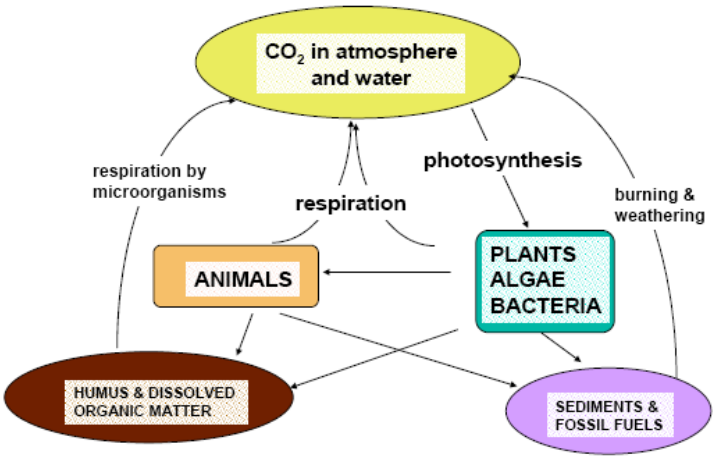
General Principles of Metabolism

Introduction

- **Living organisms** require a continual input of **free energy** for three major purposes:
    - The performance of **mechanical work** in **muscle contraction** and **cellular movement**.
    - The **active transport** of **molecules** and **ions**.
    - The **synthesis of macromolecules** and other **biomolecules** from **precursors**.
- The **free energy** used in these processes maintain an organism **far from equilibrium**.
- **Phototrophs** obtain this energy by **trapping sunlight**, whereas **chemotrophs**, which includes animals, obtain energy through the **oxidation** of **foodstuffs** generated by **phototrophs**.
- **Metabolism** is essentially a **linked series of chemical reactions** forming **metabolic pathways**. We can divide these pathways into **two broad classes**:
    - Pathways that **convert energy** from **fuels** into a **biologically useful form** are called **catabolic pathways** (and are part of **catabolism**).
    - Pathways that **require energy** are called **anabolic pathways** (and are part of **anabolism**).
    - Some can be **either**, depending on **energy conditions** in the cell – these are **amphibolic pathways**.

Metabolic Strategies

Organisms **obtain carbon** via several alternative **metabolic strategies**. Here's a rather poor representation of the carbon cycle:



		Source of carbon	
		Organic compounds (Heterotrophs)	**CO_2 (Autotrophs)**
Source of energy	**Light**	Photo-organotrophs (eg: nonsulphur purple bacteria)	Photholitotophs (eg: plants, algae, bacteria)
	Organic compounds	Chemoorganotrophs (eg: animals, fungi, bacteria)	
	Inorganic compounds		Chemolithotrophs

Chemolithotrophs are able to live in **very hostile conditions**, and are believed to be some of the **first organisms** to live on earth. They **oxidise inorganic compounds** to **obtain energy**. For example:

- **Nitrifying bacteria** use energy from the oxidation of ammonia and nitrite [the electrons released can then be used for oxidative phorphorylation]
  - $NH_3 + ½O_2 + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$
  - $NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$

  This is why nitrogen on earth predominantly exists as **nitrates**. [These electrons are also then used to fix CO_2, but not very fast!]

- **Sulphur oxidising bacteria** use energy from **oxidation of H_2S**, **elemental sulphur** and **$S_2O_3^{2-}$**. For example, *Thiobacillus concretivorous*, which corrodes concrete [note the sulphuric acid produced!]
  - $S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+ + 2e^-$
  - $S + H_2O + 1.5\ O_2 \rightarrow SO_4^{2-} + 2H^+$
- **Hydrogen bacteria** gain energy from **oxidising H_2 gas** with NAD^+ using the **hydrogenase** enzyme:
  - $H_2 + NAD^+ \rightarrow NADH + H^+$

Thermodynamics

- For a long time, so called "Vitalists" belived that there was something "magical" about the cell. **Eduard Buchner** but an end to that by showing that **yeast juice** could be used for **fermentation**.
- The **Second Law of Thermodynamics** states that the **entropy of the universe *must* increase**.
- When a reaction happens, changes in entropy occur in the **system** (the reactants, products, .etc…) and in the **surroundings**:
  - The **change in entropy** of the surroundings is **proportional** to the **heat released into the surroundings** (which is ***minus*** the heat changes in the system), and **inversely proportional** to the **temperature** of the surroundings. So, $\Delta S_{surroundings} = -\frac{\Delta H_{system}}{T}$.
  - The **change in entropy** of the **system** depends on the **reagents** and **products**.
- Thus, the **total changes in entropy** is given by $\Delta S_{total} = \Delta S_{system} - \frac{\Delta H_{system}}{T}$.
- Now, for the **total entropy** to **increase**, we must have that $\Delta S_{system} > \frac{\Delta H_{system}}{T}$, or, equivalently, that $\Delta H_{system} - T\Delta S_{system} < 0$. This function, $\Delta H_{system} - T\Delta S_{system}$, is called the **Gibbs Free Energy**, ΔG, of the reaction.
  - If $\Delta G < 0$, the reaction is **exergonic**, and **can occur spontaneously**.
  - If $\Delta G = 0$, the system is at **equilibrium**, and **no net change** can occur.

- If $\Delta G > 0$, the reaction is **endorgonic**, and **cannot occur spontaneously**.

This is, in effect, a "balance between the two laws".

- The Gibbs Energy, however, tells us **nothing** about the **rate** of the reaction. A reaction could have a **positive** ΔG, but still be occurring **imperceptibly slowly**.
- The ΔG of a reaction **only** depends on the free energy of the **reactants** and on the free energy of the **products** – *not* on the path taken to convert one to the other. It is a **state function**.
- The ΔG of a reaction is given by:

$$\Delta G = \Delta G^\circ + RT \ln \frac{\prod[\text{Products}]}{\prod[\text{Reactants}]}$$

Where ΔG° is the **standard free-energy change**, when each of the reactants are present in a concentration of 1 M (or 1 atm for a gas), and $\prod[\text{Products}]$ is the **product** of the **concentrations** (more accurately: **activities**) of the **products**.

- To **simplify biochemical calculations**, the **standard free-energy change** is taken at **pH 7** and denoted $\Delta G^{\circ'}$. The **activity** of H^+ and H_2O are then always taken to be **1**.
- At **equilibrium**, $\Delta G = 0$, and

$$\Delta G^{\circ'} = -RT \ln \frac{\prod[\text{Products}]}{\prod[\text{Reactants}]} = -RT \ln K'_{eq}$$

So:

$$K'_{eq} = 10^{-\frac{\Delta G^{\circ'}}{RT \log e}}$$

Which, at a temperature of 25° C, reduces to

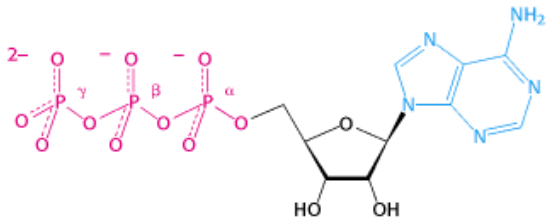
$$K'_{eq} = 10^{-\frac{\Delta G^{\circ'}}{5.69}}$$

[Conversely, $\Delta G^{\circ'}$ itself can be determined by looking at the equilibrium].

- Now, it is a fundamental principle that **the overall free-energy change for a chemically coupled series of reactions is equal to the sum of the free-energy changes of the individual steps**. Thus, a **thermodynamically unfavourable reaction can be driven by a thermodynamically favourable reaction to which it is coupled**, by making the overall ΔG of the

reaction **negative**, and so making K'_{eq} sufficiently big. Coupling can occur in various ways, for example through a **shared intermediate**. **Enzymes** also couple reactions effectively.

- **ATP is the universal currency of free energy** in the cell. The structure of ATP is:



Adenosine triphosphate (ATP)

The **active form** of **ATP** is usually a **complex** with $\mathbf{Mg^{2+}}$ or $\mathbf{Mn^{2+}}$. The P –O–P bonds are called **phosphoanhydride** bonds.

Some biosynthetic reactions are driven by other nucleoside triphosphates, such as **guanosine triphosphate** (**GTP**). **Nucleoside diphosphate kinases** catalyse the transfer of **phosphate groups** between these different molecules.

There are several properties of the ATP molecule that give it such a high **phosphoryl transfer potential**:

- **ADP**, and **particularly** $\boldsymbol{P_i}$ have greater **resonance stabilisation** than does ATP.
- At **pH 7**, the **triphosphate part** of ATP has **four negative charges** – these **repel each other**.
- More **water** can bind **more effectively** to **ADP** and $\boldsymbol{P_i}$ than can bind to the **phosphoanhydride** part of ATP, thus stabilising ADP by **hydration**.

The ΔG of hydration of ATP under typical cellular conditions is roughly –50 kJ mol^{-1} [$\Delta G^{\circ'} = -30.5$ kJ mol^{-1}]. Thus, coupling a reaction with the hydrolysis of ATP increases K_{eq} by a factor of roughly 10^8 (10^5 if using $\Delta G^{\circ'}$][1].

[1] The difference has something to do with the concentration of Mg^{2+}, Ca^{2+} and H_2O. In cellular conditions, [H_2O] is so high (~55 M) that the logarithmic term is very large and negative.

- Some compounds, however, have **phosphoryl transfer potentials** *higher* than that of ATP, and can therefore transfer phosphate groups **to** ADP during substrate-level phosphorylation (eg: phosphoenolpyruvate, 1,3-bisphosphoglycerate, creatine phosphate, etc…). This **intermediate value** of its phosphorylation potential (compared to other biologically important phosphorylated molecules) makes **ATP** an **ideal carrier of phosphoryl groups**.

  Under **high energy demand** or **metabolic crisis**, phosphocreatine initially acts as a "reservoir" of high-energy phosphates and buffers ATP. We cam measure this in a tissue using ^{31}P NMR spectroscopy.

Oxidation of Carbon Compounds

- The **oxidation of carbon compounds** is an important **source of cellular energy**.
- Carbon compounds are oxidised, and the **energy released** is used to make **ATP** from **ADP** and $\boldsymbol{P_i}$.
- In **aerobic organisms**, the **ultimate electron acceptor** from this oxidation is $\mathbf{O_2}$ to make $\mathbf{CO_2}$.
- In **substrate-level phosphorylation**, energy is **first trapped** as a **high phosphoryl-transfer-potential** compound, and then **used to form ATP**.
- In **oxidative phosphorylation**, energy is first **converted** into an **ion gradient**, which is then **used** to produce **ATP**.
- Energy from foodstuffs is usually extracted in three stages:
  1) **Large molecules of food** are **broken down** into **smaller units**. No useful energy is captured here.
  2) The **numerous small molecules** are **degraded** into a few, **simple units** that play a central role in metabolism. In fact, most of then are converted to **Acetyl CoA**.
  3) ATP is produced from the **complete oxidation** of the **Acetyl unit** in **Acetyl CoA**. This consists of the **citric acid cycle** and **oxidative**

phosphorylation. The Acetyl unit is **completely oxidised** to CO_2 and the electrons harvested are used to set up an ion gradient.

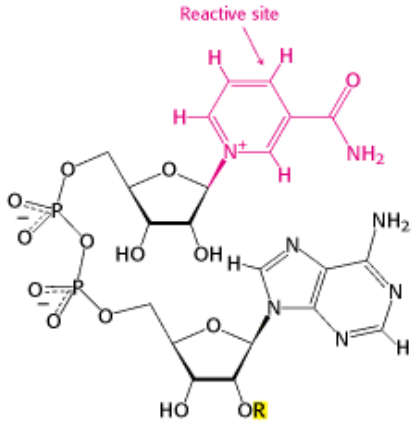
Recurring Motifs in Metabolic Pathways

Metabolic pathways contain many **recurring motifs**.

The first we consider is the recurring use of **activated carriers**:

- **ATP** is an **activated carrier** of **ATP groups**.
- ***Activated carriers of electrons for fuel oxidation*** – The ultimate electron acceptor, in aerobic organisms, is **O_2**. However, electrons are not transferred *directly* to O_2, but to **special carriers**, which are either **pyridine nucleotides** or **flavins**. The **reduced form** of these carriers then transfer their electrons to O_2.

  Nicotinamide adenine dinucleotide (NAD) is a major electron carrier in the oxidation of fuel molecules. The **active part** of the molecule is its **nicotinamide ring**, a **pyridine derivative** synthesised from the vitamin **niacin**. The nicotinamide ring can accept **a hydrogen ion** and **two electrons**. The reduced form is called NADH.



(R = **H**)

The other major carrier is **flavin adenine dinucleotide**. The **active site** of FAD is a derivative of the vitamin **riboflavin**.

- ***Activated carriers of electrons for reductive biosynthesis*** – High energy electrons are required in most biosyntheses – reducing power is needed in addition to ATP. In most of these reactions, the electron

carrier is $NADP^+$, which is **identical** in structure to NAD^+ apart from the fact that the **R** group above is a $\mathbf{PO_3^{2-}}$ group instead of an **H** atom.

This acts as a "**tag**" to distinguish the electron carrier for **anabolism** and those for **catabolism**. It is a clever way for the cell to be able to establish **two different redox potentials**.

- ***An activated carrier of two-carbon fragments*** – **Coenzyme A** is a carrier of **acetyl groups** (linked to CoA at its **terminal sulfhydril group** by a **thioester** bond). CoA can also carry other acyl groups, of course.

  The **hydrolysis** of this **thioester** bond is **exergonic** [$\Delta G^{\circ'} = -31.5$ kJ mol^{-1}], and so **Acetyl CoA** has a high **acetyl-group-transfer potential**. More so, in fact, than an ester, because the resonance structure formed by C=O with an ester is stronger than with a thioester.
- **Biotin** is an **activated carrier** of $\mathbf{CO_2}$.
- **Uridine diphosphate glucose** is an **activated carrier** for **glucose**.

Note, however, that these molecules are **thermodynamically stable** in the absence of an enzyme! Thus, NADH, $FADH_2$ and NADPH react **very slowly** with O_2 and **ATP** and **Acetyl CoA** are hydrolysed **very slowly** in the **absence of a catalyst**. This enables **enzymes** to **control** the **flow** of **energy** and **reducing power**.

Regulation of Metabolism

Metabolic processes are **regulated** in three **principal ways**:

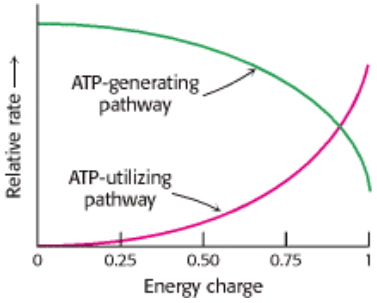
- ***Controlling the amount of enzymes*** – the amount of an enzyme depends both on its **rate of synthesis** and **rate of degradation**.
- ***Controlling catalytic activity*** – this can be done in several different ways:
  - **Reverse allosteric control**, where the **first step** in a pathway is **inhibited** by the **product** of the cycle. This type of control can be **almost instantaneous** (eg: **aspartate transcarbamoylase** is inhibited by **cytidine triphosphate**).

- **Reversible covalent modification** – for example, **glycogen phosphorylase** (glycogen → glucose) is **activated** by the **phosphorylation** of a **particular serine residue** when **glucose is sparse**.
- **Hormones coordinate metabolic relations between different tissues**, often by regulating the reversible modification of key enzymes.
- The **energy charge** of a cell is defined as

$$\text{Energy charge} = \frac{[\text{ATP}]+\frac{1}{2}[\text{ADP}]}{[\text{ATP}]+[\text{ADP}]+[\text{AMP}]}$$

[0 if all AMP and 1 if all ATP]

Daniel Atkinson showed that **catabolic pathways** are **inhibited** by **high energy charge**, and vice-versa. In a plot of *Rate* vs. *Energy Charge*, the graphs of the two pathways are **steep** near an energy charge of **about 0.8-0.95**, where they **usually intersect**. Clearly, this is designed to **keep energy charge within narrow limits** (**buffer it**):



- ***Controlling the accessibility of substrates*** – in **Eukaryotes**, **metabolic regulation** and **flexibility** are enhanced by **compartmentalisation**. For example, **fatty acid oxidation** takes place in the **mitochondria**, whereas **fatty acid synthesis** takes place in the **cytoplasm**. Thus, **compartmentalisation segregates opposed reactions**.

  Controlling the **flux of substrates** is also a useful strategy; **glucose breakdown** can only take place in a cell if **insulin** is **present** to **promote the entry** of glucose into the cell.

Reduction and Oxidation

REDUCTION is the **GAIN** of **ELECTRONS** [sometimes **GAIN** of **HYDROGEN**]

OXDIATION is the **LOSS** of **ELECTRONS** [sometimes **LOSS** of **HYDROGEN**]