L1 Citric acid cycle

Key Notes

Role

The cycle oxidizes pyruvate (formed during the glycolytic breakdown of glucose) to CO_2 and H_2O, with the concomitant production of energy. Acetyl CoA from fatty acid breakdown and amino acid degradation products are also oxidized. In addition, the cycle has a role in producing precursors for biosynthetic pathways.

Location

The citric acid cycle occurs within the mitochondria of eukaryotes and the cytosol of prokaryotes.

The cycle

The citric acid cycle has eight stages:

1. Production of citrate from oxaloacetate and acetyl CoA (catalyzed by citrate synthase).
2. Isomerization of citrate to isocitrate (catalyzed by aconitase).
3. Oxidation of isocitrate to α-ketoglutarate (catalyzed by isocitrate dehydrogenase; the reaction requires NAD^+).
4. Oxidation of α-ketoglutarate to succinyl CoA (catalyzed by the α-ketoglutarate dehydrogenase complex; the reaction requires NAD^+).
5. Conversion of succinyl CoA to succinate [catalyzed by succinyl CoA synthetase; the reaction requires inorganic phosphate and GDP (or ADP)].
6. Oxidation of succinate to fumarate (catalyzed by succinate dehydrogenase; the reaction involves FAD).
7. Hydration of fumarate to malate (catalyzed by fumarase).
8. Oxidation of malate to oxaloacetate (catalyzed by malate dehydrogenase; the reaction requires NAD^+).

Energy yield

For each turn of the cycle, 12 ATP molecules are produced, one directly from the cycle and 11 from the re-oxidation of the three NADH and one $FADH_2$ molecules produced by the cycle by oxidative phosphorylation.

Regulation

The citric acid cycle is regulated at the steps catalyzed by citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase via feedback inhibition by ATP, citrate, NADH and succinyl CoA, and stimulation of isocitrate dehydrogenase by ADP. Pyruvate dehydrogenase, which converts pyruvate to acetyl CoA to enter the cycle, is inhibited by acetyl CoA and NADH. In addition, this enzyme is inactivated by phosphorylation, a reaction catalyzed by pyruvate dehydrogenase kinase. A high ratio of $NADH/NAD^+$, acetyl CoA/CoA or ATP/ADP stimulates phosphorylation of pyruvate dehydrogenase and so inactivates this enzyme. Pyruvate inhibits the kinase. Removal of the phosphate group (dephosphorylation) by a phosphatase reactivates pyruvate dehydrogenase.

Biosynthetic pathways

Amino acids, purines and pyrimidines, porphyrins, fatty acids and glucose are all synthesized by pathways that use citric acid intermediates as precursors.

Related topics

- Glycolysis (J3)
- Gluconeogenesis (J4)
- Fatty acid breakdown (K2)
- Fatty acid synthesis (K3)
- Electron transport and oxidative phosphorylation (L2)
- Amino acid metabolism (M2)

Role

The citric acid cycle, also known as the **TCA (tricarboxylic acid) cycle** or **Krebs cycle** (after its discoverer in 1937), is used to oxidize the pyruvate formed during the glycolytic breakdown of glucose into CO_2 and H_2O. It also oxidizes acetyl CoA arising from fatty acid degradation (Topic K2), and amino acid degradation products (Topic M2). In addition, the cycle provides precursors for many biosynthetic pathways.

Location

The citric acid cycle operates in the mitochondria of eukaryotes and in the cytosol of prokaryotes. Succinate dehydrogenase, the only membrane-bound enzyme in the citric acid cycle, is embedded in the inner mitochondrial membrane in eukaryotes and in the plasma membrane in prokaryotes.

The cycle

The cycle forms the central part of a three-step process which oxidizes organic fuel molecules into CO_2 with the concomitant production of ATP.

Step 1 – Oxidation of fuel molecules to acetyl CoA

A major source of energy is glucose which is converted by glycolysis (see Topic J3) into pyruvate**. Pyruvate dehydrogenase** (a complex of three enzymes and five coenzymes) then oxidizes the pyruvate (using NAD^+ which is reduced to NADH) to form acetyl CoA and CO_2. Since the reaction involves both an oxidation and a loss of CO_2, the process is called **oxidative decarboxylation**.

Step 2 – The citric acid cycle

The cycle carries out the oxidation of acetyl groups from acetyl CoA to CO_2 with the production of four pairs of electrons, stored initially in the reduced electron carriers NADH and $FADH_2$ (*Fig. 1*).

The cycle has eight stages:

1. Citrate (6C) is formed from the irreversible condensation of acetyl CoA (2C) and oxaloacetate (4C) – catalyzed by **citrate synthase**.
2. Citrate is converted to isocitrate (6C) by an isomerization catalyzed by **aconitase**. This is actually a two-step reaction during which *cis*-aconitate is formed as an intermediate. It is the *cis*-aconitate which gives the enzyme its name.
3. Isocitrate is oxidized to α-ketoglutarate (5C) and CO_2 by **isocitrate dehydrogenase**. This mitochondrial enzyme requires NAD^+, which is reduced to NADH.
4. α-Ketoglutarate is oxidized to succinyl CoA (4C) and CO_2 by the **α-ketoglutarate dehydrogenase complex**. Like pyruvate dehydrogenase, this is a complex of three enzymes and uses NAD^+ as a cofactor.
5. Succinyl CoA is converted to succinate (4C) by **succinyl CoA synthetase**. The reaction uses the energy released by cleavage of the succinyl–CoA bond to synthesize either GTP (mainly in animals) or ATP (exclusively in plants) from P_i and, respectively, GDP or ADP.
6. Succinate is oxidized to fumarate (4C) by **succinate dehydrogenase**. FAD is tightly bound to the enzyme and is reduced to produce $FADH_2$.
7. Fumarate is converted to malate (4C) by **fumarase**; this is a hydration reaction requiring the addition of a water molecule.

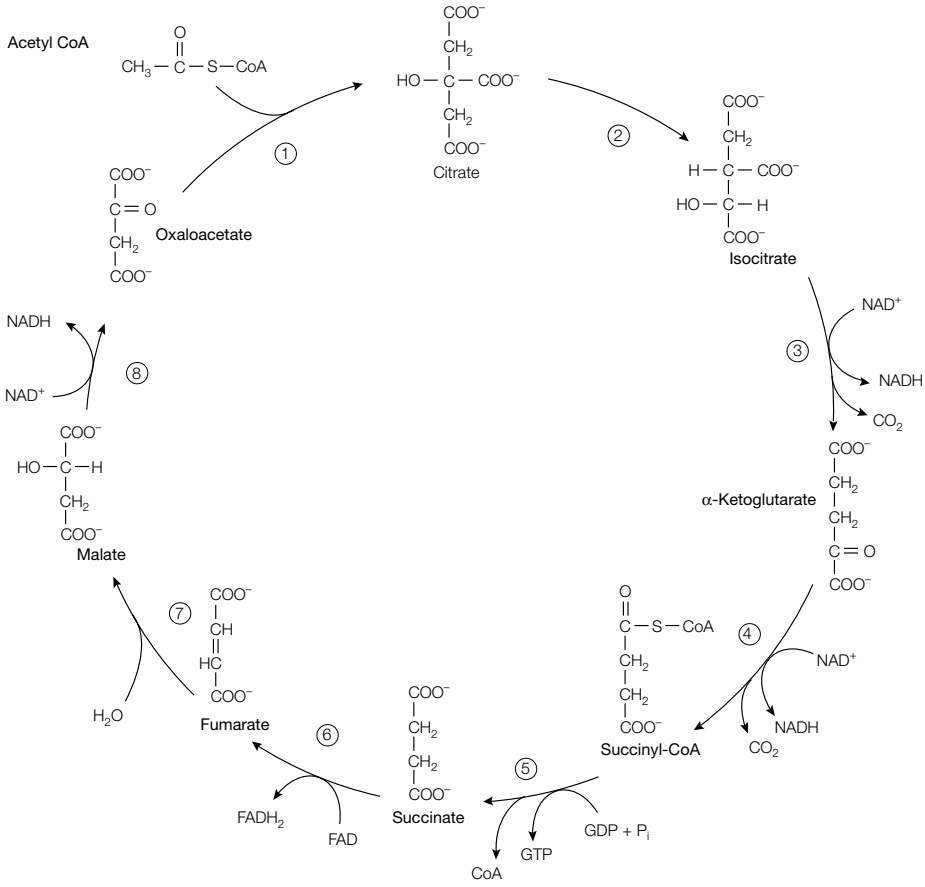

Fig. 1. The citric acid cycle (reactions 1–8 are described in the text).

8. Malate is oxidized to oxaloacetate (4C) by **malate dehydrogenase**. NAD^+ is again required by the enzyme as a co-factor to accept the free pair of electrons and produce NADH.

Step 3 – Oxidation of NADH and $FADH_2$ produced by the citric acid cycle
The NADH and $FADH_2$ produced by the citric acid cycle are re-oxidized and the energy released is used to synthesize ATP by **oxidative phosphorylation** (see Topic L2).

Energy yield

Each of the three NADH molecules produced per turn of the cycle yields 3 ATPs and the single $FADH_2$ yields 2 ATPs by oxidative phosphorylation (although some measurements indicate that the quantities are 2.5 and 1.5 respectively – see p. 380). One GTP (or ATP) is synthesized directly during the conversion of succinyl CoA to succinate. Thus the oxidation of a single molecule of glucose via the citric acid cycle produces 12 ATP molecules.

Regulation

Regulation of the cycle is governed by substrate availability, inhibition by accumulating products, and allosteric feedback inhibition by subsequent intermediates in the cycle. Three enzymes in the cycle itself are regulated (citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase) and so is the enzyme which converts pyruvate to acetyl CoA to enter the cycle, namely pyruvate dehydrogenase (*Fig. 2*):

- *citrate synthase* is inhibited by citrate and also by ATP (the K_m for acetyl CoA is raised as the level of ATP rises);
- *isocitrate dehydrogenase* is inhibited by NADH and ATP but activated by ADP;
- *α-ketoglutarate dehydrogenase* is inhibited by NADH and succinyl CoA;
- *pyruvate dehydrogenase* is inhibited by NADH and acetyl CoA (i.e. product inhibition). However, in eukaryotes the enzyme is also controlled by phosphorylation/dephosphorylation via **pyruvate dehydrogenase kinase** and a **phosphatase**. The kinase catalyzes the phosphorylation of a specific Ser residue in pyruvate dehydrogenase, using ATP as the phosphate donor, and this inactivates the enzyme. Removal of the phosphate group by the phosphatase reactivates the enzyme. At any one time, the activity of pyruvate dehydrogenase is determined by the relative balance between the kinase and phosphatase reactions. Increasing the NADH/NAD^+, acetyl CoA/CoA or ATP/ADP ratio stimulates phosphorylation and hence inactivates pyruvate dehydrogenase. As pyruvate builds up, it inhibits the kinase and hence allows the phosphatase to reactivate pyruvate dehydrogenase, thus stimulating pyruvate conversion to acetyl CoA.

Overall, the cycle speeds up when cellular energy levels are low (high ADP concentration, low ATP and NADH) and slows down as ATP (and then $NADH_2$, succinyl CoA and citrate) accumulates.

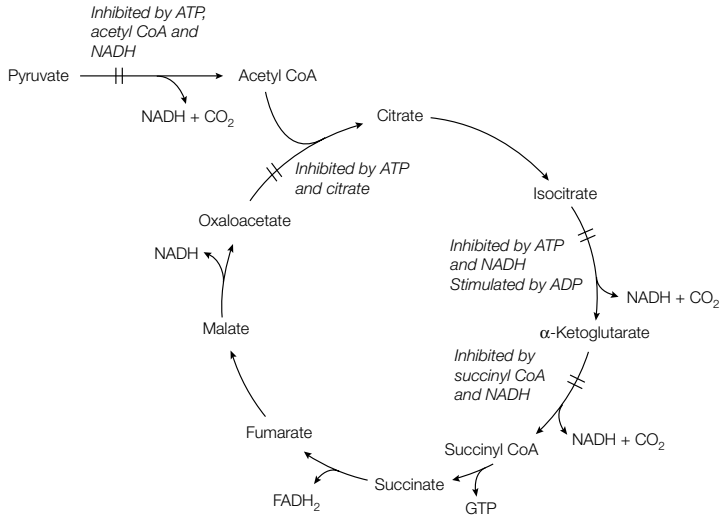

Fig. 2. Regulation points of the citric acid cycle.

Biosynthetic pathways

The intermediates in the cycle provide precursors for many biosynthetic pathways. For example:

- synthesis of fatty acids from citrate (Topic K3);
- amino acid synthesis following transamination of α-ketoglutarate (Topic M2);
- synthesis of purine and pyrimidine nucleotides from α-ketoglutarate and oxaloacetate;
- oxaloacetate can be converted to glucose by gluconeogenesis (Topic J4);
- succinyl CoA is a central intermediate in the synthesis of the porphyrin ring of heme groups (Topic M4).

L2 Electron transport and oxidative phosphorylation

Key Notes

Overview

Electron transport and oxidative phosphorylation re-oxidize NADH and $FADH_2$ and trap the energy released as ATP. In eukaryotes, electron transport and oxidative phosphorylation occur in the inner membrane of mitochondria whereas in prokaryotes the process occurs in the plasma membrane.

Redox potential

The oxidation–reduction potential, E, (or redox potential) of a substance is a measure of its affinity for electrons. The standard redox potential (E_0') is measured under standard conditions, at pH 7, and is expressed in volts. The standard free energy change of a reaction at pH 7, $\Delta G^{0\prime}$, can be calculated from the change in redox potential $\Delta E_0'$ of the substrates and products. A reaction with a positive $\Delta E_0'$ has a negative $\Delta G^{0\prime}$ (i.e. is exergonic).

Electron transport from NADH

Electrons are transferred from NADH to oxygen along the electron transport chain (also called the respiratory chain). NADH passes electrons to NADH-Q reductase (Complex I), a large protein complex that contains FMN and two types of iron–sulfur (FeS) clusters in iron–sulfur proteins. The electrons are accepted by the FMN to produce $FMNH_2$ and then passed to the iron atoms of the FeS clusters which accept and donate electrons by alternating between Fe^{3+} and Fe^{2+} states. Electrons from NADH-Q reductase are passed to ubiquinone (coenzyme Q, CoQ), converting it to ubiquinol (or $CoQH_2$), and then to Q-cytochrome *c* reductase (Complex III). This contains cytochrome *b* and cytochrome c_1, as well as an FeS protein. A cytochrome contains a heme group with a central iron atom which changes from the Fe^{3+} state to the Fe^{2+} state on accepting an electron. When the electron is donated to another component, the iron atom changes back to the Fe^{3+} state. The Q-cytochrome *c* reductase passes the electrons to cytochrome *c* which in turn passes them to cytochrome *c* oxidase (Complex IV), a complex that contains two cytochromes (cytochrome *a* and a_3) paired with copper atoms (Cu_A and Cu_B , respectively). During electron transfer, the copper atoms cycle between the Cu^{2+} and Cu^{+} states. Finally, cytochrome *c* oxidase passes four electrons to molecular oxygen to form two molecules of water.

Formation of an H^+ gradient

The change in redox potential along the chain is a measure of the free energy change at each step. At the steps involving NADH-Q reductase, Q-cytochrome *c* reductase and cytochrome *c* oxidase, the free energy change is large enough to pump H^+ ions across the inner mitochondrial membrane, from the mitochondrial matrix into the intermembrane space, to create an H^+ gradient. Therefore, each of these complexes is an H^+ pump driven by electron transport.

Electron transport from $FADH_2$

$FADH_2$ is reoxidized to FAD by donating two electrons to succinate-Q reductase (Complex II), a protein complex that contains FeS clusters. It passes the electrons on to ubiquinone in the main electron transport chain where their further transport leads to the formation of an H^+ gradient and ATP synthesis. However succinate-Q reductase does not itself pump H^+ ions.

Electron transport inhibitors

Rotenone and amytal inhibit electron transport at NADH-Q reductase, antimycin A inhibits Q-cytochrome *c* reductase, and cyanide (CN^-), azide (N_3^-) and carbon monoxide (CO) all inhibit cytochrome *c* oxidase.

Oxidative phosphorylation

Oxidative phosphorylation is ATP synthesis linked to the oxidation of NADH and $FADH_2$ by electron transport through the respiratory chain. This occurs via a mechanism originally proposed as the chemiosmotic hypothesis. Energy liberated by electron transport is used to pump H^+ ions out of the mitochondrion to create an electrochemical proton (H^+) gradient. The protons flow back into the mitochondrion through the ATP synthase located in the inner mitochondrial membrane, and this drives ATP synthesis. Approximately three ATP molecules are synthesized per NADH oxidized and approximately two ATPs are synthesized per $FADH_2$ oxidized.

ATP synthase as a rotatory engine

ATP synthase is located in the inner mitochondrial membrane. It consists of two major components, F_1 ATPase attached to component F_0 (coupling factor 0). Hence, ATP synthase is also known as F_0F_1 ATPase. In mitochondria, this complete complex uses the energy released by electron transport to drive ATP synthesis. The F_1 ATPase has a subunit structure of $\alpha_3\beta_3\gamma\delta\varepsilon$ whereas F_0 consists of a ring of 10–14 c subunits. During ATP synthesis, protons flow through a channel between the α subunits and c ring which causes the c ring to rotate. The rotating c ring turns the γ subunit of F_1 ATPase within a ring formed by the α and β subunits. Conformational changes to the β subunits now lead to ATP synthesis.

Coupling and respiratory control

Electron transport is normally tightly coupled to ATP synthesis; electrons do not flow through the electron transport chain to oxygen unless ADP is simultaneously phosphorylated to ATP. If ADP is available, electron transport proceeds and ATP is made; as the ADP concentration falls, electron transport slows down. This process, called respiratory control, ensures that electron flow occurs only when ATP synthesis is required.

Uncouplers

Some chemicals (e.g. 2,4-dinitrophenol; DNP) are uncoupling agents; they allow electron transport to proceed without ATP synthesis. They uncouple mitochondria by carrying H^+ ions across the inner mitochondrial membrane and hence dissipate the proton gradient. The energy derived from uncoupled electron transport is released as heat. Uncoupling also occurs naturally in some tissues (e.g. the mitochondria of brown adipose tissue are uncoupled by a protein called thermogenin). The resulting production of heat (nonshivering thermogenesis) by the adipose tissue serves to protect sensitive body tissues in newborn animals and to maintain body temperature during hibernation.

Reoxidation of cytosolic NADH

Cytosolic NADH cannot cross the inner mitochondrial membrane and enter mitochondria to be reoxidized. However, it can be reoxidized via the glycerol 3-phosphate shuttle. Cytosolic glycerol 3-phosphate dehydrogenase oxidizes the NADH and reduces dihydroxyacetone phosphate to glycerol 3-phosphate. The glycerol 3-phosphate enters the mitochondrion and is converted back to dihydroxyacetone phosphate by mitochondrial glycerol 3-phosphate dehydrogenase (an FAD-linked enzyme). The dihydroxyacetone phosphate diffuses back to the cytosol. The enzyme-linked $FADH_2$ is reoxidized by transferring its electrons to ubiquinone in the electron transport chain. Since the electrons enter the electron transport chain from $FADH_2$, only about two ATPs are synthesized per molecule of cytosolic NADH. In heart and liver, cytosolic NADH can be reoxidized via the malate–aspartate shuttle. Oxaloacetate in the cytosol is reduced to malate by NADH and enters the mitochondrion via a malate–α-ketoglutarate carrier. In the matrix, the malate is re-oxidized to oxaloacetate by NAD^+ which is converted to NADH, resulting in a net transfer of electrons from cytosolic NADH to matrix NADH. The oxaloacetate is converted to aspartate by transamination, leaves the mitochondrion and is reconverted to oxaloacetate in the cytosol, again by transamination.

Related topics

Glycolysis (J3)
Citric acid cycle (L1)
Photosynthesis (L3)

Overview

In eukaryotes, electron transport and oxidative phosphorylation occur in the inner membrane of mitochondria. These processes re-oxidize the NADH and $FADH_2$ that arise from the citric acid cycle (located in the mitochondrial matrix; Topic L2), glycolysis (located in the cytoplasm; Topic J3) and fatty acid oxidation (located in the mitochondrial matrix; Topic K2) and trap the energy released as ATP. Oxidative phosphorylation is by far the major source of ATP in the cell. In prokaryotes, the components of electron transport and oxidative phosphorylation are located in the plasma membrane (see Topic A1).

Redox potential

The oxidation of a molecule involves the loss of electrons. The reduction of a molecule involves the gain of electrons. Since electrons are not created or destroyed in a chemical reaction, if one molecule is oxidized, another must be reduced (i.e. it is an **oxidation–reduction reaction**). Thus, by definition, oxidation–reduction reactions involve the transfer of electrons. In the oxidation–reduction reaction:

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons NAD^+ + H_2O$$

when the NADH is oxidized to NAD^+, it loses electrons. When the molecular oxygen is reduced to water, it gains electrons.

The **oxidation–reduction potential**, E, (or **redox potential**) is a measure of the affinity of a substance for electrons and is measured relative to hydrogen. A positive redox potential means that the substance has a higher affinity for electrons than does hydrogen and so would accept electrons from hydrogen. A substance with a negative redox potential has a lower affinity for electrons than does hydrogen and would donate electrons to H^+, forming hydrogen. In the example above, NADH is a strong reducing agent with a negative redox potential and has

a tendency to donate electrons. Oxygen is a strong oxidizing agent with a positive redox potential and has a tendency to accept electrons.

For biological systems, the **standard redox potential** for a substance (E_0') is measured under standard conditions, at pH 7, and is expressed in volts. In an oxidation–reduction reaction, where electron transfer is occurring, the total voltage change of the reaction (change in electric potential, ΔE) is the sum of the voltage changes of the individual oxidation–reduction steps. The standard free energy change of a reaction at pH 7, $\Delta G^{0\prime}$, can be readily calculated from the change in redox potential $\Delta E_0'$ of the substrates and products:

$$\Delta G^{0\prime} = -nF\,\Delta E_0'$$

where n is the number of electrons transferred, $\Delta E_0'$ is in volts (V), $\Delta G^{0\prime}$ is in kilocalories per mole (kcal mol^{-1}) and F is a constant called the Faraday (23.06 kcal V^{-1} mol^{-1}). Note that a reaction with a **positive** $\Delta E_0'$ has a **negative** $\Delta G^{0\prime}$ (i.e. is exergonic).

Thus for the reaction:

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons NAD^+ + H_2O$$

$$\Delta E_0' = +1.14\ V$$

$$\Delta G^{0\prime} = -52.6\ kcal\ mol^{-1}.$$

Electron transport from NADH

Comparing the energetics of the oxidation of NADH:

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons NAD^+ + H_2O \qquad \Delta G^{0\prime} = -52.6\ kcal\ mol^{-1}$$

and the synthesis of ATP:

$$ADP + P_i + H^+ \rightleftharpoons ATP + H_2O \qquad \Delta G^{0\prime} = +7.3\ kcal\ mol^{-1}$$

it is clear that the oxidation of NADH releases sufficient energy to drive the synthesis of several molecules of ATP. However, NADH oxidation and ATP synthesis do not occur in a single step. Electrons are not transferred from NADH to oxygen directly. Rather the electrons are transferred from NADH to oxygen along a chain of electron carriers collectively called the **electron transport chain** (also called the **respiratory chain**).

Most of the protein components of the electron transport chain exist as four large protein complexes embedded in the inner mitochondrial membrane called:

- NADH-Q reductase (Complex I)
- Succinate-Q reductase (Complex II)
- Q-cytochrome *c* reductase (Complex III)
- Cytochrome *c* oxidase (Complex IV)

Electrons flow from NADH to oxygen through three of these complexes as shown in *Fig. 1*. Each complex contains several electron carriers (see below) that

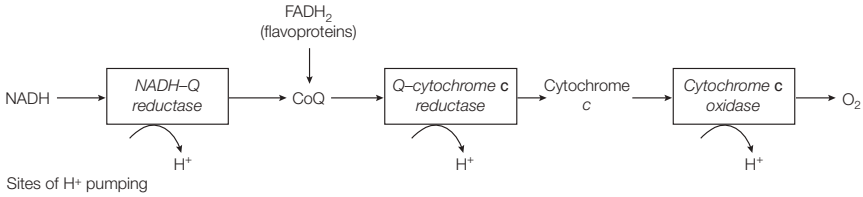

Fig. 1. Overview of the electron transport chain (respiratory chain).

work sequentially to carry electrons down the chain. Two small electron carriers are also needed to link these large complexes; **ubiquinone**, which is also called **coenzyme Q** (abbreviated as **CoQ**), and **cytochrome *c*** (*Fig. 1*).

The flow of electrons from NADH down the respiratory chain can be summarized as follows:

- **NADH-Q reductase** (**Complex I**), which consists of at least 30 polypeptides, binds the NADH and re-oxidizes it to NAD^+, passing the two electrons from NADH to a prosthetic group called **FMN** (**flavin mononucleotide**) (*Fig. 2*) to produce **$FMNH_2$** (see Topic C1 for structure of FMN). Each electron is accepted together with a hydrogen ion, H^+, such that two electrons and two H^+ are accepted in total. The electrons are then transferred, within Complex I, to **iron–sulfur clusters** (FeS) in **iron–sulfur proteins** (also called **nonheme iron proteins**). Several types of FeS clusters exist but in each case the iron atoms are coordinated to inorganic sulfur atoms and the sulfur of cysteine side-chains in the protein. Within an FeS cluster, an electron is carried by the iron atom which, on accepting the electron, changes from the Fe^{3+} (ferric) state to the Fe^{2+} (ferrous) state (*Fig. 2*). As the electron is passed to another electron carrier, the iron atom of the FeS cluster changes back again to the Fe^{3+} state.
- Electrons from the FeS clusters of Complex I are passed on to ubiquinone (CoQ), a small lipid-soluble molecule in the inner mitochondrial membrane. This molecule can act as an electron carrier by accepting up to two electrons and two H^+ ions. In so doing, ubiquinone (CoQ) is converted to ubiquinol ($CoQH_2$).
- When ubiquinol ($CoQH_2$) donates its two electrons to the next carrier in the chain, the **Q-cytochrome *c* reductase** (**Complex III**), the H^+ ions are released once more. This complex contains two types of cytochromes, **cytochrome *b*** and **cytochrome c_1**, as well as an FeS protein (*Fig. 2*). A cytochrome is a protein with a bound **heme group** that contains an iron atom (see Topic M4, *Fig. 1*). Different cytochromes have different heme groups, but all cytochromes have the ability to act as electron carriers. As the electron is accepted, the iron atom of the heme group changes from the Fe^{3+} (ferric) state to the Fe^{2+} (ferrous) state. *Fig. 2* shows the electrons passing from ubiquinol ($CoQH_2$) through the cytochrome *b*, FeS and cytochrome c_1 components of the Q-cytochrome *c* reductase complex to the next electron carrier, cytochrome *c*. Since ubiquinol is a two-electron carrier whereas cytochromes are one-electron carriers, the pathway of electron transfer within the Q-cytochrome *c* reductase complex is complicated and involves ubiquinol ($CoQH_2$) releasing first one electron and an H^+ ion to become ubisemiquinone ($CoQH^\bullet$) and then the second electron and H^+ ion to become ubiquinone (CoQ).
- Cytochrome *c* is a peripheral membrane protein that is loosely bound to the outer surface of the inner mitochondrial membrane. It binds to the Q-cytochrome *c* reductase complex and accepts an electron via an Fe^{3+} to Fe^{2+} transition. Then it binds to the cytochrome *c* oxidase complex (Complex IV) and donates the electron, with the iron atom of the heme of cytochrome *c* then reverting to the Fe^{3+} state (*Fig. 2*).
- **Cytochrome *c* oxidase** (**Complex IV**) contains two cytochromes (cytochrome *a* and a_3). Cytochrome *a* is paired with a copper atom, Cu_A, and cytochrome a_3 is paired with a different copper atom, Cu_B. During electron transfer, the iron atoms of the cytochromes cycle between the Fe^{3+} and Fe^{2+} states whilst the

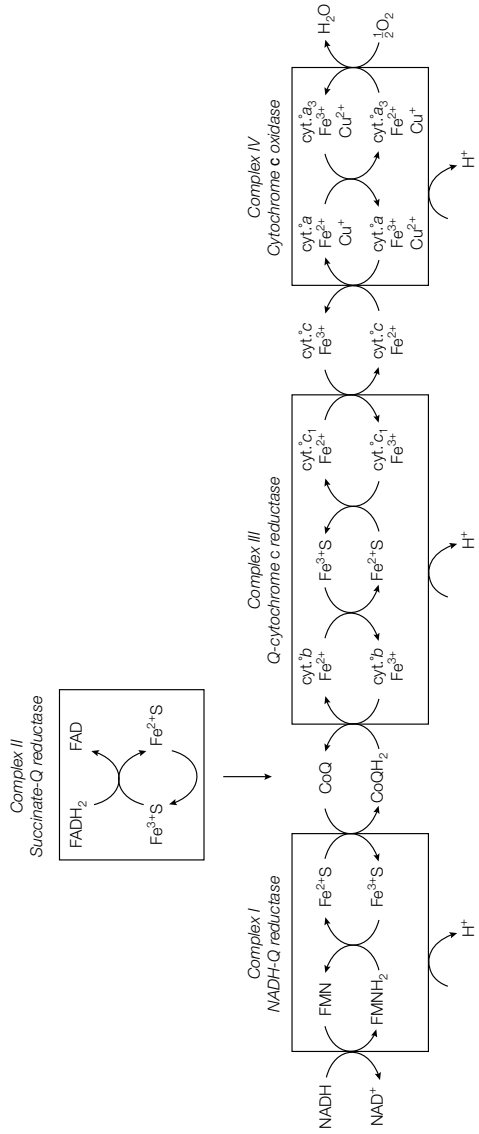

Fig. 2. Details of electron transport.

copper atoms cycle between Cu^{2+} and Cu^{+}. The cytochrome *c* oxidase reaction is complex; it transfers four electrons from four cytochrome *c* molecules and four H^+ ions to molecular oxygen to form two molecules of water:

$$4 \text{ cyt. } c\ (Fe^{2+}) + 4\ H^+ + O_2 \xrightarrow{\textit{Cytochrome c oxidase}} 4 \text{ cyt. } c\ (Fe^{3+}) + 2\ H_2O$$

Formation of an H^+ gradient

All of the electron carriers in the electron transport chain interact according to their redox potentials. Every time that an electron transfer occurs, the accepting carrier has a higher affinity for electrons than the donating carrier. Thus there is a net flow of electrons from NADH (most negative redox potential, least affinity for electrons) to oxygen (most positive redox potential, highest affinity for electrons). This ensures a unidirectional flow of electrons. However, note that each cytochrome, each FeS center and each copper atom can carry only one electron but each NADH donates two electrons. Furthermore, each molecule of oxygen (O_2) needs to accept four electrons to be reduced to a molecule of water, H_2O. The various components are arranged in such a manner as to allow their different electron-handling properties to work in harmony.

The change in redox potential along the chain is a measure of the free energy change occurring (see above). The potential falls (i.e. becomes more positive) throughout the chain but mainly in three large steps that correspond to the three main protein complexes: Complex I, III and IV. The large free energy change at each of these three steps, and only these three steps, is large enough to pump H^+ ions from the mitochondrial matrix across the inner mitochondrial membrane and into the intermembrane space. Thus, each of these three complexes is an **H^+ pump** driven by electron transport (*Figs 1* and *2*). Overall, therefore, electron transport along the chain from NADH releases energy that is used to create an **H^+ gradient**.

Electron transport from $FADH_2$

Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate in the citric acid cycle (Topic L1). The succinate dehydrogenase contains bound FAD that is reduced to $FADH_2$ in the reaction. The re-oxidation of the $FADH_2$ occurs via **succinate–Q reductase** (**Complex II**), an integral protein of the inner mitochondrial membrane. Succinate dehydrogenase is part of this complex but it also contains FeS clusters. During re-oxidation of $FADH_2$, the two electrons pass from the $FADH_2$ to the FeS clusters and are then passed on to ubiquinone (CoQ; see *Fig. 2*). They then enter the main electron transport chain and cause H^+ ions to be pumped out of the mitochondrion as for the oxidation of NADH. However, succinate–Q reductase itself is *not* an H^+ pump because the free energy change of the overall reaction is too small. The $FADH_2$ of other **flavoproteins,** such as mitochondrial glycerol 3-phosphate dehydrogenase in the glycerol 3-phosphate shuttle (see below) and fatty acyl CoA dehydrogenase in fatty acid oxidation (Topic K2), also feed their electrons into the electron transport chain at ubiquinone.

Electron transport inhibitors

Several inhibitors of specific electron carriers are known and were used in the original studies to determine the order of the components in the respiratory chain. For example:

- **rotenone** and **amytal** inhibit electron transport at NADH-Q reductase and so prevent NADH oxidation but the oxidation of $FADH_2$ can still occur since this feeds electrons into the chain at CoQ (see *Fig. 1*) (i.e. past the point of inhibition);

- **antimycin A** inhibits electron transport at the Q-cytochrome *c* reductase complex;
- **cyanide** (CN^-), **azide** (N_3^-) and **carbon monoxide** (CO) all inhibit cytochrome *c* oxidase.

Oxidative phosphorylation

Oxidative phosphorylation is the name given to the synthesis of ATP (*phosphorylation*) that occurs when NADH and $FADH_2$ are oxidized (hence *oxidative*) by electron transport through the respiratory chain. Unlike substrate level phosphorylation (see Topics J3 and L1), it does not involve phosphorylated chemical intermediates. Rather, a very different mechanism was proposed by Peter Mitchell in 1961, the **chemiosmotic hypothesis**. This proposes that energy liberated by electron transport is used to create a proton gradient across the mitochondrial inner membrane and that it is this that is used to drive ATP synthesis. Thus the proton gradient couples electron transport and ATP synthesis, not a chemical intermediate. The evidence is overwhelming that this is indeed the way that oxidative phosphorylation works. The actual synthesis of ATP is carried out by an enzyme called **ATP synthase** located in the inner mitochondrial membrane (*Fig. 3*).

In summary, the process is as follows. Electron transport down the respiratory chain from NADH oxidation causes H^+ ions to be pumped out of the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space by the three H^+ pumps; Complex I, III and IV (see above). [Because $FADH_2$ is reoxidized via ubiquinone (see *Figs 1* and 2), its oxidation causes H^+ ions to be pumped out only by Complex III and IV and so the amount of ATP made from $FADH_2$ is less than from NADH.] The free energy change in transporting an electrically charged ion across a membrane is related both to its electrical charge and the concentration of the species. The pumping out of the H^+ ions generates a higher concentration of H^+ ions in the intermembrane space and an electrical potential, with the side of the inner mitochondrial membrane facing the intermembrane space being positive (*Fig. 3*). Thus, overall, an **electrochemical proton**

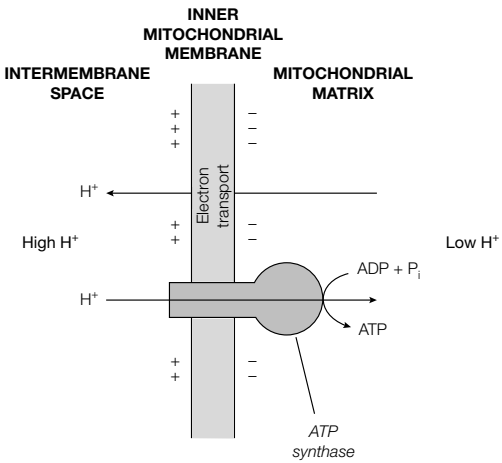

Fig. 3. The mechanism of oxidative phosphorylation.

gradient is formed. The protons flow back into the mitochondrial matrix through the ATP synthase and this drives ATP synthesis. The ATP synthase is driven by **proton-motive force**, which is the sum of the pH gradient (i.e. the chemical gradient of H^+ ions) and the membrane potential (i.e. the electrical charge potential across the inner mitochondrial membrane). There is some debate over the exact stoichiometry of ATP production; in past years it was believed that 3 ATPs were generated per NADH and 2 ATPs per $FADH_2$ but some recent measurements have indicated that the numbers of ATP molecules generated may be 2.5 and 1.5, respectively.

ATP synthase as a rotatory engine

The ATP synthase can be seen as spherical projections from the inner membrane (*Fig. 4*). If mitochondria are subjected to sonic disruption, submitochondrial vesicles are formed in which the spheres of the ATP synthase point outward (*Fig. 4*). In 1960, Racker showed that the spheres can be removed and that the isolated spheres *hydrolyze* ATP, that is, the spheres have ATPase activity (called **F_1 ATPase**; *Fig. 5*). The stripped submitochondrial vesicles, devoid of the F_1 ATPase, can still transport electrons along the electron transport chain but cannot synthesize ATP. These stripped submitochondrial vesicles contain the other major part of the ATP synthase, called **F_0** (coupling factor 0) which spans the inner mitochondrial membrane (*Fig. 5*). Since it is composed of these two major component parts, ATP synthase is also known as **F_0F_1 ATPase**. The complete complex harnesses the energy released by electron transport to drive ATP synthesis whereas alone, without coupling to electron transport, the F_1 component hydrolyzes ATP.

The F_1 ATPase consists of five types of polypeptides in the following ratio: $\alpha 3$, $\beta 3$, γ, δ, ε. The six α and β subunits are arranged alternately in a ring, with a central stalk formed by the γ and ε subunits (*Fig. 5*). F_0 consists of a ring of 10 to 14 **c subunits** sitting in the inner mitochondrial membrane. This contacts a single **a subunit** that links to **two b subunits** and the single δ subunit to form a long column that connects to the head of the F_1 ATPase (*Fig 5*). This overall structure, the F_0F_1 ATPase, is a remarkable molecular motor. During ATP synthesis, protons flow through a channel created at the interface between the **a** subunit and **c** subunits and this causes the **c** subunit ring to spin relative to the static **a** subunit. Thus the **c** ring acts as a 'rotor' and the **a** subunit as a 'stator'. As

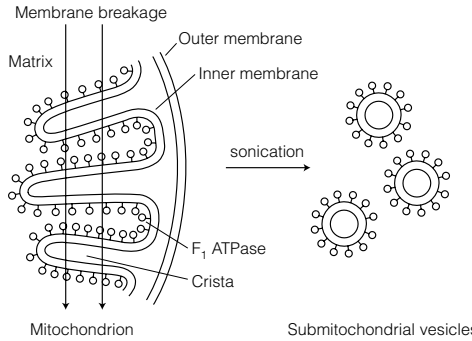

Fig. 4. Sonic disruption (sonication) of mitochondria produces submitochondrial vesicles.

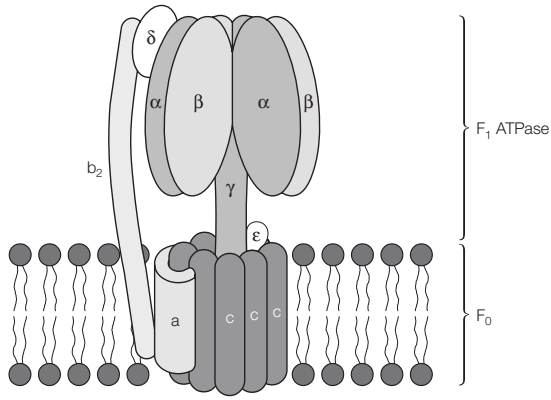

Fig. 5. Schematic representation of the ATP synthase complex.

the **c** subunit ring rotates, it turns the γ subunit stalk, which therefore turns rapidly inside the αβ ring. The αβ ring cannot rotate since it is held in place by the arm of the two **b** subunits and δ subunit (Fig 5).

The three β subunits in the αβ ring can bind ADP and inorganic phosphate. As the γ subunit rotates inside the αβ ring, the mechanical energy is used to drive ATP synthesis; three molecules of ATP are synthesized for each 360 degree rotation of the γ subunit (which equates to over 1000 molecules per second!). How is the ATP made? According to the **binding-charge mechanism** of Paul Boyer, as the rotation causes the γ subunit to turn past the three β subunits, the protein conformation of the three nucleotide binding sites of the β subunits changes through different states which causes ADP to be bound, then phosphorylated and then released as ATP. So the mechanical energy of the rotating γ subunit is used to drive protein conformational changes that in turn lead to ATP synthesis.

Coupling and respiratory control

Electron transport is normally tightly **coupled** to ATP synthesis (i.e. electrons do not flow through the electron transport chain to oxygen unless ADP is simultaneously phosphorylated to ATP). Clearly, it also follows that ATP is not synthesized unless electron transport is occurring to provide the proton gradient. Thus oxidative phosphorylation needs NADH or $FADH_2$, oxygen, ADP and inorganic phosphate. The actual rate of oxidative phosphorylation is set by the availability of ADP. If ADP is added to mitochondria, the rate of oxygen consumption rises as electrons flow down the chain and then the rate of oxygen utilization falls when all the ADP has been phosphorylated to ATP; a process called **respiratory control**. This mechanism ensures that electrons flow down the chain only when ATP synthesis is needed. If the level of ATP is high and the ADP level is low, no electron transport occurs, NADH and $FADH_2$ build up, as does excess citrate, and the citric acid cycle (Topic L1) and glycolysis (Topic J3) are inhibited.

Uncouplers

Some chemicals, such as 2,4-dinitrophenol (DNP), act as **uncoupling agents**, that is, when added to cells, they stop ATP synthesis but electron transport still continues and so oxygen is still consumed. The reason is that DNP and other

uncoupling agents are lipid-soluble small molecules that can bind H^+ ions and transport them across membranes (i.e. they are **H^+ ionophores**). Electron transport occurs and pumps out H^+ ions across the inner mitochondrial membrane but DNP in the same membrane carries the H^+ ions back into the mitochondrion, preventing formation of a proton gradient. Since no proton gradient forms, no ATP can be made by oxidative phosphorylation. Rather the energy derived from electron transport is released as heat.

The production of heat by uncoupling is called **nonshivering thermogenesis.** It is important in certain biological situations. For example, uncoupling occurs naturally in brown adipose tissue. This tissue is rich in mitochondria, the inner mitochondrial membranes of which contain a protein called **thermogenin** (or **uncoupling protein**). Thermogenin allows H^+ ions to flow back into mitochondria without having to enter via the ATP synthase and so uncouples electron transport and oxidative phosphorylation, generating heat. The importance of this natural phenomenon is that brown adipose tissue is found in sensitive body areas of some newborn animals (including humans) where the heat production provides protection from cold conditions. In addition, thermogenesis by brown adipose tissue plays a role in maintaining body temperature in hibernating animals.

Reoxidation of cytosolic NADH

The inner mitochondrial membrane is impermeable to NADH. Therefore NADH produced in the cytoplasm during glycolysis must be reoxidized via a **membrane shuttle**, a combination of enzyme reactions that bypass this impermeability barrier. *Fig. 6* shows the **glycerol 3-phosphate shuttle**. Dihydroxyacetone phosphate in the cytosol is reduced to glycerol 3-phosphate, and NADH reoxidized to NAD^+, by cytosolic glycerol 3-phosphate dehydrogenase. The glycerol 3-phosphate diffuses across the inner mitochondrial membrane where it is converted back to dihydroxyacetone phosphate by mitochondrial glycerol 3-phosphate dehydrogenase, a transmembrane protein of the inner mitochondrial membrane. The dihydroxyacetone phosphate then diffuses back to the cytosol. The mitochondrial glycerol 3-phosphate dehydrogenase does not use NAD^+ but instead uses FAD. The enzyme-linked $FADH_2$ ($E.FADH_2$) is then reoxidized by transferring its electrons to ubiquinone in the same inner mitochondrial membrane (see above). Note that the shuttle does not allow cytoplasmic NADH to enter the mito-

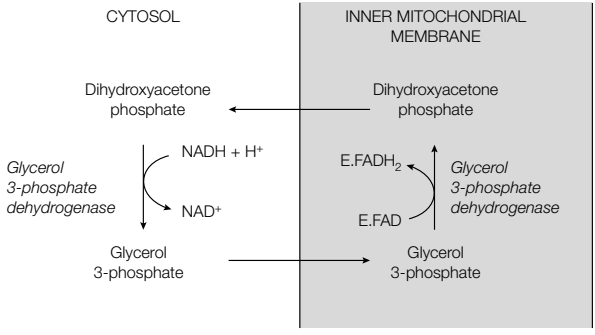

Fig. 6. The glycerol 3-phosphate shuttle.

chondrion but its operation effectively transports the two electrons from the NADH into the mitochondrion and feeds them into the electron transport chain. Since the electrons from cytoplasmic NADH actually enter the electron transport chain from $FADH_2$, only about two ATPs are synthesized instead of approximately three ATPs from each NADH that arises inside the mitochondrion from the citric acid cycle (Topic L1) and fatty acid oxidation (Topic K2).

A similar shuttle, the **malate–aspartate shuttle**, operates in heart and liver (*Fig. 7*). Oxaloacetate in the cytosol is converted to malate by cytoplasmic malate dehydrogenase, reoxidizing NADH to NAD^+ in the process. The malate enters the mitochondrion via a **malate–α-ketoglutarate carrier** in the inner mitochondrial membrane. In the matrix the malate is reoxidized to oxaloacetate by NAD^+ to form NADH. Oxaloacetate does not easily cross the inner mitochondrial membrane and so is transaminated to form aspartate which then exits from the mitochondrion and is reconverted to oxaloacetate in the cytosol, again by transamination. The net result of this cycle of reactions is to transfer the electrons from NADH in the cytosol to NADH in the mitochondrial matrix which is then reoxidized by the electron transport chain.

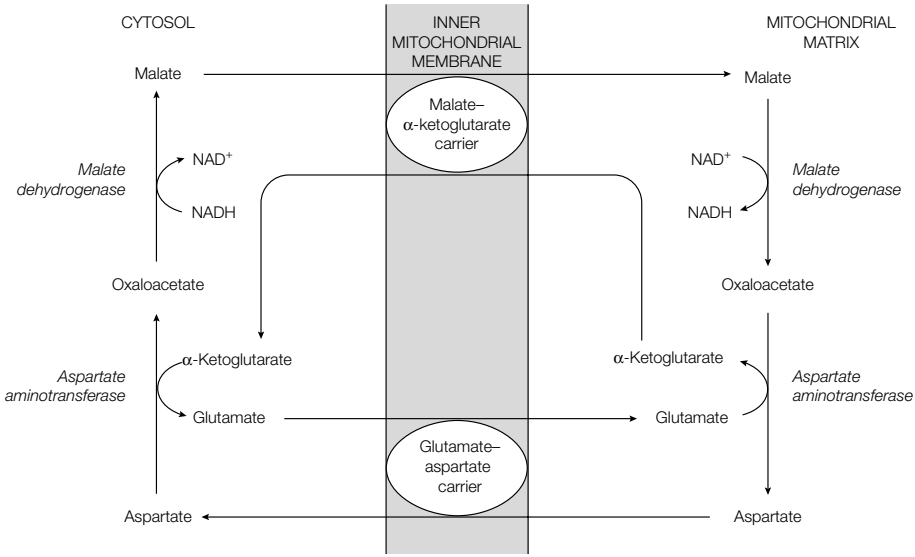

Fig. 7. The malate–aspartate shuttle.

L3 PHOTOSYNTHESIS

Key Notes

Overview

Photosynthesis uses solar energy to synthesize carbohydrate from carbon dioxide and water. In the light reactions, the light energy drives the synthesis of NADPH and ATP. In the dark reactions (carbon-fixation reactions), the NADPH and ATP are used to synthesize carbohydrate from CO_2 and H_2O.

Location

In green plants and algae, photosynthesis takes place in chloroplasts. The light reactions occur in the thylakoid membranes and the dark reactions take place in the stroma. In photosynthetic bacteria the light reactions take place in the bacterial plasma membrane, or in invaginations of it (chromatophores).

Light harvesting in green plants

Sunlight is absorbed by chlorophyll molecules, each of which is a magnesium porphyrin. Accessory pigments, such as carotenoids, absorb light at other wavelengths so maximizing light absorption. The pigments are arranged as photosystems, each photosystem consisting of an antenna complex and a photosynthetic reaction center. An antenna complex has several hundred chlorophyll molecules and accessory pigments clustered together in the thylakoid membrane. The absorption of a photon of light by a chlorophyll molecule raises an electron to a higher energy orbital. The excited chlorophyll can pass its extra energy on to another chlorophyll molecule in the complex by exciton transfer. The energy is channeled to two special chlorophyll molecules in the photosynthetic reaction center.

Photosystems I and II

Green plants and algae use two types of photosystem, photosystem I with chlorophyll P700 in its reaction center and photosystem II with P680 in its reaction center. The two photosystems are linked by a chain of electron carriers. When arranged in order of their redox potentials, the components form the so-called Z scheme. Light excites P680 of photosystem II to P680*. The excited $P680^*$ passes a high-energy electron to pheophytin, and is oxidized to $P680^+$. The $P680^+$ accepts an electron from water and returns to the ground state. Overall, the removal of four electrons from two molecules of water generates four H^+ ions and one molecule of O_2. The high-energy electrons accepted by pheophytin are passed in order to plastoquinone (PQ), the cytochrome *bf* complex (also called cytochrome b_6f complex) and plastocyanin. Light excites P700 of photosystem I to P700*. The excited $P700^+$ passes a high-energy electron to ferredoxin, and becomes oxidized to $P700^+$. The $P700^+$ accepts an electron from plastocyanin and returns to the ground state. Finally, two electrons from two molecules of reduced ferredoxin are transferred to $NADP^+$ to form NADPH.

Noncyclic photophosphorylation

The cytochrome *bf* complex is a proton pump and, during electron transport, pumps H^+ ions from the stroma into the thylakoid space, creating an H^+ gradient. H^+ ions are also released into the thylakoid space when photosystem II oxidizes water to produce oxygen whilst the H^+ ions used to reduce $NADP^+$ to NADPH are taken up from the stroma. Both effects contribute to the H^+ gradient. The proton gradient drives ATP synthesis via an ATP synthase located in the thylakoid membrane (photophosphorylation). Since the electron transport involves a linear array of electron carriers, the system is called noncyclic photophosphorylation.

Cyclic photophosphorylation

When little $NADP^+$ is available to accept electrons, an alternative electron transport pathway is used. The high-energy electron donated by photosystem I passes to ferredoxin, then the cytochrome *bf* complex, then plastocyanin and back to the P700 of photosystem I. The resulting proton gradient generated by the cytochrome *bf* complex drives ATP synthesis (cyclic photophosphorylation) but no NADPH is made and no O_2 is produced.

Bacterial photosynthesis

Cyanobacteria use two photosystems as in green plants. The purple photosynthetic bacterium, *Rhodospirillum rubrum*, has only a single photosystem reaction center. This can carry out cyclic electron transport, synthesizing ATP (cyclic photophosphorylation). Alternatively, noncyclic electron transport can be used, producing NADH. Hydrogen sulfide (H_2S) can act as electron donor, generating sulfur (S). Hydrogen gas (H_2) and a variety of organic compounds can also be used as electron donors. Water is *not* used as electron donor and so no oxygen is produced.

The dark reactions

The dark reactions (carbon-fixation reactions) use the ATP and NADPH produced by the light reactions to 'fix' carbon dioxide as carbohydrate; sucrose and starch. The reactions form a cycle (the Calvin cycle) in which the enzyme ribulose bisphosphate carboxylase (rubisco), located in the stroma, condenses a CO_2 molecule with ribulose 1,5-bisphosphate to produce two molecules of 3-phosphoglycerate. Other reactions then regenerate the ribulose 1,5-bisphosphate. The fixation of three molecules of CO_2 requires six NADPH and nine ATP and leads to the net production of one molecule of glyceraldehyde 3-phosphate. For the synthesis of sucrose, glyceraldehyde 3-phosphate exits to the cytosol and is converted to fructose 6-phosphate and glucose 1-phosphate. The latter is then converted to UDP-glucose and reacts with fructose 6-phosphate to form sucrose 6-phosphate. Hydrolysis of the sucrose 6-phosphate yields sucrose. The glyceraldehyde 3-phosphate from the Calvin cycle is also used to synthesize glucose 1-phosphate which generates ADP-glucose, CDP-glucose or GDP-glucose as precursors for starch synthesis.

The C4 pathway

When the CO_2 concentration is low, rubisco can add O_2 to ribulose1,5-bisphosphate (oxygenase activity) instead of CO_2 (carboxylase activity) producing phosphoglycolate and 3-phosphoglycerate. Metabolism of phosphoglycolate releases CO_2 and NH_4^+ and wastes energy. This consumption of O_2 and release of CO_2 is called photorespiration. Plants in hot climates close their stomata to reduce water loss. This causes a drop in the CO_2 concentration in the leaf, favoring photorespiration. To avoid this problem, these plants carry out the Calvin cycle only in bundle-sheath cells that are protected from the O_2 in air by mesophyll cells. The CO_2 is transported from the air via the mesophyll cells to the bundle-sheath cells by combining with three-carbon molecules (C3) to produce four-carbon molecules (C4). This C4 pathway ensures a high CO_2 concentration for carbon fixation by rubisco in the bundle-sheath cells.

Related topics

Eukaryote cell structure (A2)
Electron transport and oxidative phosphorylation (L2)
Hemes and chlorophylls (M4)

Overview

Photosynthesis occurs in green plants, algae and photosynthetic bacteria. Its role is to trap solar energy and use this to drive the synthesis of carbohydrate from carbon dioxide and water. Using (CH_2O) to represent carbohydrate, the overall reaction is:

$$H_2O + CO_2 \xrightarrow{Light} (CH_2O) + O_2$$

The reactions of photosynthesis occur in two distinct phases:

- **the light reactions**: which use light energy to synthesize NADPH and ATP;
- **the dark reactions**: that use the NADPH and ATP to synthesize carbohydrate from CO_2 and H_2O. In fact, the term 'dark reactions' is a misnomer; these **carbon-fixation reactions** should really be called light-independent reactions.

Location

In green plants and algae, photosynthesis takes place in chloroplasts (see Topic A2). Similar to a mitochondrion, a chloroplast has a highly permeable **outer membrane** and an **inner membrane** that is impermeable to most molecules and ions. Within each chloroplast lies the **stroma**, containing soluble enzymes (analogous to the matrix of a mitochondrion). However, whereas the inner membrane of a mitochondrion contains the electron transport chain and ATP synthase (see Topic L2), in a chloroplast these are located, together with photosynthetic light-absorbing systems, in stacks of flattened membranes within the stroma called **thylakoids** (see Topic A2). Thus the primary events of trapping solar energy in photosynthesis, the light reactions, occur in the thylakoid membranes. The dark reactions take place in the stroma. In photosynthetic bacteria the light reactions take place in the bacterial plasma membrane, or in invaginations of it called **chromatophores**.

Light harvesting in green plants

Sunlight is absorbed by **chlorophyll** molecules. **Chlorophyll** is a porphyrin in which nitrogen atoms are coordinated to a magnesium ion (see Topic M4, *Fig. 1*) (i.e. it is a **magnesium porphyrin**). This contrasts with a heme in which the nitrogen atoms are coordinated to an iron atom to form an iron porphyrin (see Topics L2 and M4). Green plants contain two types of chlorophyll molecules, **chlorophyll *a*** and **chlorophyll *b***, that differ slightly in structure (see Topic M4, *Fig. 1*) and in the wavelength of light they can absorb. Although light is trapped by **chlorophyll** molecules directly, several **accessory pigments** also exist that absorb light and pass the excitation energy on to chlorophyll molecules. Thus the **carotenoids** are important accessory pigments in green plants whilst **phycobilins** are accessory pigments in photosynthetic bacteria. These pigments absorb light at wavelengths different from that of chlorophyll and so act together to maximize the light harvested.

When a chlorophyll molecule is excited by a quantum of light (a **photon**), an electron is excited to a higher energy orbital. The excited chlorophyll can pass on its extra energy to a neighboring chlorophyll molecule by **exciton transfer** (also called **resonance energy transfer**) and so return to the unexcited state. Alternatively, the high-energy electron itself may be passed on, with the chlorophyll taking up a low-energy electron from another source.

The capture of solar energy occurs in **photosystems**. Each photosystem consists of an **antenna complex** and a **photosynthetic reaction center**. The antenna complex is composed of several hundred chlorophyll molecules and accessory pigments clustered together in the thylakoid membrane. When a chlorophyll molecule in the antenna complex absorbs light and is excited, the

energy is passed by exciton transfer, from molecule to molecule, and is finally channeled to two special chlorophyll molecules in the photosynthetic reaction center. The reaction center passes on the energy as a high-energy electron to a chain of electron carriers in the thylakoid membrane (see below).

Photosystems I and II

Green plants and algae use two types of photosystem called **photosystem I** (**PSI**) and **photosystem II** (**PSII**). The chlorophyll in the reaction center of PSI has an absorption maximum at 700 nm and so is called **P700** (P for pigment) and that in the reaction center of PSII has an absorption maximum at 680 nm and so is called **P680**. The two photosystems are linked by other electron carriers. When arranged according to their redox potentials (see Topic L2) the various components form the so-called **Z scheme** (*Fig. 1*) because the overall shape of the redox diagram looks like a Z.

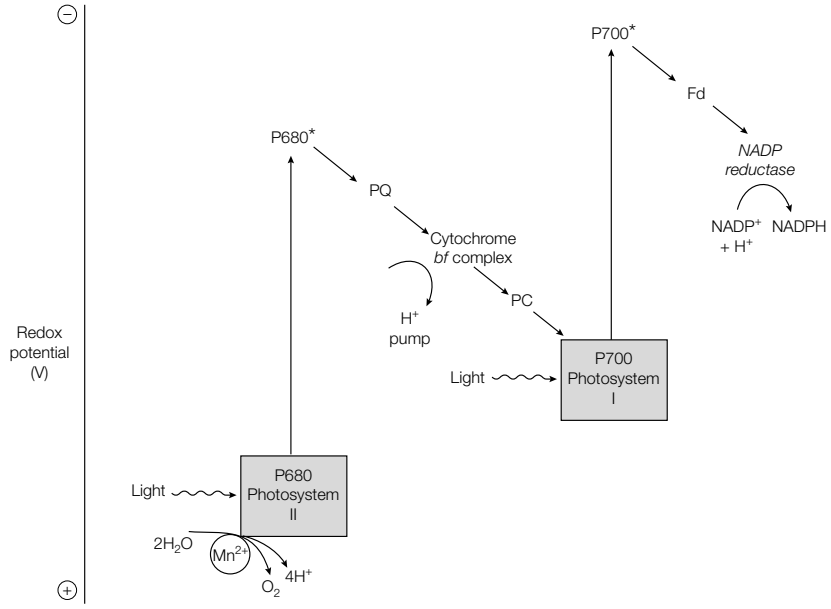

Fig. 1. The Z scheme of noncyclic photophosphorylation in green plants. Note that the arrows in this diagram represent the sequence of events during photosynthesis, as described in the text, and are not metabolic interconversions.

The sequence of reactions during light absorption (*Fig. 1*) is as follows:

1. Light is harvested by the antenna complex chlorophylls of PSII and the energy is channeled towards the reaction center at which P680 is located.
2. The excited P680 (P680*) emits a high-energy electron that passes to **plastoquinone** (PQ), a mobile quinone in the thylakoid membrane. This leaves P680 as the **$P680^+$ cation**. Plastoquinone accepts a total of two electrons and two H^+ ions to form PQH_2.
3. $P680^+$ extracts an electron from water, returning to its unexcited state. The removal of four electrons from two molecules of water requires four quanta

of light to fall on PSII and leads to the production of four H^+ ions and one molecule of O_2:

$$2\,H_2O \xrightarrow{\textit{4 photons}} 4e^- + 4\,H^+ + O_2$$

This reaction is mediated by a cluster of four manganese ions (Mn^{2+}) in PSII.

4. The electrons are now passed from PQH_2 via the **cytochrome *bf* complex** (also called **cytochrome b_6f complex**) to **plastocyanin** (PC). PC is a copper-containing protein that accepts electrons by the copper cycling between Cu^{2+} and Cu^+ states:

$$PQH_2 + 2\,PC\,(Cu^{2+}) \xrightarrow{\textit{Cytochrome } b_6f \textit{ complex}} PQ + 2\,PC\,(Cu^+) + 2\,H^+$$

5. Light energy falling onto the antenna complex of PSI is funneled to the reaction center. Here P700 is excited (to P700*) and emits a high-energy electron to **ferredoxin**, a protein that contains at least one FeS cluster (see Topic L2), becoming the **$P700^+$ cation**. The $P700^+$ receives the electron from PC (see step 4 above) and so returns to the unexcited state.
6. Two high-energy electrons from two molecules of reduced ferredoxin are now transferred to $NADP^+$ to form NADPH. The reaction is carried out by **NADP reductase**.

$$NADP^+ + 2\,e^- + H^+ \xrightarrow{\textit{NADP reductase}} NADPH$$

Taking account of the entire sequence of electron transport, the reaction can be written as:

$$2\,H_2O + 2\,NADP^+ \xrightarrow{\textit{Light}} 2\,NADPH + 2\,H^+ + O_2$$

showing that electrons flow from H_2O to $NADP^+$, reducing it to NADPH.

Noncyclic photophosphorylation

During operation of the Z scheme, high-energy electrons are created by energy-input via the two photosystems and the electrons then travel along a chain of carriers that decrease in redox potential (*Fig. 1*). This is analogous to the passage of electrons along the respiratory chain in mitochondria (Topic L2). In a further analogy, the cytochrome *bf* complex is a **proton pump** (*Fig. 1*) and pumps H^+ ions from the stroma into the thylakoid space (*Fig. 2*). Thus an H^+ gradient is

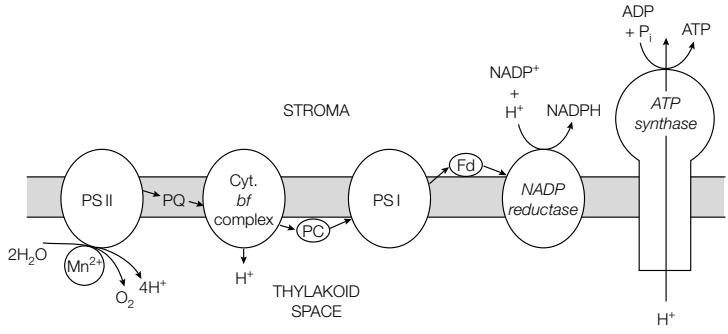

Fig. 2. Formation of the proton gradient and ATP synthesis.

formed during electron transport. Because of the orientation of the various electron transport components in the thylakoid membrane (*Fig. 2*), the H^+ ions released when PSII oxidizes water to produce oxygen are released into the thylakoid space whilst the H^+ used to reduce $NADP^+$ to NADPH by NADP reductase are taken up from the stroma. Thus these two reactions also contribute to the proton gradient. The proton gradient drives ATP synthesis via an ATP synthase located in the thylakoid membrane (*Fig. 2*). This is called **photophosphorylation** and is analogous to ATP synthesis via a proton gradient during oxidative phosphorylation in mitochondria (see Topic L2). The difference is that protons are pumped *out* of mitochondria but *into* a subcompartment, the thylakoid space, in chloroplasts. Because of the alternative ('cyclic') pathway for electron transport and ATP synthesis (see below), the formation of ATP via the joint operation of PSI and PSII (*Fig. 1*; the Z scheme) is called **noncyclic photophosphorylation**.

Cyclic photophosphorylation

When the NADPH/$NADP^+$ ratio is high and little $NADP^+$ is available to accept electrons, an alternative electron transport pathway is used that involves only PSI and a few electron carriers (*Fig. 3*). Here the high-energy electron is passed by ferredoxin to the cytochrome *bf* complex instead of to $NADP^+$. It then flows to plastocyanin and back to the P700 of PSI. The resulting proton gradient generated from the H^+ pump, cytochrome *bf* complex, then drives ATP synthesis. During this **cyclic photophosphorylation**, ATP is formed but no NADPH is made. Furthermore, since PSII is not involved, no O_2 is produced.

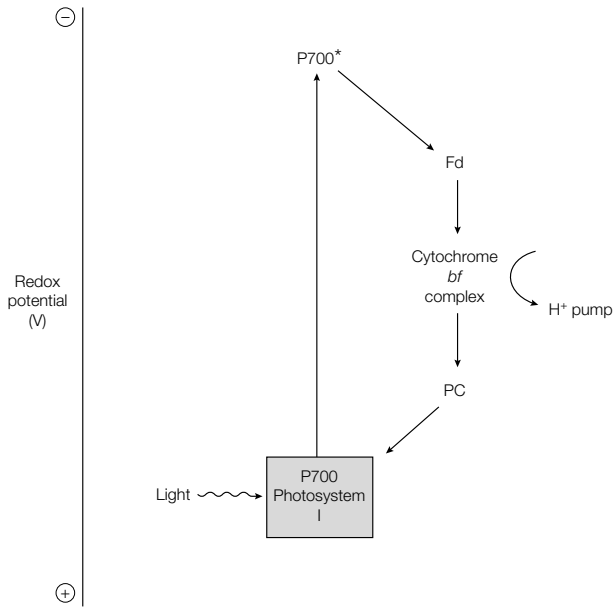

Fig. 3. Cyclic photophosphorylation in green plants. Note that the arrows in this diagram represent the sequence of events during photosynthesis, as described in the text, and are not metabolic interconversions.

In summary, when electron transport is operating in noncyclic mode, via PSI and PSII, the products are NADPH and ATP. In cyclic electron transport, on the other hand, the sole product is ATP.

Bacterial photosynthesis

Cyanobacteria carry out photosynthesis using two photosystems as in green plants. However, other photosynthetic bacteria, such as the **purple photosynthetic bacterium** *Rhodospirillum rubrum*, have only a single photosystem reaction center. This can carry out cyclic electron transport, generating a proton gradient and hence synthesizing ATP (cyclic photophosphorylation). Alternatively, a noncyclic pattern of electron transport can be carried out in which the electrons from the cytochromes pass to NAD^+ (rather than $NADP^+$ as in green plants) to produce NADH. The electron donor is, for example, hydrogen sulfide (H_2S), which generates sulfur (S). Hydrogen gas (H_2) and a variety of organic compounds can also be used as electron donors by certain photosynthetic bacteria. Since H_2O is *not* used as electron donor, no oxygen is produced.

The dark reactions

The dark reactions (also called the **carbon-fixation reactions**) use the ATP and NADPH produced by the light reactions to convert carbon dioxide into carbohydrate. The final products are sucrose and starch.

The key carbon fixation reaction is catalyzed by a large enzyme called **ribulose bisphosphate carboxylase** (often abbreviated to **rubisco**) that is located in the stroma. The reaction condenses a CO_2 molecule with **ribulose 1,5-bisphosphate** (a five-carbon molecule) to produce a transient six-carbon intermediate that rapidly hydrolyzes to two molecules of 3-phosphoglycerate (*Fig. 4*):

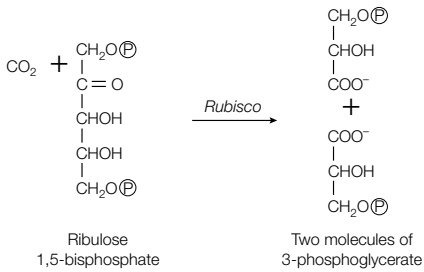

Fig. 4. The rubisco reaction.

Rubisco is a very slow enzyme, fixing only three molecules of its substrate every second and hence a large amount of this enzyme is needed by each plant. Typically, rubisco accounts for 50% or so of the total protein in a chloroplast. Indeed, it is probably the most abundant protein on earth!

The rubisco reaction forms part of a cycle of reactions, called the **Calvin cycle**, that leads to the regeneration of ribulose 1,5-bisphosphate (ready to fix another CO_2) and the net production of glyceraldehyde 3-phosphate for the synthesis of sucrose and starch. Three molecules of CO_2 must be fixed to generate one molecule of glyceraldehyde 3-phosphate (a three-carbon molecule). The overall reaction for this is:

$$3\,CO_2 + 6\,NADPH + 9\,ATP \rightarrow \underset{3\text{-phosphate}}{\text{glyceraldehyde}} + 6\,NADP^+ + 9\,ADP + 8\,Pi$$

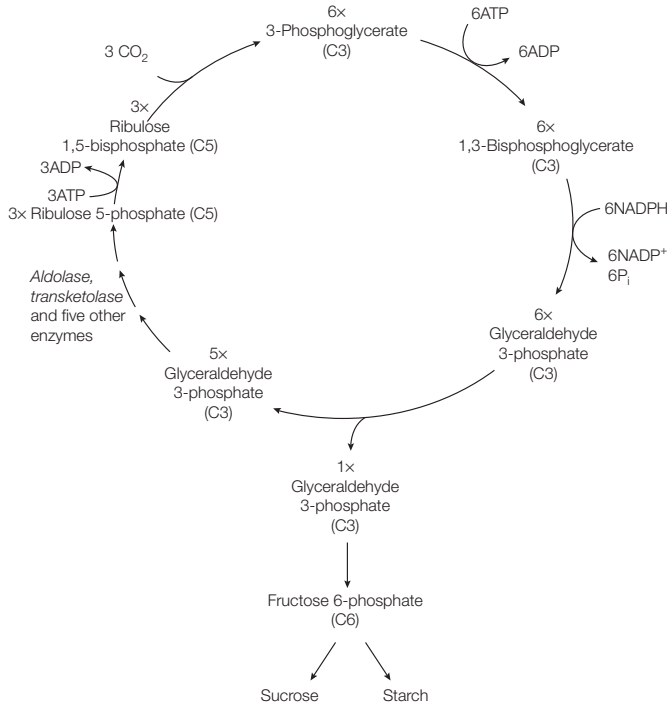

Fig. 5. The Calvin cycle.

The various reactions are shown in *Fig. 5*. Following the formation of six molecules of 3-phosphoglycerate, six ATPs and six NADPHs are used to generate six molecules of glyceraldehyde 3-phosphate. Only one of these molecules of glyceraldehyde 3-phosphate is then used to form fructose 6-phosphate and, eventually, starch. The other five molecules of glyceraldehyde 3-phosphate are converted in a series of steps (involving aldolase, transketolase and five other enzymes) to three molecules of ribulose 5-phosphate which are then phosphorylated (using three molecules of ATP) to form three molecules of ribulose 1,5-bisphosphate, ready for another turn of the cycle. Hence one complete turn of the cycle, trapping three molecules of CO_2 as one molecule of glyceraldehyde 3-phosphate, requires 6 +3 = 9 ATPs, and 6 NADPHs.

Synthesis of sucrose

Much of the glyceraldehyde 3-phosphate produced by the Calvin cycle in chloroplasts is exported to the cytosol and used to produce the disaccharide, sucrose. First the glyceraldehyde 3-phosphate is converted to fructose 6-phosphate and glucose 1-phosphate. The chemical reactions involved are essentially a reversal of glycolysis (see Topic J3). The glucose 1-phosphate is then converted to UDP-glucose and this reacts with fructose 6-phosphate to synthesize sucrose 6-phosphate:

$$\text{UDP-glucose} + \text{fructose 6-phosphate} \rightarrow \text{sucrose 6-phosphate} + \text{UDP}$$

Hydrolysis of the sucrose 6-phosphate yields sucrose. This is the major sugar that is transported between plant cells, analogous to the supply of glucose via the bloodstream to animal tissues (see Topic J4).

Synthesis of starch

Whereas animals store excess carbohydrate as glycogen (see Topics J2 and J6), plants do so in the form of starch (Topic J2). Starch is produced in the stroma of chloroplasts and stored there as starch grains. Starch synthesis occurs from ADP-glucose, CDP-glucose or GDP-glucose (but *not* UDP-glucose). The pathway involves the conversion of glyceraldehyde 3-phosphate (from the Calvin cycle) to glucose 1-phosphate which in turn is used to synthesize the nucleotide sugar derivatives.

The C4 pathway

Under normal atmospheric conditions, rubisco adds CO_2 to ribulose 1,5-bisphosphate. However, when the CO_2 concentration is low, it can add O_2 instead. This produces phosphoglycolate and 3-phosphoglycerate. The phosphoglycolate can be salvaged and used for biosynthetic reactions but the pathway for achieving this releases CO_2 and NH_4^+ and wastes metabolic energy. Because the net result of this process is to consume O_2 and release CO_2, it is known as **photorespiration**. This is a major problem for plants in hot climates. The plants close the gas exchange pores in their leaves (stomata) to conserve water but this leads to a drop in the CO_2 concentration within the leaf, favoring photorespiration. In addition, as temperature rises, the oxygenase activity of rubisco (using O_2) increases more rapidly than the carboxylase activity (using CO_2), again favoring photorespiration. To avoid these problems, some plants adapted to live in hot climates, such as corn and sugar cane, have evolved a mechanism to maximize the carboxylase activity of rubisco. In these plants, carbon fixation using the Calvin cycle takes place only in **bundle-sheath cells** that are protected from the air by **mesophyll cells**. Since the bundle-sheath cells are not exposed to air, the O_2 concentration is low. The CO_2 is transported from the air via the mesophyll cells to the bundle-sheath cells by combining with three-carbon molecules (C3) to produce four-carbon molecules (C4). These enter the bundle-sheath cells where they are broken down to C3 compounds, releasing CO_2. The C3 molecules return to the mesophyll cell to accept more CO_2. This cycle ensures a high CO_2 concentration for the carboxylase activity of rubisco action in the bundle-sheath cells. Since it relies on CO_2 transport via four-carbon molecules, it is called the **C4 pathway** and plants that use this mechanism are called **C4 plants**. All other plants are called **C3 plants** since they trap CO_2 directly as the three-carbon compound 3-phosphoglycerate (*Fig. 4*).

Details of the C4 pathway are shown in *Fig. 6*. The steps involved are as follows:

- in the mesophyll cell, phosphoenolpyruvate (C3) accepts CO_2 to form oxaloacetate (C4); a reaction catalyzed by **phosphoenolpyruvate carboxylase**
- oxaloacetate is converted to malate (C4) by **$NADP^+$-linked malate dehydrogenase**
- malate enters the bundle-sheath cell and releases CO_2, forming pyruvate (C3); catalyzed by **$NADP^+$-linked malate enzyme**
- pyruvate returns to the mesophyll cell and is used to regenerate phosphoenolpyruvate. This reaction, catalyzed by **pyruvate-P_i dikinase**, is unusual in that it requires ATP and P_i and breaks a high-energy bond to generate AMP and pyrophosphate.

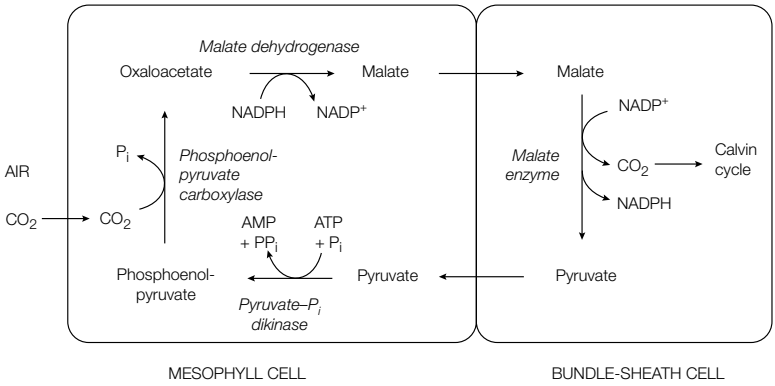

Fig. 6. The C4 pathway.

The pyrophosphate from the pyruvate-P_i dikinase is rapidly degraded so that, overall, the net price the plant pays for operation of this CO_2 pump is the hydrolysis of two high-energy phosphate bonds for every molecule of CO_2 transported:

$$CO_2\,(\text{in air}) + \text{ATP} \rightarrow CO_2\,(\text{bundle-sheath cell}) + \text{AMP} + 2\,P_i$$