

Chapter 18 Molecular Biology and Medicine

I Protein as Phenotype

- Genetic mutations can cause biochemical-related diseases.
- Alkaptonuria is a genetic disease that prevents usual phenylalanine metabolism.

A. Many genetic diseases result from abnormal or missing proteins

- Proteins have a variety of roles including being enzymes, receptors, transporters, carriers and structural proteins.
- Enzymes:
 - A defect in the gene that codes for phenylalanine hydroxylase causes phenylketonuria, also call PKA. *See Figure 18.1.*
 - This enzyme catalyzes the conversion of dietary phenylalanine to tyrosine in the liver.
 - At position 408 of a 451 amino acid peptide, most affected individuals have the amino acid tryptophan and not arginine.
 - Those with PKU have low levels of the amino acid tyrosine because the defective enzymes fail to convert phenylalanine to tyrosine. Tyrosine is converted to the skin and hair pigment, melanin.
 - The disease causes high levels of phenylalanine in the blood.
 - Hundreds of human genetic diseases that result from enzyme abnormalities have been discovered.
 - Many proteins show variation in amino acid sequence.
 - Not all changes cause problems with function.
 - If the most frequent form of a certain protein is present in less than 99% of the population, the protein is said to be polymorphic.
- Hemoglobin:
 - Sickle-cell anemia is a disease caused by a mutation that affects hemoglobin.
 - Of the α and β subunits of hemoglobin, it is the β -globin that differs in those affected.
 - Of the 146 amino acids in β -subunits, the sixth is changed from a glutamic acid to a valine.
 - Another disease, called hemoglobin C disease, has a changed amino acid at the same location, but instead of glutamic acid, there is lysine.
 - Whereas sickle-cell disease is severe in homozygotes, hemoglobin C disease is much less so. (*See Figure 18.2*)
- Receptor and transport proteins:
 - Familial hypercholesterolemia (FH) is a disease where blood cholesterol levels are several times higher than normal. (*See Figure 18.3*)
 - The biochemical basis is an altered cell surface receptor for the lipid carrier protein, LDL (low-density lipoprotein).
 - Normally, the receptor is functional and involved in the transport of lipoprotein, via endocytosis in liver cells.
 - Those with FH have an altered amino acid of the 840 that make up the receptor.
 - Cystic fibrosis is another genetic disease.

- Its symptoms include unusually thick and dry mucus in the tubes of the respiratory system.
- This interferes with the normal functioning of the cilia.
- The defect has been traced to a chloride transporter.
- Normally, the imbalance of Cl⁻ ions, more outside than inside, causes cellular water loss and moist extracellular mucus.
- The lack of function of transporters changes the normal imbalance and the mucus is dry in those affected with the disease.
- Structural Proteins:
 - About one in 3,000 people are born with *Duchenne's muscular dystrophy*.
 - Patients usually die in their twenties, when the muscles that serve their respiratory system fail.
 - Dystrophin, which attaches actin to plasma membrane in muscle cells, is missing or non-functional.
 - Hemophilia is a genetic disease caused by a lack of one of the coagulation proteins. Affected people risk bleeding to death from even minor cuts.

B. Prion diseases are disorders of protein conformation

- *Transmissible Spongiform encephalopathies* (TSE's) are degenerative brain diseases that occur in mammals, including humans. (See Figure 18.4)
- Scrapie is the TSE found in sheep.
- In the 1980's, TSE transferred to cattle from sheep.
- In the 1990's, people who had eaten beef from cows with TSE, got TSE.
- Kuru is a TSE disease found in the Fore tribe of New Guinea. They got it from eating dead relatives' brains.
- Tikva Alper provided evidence that the infectious agent was a protein.
- Stanley Prusiner purified the protein and proved it was free of DNA and RNA.
 - Normal brain cells have a membrane protein called P^rP^c.
 - Abnormal TSE affected brain cells have the same protein but with an altered shape.
 - The amino acid sequence is the same but the shape of the protein has been altered.
 - Insoluble P^rP^{sc} accumulates as fibers and causes cell death.

C. Most diseases are caused by both heredity and environment

- Estimates suggest that up to 60 percent of all people have diseases that are genetically influenced.
- About 1 percent of those in the human population have a disease caused by a single gene.

D. Human genetic diseases have several patterns of inheritance

- Autosomal Recessives:
 - PKU, sickle-cell anemia and cystic fibrosis are autosomal recessive.
 - Those homozygous for the mutant allele are affected.
 - Those heterozygous might have less of the gene product, but enough to have a normal phenotype.
- Autosomal Dominants:
 - This is when the presence of just one mutant allele is enough to produce the clinical phenotype.

- An example in humans is familial hypercholesterolemia. Having half the receptors for LDL is inadequate to prevent accumulation of cholesterol.
- X-linked Inheritance:
 - Both hemophilia and Duchenne's muscular dystrophy are inherited as X-linked recessive diseases.
 - Sons inherit them from their mothers.
 - Affected fathers' daughters are all carriers (or affected, if homozygous).
 - Males are affected more frequently (the frequency of the allele in the population) than females (the square of the frequency of the allele in the population).
- Chromosomal Abnormalities:
 - Chromosomal abnormalities include loss or gain of one or more chromosomes, loss or gain of a piece of a chromosome, or transfer of a piece from one to another chromosome.
 - Some are inherited. Some are the result of non-disjunction during meiosis (or early mitosis).
 - Approximately 20 percent of pregnancies that spontaneously abort during the first 3 months of human development are chromosomally aberrant.
 - Estimated 90 percent of human zygotes that have just one X chromosome and no Y fail to survive beyond four months.
 - A common cause of mental retardation is fragile-X syndrome.
 - About one male in 1,500, and one female in 2,000, are affected.
- Near the tip of the abnormal X chromosome, constrictions are observed, which tend to break. (See Figure 18.5)
- Not all people with fragile-X abnormality are mentally retarded (explained below).

II Mutations and Human Diseases

- Some mutations associated with human diseases are easy to clone. Hemoglobin abnormalities are an example.
- Finding the troublesome gene is much more difficult when the molecular causes are unknown.
- See Figure 18.6.

A. The logical way to identify a gene is to start with its protein

- Sickle-cell anemia is caused by a defect in the β -globin subunits of hemoglobin.
- It was possible to find the exact cause because the protein involved was known.

B. Chromosome deletions can lead to gene and then protein isolation

- Deletions can help identify the gene identification and the protein defect associated with disease.
- This was the case of the discovery of the cause of Duchenne's muscular dystrophy.
- Several boys with the disease were found to have small deletion in their X chromosome.

C. DNA markers can point the way to important genes

- See Figure 18.7.
- An approach called positional cloning can be used when no candidate protein or deletion is known for a gene.
- RFLP's have been found at more than 1000 sites for the human genome.
- The RFLP's can be used as landmarks.
- Marker types and pedigree analysis information are compared. If a marker is found to correspond to a phenotype, they must be near each other.
- The neighborhood around the RFLP can be screened for further RFLP's. If one is linked directly, a DNA fragment from the region can be used to identify a cDNA sequence.
- The gene from affected and unaffected people is compared to determine the genetic difference responsible for the disease.

D. Human gene mutations come in many sizes

- Some cystic fibrosis sufferers have a nonsense mutation such that a codon near the beginning has changed to a stop codon.
- 5-methylcytosin loses its amino group and becomes thymine. Regions of DNA with methylated cytosine are prone to mutation and are called "hot spots".
- See Figure 18.8.
- Larger mutations include deletions of regions of chromosomes.

E. Expanding triplet repeats demonstrate the fragility of some human genes

- About one-fifth of all males that have a fragile-X chromosome are phenotypically normal.
- However, many of their daughters' sons are mentally retarded.
- Fragile-X syndrome is related to the condition of the DNA sequence FMR1.
- It contains a repeated triplet sequence: CGG.
- In normal people, this triplet is repeated 6 to 54 times. In those affected, CGG is repeated 200 to 1,300 times.
- Those males with no symptoms have 52-200 repeats. These become more numerous in the daughters, and their sons then get more than 200 copies.
- See Figure 18.9.

F. Genomic imprinting shows that mammals need both a mother and father

- It is possible to make a mammalian embryo with two pronuclei that are from males (two sperm) or females (two female pronuclei).
- These fail to produce development beyond the embryonic stages.
- This shows that the DNA from fathers and mothers are expressed differently.
- A genetic disease caused by a small deletion in chromosome 15 produces completely different results, depending on whether it comes from the mother or father.
- From the father, the child is short and obese, with small hands and feet (Prader-Willi syndrome); if from the mother, Angelman syndrome develops, which is a thin child with a wide mouth and prominent jaw.

III Detecting Human Genetic Variations

- Learning the molecular basis for human genetic diseases has helped increase knowledge of normal cell physiology.
- Specific biochemical treatments and possible cures depend on the acquired knowledge.
- Diagnosis may be possible before symptoms first appear, thus making medical intervention possible.
- Genetic screening is the application of a test to identify people who are predisposed to certain diseases.
 - This knowledge can be used to the best interest of the individual.
 - This information might be used by insurance companies to exclude certain people for full medical coverage.

A. Screening for abnormal phenotypes can make use of protein expression

- Screening for PKU is legally required in many countries including the USA. (*See Figure 18.10*)
- Proteins consumed by babies with the disease contain phenylalanine, which accumulates and causes brain damage.
- Auxotrophic bacteria can be used to detect the presence of phenylalanine.
- If the test is positive, additional biochemical tests are run.
- Treatment begins by the second week of life.
- Tay-Sachs disease is an autosomal recessive lethal disease. Carriers can be identified.
- Widespread screening among Ashkenazic Jews has reduced the new diagnosis among newborns from 65 to fewer than 5.

B. There are several ways to screen for abnormal genes

- DNA testing is the most accurate way to test for an abnormal gene.
- This works best if just a few different allelic forms of the disease gene exist.
- PCR allows testing on just a single cell.
- This can be done on an embryo.
- If both parents are heterozygous for a recessive gene, a cell from an embryo can be tested for the presence of the disease.
- Normal embryos are transferred
- There are genetic tests for many diseases now.
- The genetic tests work one of several ways.
 - Allele-specific cleavage differences:
 - Changes responsible for the gene defect also change a restriction site.
 - The fragments generated between normal and mutant have different lengths. (*See Figure 18.11*)
 - Sickle alleles can be detected this way.
 - Allele-specific oligonucleotide hybridization:
 - The probe will hybridize if enough bases complement.
 - If there are differences, hybridization fails to occur.
 - See Figure 18.12.

IV Cancer: A Disease of Genetic Changes

- One in three Americans will have some form of cancer in their lifetime. One in four will die of it.

- Cancer is more frequent than in the past, in part due to longer life spans.
- Cancer is caused primarily by genetic changes.
- See Figure 18.14.

A. Cancer cells differ from their normal counterparts

- Cancer cells have lost control over appropriate cell division.
- They form tumors.
- A benign tumor resembles the tissue it comes from.
- They remain localized.
- Malignant tumors do not look like parent tissues.
- They often have irregular structures, such as variable sizes and shapes of nuclei.
- Spreading of cancer is called metastasis.
 - The malignant tumor secretes chemical signals that cause blood vessels to grow into it. This is called angiogenesis.
 - Next, the cells of the tumor secrete enzymes that digest and disintegrate other surrounding tissues.
 - Then, they erode blood vessels. Some cells gain the ability to divide free from the tumor.
 - These enter the bloodstream or lymphatic system. A few of these survive and form additional tumors.
- About 85 percent of all human tumors are carcinomas.
 - They form from epithelial cells.
 - Some skin cancers are of this type.
 - Lung, breast, colon and liver cancers are carcinomas.
- Sarcomas are cancers of tissues such as bone, blood vessels and muscle.
- Leukemias and lymphomas affect the cells that give rise to blood cells.

B. Some cancers are caused by viruses

- Viruses cause at least five types of human cancer. (See Table 18.2)
- Hepatitis B virus is associated with liver cancers in Asians and Africans. Its role is unclear. Papilloma viruses cause genital and anal warts that can often develop into tumors. These can cause cancer without the cell mutating.

C. Most cancers are caused by genetic mutations

- About 85 percent of cancers are caused by genetic changes in cells.
- Carcinogens generally cause mutations that lead to cancer.
- Tobacco smoke, meat preservatives, ultraviolet light from the sun and ionizing radiation are common carcinogens.
- Natural carcinogens are found in foods people eat.
- It is estimated that 80 percent of human exposure to carcinogens come from the substances plants and fungi produce, which humans consume as food.
- *See Figure 18.14.*

D. Two kinds of genes are changed in many cancers

- Oncogenes and tumor suppressor genes are the two kinds of genes involved in cancer.
- Oncogenes act to stimulate cell division and were the genes carried by cancer causing viruses.

- Proto-oncogenes are the normal cellular counterparts that if mutated can also contribute to cancer development. (*See Figure 18.15*)
- Tumor suppressor genes when functioning normally prevent cell division. It takes two defective alleles to enable tumor suppressor genes. (*See Figure 18.16*)
- About 10 percent of cancers are caused by defective tumor suppressor genes.
- An inherited form of breast cancer is linked to tumor suppressor genes
 - Women with one mutant *BRCA1* gene have a 60 percent chance of having breast cancer by age 50.
 - Women with two normal genes have a 2 percent chance.
- See Figure 18.17 for a model on how tumor suppressor genes normally act in cells.
- The protein product of *p53* also stops cells during G1. Mutations in this gene is associated with many cancers. Mutations of *p53* actually increase mutation frequencies by failing to perform one of its functions, stopping DNA replication when DNA has been damaged.

E. The pathway from normal cell to cancerous cell is complex

- Figure 18.18 outlines the progress of the formation of colon cancers.
- Genetic tests have begun to be applied to cells of tumors.

V Treating Genetic Diseases

- To treat genetic disease, physicians must have the disease correctly diagnosed, know the molecular mechanisms of the disease, and be able to intervene early, before disease causes damage.
- Progress is being made toward these objectives.

A. One approach to treatment is to modify the phenotype

- There are three ways to alter the outcome of genetic disease to benefit patients: restricting the substrate, using metabolic inhibitors or supplying the missing protein. (*See Figure 18.22*)
- Restricting the substrate:
 - Reduced dietary phenylalanine for PKU sufferers is an example.
- Metabolic inhibitors:
 - Cholesterol synthesis by the liver can be lowered by metabolic inhibitors such as mevinolin.
 - This helps those with familial hypercholesterolemia.
- Supplying the missing protein:
 - An example is treatment of hemophilia with clotting factor protein produced using biotechnology.
 - Often a single-gene mutation causes numerous problems which are difficult to treat with a single product.
 - Many problems are intracellular and therefore difficult to treat.

B. Gene therapy offers the hope of specific treatments

- Gene therapy is when a new gene is inserted into the patient's cells.
- Varieties of different methods are being tried to get cells to take up and incorporate the DNA.

- Cells have been removed from patients, genetically modified and then reintroduced back into the same patient.
- A girl without the enzyme adenosine deaminase had white blood cells modified and then reintroduced. She lived for a short time. (*See Figure 18.20*)
- Attempts have been made at genetic modification of cells within patients.
 - This approach has been tried as a cancer treatment.
 - Tumor suppressor genes have been put in vectors and targeted at tumors.
 - See Figure 18.19.

VI Sequencing the Human Genome

- The Human Genome Project is an internationally funded program to determine the sequences of the human genome. Private industry has also made rapid progress.
- During the summer of 2000, "draft" sequences of the entire genome were ready.
- Final sequences will be ready by 2003.
- *See Figure 18.21.*

A. There are two approaches to genome sequencing

- Chromosomes can be sorted by a machine based on their different sizes.
- The DNA of a chromosome is too long to be sequenced directly.
- The DNA must be fragmented using restriction enzymes, and restriction maps must be constructed.
- Hierarchical Sequencing:
 - See Figure 18.21 for a description of Hierarchical sequencing.
 - Chromosomes are fragmented. A large piece is isolated.
 - This DNA is cut into smaller large pieces with restriction enzymes.
 - These fragments are cloned into bacterial artificial chromosome (BAC) vectors.
 - Sequence-tagged sites (STS) are identified on the fragments. (About 41,000 STS's have been mapped on the human chromosomes.)
 - If two large fragments, cut with restriction enzymes, both have the same STS, they must overlap.
 - This makes fragment ordering possible.
- Shotgun sequencing:
 - Human DNA is randomly broken into 500-800 base pair fragments.
 - Each fragment is sequenced.
 - A computer finds and uses overlapping sequences shared by fragments to align them.
 - The entire 180 million base pair fruit fly genome was sequenced by the shotgun method.

B. The human genome is more than just a sequence

- Many simple organisms have gene sequences in common with humans. Determining their functions is useful to understanding their function in humans.
- Mapping technologies make isolation of genes easier.
- Better drug treatments based on genetic make-up might be possible.

- Differential gene expression can be studied using DNA chips.
- The Human Genome Diversity Project is looking for sub-population differences.

C. How should genetic information be used?

- Many people are mostly uninterested in their genetic make-up, except if they or a close relative is known to have a genetic disease.
- Insurance companies might take a frightful interest, and try to use the information for health insurance exclusions.
- Who will profit from the Project?