STRATEGIE METABOLICHE DI BASE

Produrre ATP e potere riducente, NADH, NADPH, per:

- Sintetizare i precursori delle macromolecole, zuccheri, amino acidi, nucleotidi, acidi grassi ecc.
- Sintetizzare vitamine, ormoni, pigmenti fotosintetici, ecc.
- Sintetizzare proteine, lipidi, acidi nucleici, amido, cellulosa, lignina ecc.

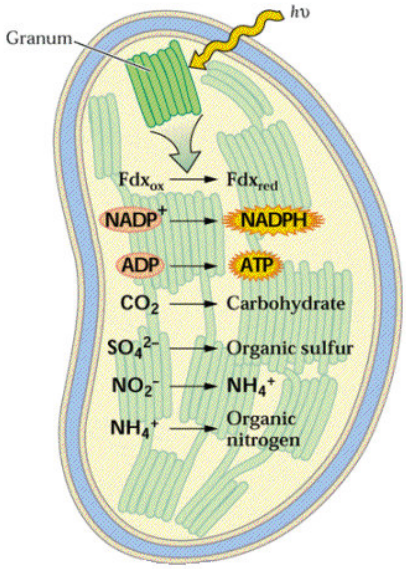
I pathways che generano ATPe NADH generano anche i precursori.
Esempi: DAHP e G3P, PEP, AcCoA, SuccCoA…

Altri utilizzi di ATP e NADH

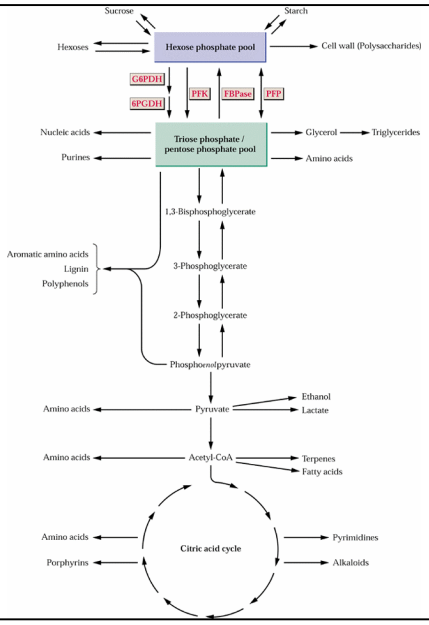
- Per il trasporto attivo, l'assimilazione riduttiva (N, S) e l'organicazione dei nutrienti minerali
- Per il lavoro meccanico nella divisione cellulare
- Per il "traffico" intracellulare
- Per la secrezione ed i trasporti inter cellulari
- Altri scopi specifici

Il "motore" delle piante, che rende possibile il modo di vita autotrofo, è costituito dalla fotosintesi che, grazie all'energia luminosa permette alla pianta di utilizzare l'anidride carbonica come fonte di carbonio per la sintesi di zuccheri che in parte vengono a loro volta utilizzati come fornitori del carbonio di tutte le molecole organiche cellulari, in parte vengono "bruciati" (ossidati) come "combustibile" per ricavarne l'energia ed il potere riducente per tutte le attività cellulari sopra ricordate

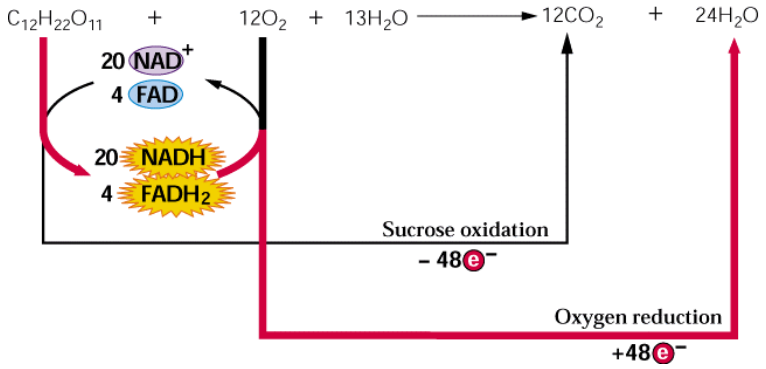
Durante la fase luminosa della fotosintesi si generano ATP e riducenti da usare per assimilare il C, ma anche N ed S

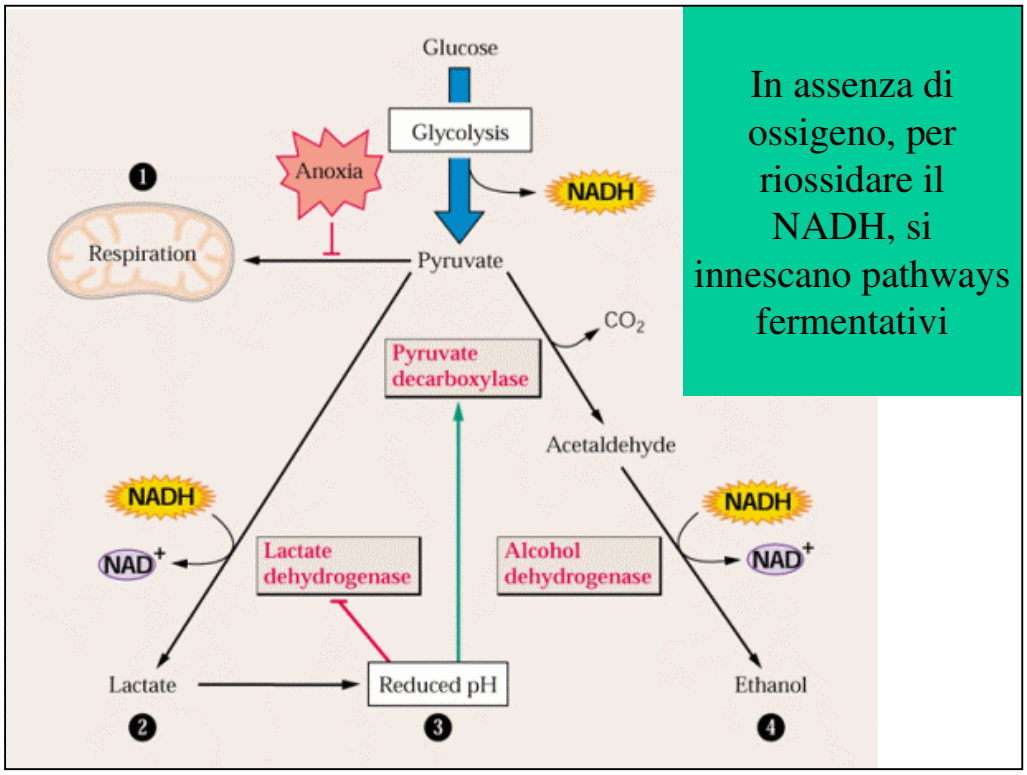


Ruolo centrale del metabolismo dei carboidrati nel fornire gli scheletri carboniosi per le reazioni biosintetiche

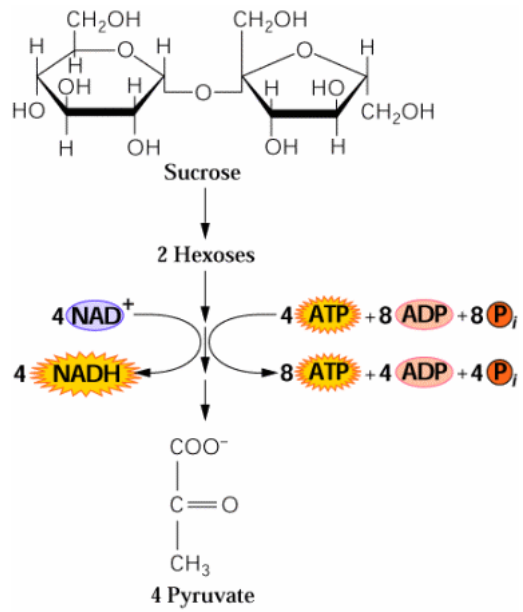


Ossidazione del saccarosio

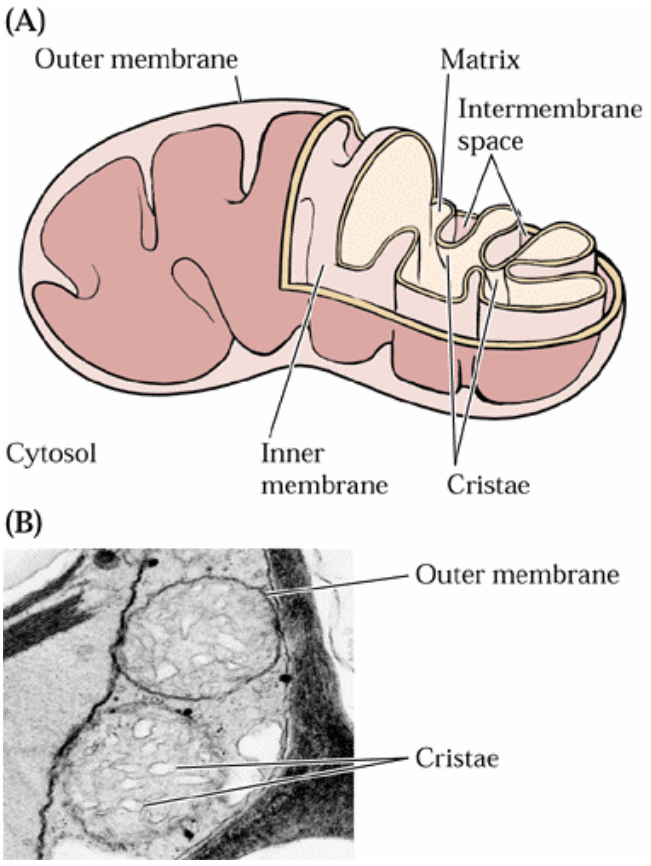




In assenza di ossigeno, per riossidare il NADH, si innescano pathways fermentativi



Conversion of sucrose to pyruvate during glycolysis in plants. The conversion of sucrose to pyruvate involves an initial input of ATP but leads to formation of an even greater amount of ATP via substrate-level phosphorylation, Also involved is an oxidation coupled to the reduction of NAD' to NADH. The products of sucrose catabolism are described here as two hexoses. Depending on the enzyme activity involved, sucrose can be converted to fructose and glucose or to fructose and UDP-glucose



The mitochondrion is the principal organelle of eukaryotic respiration. Plant mitochondria appear as spherical or rod-shaped entities 0.5 to 1.0 4m in diameter and 1 to 3 4m long. The number of mitochondria per plant cell varies and is related primarily to the metabolic activity of the particular tissue. However, the concentration of mitochondria per unit volume of cytoplasm usually remains similar during different developmental stages of the same cell type. For example, the small cells of the root cap of maize seedlings contain approximately 200 mitochondria each, whereas the much larger mature root tip cells may have approximately 2000 mitochondria each. In very active cells, such as phloem companion cells, secretory cells, and transfer cells, a large fraction (up to 20%) of the volume of the cytoplasm may be occupied by mitochondria. On the other hand, some unicellular algae (such as Chlatnydomonas) contain only a few mitochondria per cell. With a few exceptions (e.g., the very active cell types mentioned above), the density of mitochondria observed in most plant cells is less than that found in a typical animal cell. However, the respiratory rates of mitochondria isolated from plants are generally higher than in those isolated from animals.

Analogie tra mitocondri animali e vegetali

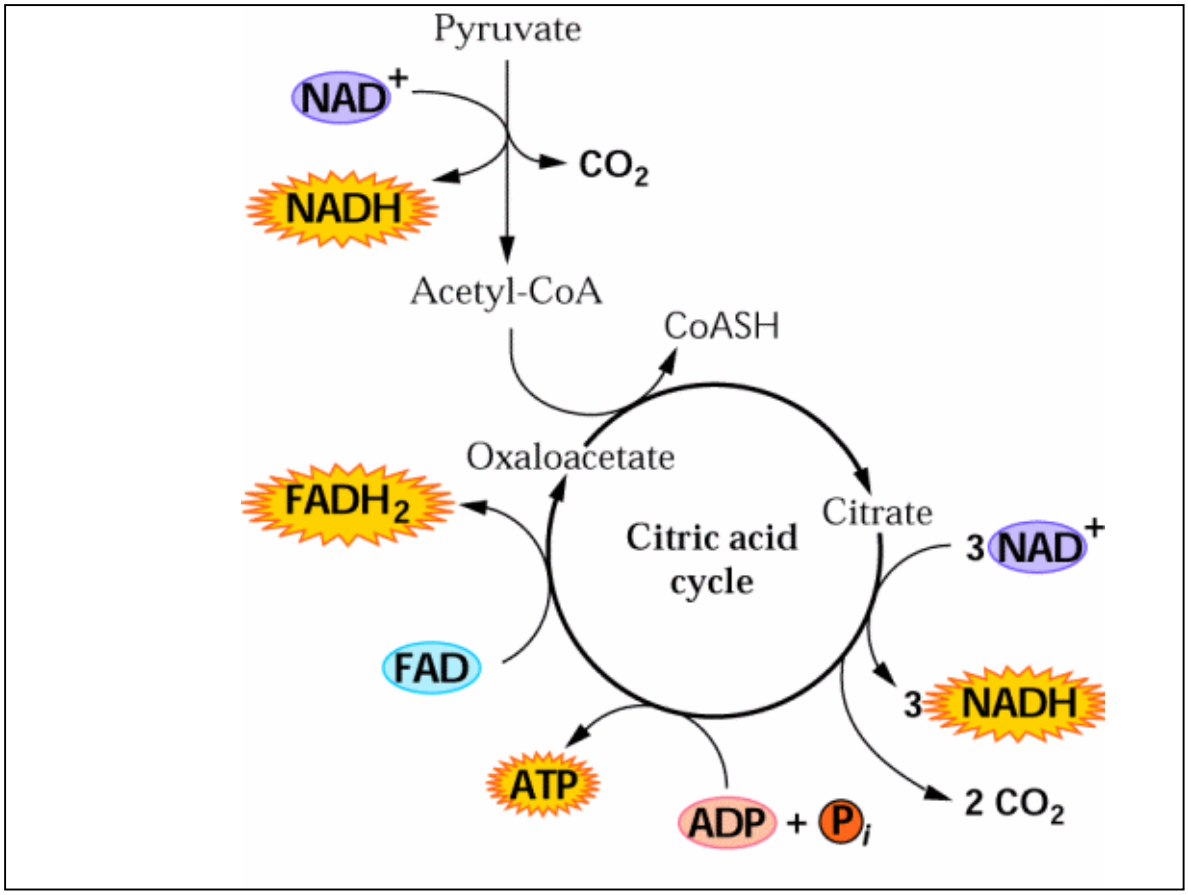
- Morfologia
- Fosfolipidi di membrana
- Sequenza trasportatori di elettroni
- Sistemi di sintesi di ATP
- Presenza della “porina” sulla membrana esterna
- Enzimi del ciclo di Krebs

Peuliarità dei mitocondri vegetali

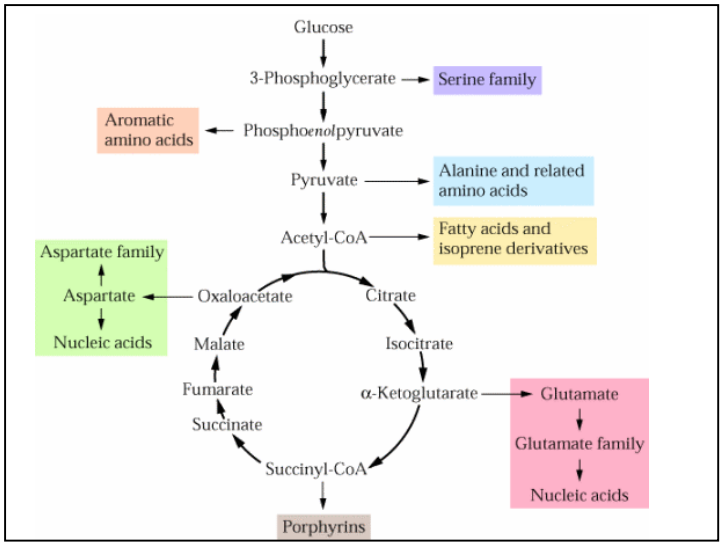
Le differenze fra mitocondri animali e vegetali si riferiscono più ad aspetti biochimico-funzionali che a caratteristiche morfologiche e strutturali.

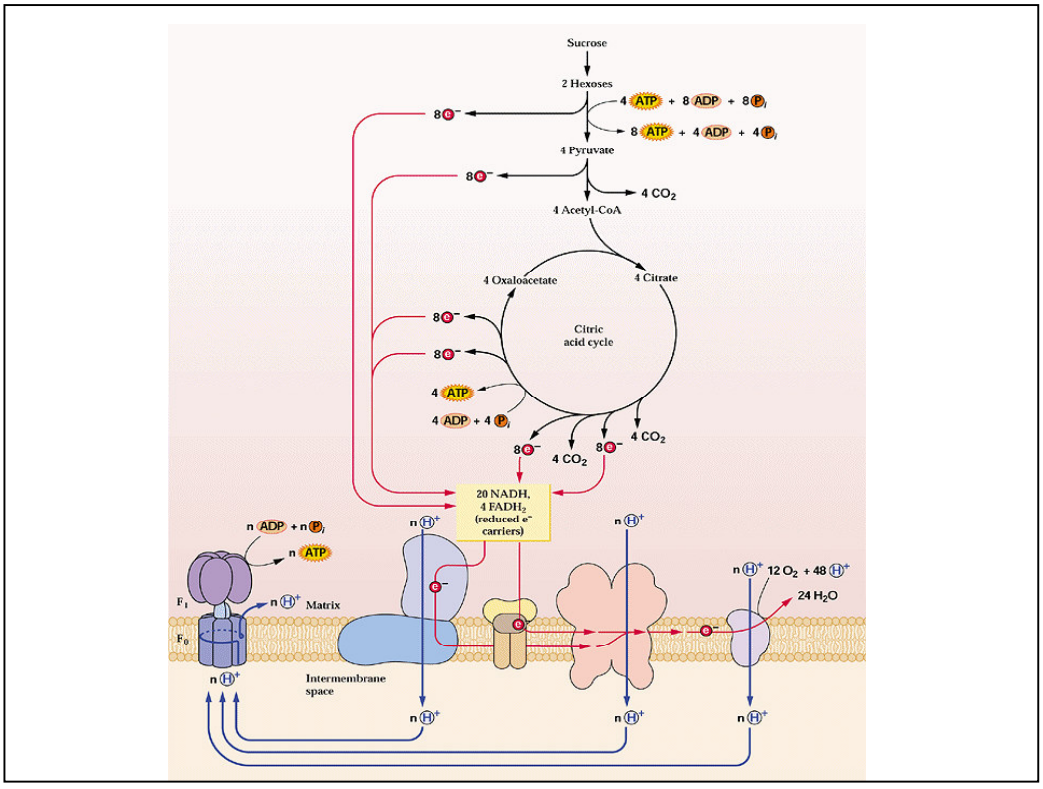
In particolare, riguardano:

- la capacità di utilizzare più substrati respiratori,
- la presenza di deidrogenasi ed ossidasi aggiuntive,
- la dimensione, la ridondanza e l’eterogeneità del DNA mitocondriale,
- La velocità (maggiore) di consumo di ossigeno,
- L’assenza della beta-ossidazione degli acidi grassi.



The citric acid cycle leads to the complete oxidation of pyruvate. As pyruvate is progressively oxidized to three molecules of C021 its electrons are transferred to redox cofactors, reducing four molecules of NAD' to NADH and one molecule of FAD to FADH2. This series of reactions takes place entirely within the mitochondrial matrix. In addition, one molecule of ATP is synthesized directly from ADP and Pi.

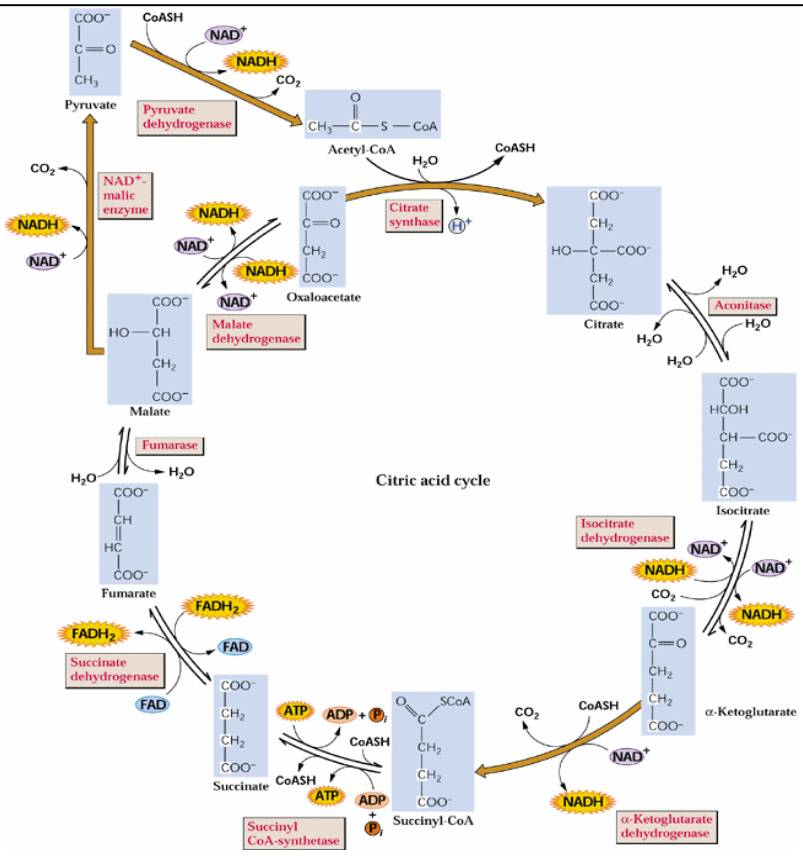


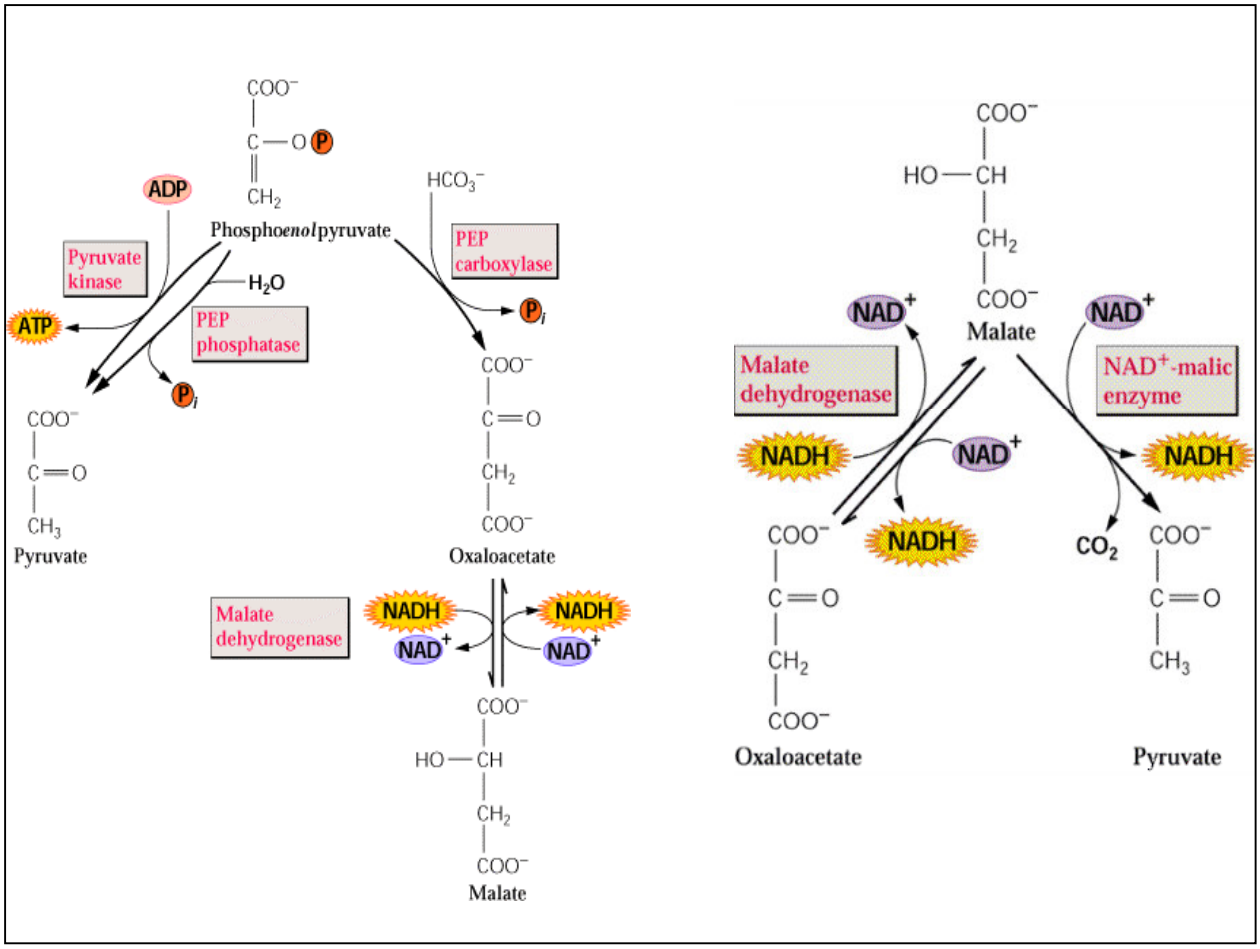


The general mechanism of oxidative phosphorylation in mitochondria. Electrons released during the oxidative steps of glycolysis and the citric acid cycle produce 20 molecules of NADH and 4 molecules of FADH2. These reduced coenzymes are subsequently oxidized by the mitochondrial electron transfer chain. The free energy released during mitochondrial electron transfer is coupled to the translocation of protons across the inner mitochondrial membrane from the matrix into the intermembrane space, generating an electrochemical proton gradient across the inner membrane. The free energy subsequently released by the movement of protons back across the inner membrane through the proton channel of the ATP synthase complex is used by the catalytic site on the component of the complex to convert ADP and Pi to ATP in the mitochondrial matrix.

64 ATP da una molecola di saccarosio

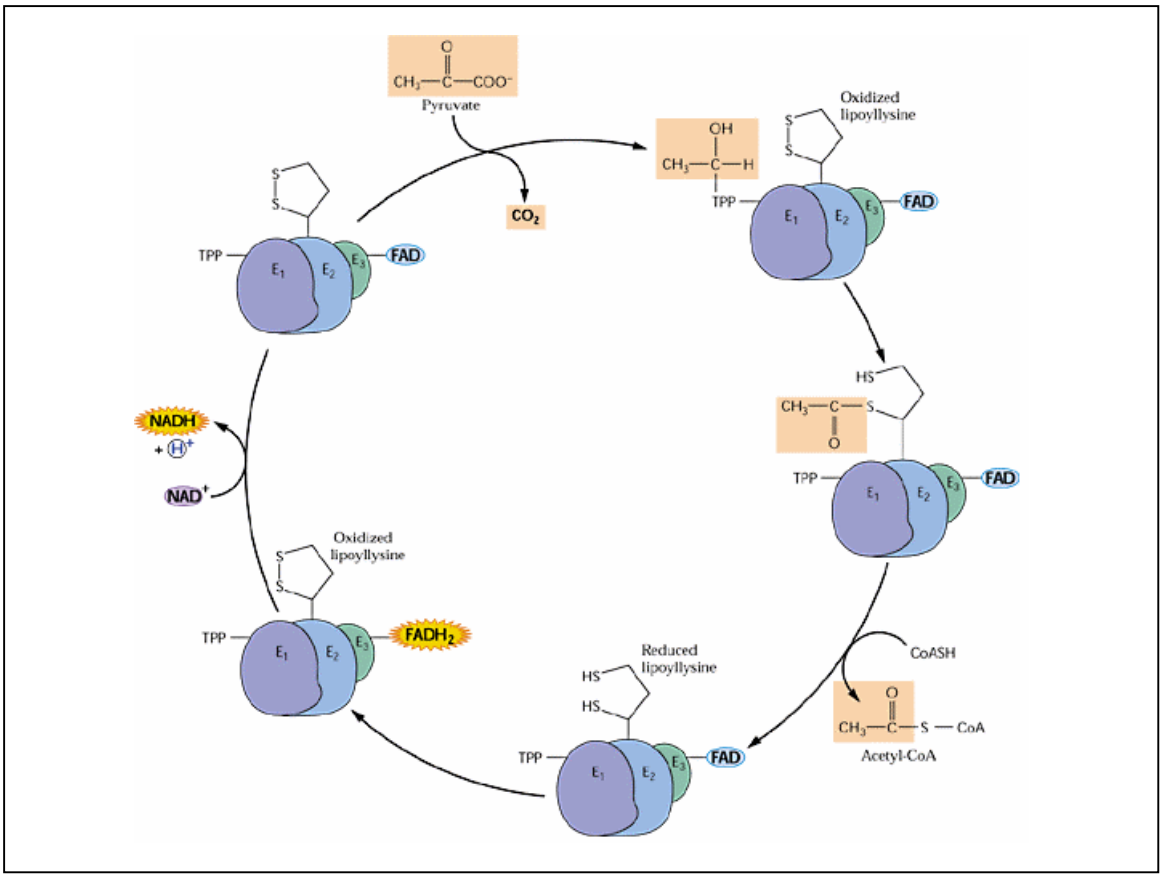
- **Nel citosol, da saccarosio a piruvato**:
  -4ATP per la fosforilazione degli esosi; +8ATP dalla defosforilazione di 4BPG e 4PEP;
  4NADH dall'ossidazione di 4G3P
- **Nella matrice del mitocondrio:**
  4 NADH dalla conversione di piruvato ad AcCoA
  4GTP(ATP) dall'ossidazione di 4 succinilCoA nel ciclo di Krebs
  12 NADH dall'ossidazione di 4 molecole di isocitrato 4 di alfaKG e 4 di malato nel ciclo di Krebs
  4 FADH2 dall'ossidazione di 4 molecole di succinato nel ciclo di Krebs
- **Nella fosforilazione ossidativa:**
  8ATP dai 4 NADH formati nella glicolisi
  8ATP dai 4 FADH2 del ciclo di Krebs
  12ATP dai 4 NADH formatisi dalla conversione piruvato-AcCoA
  36ATP dai 12 NADH formatisi nel ciclo di Krebs





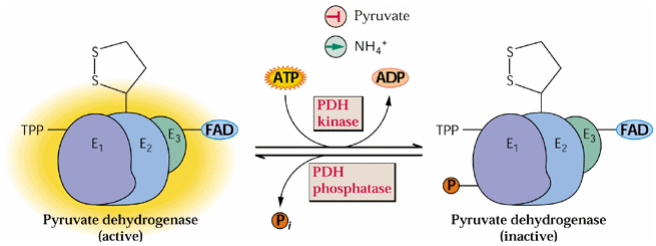
Conversion of phosphoenolpyruvate (PEP) to pyruvate or malate during plant respiration. The conversion of PEP to pyruvate by the enzyme pyruvate kinase is coupled to phosphorylation of ADP. PEP phosphatase can bypass this ATP synthesis step, releasing inorganic phosphate. Alternatively, PEP can react with HC03- via the enzyme PEP carboxylase, releasing the phosphate and producing the C4 product, oxaloacetate (OAA). OAA is commonly reduced to malate by NADH through the action of malate dehydrogenase. Both pyruvate and malate can be readily transported into mitochondria via carriers located in the inner mitochondrial membrane.

Once transported into the mitochondrion, malate can be acted on either by malate dehydrogenase, which couples the oxidation of malate to the reduction of NAD, producing OAA and NADH, or bv NAD-malic enzvme. which catalvzes the oxidative decarboxylation of malate to pyruvate and CO2 and reduces NAD+



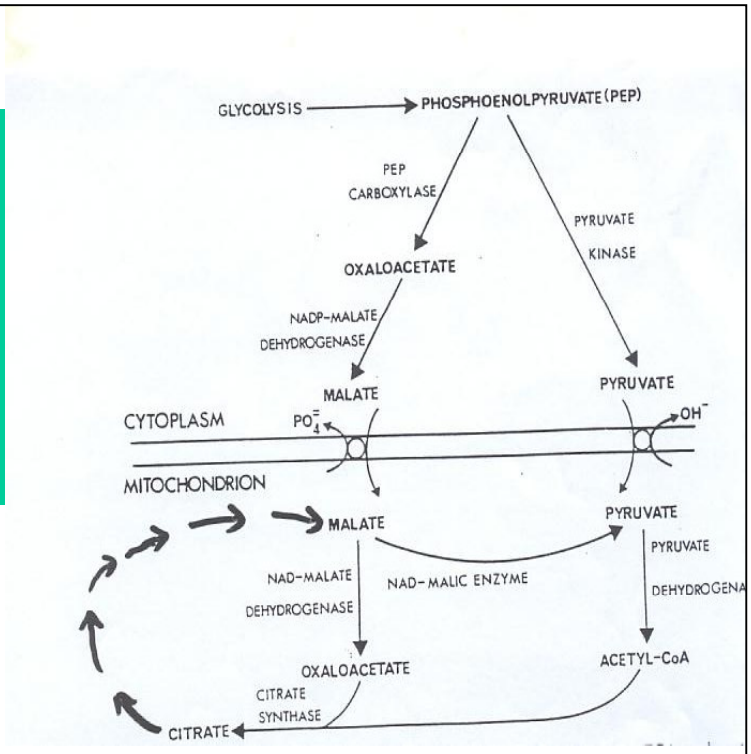
Reaction sequence catalyzed by the pyruvate dehydrogenase complex. The overall reaction catalyzed by this complex involves the oxidative decarboxylation of pyruvate and leads to the formation of acetyl-CoA, C02, and NADH. The enzyme pyruvate dehydrogenase (El) initially catalyzes the decarboxylation of pyruvate to produce acetaldehyde, a C2 product that reacts with bound thiamine pyrophosphate (TPP) to form hydroxyethyl-TPP. The hydroxyethyl derivative is transferred to the oxidized lipoamide group of dihydrolipoyl transacetylase (E2), forming the acetyl thioester of reduced lipoic acid. E2 next transfers the acetate to the thiol moiety of coenzyme A (CoASH) to give acetylCoA and a fully reduced lipoamide group on the dihydrohpoyl transacetylase. Dihydrolipoyl dehydrogenase (E3) then catalyzes the transfer of two electrons and two protons from the reduced lipoarnide group of E2 to the bound FAD of E3, oxidizing the lipoaamide and producing FADH2. In the final reaction of the series, E3 transfers two electrons and a proton to NAD+, yielding NADH.

Regolazione del complesso PDH

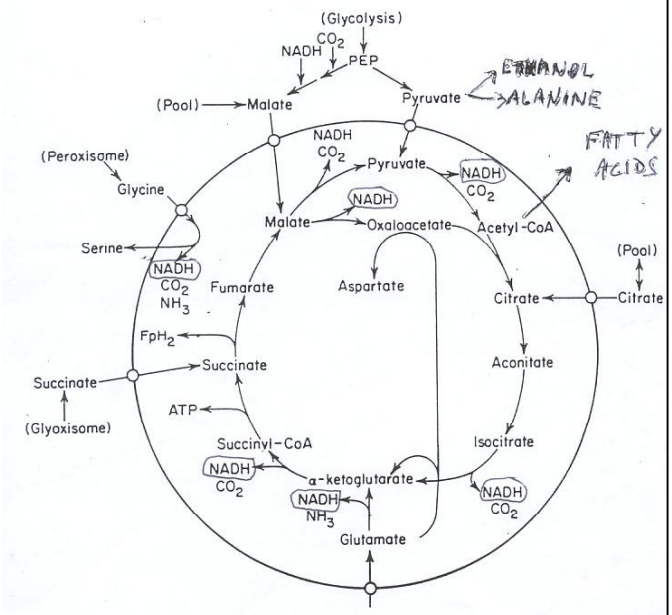


Regulation of the pyruvate dehydrogenase (PDH) complex by phosphorylation/dephosphorylation. PDH kinase, which is activated by ammonia and inhibited by pyruvate, inactivates the PDH complex by catalyzing the phosphorylation of El. Reactivation of PDH involves subsequent dephosphorylation by a PDH phosphatase.

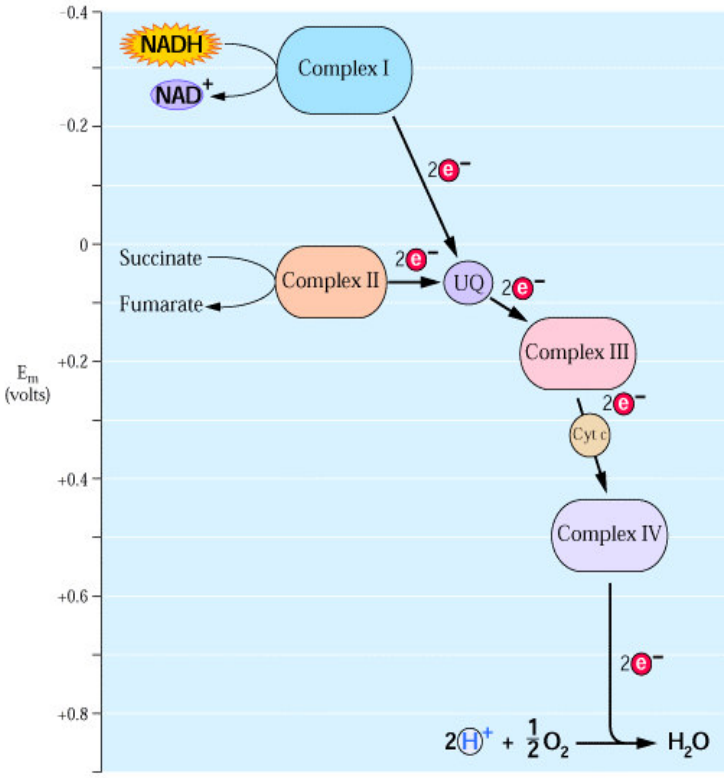
Bypass malico - piruvico



Capacità di utilizzazione di substrati da parte dei mitocondri vegetali

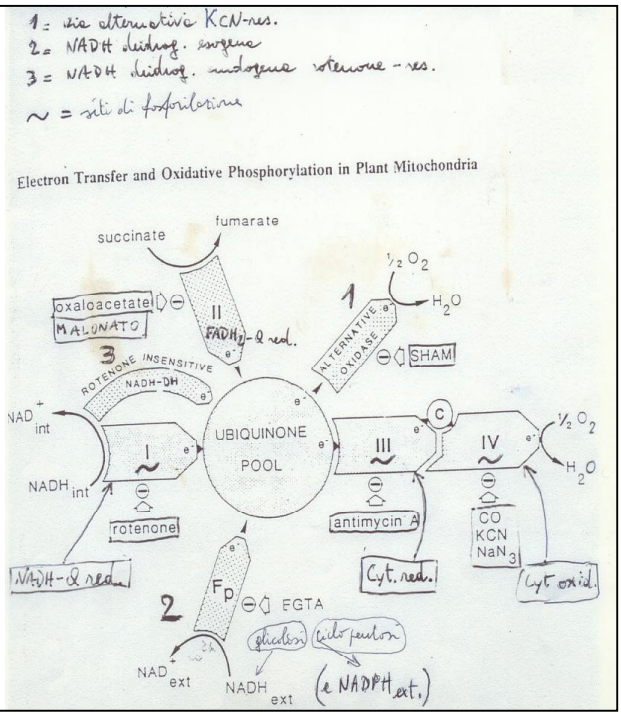


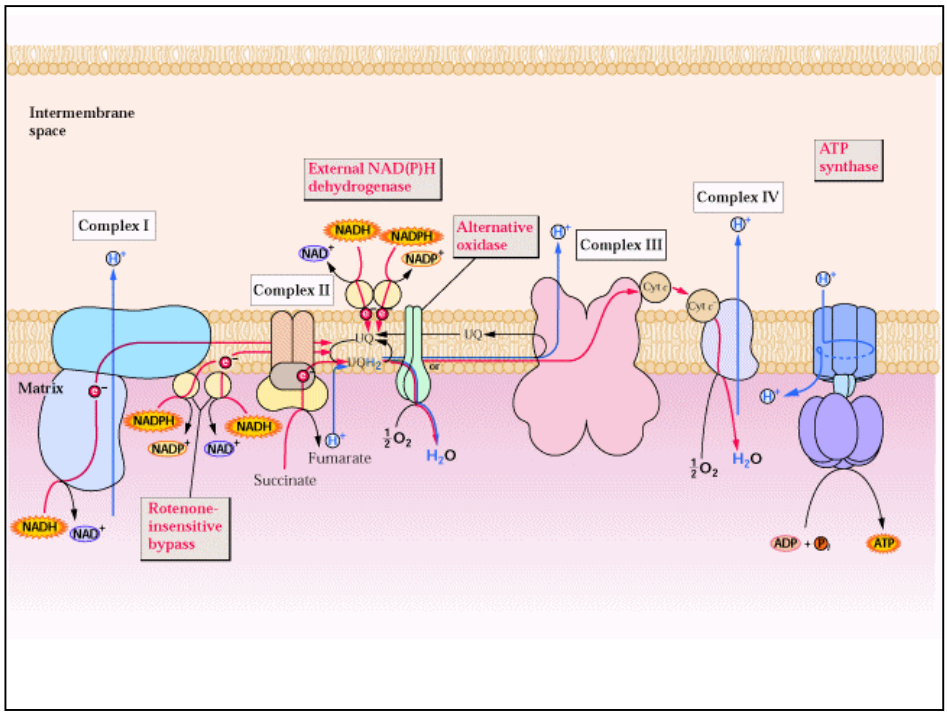
Schematic diagram of the primary sources and pathways of respiratory carbon plant mitochondria.



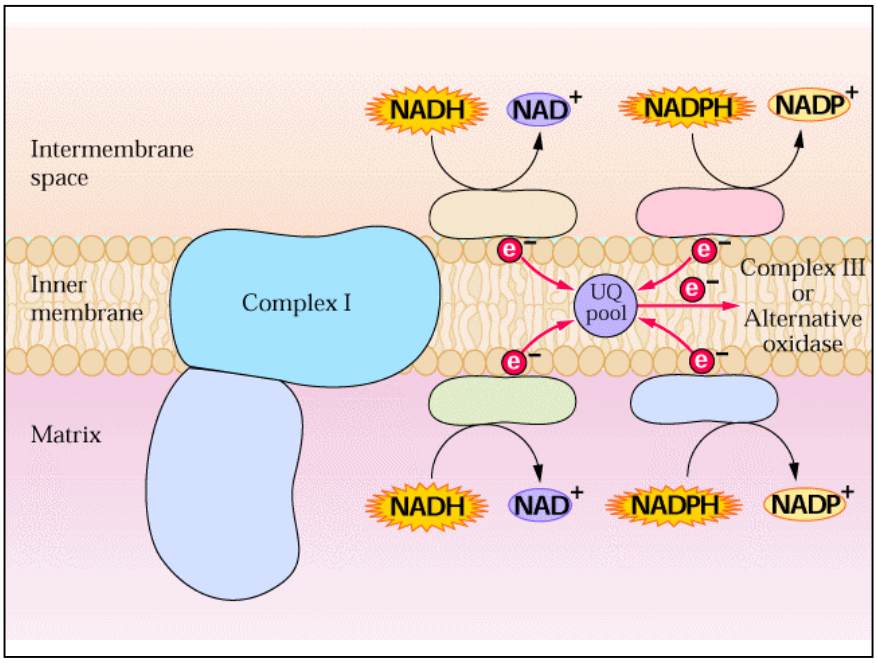
Posizione approssimativa dei componenti della catena respiratoria su una scala di potenziale redox

Peculiarità dei mitocondri vegetali a livello della catena di trasporto degli elettroni



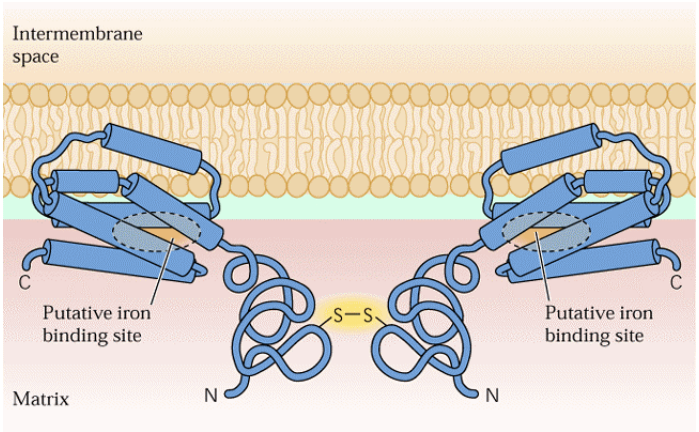


Organization of the plant mitochondrial electron transfer chain in the inner mitochondrial membrane. Electron transfer Complexes I-IV, ATP synthase, four additional rotenone-resistant NAD(P)H dehydrogenases, and the alternative oxidase are shown in diagrammatic fashion, incorporating what is currently known about their topological orientation in the inner membrane. A large pool of oxidized ubiquinone (UQ) and reduced ubiquinol (UQH2) freely
diffuses within the inner membrane and transfers electrons derived from the dehydrogenases to either Complex III or to the alternative oxidase. Red arrows indicate electron transfer; blue arrows indicate proton translocation. Transmembrane proton movement generates a proton electrochemical gradient that drives the synthesis of ATP from ADP and P, via the ATP synthase.



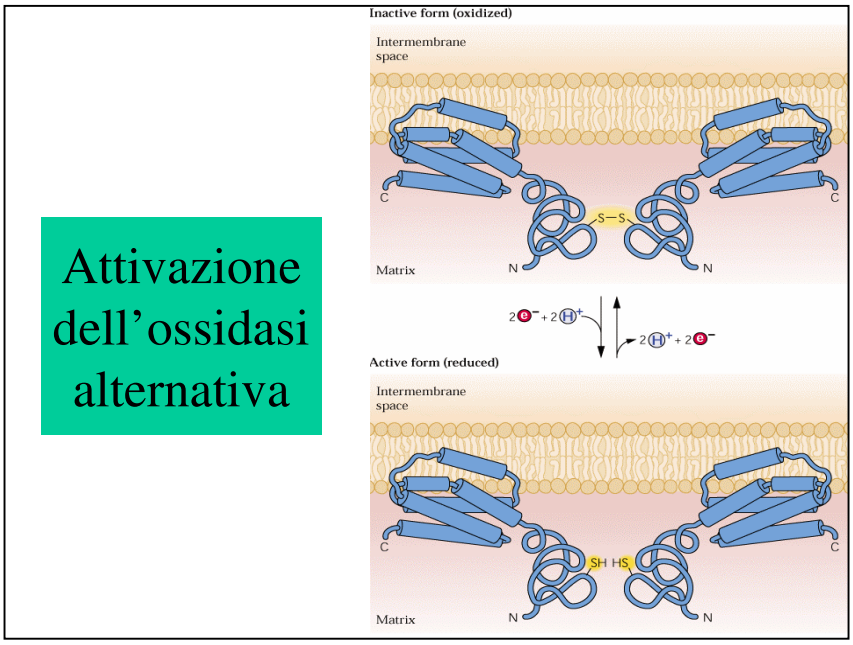
Rotenone-insensitive NADH and NADPH dehydrogenases of the inner mitochondrial membrane. In addition to Complex 1, plant mitochondria possess simpler (single polypeptide) dehydrogenases on both surfaces of the mitochondrial inner membrane. These do not pump protons and are insensitive to Complex I inhibitors such as rotenone. Four dehydrogenases have been described, although not all of these may occur in all plant tissues. The two external dehydrogenases are thought to oxidize cytosolic NAD(P)H and feed electrons into the UQ pool. The two enzymes on the inner surface provide additional routes for oxidation of the NADH and NADPH formed in the matrix. The proteins involved in these processes have not yet been firmly identified.

Struttura dell'ossidasi alternativa



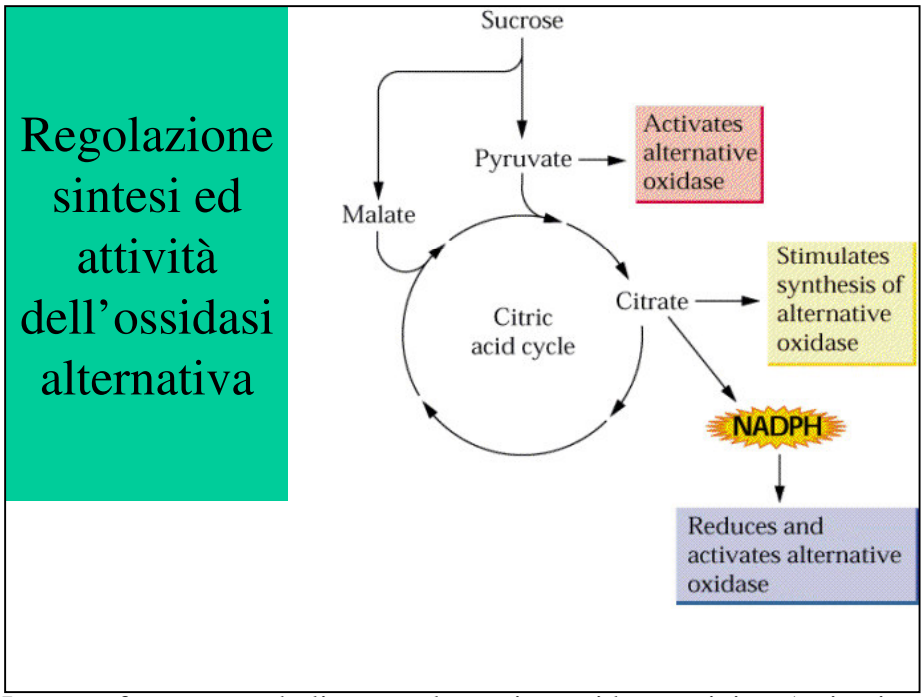
Proposed structure and membrane topography of the alternative oxidase dimer, as deduced from derived amino acid sequences. In plants, the alternative oxidase exists in the membrane as a dimer. Two large hydrophilic domains extend into the mitochondrial matrix. The amino acids that make up the iron-binding motif of the putative di-iron center are located in the C terminal hydrophilic domain.

Attivazione dell'ossidasi alternativa

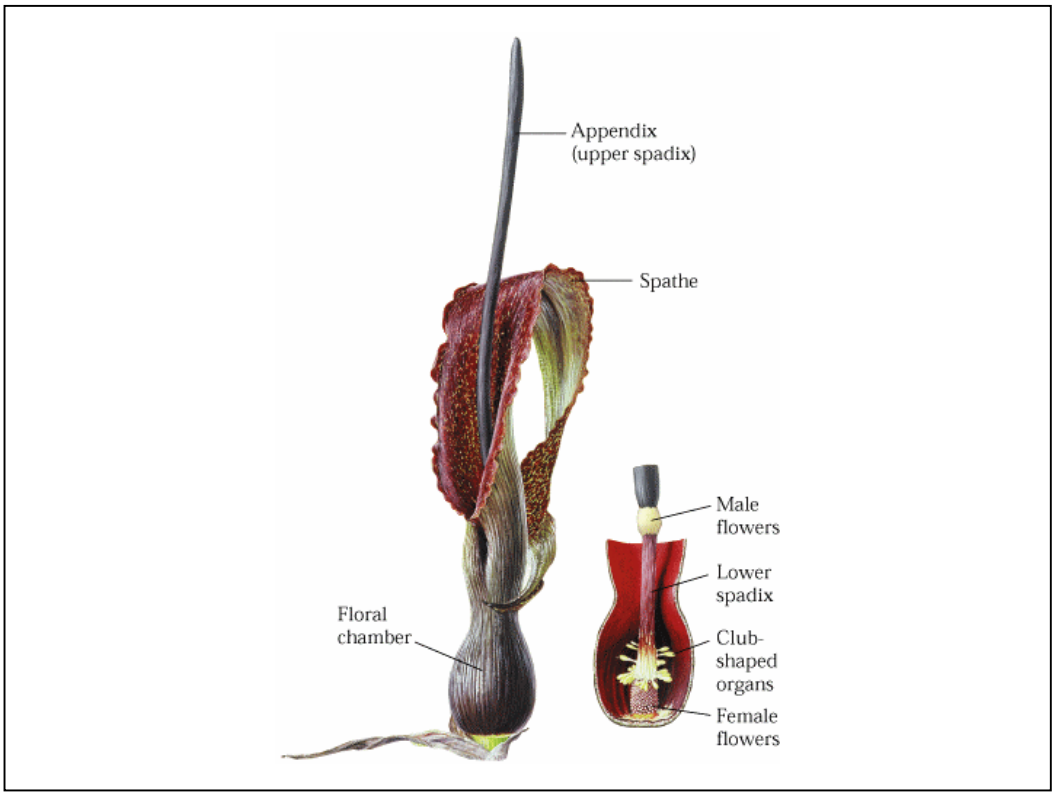


Although the nature of the protein-protein interactions that maintain the dimeric structure when the intermolecular disulfide is reduced remain unknown, the proximity of the N-terminal hydrophilic domains required to form the intermolecular disulfide bond suggests their possible involvement.

Regolazione sintesi ed attività dell'ossidasi alternativa



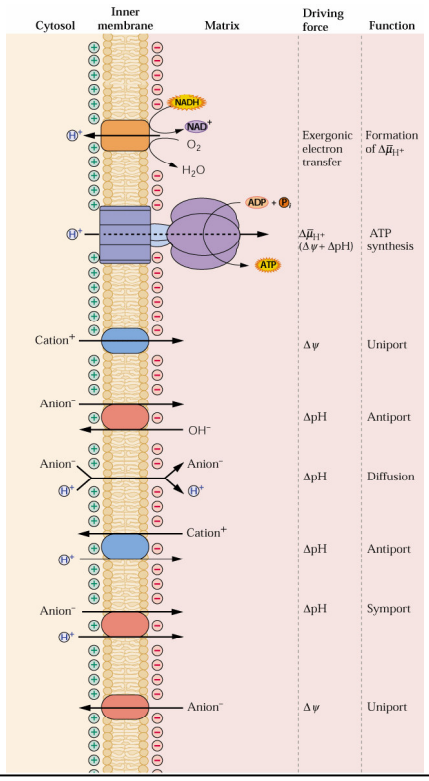
Impact of sugar metabolism on alternative oxidase activity. Activation and synthesis of the alternative oxidase are linked to the carbon status of the cell. When carbon flux to the mitochondrion exceeds the capacity of the electron transport chain to accept electrons, carbon intermediates such as pyruvate and citrate accumulate and the matrix pyridine nucleotide pool becomes highly reduced. Accumulation of citrate leads to synthesis of more alternative oxidase protein, whereas accumulated pyruvate and NADPH activate the enzyme. This feed-forward control ensures that the potentially wasteful alternative oxidase is active only when the carbon supply



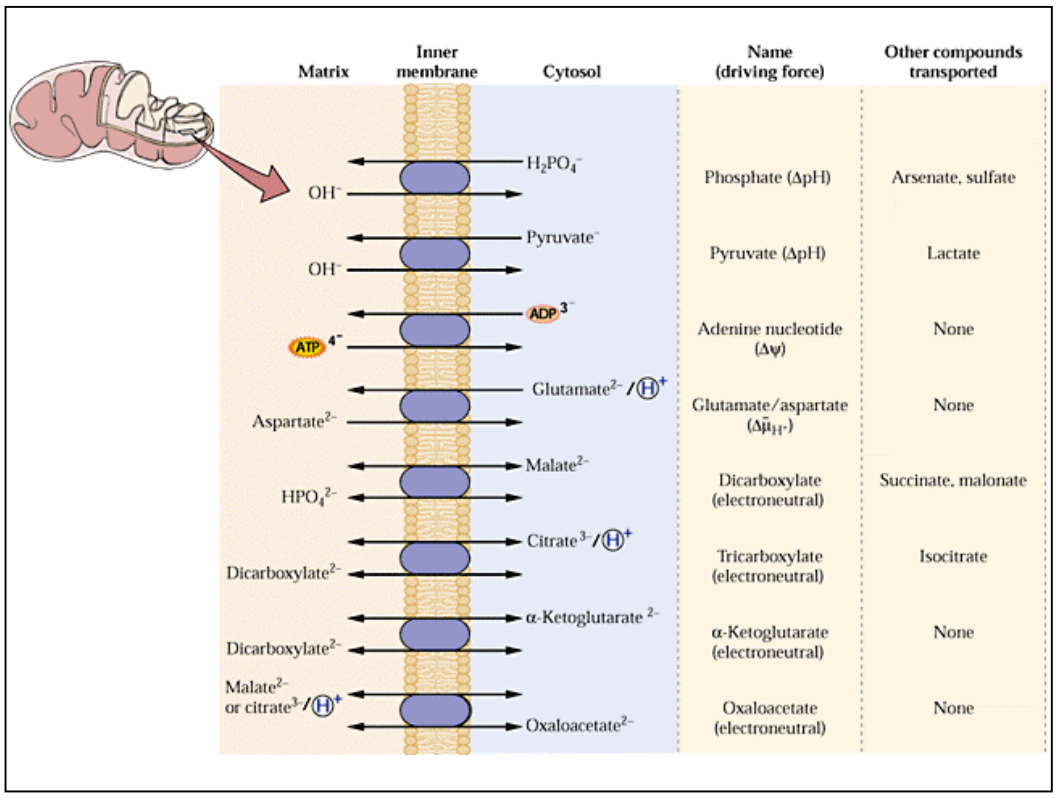
The alternative oxidase has one documented role: thermogenesis during flowering in a few plants, mostly members of the family Araceae. These plants, which include skunk cabbage and voodoo lily produce a club-like structure (called an appendix) on the developing floral apex (see illustration). The outer layer appendix contain many more mitochondria than most plant tissues do. During anthesis, mitochondria in the appendix use the alternative oxidase to respire at very high rates. The free energy is realeased as heat raising tissue temperature to 10°C to 25°C above ambient conditions and volatilizes odorous compounds that attract pollinators. In some cases, this mechanism can mimic the scent of rotting flesh and is used to deceive insects that lay their eggs on carrion.

The alternative oxidase is unlikely to promote thermogenesis in other plant tissues, for plants are not generally thermogenic. Observed rates of plant respiration are simply too low to generate significant amounts of heat, even if all electron flow were to be shunted through the alternative oxidase. Moreover, the high respiration rates observed in aroid appendices would release considerable heat even if the electrons were to travel through the cytochrome pathway; if thermogenesis were linked to ATP synthesis, however, the tissue would need to utilize large amounts of ATP very rapidly, or insufficient ADP would soon limit respiration.

Trasporto ionico attraverso la membrana interna del mitocondrio



The proton motive force established by proton pumping of the respiratory chain can be used to drive ion transport across the inner rnitochondrial membrane. Electrogenic transport involves the net movement of charge across the membrane; transport without net charge transfer is termed electroneutral. Inner membrane carriers that move only one ion (uniporters) are linked directly to the gradient of electric potential. Two chemical species may be cotransported in the same direction by symporters, or in opposite directions by antiporters. In the mitochondrial membrane, such coupled transport may be driven by the deltapH, if one of the ions is a H+ or OH-; by the gradient of electric potential, if the combined transport is electrogenic; or by a combination of the two delta.



Carriers (substrate transporters) of the inner mitochondrial membrane. All of these secondary active transporters are indirectly linked to the proton motive force across the membrane and are capable of accumulating substrates inside the mitochondrial matrix. Uptake of pyruvate and Pi are linked to the ApH and exchange for hydroxide ions. The Pi gradient so generated can then be used to drive uptake of dicarboxylate anions via exchange on the dicarboxylate carrier. Dicarboxylates in turn can exchange for alpha-ketoglutarate or tricarboxylates; in the latter exchange, electroneutrality is maintained by cotransport of a proton with the citrate. This is also true for the exchange catalyzed by the OAA transporter. The exchange of ADP for ATP and glutamate for aspartate are electrogenic and driven by the electric potential delta across the inner membrane.

Mitochondrial DNA from different organisms

	Number of base pairs	Number of different molecules per organelle
Higher plants		
Brassica sp.	218 000	3 (circular) 218kb, 135kb, 83kb
Maize	570 000 plus a variable number of plasmid-like DNAs from 1 400–6 000 bp	7 (circular) from 570kb–47kb up to 4 (circular or linear)
Muskmelon	2 400 000	?
Fungi		
Podospera anserina	juvenile 95 000	1 (circular)
	senescent 30 000 + 2 400	2 (circular)
Saccharomyces cerevisiae	75 000	1 (circular)
Others		
Cow and man	16 000	1 (circular)

Genes located on plant mitochondrial DNA

one gene for each of the rRNAs:
- 26S mol. wt. $1.12 - 1.16 \times 10^6$
- 18S mol. wt. $0.69 - 0.78 \times 10^6$
- 5S mol. wt. 39.000

} closely linked

tRNA genes

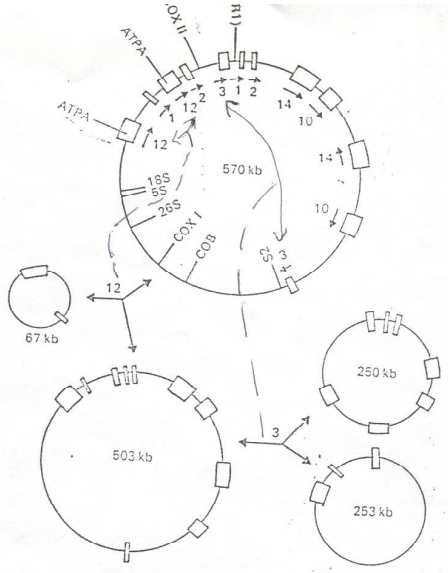
genes for 20–30 hydrophobic membrane-associated proteins including:

	mol. wt.
α-subunit of the F_1-ATPase	58 000
Proteolipid proton channel of F_0 (DCCD binding protein)	8 000
apoprotein of cytochrome b	42 900
cytochrome c oxidase subunit I	38 000
subunit II	34 000

one ribosomal protein

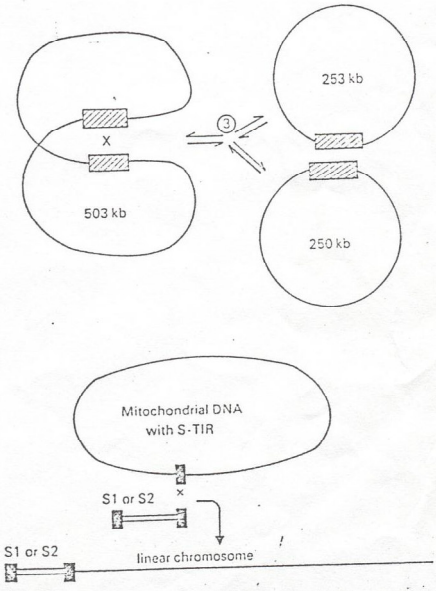
chloroplast genes (in maize)
- 16S rRNA
- tRNA
- large subunit of rubisco

Posizione di alcuni geni sul genoma di mitocondri di mais



Location of some of the genes on the mitochondrial genome of fertile (N) maize. The location and orientation of six repeated DNA sequences (the 1, 2, 3, 10, 13 and 14 kilobase repeats) are shown, together with the positions of the integrated S1 (sometimes called R1) and S2 DNA sequences. Subgenomic molecules are generated from the master circle of 570 kilobase-pairs by recombination across the repeated sequences. Examples shown are for recombination across the 12- and 3-kilobase repeats. After Bailey–Serres (1987).

Ricombinazione di molecole di DNA mitocondriale a formare molecole circolari o lineari



Recombination of mitochondrial DNA molecules to generate linear or circular DNAs in male-sterile maize carrying S cytoplasm. (*A*) Reversible recombination across the 3-kilobase repeats can give rise to two smaller circles from the main genome. (*B*) Recombination between S1 or S1 linear DNAs and related sequences (S-TIR) 5′ to the COX I gene can give rise to linear chromosomes, with S1 or S2 at the ends. After Bailey–Serres (1987).