium is kept at the correct temperature and pH. There are two ways of obtaining the products:
   a. Continuous fermentation – the products are tapped off continuously and the fermenter is kept going all the time.
   b. Batch fermentation – a batch of the product is produced, and then the fermenter is shut down to separate it.
12. The polymerase chain reaction (PCR) makes millions of copies of DNA from a single source to give an amount that can be manipulated:
   a. Take the original DNA, and heat to 90°C to break the hydrogen bonds.
   b. Add primers (short lengths of DNA) and cool to 40°C so that they attach to the DNA.
   c. Add DNA polymerase enzyme and heat to 70°C. DNA polymerase is thermostable, and so will work at this temperature – the DNA is replicated.
   d. The cycle is repeated until the amount of DNA needed has been produced.
13. Electrophoresis is used to create a genetic fingerprint:
   a. A gel is prepared with a series of wells at one end, and is placed across a voltage with the wells being at the cathode end.
   b. The DNA obtained from the PCR is incubated with a restriction endonuclease enzyme, to break the DNA into fragments. Other DNA samples will use the same restriction enzyme to make it a fair comparison.
   c. The samples of DNA are placed in the wells prepared in the gel. An ionic buffer solution is placed around the gel.
   d. The current is allowed to flow through the gel. As DNA is always negative, it is attracted towards the anode. Different lengths of DNA will move at different rates due to having different masses.
   e. A nitrocellulose membrane is placed on the gel, to transfer the DNA fragments onto it.
   f. The nitrocellulose membrane is then incubated with a radioactive probe, which will bind to the DNA fragments.
   g. A photographic plate is then placed next to the membrane, to expose it where the DNA fragments are using autoradiography. This produces the characteristic bands of DNA.
14. There are various ethical considerations when using genetic engineering:
   a. Is it like ‘playing God’?
   b. Who ‘owns’ the product that is produced?
   c. What are the side effects of interfering with the genome (e.g. toxicity)?
   d. What are the effects on the ecosystem?
   e. When does an embryo become a human being?
   f. What are the psychological problems with cloning?
   g. Should other animals be genetically modified for our gain (animal rights etc.)?
   h. If the treatment is expensive, then will only the rich get it?

Genes And Medicine

1. AAT (alpha-1-antitrypsin) is a glycoprotein that inhibits the enzyme elastase (produced by white blood cells). Elastase breaks down elastic tissue in the lungs, causing emphysema. The Z-allele of AAT is a mutant gene, resulting in the production of faulty AAT and therefore emphysema.
2. Working AAT can be produced using transgenic organisms:
   a. Mature eggs are removed from the ovary of a sheep and fertilised in vitro.
   b. Plasmids containing the gene for AAT are inserted into the cells with a micropipette.
   c. The embryos are developed to the 16-cell stage and inserted into surrogate mothers.
   d. Some of the lambs produced will (hopefully) be transgenic. AAT will be produced in the milk of the transgenic sheep.

3. People with the Z-allele can be treated using an aerosol spray containing the AAT in liposomes. This will temporarily inhibit the elastase and give temporary relief from emphysema. There is, however, no permanent cure, and lifelong treatment would be needed.
4. Cystic fibrosis is caused by a faulty gene for the production of CFTR (cystic fibrosis transmembrane regulator). CFTR is a protein pore in the membrane of epithelial cells that 'pumps' Cl^- ions out of the cells onto the layer of mucus coating them. This attracts water from the cells in order to keep the mucus layer liquid, so it can drain away. Without CFTR the mucus becomes thick and sticky, and so the sufferer needs intensive physiotherapy, and is prone to disease.
5. The working CFTR gene is incorporated into a bacterial plasmid. This is taken as an aerosol spray, and can be incorporated into the epithelial cells of the patient in two ways:
    a. Liposomes – The vector DNA is placed into small hollow spheres of lipid, which fuse with the epithelial cells' plasma membranes, releasing the contents into the cell. Consequently the cells take up the working CFTR gene.
    b. Adenoviruses – The CFTR gene is incorporated into the DNA of a virus, and the disease-causing DNA in the virus is removed (i.e. it is a harmless virus as it cannot replicate and harm the cells). There is always the chance of the virus mutating into a harmful form though.
6. Both of these methods relieve the symptoms of cystic fibrosis, but are only short-term solutions as the epithelial cells are continually being worn away and replaced. Adenoviruses are more effective than liposomes, but carry more dangers with them.