Hershey & Chase

- 1952, experiments that confirmed that DNA is the genetic material of life
- They used radioisotopes of phosphorus and sulfur to mark the protein and the DNA of bacteriophage viruses
  - *Radioisotopes* are radioactive forms of elements that decay over time at a predictable rate
    - For the experiment ^{32}P and ^{35}S were used
  - T2 bacteriophage virus and E.coli were used
    - A *bacteriophage virus* is a virus composed of a protein outer coat and an inner core of DNA, or sometimes RNA
    - When this virus infects a cell, it takes over the metabolism of the cell resulting in multiple viruses of its kind being formed
  - Phosphoru marked the DNA of the virus and sulfur the protein of which the virus was made/had on the outer core
- One culture had radioactive phosphorus and the other radioactive sulfur
- Then they were allowed to infect the bacterium E.coli
  - In this way the virus could reproduce and the researchers could see whether the genetic material is the DNA or the protein
    - If it was the DNA, then E.coli would contain phosphorus, but if it contained sulfur, then the protein would be the genetic material
- They put the cultures in centrifugal and observed that the pellet which had formed on the bottom, contained phosphorus

DNA structure

- Each strand of DNA is composed of a backbone of alternating phosphate and deoxyribose molecules
  - These two molecules are held together by a covalent bond called *phosphodiester bond* or *linkage*
  - It forms between a hydroxyl group of the 3’ carbon of deoxyribose and the phosphate group attached to the 5’ carbon of the deoxyribose
    - Condensation reaction
    - Each nucleotide is attached to the previous one by this kind of bond
- New nucleotides are always added to the 3’ end
- DNA molecules always have a non-bonding 5’ carbon at the top and a free 3’ carbon at the bottom with which other nucleotides can bond

- The DNA molecule is negatively charged
- The two strands of DNA are *antiparallel* to each other
  - They run in opposite directions
  - One strand has the 5' carbon on the top and the 3' carbon on the bottom and the other strand has the 3' on top and the 5' on the bottom
- The nitrogenous bases on the nucleotides form *hydrogen bonds* with the bases of the other strand
  - A with T => double bond and C and G => triple bond
    - Complementary bases

Complementary bases

- Adenine and guanine are double-ring structures known as *purines*
- Cytosine and thymine are single-ring structures known as *pyrimidines*
- A double-ring nitrogenous base always pairs with a single-ring nitrogenous base
- Complementary pairing occurs, because of the specific distance that exists between the two sugar-phosphate chains

DNA packaging

- In eukaryotic cells, DNA molecules are paired with a type of protein called *histone*
  - There are 4 different histones and one fifth for a different use
  - DNA and histones make up a *nucleosome*
- A nucleosome consists of two molecules of each of the 4 different histones
- The DNA wraps twice around these 8 histones and one molecule of the fifth type is attached to the nucleosome acting as tape which helps the structure to maintain its shape
  - The DNA is attracted to the histones, because DNA is negatively charged while histones are positively charged
- Between the nucleosomes is a single string of DNA
- The fifth histon leads to further wrapping of the DNA
- When DNA is wrapped around histones and then in more elaborate structures, it cannot be transcribed
  - This regulates the process
  - Allows only certain areas of DNA to be involved in protein synthesis

Types of DNA sequences

- *Genomics* involves the science of sequencing, interpreting and comparing whole genomes
- The International Human Genome Project, started in 1970, had the assignment to determine the whole human genome
  - First publication in 2001
- It was found that only 2% of our genes code for proteins
  - The rest are:
    - Regulators for gene expression
      - Sequences of DNA regulating if and when DNA should be unwinded from the histones
    - Introns (24%)
      - Highly repetitive sequences that do not have any coding function
      - 5-300 base pairs per repetitive sequence
      - Can be as many as 100 000 replicates of a certain type per genome
        - If it is in discrete areas, it is referred to as *satellite DNA*
        - They are mostly dispersed
      - They are transportable elements, called *jumping genes*
        - Can move from one genome location to another
        - Never detach from the DNA molecule they are part of
      - The centromere of chromosomes is largely made up of them
    - Telomeres
      - Highly coiled DNA that does not have a coding function as *pseudogenes*, due to mutation involving base sequence change
      - Occur on the ends of chromosomes
      - Consist of 6-8 base pair sequence that is repeated up to hundreds of thousands of times
        - Number of pairs and number of their repetition depend of the species
    - Genes for tRNA
      - The genetic code to produce tRNA molecules

- The promoter region for a particular gene determines which DNA strand is the antisense strand
  - Usually on the same strand, but there are some genes on the other strand
  - The promoter is not transcribed

Splicing

- In eukaryotic cell DNA there are regions that do not code for any proteins, called *introns*
  - These will also be transcribed
  - Prokaryotic DNA does not have them
- The mRNA that contains both exons and introns is called *pre-mRNA* or *primary RNA transcript*
  - *Splicing* is the process during which the introns are removed
  - Those sequences of mRNA remaining after splicing are called *exons*
- *Spliceosomes* cut the introns out
  - They consist of small nuclear RNAs called *snRNAs* or *snurps*
- When introns are removed, the exons might be rearranged, resulting in different possible proteins
- In some higher eukaryotes different sections of a gene act as introns at different times
  - This increases the number of possible proteins produced by one gene

Protection

- The final mRNA (*mature RNA)* is also protected by a cap and a tail
  - The *cap* exists on the 5' end and it is made of modified guanine nucleotide with three phosphates
  - The *tail* is on the 3' end and consists of 50-250 adenine nucleotides
- The cap and tail protect the mRNA from degradation in the cytoplasm
  - The cell has mechanisms that break down nucleotides that are outside the nucleus
- They also enhance the translation process

Regulators of transcription

- Nucleosomes
  - If DNA is wrapped around the histones it cannot be transcribed
  - A *methyl group (CH_3)* is an organic functional group that causes a section of DNA to wrap more tightly around histones
    - Prevents transcription of that particular allele
    - The gene becomes inactive, not expressed
    - The methylation patterns persist through cell division = keep on being methylated
  - Example: mammalian females have two X chromosomes, one become heavily methylated = inactive
  - Methylation may regulate the expression of either the maternal or paternal allele of a gene
  - Some methylation patterns are also associated to cancer
    - *Hypermethylation* and *hypomethylation*
      - Both due to carcinogens and toxic substances
    - Can be used to diagnose cancer
  - Sometimes methylation goes wrong and genes that were supposed to be active are inactive and vice versa
- Binding proteins
  - Proteins can regulate transcription by assisting or hindering the binding of RNA to the promoter
  - Transcription *activators* cause looping of DNA, which results in a shorter distance between the activator and the promoter region of the gene
    - Assist the binding of RNA polymerase
  - The *repressors* bind to segments of DNA called *silencers* preventing transcription of that segment
- The environment of the organism
  - Recent science has shown that the environment of the organism influences genes
    - The science is called *epigenetics*
    - Example: people in urban areas show expression of more respiratory genes than people from rural areas
    - Genes can be methylated or unmethylated

- *Translation* is the process when the processed mRNA is translated to an amino acid sequence
  - This occurs at the ribosomes
- Free ribosomes synthesize proteins for use within the cell
  - These proteins that are produced are water soluble
- Bound ribosomes (rough endoplasmic reticulum) synthesize proteins for secretion or for use in lysosomes

Ribosomes

- Consist of a *large subunit* and *small subunit*
  - There subunits are composed of ribosomal RNA (rRNA) molecules and many proteins
    - These proteins are small and associated with the core of the RNA subunits
- ⅔ of their mass is rRNA (80S for eukaryotes and 70S for prokaryotes)
- The molecules of the ribosomes are constructed in the nucleolus of eukaryotic cells and exit the nucleus through the membrane pores
  - Prokaryotes have no nuclear membrane
- There is also a difference in molecular makeup between eukaryotes and prokaryotes
- The decoding of a strand of mRNA to produce a polypeptide occurs in the space between the 2 subunits
  - There are binding sites for mRNA
  - There are 3 binding sites for tRNA
- Sites and function:
  - A
    - Holds the tRNA carrying the next amino acid to be added to the polypeptide chain
  - P
    - Holds the tRNA carrying the growing polypeptide chain
  - E
    - Site from which tRNA that has lost its amino acid is discharged
- The triplet bases of the mRNA codon pair with the complementary bases of the triplet anticodon of the tRNA
- The binding between mRNA and tRNA occurs in the cavity between the 2 subunits

- The amino acids have different properties which lead to different interactions
  - Side chains:
    - Contain sulfur
    - Are polar/hydrophilic
    - Are non-polar/hydrophobic
    - Have an electrical charge
- Covalent bonds can be formed between sulfur atoms on different amino acids
  - Create *disulfide bonds*
    - Sometimes called *bridges* because they are strong (the strongest)
- Ionic bonds between positively and negatively charged side chains
- Hydrogen bonds between polar side chains

- Quaternary
  - Some proteins also include a quaternary structure
    - All the bonds mentioned in the first 3 levels of protein structure are involved here
  - It involves multiple polypeptide chains that combine to form a single structure
  - This is also due to interactions between the R-groups
  - Some proteins with a quaternary structure include a *prosthetic group*
    - These proteins are called *conjugated proteins*
    - The prosthetic group is a *non-polypeptide group*, usually metal
    - Example: haemoglobin
      - 4 polypeptide chains bonded to each other (2 alpha and 2 beta chains) = a tetramer
      - Each chain contains a non-polypeptide group called *haem*
        - It contains iron ions that binds to oxygen

Note

- *Dimers* are proteins with 2 polypeptide subunits
- *Tetramers* have 4 such units
- In some cases the units are the same, but they may be different

Fibrous and globular proteins

- *Fibrous proteins* are long and narrow and usually insoluble in water
  - Example: collagen, actin
- *Globular proteins* have a three-dimensional shape and are mostly water soluble
  - Example: haemoglobin, insulin

Amino acids

- Are grouped based on the properties of their side chains
- Amino acids with non-polar side chains are hydrophobic
  - Non-polar amino acids are found in the regions of proteins that are linked to the hydrophobic area of the cell membrane
- Polar amino acids have hydrophilic properties
  - Found in regions of proteins that are exposed to water
  - Amino acids in membrane proteins create hydrophilic channels through which polar substances can move
- Polar and non-polar amino acids are important in determining the *specificity of an enzyme*
  - Only specific substrates can fit in the active site of an enzyme
    - The fitting involves the general shape and polar properties of the substrate and of the amino acids exposed at the active site

Metabolism

- *Metabolism* is the sum of all chemical reactions occurring within a living organism
- *Anabolism* is the type of reaction that uses energy to build complex organic molecules from simpler ones
- *Catabolism* is the type of reaction that breaks down complex organic molecules with the release of energy

Anabolic reactions	Catabolic reactions
Build complex molecules	Breaks down complex molecules
Are endergonic	Are exergonic
Are biosynthetic	Are degradative
Example: photosynthesis	Example: cellular respiration

- *Endergonic reactions* occur when the products of a chemical reaction have more energy than the reactants or the substrates of the reaction
  - Tend to occur in *biosynthetic reactions* in which more complex molecules are produced
  - Energy flows into a system
- *Exergonic reactions* occur when the products of a chemical reactions have less energy than the reaction's reactants or substrates
  - Tend to occur in *degradative reactions* in which complex molecules are broken down into simpler materials
  - Energy flows out of a system

Metabolic pathways

- Almost all metabolic reactions in organisms are catalysed by enzymes
- *Metabolic* or *biochemical pathways* are specific sequences in which many of the metabolic reactions occur
- Enzymes cause one substrate to be changed to another until the final product of the pathway is formed
- Some metabolic pathways consist of cycles of reactions and some of chains of reactions. Others include both

Cellular respiration and mitochondria

- ➢ Cell respiration is the most important catabolic process in life
- ➢ It is the process by which ATP is provided to the organism so that it can live
  - ○ It is a very complex series of chemical reactions, most of which occur in the mitochondria

Feature	Role
Outer membrane	Separates the contents of mitochondria from the rest of the cell
Matrix	Contains the enzymes for the link reaction and the Krebs cycle
Cristae	Increase the surface area for oxidative phosphorylation
Inner membrane	Contains the carriers for the ETC and ATP synthase for chemiosmosis
Intermembrane space	Contains hydrogen ions/protons

- ➢ Mitochondria can be seen using microscopes of different technologies
- ➢ We have seen electron micrographs made by electron tomography
  - ○ A method to get a three-dimensional image of an active mitochondrion

Oxidation and reduction

- ➢ cell respiration contains many oxidation and reduction reactions
  - ○ There 2 reactions occur together during chemical reactions
  - ○ Called *redox reactions* for short

Oxidation	Reduction
Loss of electrons	Gain of electrons
Gain of oxygen	Loss of oxygen
Loss of hydrogen	Gain of hydrogen
Results in many C–O bonds	Results in many C–H bonds

Results in a compound with lower potential energy	Results in a compound with higher potential energy

- The reduced form of a molecule always has more potential energy than the oxidized form of the molecule
- Redox reactions play a key role in the flow of energy through living systems
  - Because the electrons that are flowing from one molecule to the next are carrying energy with them

Example: $C_6H_{12}O_6 + 6O_2 \Rightarrow 6CO_2 + 6H_2O$ + energy

  - $C_6H_{12}O_6$ was oxidized since it lost hydrogen atoms to form carbon dioxide
  - $6O_2$ was reduced since it gained hydrogen atoms to form water

Glycolysis

- Uses no oxygen and occurs in the cytoplasm
  - No organelles are required
  - It proceeds efficiently in both aerobic and anaerobic environments
- Occurs in both prokaryotic and eukaryotic cells
- Steps:
  - Two molecules of ATP are used to begin glycolysis
    - The phosphates from the ATPs are added to glucose to form another sugar
      - Less stable molecule
      - The process is called *phosphorylation*
  - The less stable 6-carbon sugar is split into two 3-carbon sugars
    - This splitting process is known as *lysis*
  - These sugars enter an oxidation phase involving ATP formation and the production of the reduced coenzyme NAD
    - Each of those phosphate molecules undergoes oxidation to form a reduced NAD^+ which becomes NADH
      - Energy is released and it is used to add an inorganic phosphate to the 3-carbon compound
  - Enzymes remove the phosphate groups so that they can be added to ADP to produce ATP

        - The end result is the formation of 4 molecules of ATP, 2 molecules of NADH and 2 molecules of pyruvate (3-carbon)
- *Pyruvate* is the ionized form of pyruvic acid
- Once pyruvate is obtained, the next pathway is determined by the presence of oxygen
    - If oxygen is present, pyruvate enters the mitochondria and aerobic respiration occurs
    - If not, anaerobic respiration occurs in the cytoplasm
        - Later, pyruvate is converted to lactase in animals and ethanol and carbon dioxide in plants
- High levels of ATP in the cytoplasm will inhibit the first enzyme in the pathway by end-product inhibition
- The way of producing ATP in glycolysis is called *substrate-level phosphorylation*, because the phosphate group is transferred directly to ADP from the original phosphate-bearing molecule

The link reaction

- Pyruvate enters the matrix of the mitochondria via active transport
- *Link reaction* is the first process that occurs in the mitochondria, that is decarboxylation
- *Decarboxylation* is the removal of a carbon atom
- Pyruvate is decarboxylated and a 2-carbon acetyl group is formed
    - The removed carbon is released as carbon dioxide
- The acetyl group is oxidised by NAD^+ giving another NADH
- The acetyl group combines with coenzyme A (CoA) to form acetyl-CoA
- The link reaction is controlled by a system of enzymes
- Acetyl CoA enters the Krebs cycle to continue the aerobic respiration process
- Acetyl CoA can be produced from most carbohydrates and lipids
- Acetyl CoA can be synthesized into a lipid for storage purposes when ATP levels in the cell are high

Krebs cycle

- If cellular ATP levels are low, the acetyl CoA enters the Krebs cycle
    - The cycle is also called *tricarboxylic acid cycle* and is a cycle because it begins and ends with the same substance