

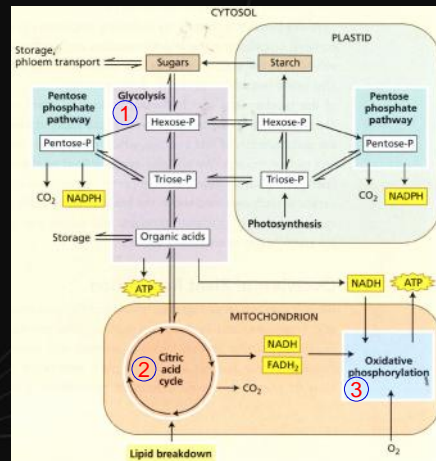


<http://smtom.lecture.ub.ac.id/>
Password:

<https://syukur16tom.wordpress.com/>
Password:



LECT 6. RESPIRATION



Cellular respiration is **critical** for the **survival** of most organisms due to its role in the provision of metabolic energy (ATP)



COMPETENCIES

Students, after mastering materials of the present lecture, should be able:

1. To explain the process of respiration (the oxidation of substrates particularly carbohydrates or the synthesis of metabolic energy used for plant growth and maintenance)
2. To explain reactions, enzymes and products involved the respiration
3. To explain specifically glycolysis, gluconeogenesis, pentose phosphate pathway, citric acid cycle and oxidative phosphorylation.



9/19/2018

2



LECTURE FLOW

1. INTRODUCTION

Definition
Site of Respiration
Electron Carriers
Stage of Respiration

2. GLYCOLYSIS

Initial Phase
Energy-Conserving Phase
Gluconeogenesis
Fermentation
Oxidative Pentose
Phosphate Pathway

3. CITRIC ACID CYCLE

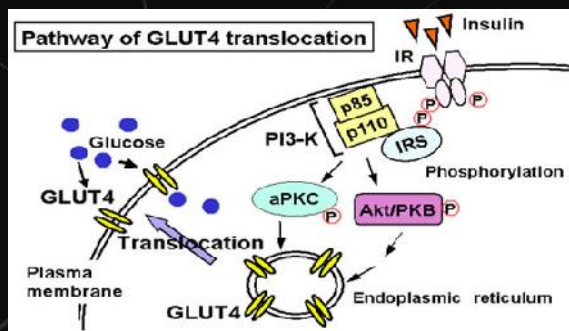
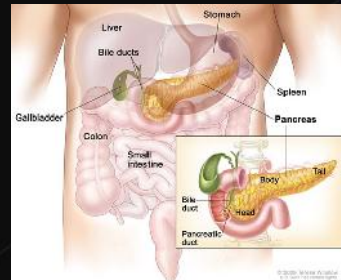
Discovery
Mithochondria
Pyruvate Oxidation

4. OXIDATIVE PHOSPHOTYLATION

Electron Transport Chain
Multiprotein Complexes
ATP Synthesis

Glucose is the most commonly cited substrate of respiration

Glucose serves as the primary energy source for the brain and is also a source of energy for cells throughout the body



Blood glucose is normally maintained between 70 mg/dl and 110 mg

GLUT4: glucose transporters (protein)

<http://www.ans.kobe-u.ac.jp/english/gakka/seibutsukinou/seibutu.html>

What is respiration ?

1. INTRODUCTION

1. Definition

- **Photosynthesis** provides the **organic building blocks** that plants (and nearly all other organisms) depend on.
- **Respiration** is the process whereby the energy stored in carbohydrates, produced during photosynthesis, is released in a controlled manner for cellular use.
- The energy (**free energy**) released during respiration is incorporated into a form (**ATP**) that can be readily utilized for the growth, development and maintenance of the plant.
- At the same time it generates many carbon precursors for biosynthesis.
- Therefore, respiration is tightly coupled to other pathways (Fig. 1).

9/19/2018

5

Chemical Changes:
Metabolism
(145 pathways)

1. Carbohydrate Metabolism (17)
2. Energy Metabolism (8)
3. Lipid Metabolism (14)
4. Nucleotide Metabolism (2)
5. Amino Acid Metabolism (16)
6. Metabolism of Other Amino Acids (9)
7. Glycan Biosynthesis and Metabolism (18)
8. Biosynthesis of Polyketides and Nonribosomal Peptides (9)
9. Metabolism of Cofactors and Vitamins (11)
10. Biosynthesis of Secondary Metabolites (21)
11. Biodegradation of Xenobiotics (21)

U.L.B.U. 5/25/04 Image source from KEGG

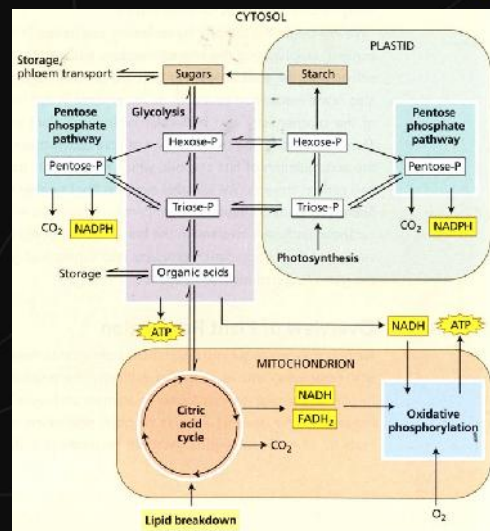
<http://manet.illinois.edu/pathways.php>

- From a chemical standpoint, respiration is most commonly expressed in terms of the oxidation of the six-carbon sugar glucose.

$$\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 6\text{H}_2\text{O} \longrightarrow 6\text{CO}_2 + 12\text{H}_2\text{O}$$

- This equation represents a coupled redox reaction that oxidizes completely glucose to CO_2 with oxygen serving as the ultimate electron acceptor and reduced to water.
- Glucose is most commonly cited as the substrate for respiration.
- In a functioning plant cell, however, reduced carbon is derived mainly from sources such as the disaccharide sucrose, triose phosphates from photosynthesis, fructose-containing polymers (fructans), and other sugars, as well as from lipids (primarily triacylglycerols), organic acids, and on occasion, proteins (Fig. 11.1).

Fig. 11.1 Overview of respiration. Substrates for respiration are generated by other cellular processes and enter the respiratory pathways.



Glycolysis

- Therefore, plant respiration can be expressed as **the oxidation** of the 12-carbon molecule **sucrose** and **the reduction** of 12 molecules of O_2 :

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e^-$$

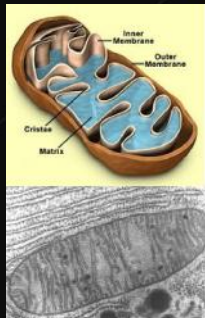
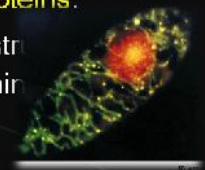
$$12O_2 + 48H^+ + 48e^- \rightarrow 24H_2O$$
 giving the following net reaction

$$C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12CO_2 + 11H_2O$$
- This reaction is **the reversal of the photosynthetic process**; it represents a coupled redox reaction in which **sucrose is completely oxidized to CO_2** while **oxygen serves as the ultimate electron acceptor** and is reduced to water in the process.
- The change in standard **Gibbs free energy** (ΔG^0) for the net reaction is **-5760 kJ** per mole (342 g) of sucrose oxidized.

9/19/2018

2. Site of Respiration

- Cytosol** and **plastids** are involved in the process of respiration.
- Mitochondria are the main site of ATP synthesis in eukaryote cells and as such are vital for the health and survival of the cell. Numbers of mitochondria per cell vary but usually 100s/cell
- Mitochondria have some of their own DNA, ribosomes and tRNA; 22 tRNAs & rRNAs (16S and 12S), so **mitochondria can make many of their own proteins**.
- The DNA is circular and lies in the matrix in str called "nucleoids". Each nucleoid may contain of the mitochondrial DNA (mtDNA).

9/19/2018

3. Electron Carriers

- As respiration can be regarded as the redox reactions, then electron carriers are required to support the process.
- Nicotinamide adenine dinucleotide (NAD⁺/NADH) is an **organic cofactor** (coenzyme) associated with many enzymes that catalyze cellular redox reactions.
- NAD⁺ is the oxidized form of the cofactor, which undergoes a reversible two-electron reaction that yields NADH (Fig. 11.2):



- The standard reduction potential for this redox couple is about -320 mV, which makes it a relatively strong reductant (i.e., electron donor).
- NADH is thus a good molecule in which to conserve the free energy carried by the electrons released during the stepwise oxidations of glycolysis and the citric acid cycle.



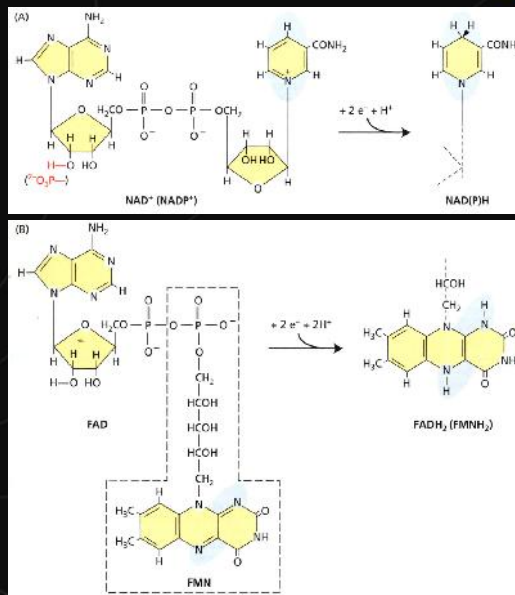
9/19/2018

11



Fig. 11.2 Structures and reactions of the **major electron-carrying nucleotides** involved in respiratory bioenergetics.



- (A) Reduction of NAD(P)⁺ to NAD(P)H. The hydrogen (in red) in NAD⁺ is replaced by a phosphate group (also in red) in NADP⁺.
- (B) Reduction of FAD to FADH₂. FMN is identical to the flavin part of FAD and is shown in the dashed box. Blue shaded areas show the portions of the molecules that are involved in the redox reaction.



9/19/2018


12






4. Stage of Respiration

- The cells mobilize the large amount of free energy released in the oxidation of sucrose in a series of step-by-step reactions to prevent damage (incineration) of cellular structures.
- These reactions can be grouped into four major processes:
 1. **glycolysis**,
 2. the **oxidative pentose phosphate pathway**,
 3. the **citric acid cycle**, and
 4. **oxidative phosphorylation**.
- These pathways do not function in isolation, but exchange metabolites at several levels.



9/19/2018


13

2. GLYCOLYSIS


1. Initial Phase

- Glycolysis (from the Greek words glykos, "sugar," and lysis, "splitting") occurs in all living organisms (prokaryotes and eukaryotes).
- The principal reactions associated with the classic glycolytic pathway in plants are almost identical to those in animal cells (Fig. 11.3).
- In animals, the substrate of glycolysis is **glucose**, and the end product is **pyruvate**.
- **Sucrose** (not glucose) can be argued to be **the true sugar substrate for plant respiration** as the fact that:
 - sucrose is the major translocated sugar in most plants, and is therefore the form of carbon that most nonphotosynthetic tissues import.



9/19/2018

14



- Substrates from different sources are channeled into **triose phosphate** (Fig. 11.3).
- In the early steps of glycolysis, sucrose is split into its two monosaccharide units—**glucose** and **fructose**—which can readily enter the glycolytic pathway.
- Two pathways for the splitting of sucrose are known in plants, both of which also take part in the unloading of sucrose from the phloem:
 - Sucrose synthase pathway.** Sucrose synthase, located in the cytosol, combines sucrose with UDP to produce fructose and UDP-glucose.
 - Invertase pathway.** *Invertases* present in the cell wall, vacuole, or cytosol hydrolyze sucrose into its two component hexoses (glucose and fructose).
- Four molecules of triose phosphates** are formed for **each molecule of sucrose** that is metabolized which requires 4 ATP.

Fig. 11.3 Initial reactions of plant glycolysis and fermentation.

(A) In the main glycolytic pathway, sucrose is oxidized via **hexose phosphates** and **triose phosphates** to the organic acid **pyruvate**, but plants also carry out alternative reactions. All the enzymes included in this figure have been measured at levels sufficient to support the respiration rates observed in intact plant tissues, and flux through the pathway has been observed in vivo.

2. Energy-Conserving Phase

- Energy-conserving phase of glycolysis occur in the reactions converting triose phosphate to pyruvate as the end product.
- In the first and reactions, NAD^+ is reduced to NADH by **glyceraldehyde-3-phosphate dehydrogenase**, and ATP is synthesized in the reaction catalyzed by **phosphoglycerate kinase** and **pyruvate kinase**.
- An alternative end product, **phosphoenolpyruvate**, can be converted to malate for mitochondrial oxidation or storage in the vacuole.
- NADH can be reoxidized during fermentation by either **lactate dehydrogenase** or **alcohol dehydrogenase**.
- For **each sucrose** entering the pathway, **four ATPs** are generated by this reaction—one for each molecule of 1,3—bisphosphoglycerate.

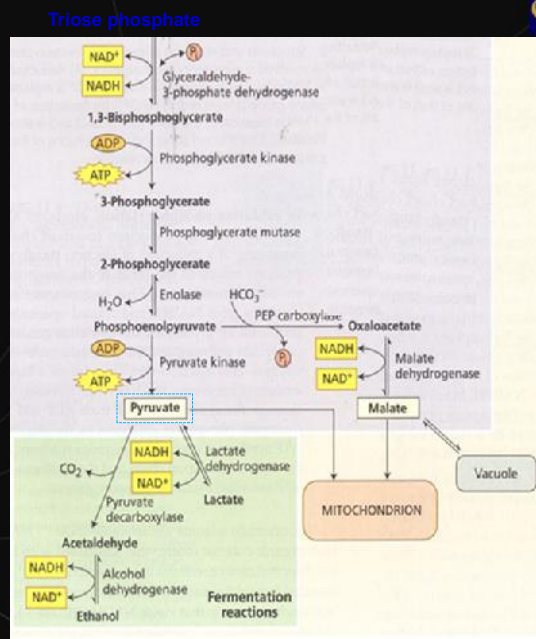


9/19/2018

17



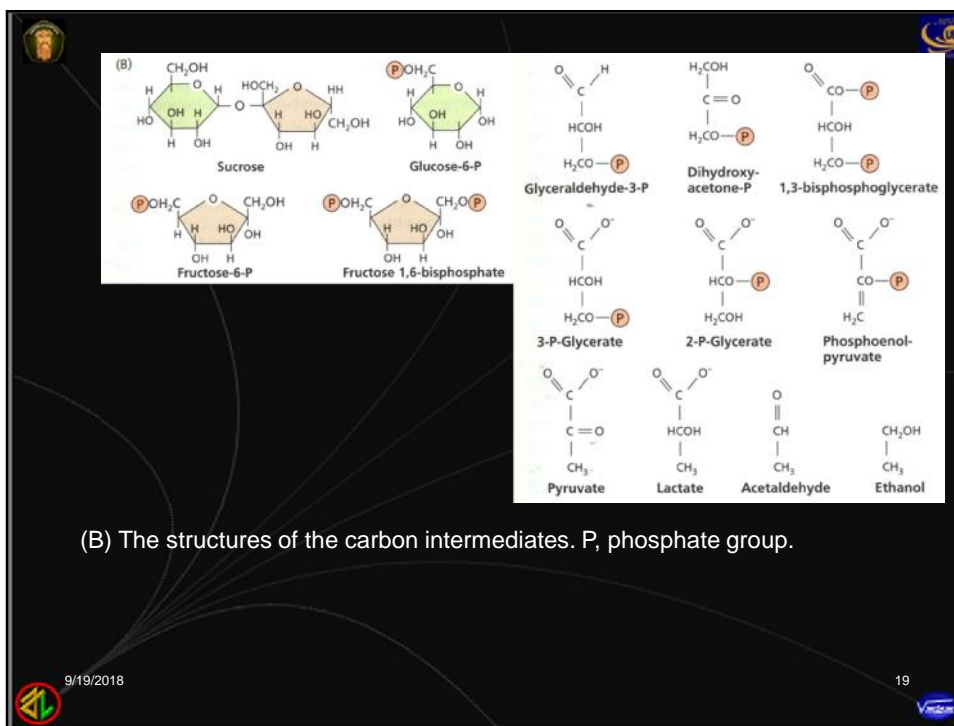
Fig. 11.3 Energy-conserving phase of glycolysis starting from the conversion of triose phosphate to 1,3-Bisphosphoglycerate with pyruvate as the end product, but **fermentation** takes place under anaerobic conditions with Ethanol or Lactate as the end products



9/19/2018

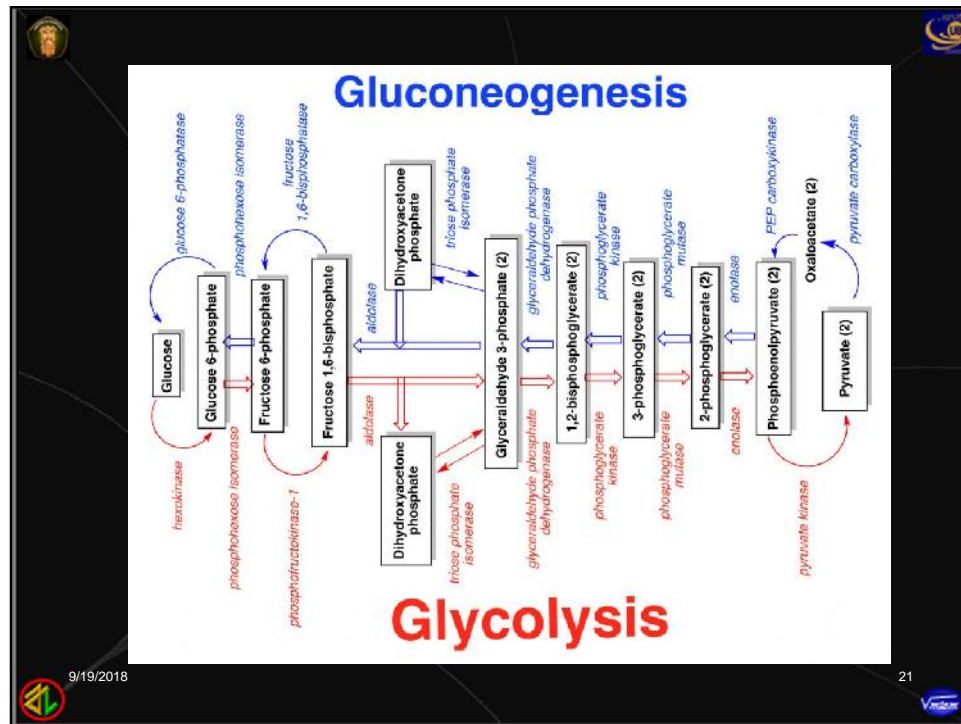
18





3. Gluconeogenesis

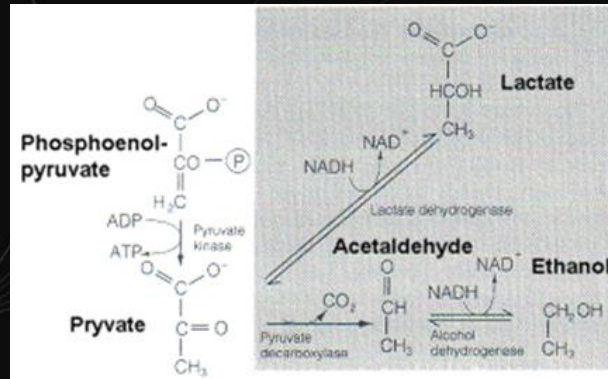
- Organisms can operate the **glycolytic pathway in the opposite direction** to synthesize **sugars** from **organic acids** which process is known as **gluconeogenesis**.
- Gluconeogenesis is particularly important in the seeds of plants (such as the castor oil plant *Ricinus communis* and sunflower) that store a significant quantity of their carbon reserves in the form of oils (triacylglycerols).
- After such a seed germinates, much of the oil is converted by gluconeogenesis into sucrose, which is then used to support the growing seedling.
- In the initial phase of glycolysis, **gluconeogenesis overlaps with the pathway** for **synthesis of sucrose** from photosynthetic triose phosphate.



4. Fermentation

- Oxidative phosphorylation does not function in the absence of oxygen.
- Glycolysis thus cannot continue to operate because the cell's supply of NAD^+ is limited and once all the NAD^+ becomes tied up in the reduced state (NADH), the catalytic activity of glyceraldehyde-3-phosphate dehydrogenase comes to a halt.
- To overcome this limitation, plants and other organisms can further metabolize pyruvate by carrying out one or more forms of **fermentation** (Fig. 11.3).
- Alcoholic fermentation is common in plants, although more widely known from brewer's yeast.
- Two enzymes, **pyruvate decarboxylase** and **alcohol dehydrogenase**, act on pyruvate, ultimately producing **ethanol** and CO_2 and oxidizing NADH in the process.

- In lactic acid fermentation (common in mammalian muscle, but also found in plants), the enzyme **lactate dehydrogenase** uses NADH to reduce pyruvate to lactate, thus regenerating NAD⁺.
- In **plant roots in flooded soils**—glycolysis (fermentation) can be the main source of energy for cells.





9/19/2018

23


- *Efficiency* is defined here as the energy conserved as ATP relative to the energy potentially available in a molecule of sucrose.
- The standard free-energy change (ΔG°) for the complete oxidation of sucrose to CO₂ is -5760 kJ mol⁻¹. The ΔG° for the synthesis of ATP is 32 kJ mol⁻¹.
- However, under the nonstandard conditions that normally exist in both mammalian and plant cells, the synthesis of ATP requires an input of free energy of approximately 50 kJ mol⁻¹.
- With ethanol or lactate as the final product, the efficiency of fermentation is only about 4%.
- Most of the energy available in sucrose remains in the ethanol or lactate.

9/19/2018

24







- The oxidative PP pathway plays several roles in plant metabolism:
 - **NADPH supply in the cytosol.** This NADPH drives reductive steps associated with **biosynthetic** and **defensive** reactions that occur in the cytosol and is a substrate for reactions that remove reactive oxygen species (ROS).
 - **NADPH supply in plastids.** In nongreen plastids, such as amyloplasts in the root, and in chloroplasts functioning in the dark, the PP pathway is a major supplier of NADPH.
 - **Supply of substrates for biosynthetic processes.** In most organisms, the PP pathway produces ribose 5-phosphate, which is a precursor of the ribose and deoxyribose needed in the synthesis of nucleic acids. In plants, however, ribose appears to be synthesized by another, as yet unknown, pathway (Sharpley and Fry 2007). Another intermediate in the PP pathway, the four-carbon erythrose 4—phosphate, combines with PEP in the initial reaction that produces plant phenolic compounds, including aromatic amino acids and the precursors of lignin, flavonoids, and phytoalexins.




9/19/2018

27






4. CITRIC ACID CYCLE

1. Discovery




- During the 19th century, biologists discovered that in the absence of air, cells produce **ethanol** or **lactic acid**, whereas in the presence of air, cells consume **O₂** and produce **CO₂** and **H₂O**.
- In 1937 the German-born British biochemist Hans A. Krebs reported the discovery of the **citric acid cycle**—also called the **tricarboxylic acid cycle** or **Krebs cycle**.
- This discovery explained how pyruvate is broken down into **CO₂** and **H₂O**, and highlighted the key concept of cycles in metabolic pathways.
- For his discovery, Hans Krebs was awarded the Nobel Prize in physiology or medicine in 1953.



9/19/2018

28



3. Mitochondria

- The breakdown of sucrose into pyruvate releases less than 25% of the total energy in sucrose; the remaining energy is stored in the four molecules of pyruvate. The next two stages of respiration (the citric acid cycle and oxidative phosphorylation) take place within an organelle enclosed by a double membrane, the **mitochondrion** (plural *mitochondria*).
- In electron micrographs, plant mitochondria usually look spherical or rod-like (Fig. 11.5) with **0.5 to 1.0 μm** in diameter and up to **3 μm in length** (Douce 1985).
- The number of mitochondria per plant cell varies; it is usually directly related to the metabolic activity of the tissue, reflecting the mitochondrial role in energy metabolism.

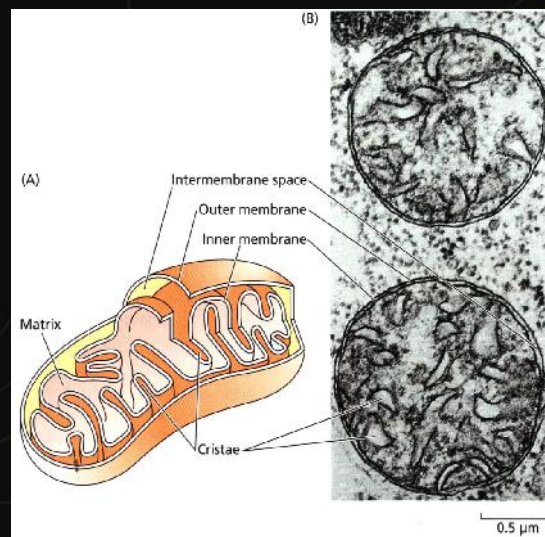


9/19/2018

29



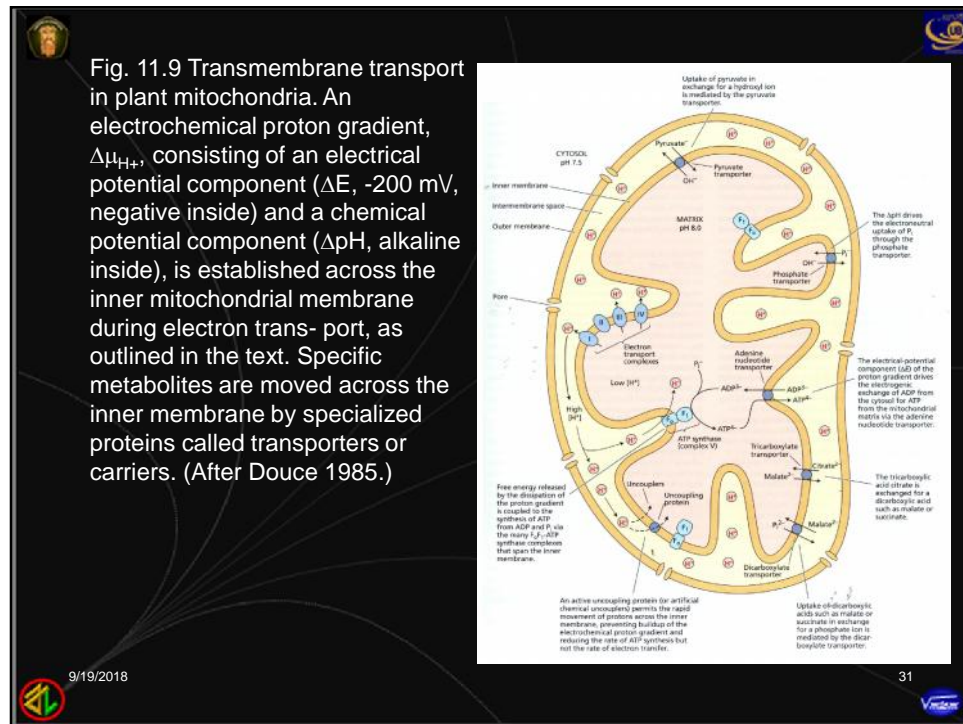
Fig. 11.5 Structure of plant mitochondria. (A) **Three-dimensional representation of a mitochondrion**, showing the invaginations of the inner membrane, called cristae, as well as the locations of the matrix and intermembrane space (see also Figure 11.9). (B) Electron micrograph of mitochondria in a mesophyll cell of **broad bean** (*Vicia faba*). Typically, individual mitochondria are 1 to 3 μm long in plant cells, which means that they are substantially smaller than nuclei and plastids. (B from Gunning and Steer 1996.)



9/19/2018

30





- Plant mitochondria have two membranes:
 - a smooth **outer membrane** completely surrounds a highly invaginated **inner membrane**.
 - The invaginations of the inner membrane are known as **cristae** (singular **crista**).
 - The region between the two mitochondrial membranes is known as the **intermembrane space**.
 - The compartment enclosed by the inner membrane is referred to as the **mitochondrial matrix**.
- The lipid fraction of both membranes is primarily made up of phospholipids:
 - 80% of which are either **phosphatidylcholine** or **phosphatidylethanolamine**.
 - About 15% is **diphosphatidylglycerol** (also called **cardiolipin**), which occurs in cells only in the inner mitochondrial membrane.

5. OXIDATIVE PHOSPHORYLATION

1. Electron Transport Chain

- For each molecule of sucrose oxidized through glycolysis and the citric acid cycle,
 - 4 molecules of NADH are generated in the cytosol, and 16 molecules of NADH plus 4 molecules of FADH₂ (associated with succinate dehydrogenase) are generated in the mitochondrial matrix.
 - These reduced compounds must be reoxidized, or the entire respiratory process will come to a halt.
- The electron transport chain catalyzes a transfer of two electrons from NADH (or FADH₂) to oxygen, the final electron acceptor of the respiratory process.



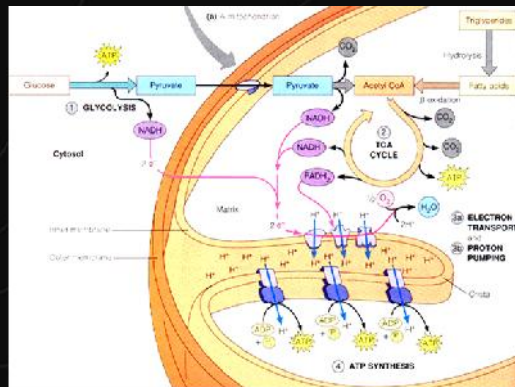
9/19/2018

33



2. Pyruvate Oxidation

- Pyruvate enters the mitochondrion and is oxidized via the citric acid cycle (Fig. 11.6).
- The products of pyruvate oxidation are and produces NADH, CO₂, and acetyl-CoA, in which the acetyl group derived from pyruvate is linked by a thioester bond to a cofactor, coenzyme A.

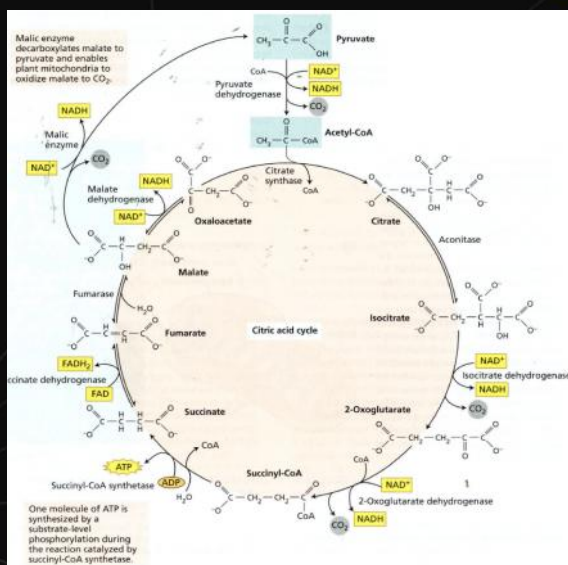


9/19/2018

34



Fig. 11.6 The plant citric acid cycle. The reactions and enzymes of the citric acid cycle are displayed, along with the accessory reactions of pyruvate dehydrogenase and malic enzyme. Pyruvate is completely oxidized to three molecules of CO_2 . The electrons released during these oxidations are used to reduce four molecules of NAD^+ to NADH and one molecule of FAD to FADH_2 .



9/19/2018

35

- For the oxidation of NADH , the reaction can be written as

$$\text{NADH} + \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O}$$
- The role of the electron transport chain is to bring about the oxidation of NADH (and FADH_2) and, in the process, utilize some of the free energy released to generate an electrochemical proton gradient, $\Delta\mu_{\text{H}^+}$, across the inner mitochondrial membrane.
- The electron transport chain (ETC) of plants contains the same set of electron carriers found in the mitochondria of other organisms (Fig. 11.8).
- The individual electron transport proteins are organized into **four transmembrane multiprotein complexes** (identified as I through IV), all of which are localized in the **inner mitochondrial membrane**.

9/19/2018

36

- Three of these complexes are engaged in proton pumping (I, III, and IV).

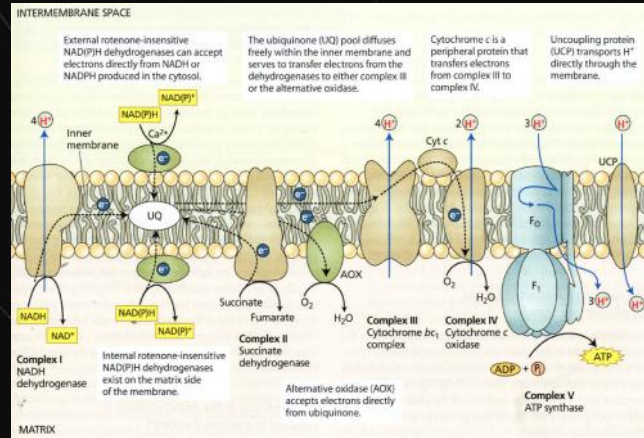
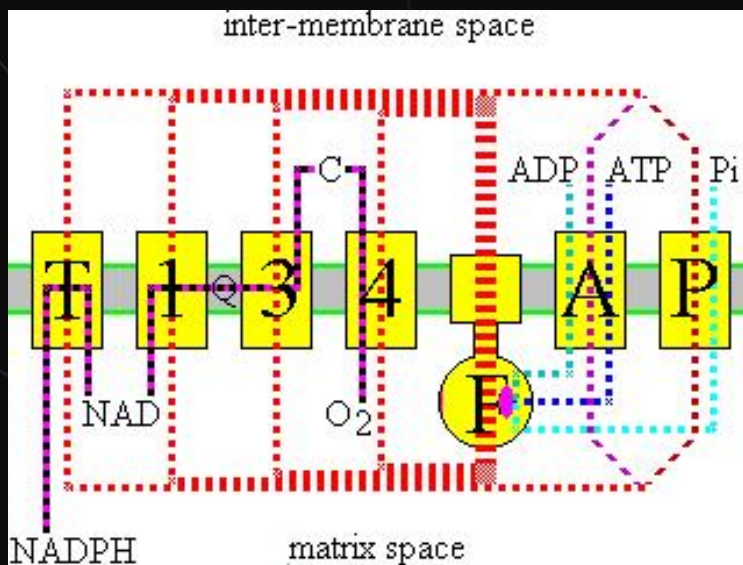


Fig. 11.8 Organization of the electron transport chain and ATP synthesis in the inner membrane of the plant mitochondrion.

9/19/2018

37



<http://www.bmb.leeds.ac.uk/illingworth/oxphos/>

2. Multiprotein Complexes

- Mitochondria from nearly all eukaryotes contain the four standard protein complexes: I, II, III, and IV.
 1. **COMPLEX I (NADH DEHYDROGENASE)** Electrons from **NADH**, generated in the mitochondrial matrix during the citric acid cycle, are oxidized by complex I (an **NADH dehydrogenase**).
 - The electron carriers in complex I include a tightly bound cofactor (**flavin mononucleotide**, or **FMN**, which is chemically similar to FAD) and several iron-sulfur centers.
 - **Ubiquinone**, a small lipid-soluble **electron and proton carrier**, is localized within the inner membrane. It is not tightly associated with any protein, and it can diffuse within the hydrophobic core of the membrane bilayer.
 2. **COMPLEX II (SUCCINATE DEHYDROGENASE)** Oxidation of **succinate** in the citric acid cycle is catalyzed by this complex, and the reducing equivalents are transferred via FADH_2 and a group of iron—sulfur centers to ubiquinone. Complex II does not pump protons.

9/19/2018

39

3. **COMPLEX III (CYTOCHROME bc_1 COMPLEX)** Complex III oxidizes **reduced ubiquinone (ubiquinol)** and transfers the electrons via an iron—sulfur center, two *b*-type cytochromes (b_{565} and b_{560}), and a membrane-bound cytochrome *c*1 to cytochrome *c*. Four protons per electron pair are pumped out of the matrix by complex III using a mechanism called the Q-cycle.
 - **Cytochrome c** is a small protein loosely attached to the outer surface of the inner membrane and serves as a mobile carrier to transfer electrons between complexes III and IV.
 4. **COMPLEX IV (CYTOCHROME *c* OXIDASE)** Complex IV contains two copper centers (Cu_A and Cu_B) and cytochromes *a* and a_3 . This complex is the terminal oxidase and brings about the four-electron reduction of O_2 to two molecules of H_2O . Two protons are pumped out of the matrix per electron pair.
- Reality may be more complex than the description above implies. Plant respiratory complexes contain a number of plant—specific subunits whose function is still unknown.

9/19/2018

40

3. ATP Synthesis

- In oxidative phosphorylation, the transfer of electrons to oxygen via complexes I, III, and IV is coupled to the synthesis of ATP from ADP and Pi via the F_0F_1 -ATP synthase (complex V).
 - The number of ATPs synthesized depends on the nature of the electron donor.
 - In experiments conducted on isolated mitochondria, electrons derived from matrix NADH (e.g., generated by malate oxidation) give **ADP:O ratios** (the number of ATPs synthesized per two electrons transferred to oxygen) of 2.4 to 2.7 (Table 11.1).

TABLE 11.1
Theoretical and experimental ADP:O ratios in isolated plant mitochondria

Substrate	ADP:O ratio	
	Theoretical ^a	Experimental
Malate	2.5	2.4–2.7
Succinate	1.5	1.6–1.8
NADH (external)	1.5	1.6–1.8
Ascorbate	1.0 ^b	0.8–0.9

9/19/2018

41



- On the basis of theoretical ADP:O values, **52 molecules of ATP** is estimated to be generated per molecule of sucrose by oxidative phosphorylation.
- The complete aerobic oxidation of sucrose (including substrate-level phosphorylation) results in a total of about **60 ATPs** synthesized per **sucrose molecule** (Table 11.2).

TABLE 11.2
The maximum yield of cytosolic ATP from the complete oxidation of sucrose to CO_2 via aerobic glycolysis and the citric acid cycle

Part reaction	ATP per sucrose ^a
Glycolysis	
4 substrate-level phosphorylations	4
4 NADH	$4 \times 1.5 = 6$
Citric acid cycle	
4 substrate-level phosphorylations	4
4 $FADH_2$	$4 \times 1.5 = 6$
16 NADH	$16 \times 2.5 = 40$
Total	60

9/19/2018

42

ENERGY PRODUCTION

Site/Process	Quantity	ADP/O	ATP
• Glycolysis	2 ATP		2
• TCA Cycle	2 ATP		2
• Cytosol	2 NADH	2.5	5
• Mitochondrial Matrix	8 NADH	2.5	20
• Mitochondrial Matrix	2 FADH ₂	1.5	3
TOTAL			32

Conversion Efficiency
Glucose:
 $(32 \times 50.2 \text{ kJ/mol}) / (2880 \text{ kJ/mol}) = 55.8\%$
Sucrose:
 $(60 \times 50.2 \text{ kJ/mol}) / (5647 \text{ kJ/mol}) = 53.3\%$







Table. ADP/O ratios in isolated plant mitochondria

Substrate	ADP/O
NADH (Malate)	2.4-2.7
NADH (Succinate)	1.6-1.8
NADH (External)	1.6-1.8
NADH (Ascorbate)*	0.8-0.9

*Artificial electron donor
ADP/O = number of ATPs synthesized per two electrons transferred to oxygen

Table 11.2 The maximum yield of cytosolic ATP from the complete oxidation of sucrose to CO₂ via aerobic glycolysis and the citric acid cycle

Part reaction	ATP per sucrose ^a
Glycolysis	
4 substrate-level phosphorylations	4
4 NADH	4 x 1.5 = 6
Citric acid cycle	
4 substrate-level phosphorylations	4
4 FADH ₂	4 X 1.5 = 6
16 NADH	16 X 2.5 = 40
TOTAL	60

Note: Cytosolic NADH is assumed oxidized by the external NADH dehydrogenase. The nonphosphorylating pathways are assumed not to be engaged.

^aCalculated using the theoretical ADP / O values from Table 11.1.
Source: Adapted from Brand 1994.