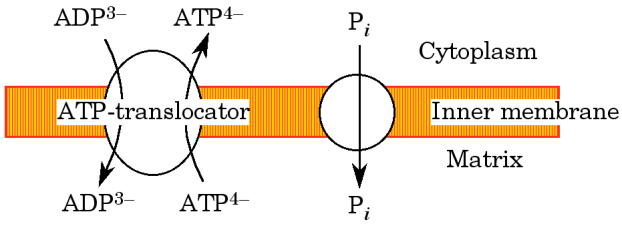
Mitochondrial Shuttles and Transporters

One major mechanism for the regulation of metabolic processes within eukaryotic cells is related to the fact that most processes are located in specific compartments within the cell. This means that separate pools of some important metabolites are maintained in different locations, allowing the movement of the molecules between these pools to act as an additional level of regulation. This separation is especially obvious for the mitochondria, where the inner membrane is a barrier to the transit of most molecules.

A few molecules can cross the mitochondrial inner membrane unassisted. These include small, uncharged molecules (*e.g.*, CO_2, O_2, and NH_3), and some small carboxylic acids, probably in their uncharged forms (*e.g.*, protonated acetic acid). Otherwise, only molecules that have specific transporter proteins are capable of crossing the mitochondrial membrane.

ATP/ADP and Phosphate pumps

The oxidative phosphorylation pathway generates ATP inside the mitochondria. However, most ATP-dependent processes occur in other compartments in the cell. Therefore, ATP must be pumped out of the mitochondria, and the ADP and inorganic phosphate generated elsewhere must be pumped in. The ATP translocator is not actually a pump; however, because ATP^{4-} has more charges than ADP^{3-}, the proton gradient tends to force the ATP out of the mitochondria even in the face of an opposing concentration gradient.



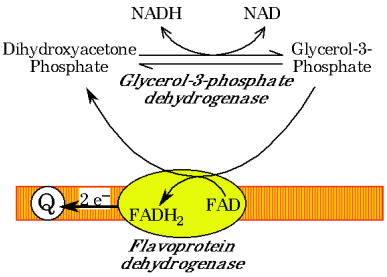
Phosphate also must be moved into the mitochondria to allow ATP synthesis; the movement of phosphate can be driven by a proton gradient-dependent pump. Phosphate also has exchangers (see below).

Shuttles

Shuttles are systems of enzymes and transporters. The enzymes convert molecules into metabolites that are capable of crossing membranes via the transporters, a process that is frequently followed by reformation of the original molecule.

The electrons of NADH produced in the cytoplasm must be transported into the mitochondria for conversion to ATP by the electron transport pathway. Because the NADH itself cannot cross the mitochondrial membrane, one important function of shuttle mechanisms is the transport of reducing equivalents across the mitochondrial membrane. Two separate methods are used for this purpose: the **Glycerophosphate shuttle** and the **Malate-Aspartate shuttle**.

Glycerophosphate shuttle



The glycolytic intermediate dihydroxyacetone phosphate can be converted to glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase; this process also results in conversion of NADH to NAD. Glycerol-3-phosphate can then be converted back to dihydroxyacetone phosphate by flavoprotein dehydrogenase (a different glycerol-3-phosphate dehydrogenase); this second enzyme is an FAD-dependent enzyme located in the mitochondrial inner membrane. Like Complex II of the electron transport chain, flavoprotein dehydrogenase donates electrons directly to Coenzyme Q without pumping protons.

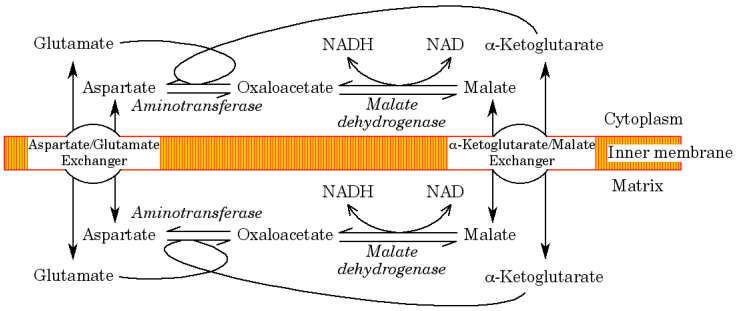
The glycerophosphate shuttle is essentially **irreversible**, and therefore can be used under essentially all conditions. Because the electrons using the glycerophosphate shuttle enter the electron transport pathway at the level of Coenzyme Q, the electrons can only be used to synthesize a maximum of **two ATP**, instead of the maximum of three ATP derived from NADH formed inside the mitochondria.

The glycerophosphate shuttle is heavily used in insect flight muscle (a tissue heavily specialized for conversion of chemical energy to mechanical energy). Some mammalian tissues use the glycerophosphate shuttle also, but tend to prefer a more energy efficient shuttle system that uses malate and aspartate.

Malate-Aspartate Shuttle

Mammalian tissues can use a shuttle system involving malate and aspartate to transport electrons across the mitochondrial inner membrane. Oxaloacetate in the cytoplasm is converted to malate by malate dehydrogenase, oxidizing NADH to NAD. The malate enters the mitochondria using an exchanger protein that must also transport α-ketoglutarate in the opposite direction. The malate is then oxidized to oxaloacetate by the mitochondrial malate dehydrogenase, resulting in formation of NADH, which can then enter the electron transport pathway.

Return of the oxaloacetate to the cytoplasm requires a separate transporter, which exchanges aspartate for glutamate. (Note that this conserves the nitrogens present in these amino acids; oxaloacetate and α-ketoglutarate are the α-keto acid counterparts of aspartate and glutamate, respectively.) This separate exchanger is necessary to allow net movement of electrons from one side of the membrane to the other.



If you follow the malate carbons in the clockwise direction in the diagram above, you will note that the malate-aspartate shuttle results only in the movement of the electrons from NADH from the outside to the inside, with no net movement of carbon or nitrogen. This conserves all of the energy in the NADH electrons, and allows the synthesis of **three ATP** (under optimum conditions) from NADH generated in the cytoplasm.

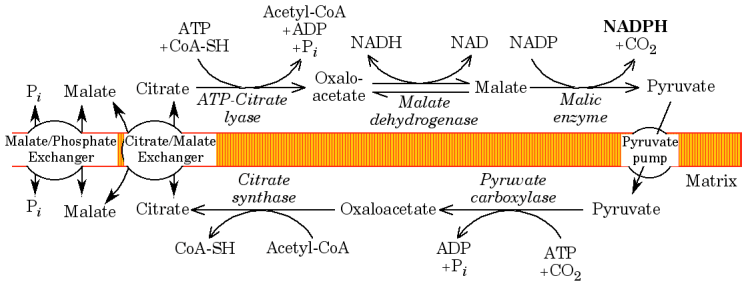
The malate-aspartate shuttle is **reversible**; during gluconeogenesis, parts of this shuttle act as one method for releasing oxaloacetate (in the form of malate) into the cytoplasm for the later reactions of the gluconeogenic pathway.

Note that this shuttle system depends on the fact that oxaloacetate cannot be transported across the mitochondrial membrane. This allows separate, independently regulated pools of oxaloacetate. The direction of the shuttle depends on the metabolic state of the cell.

Both the glycerophosphate shuttle and the malate-aspartate shuttle result in **movement of electrons** from one side of the mitochondrial inner membrane to the other, with **no net movement of metabolites**. In each case, the same amount of carbon is present inside the mitochondria at the beginning and end of the shuttling process.

Citrate-pyruvate shuttle

A related shuttle is used to move reducing equivalents out of the mitochondria. Citrate can exchange for malate. The citrate in the cytoplasm can act as a regulator of phosphofructokinase, or as a substrate for ATP-citrate lyase, an enzyme that reverses the citrate synthase reaction, and produces acetyl-CoA and oxaloacetate. (Note that ATP is required to generate the high-energy bond present in acetyl-CoA.)



The oxaloacetate produced in the ATP-citrate lyase reaction can be converted to malate. Both the oxaloacetate and malate can be used for a variety of reactions; for malate one additional reaction, catalyzed by malic enzyme, results in the formation of NADPH and pyruvate and carbon dioxide. The pyruvate produced by malic enzyme can return to the mitochondria to complete the cycle.

Note that pyruvate has a proton-dependent pump, and generally cannot leave the mitochondria directly.

The net reaction for this shuttle reveals that two ATP are being used to transport acetyl-CoA out of the mitochondria and to transfer electrons from NADH to NADPH.

The citrate-pyruvate shuttle as drawn here is irreversible, because four of the enzymes (pyruvate carboxylase, citrate synthase, ATP-citrate lyase, and malic enzyme) and the pyruvate pump mediate irreversible processes. Note, however, that the citrate and malate are transported across the membrane by exchangers that allow movement in either direction, and that malate has several mechanisms for both entering and leaving the mitochondria.

The shuttle mechanisms presented here are examples of commonly used processes. The discussion above does not include a complete list of the exchangers and pumps used in the mitochondria membrane. In addition, note that malate is used in both the citrate-pyruvate and malate-aspartate shuttles; cells can use mixtures of these processes to move a variety of carbon units or electrons from one side of the mitochondrial membrane to the other.

Calcium pumping

The mitochondria contain a proton gradient-dependent calcium pump. When cytoplasmic calcium concentrations rise, much of this calcium is then pumped into the mitochondria. This has two functions: 1) it decreases cytoplasmic calcium concentration, and 2) it raises mitochondrial calcium concentration. High mitochondrial calcium levels stimulate pyruvate dehydrogenase, isocitrate dehydrogenase, and □-ketoglutarate dehydrogenase, and therefore increase the rate of the TCA cycle. This is especially important in muscle, where increased cytoplasmic calcium concentration is a signal for the heavily energy-requiring events of muscle contraction.

Summary

Electrons need to cross the mitochondrial membrane for a variety of purposes. The nicotinamide electron carrier cofactor cannot cross the membrane directly. Two major shuttles are involved in this electron transfer: the glycerophosphate shuttle, which is only used to transfer electrons into the mitochondria, and the malate-aspartate shuttle, which can be used to transfer electrons in either direction.

Transporters also allow molecules like citrate to cross the membrane; citrate has several roles in the cytoplasm, including regulation of the glycolytic pathway.

Some molecules, such as oxaloacetate, cannot cross the mitochondrial membrane. This results in separate, independently regulated, pools of these compounds, which play important roles in metabolism.

The specific transporters and pumps function as an elegant mechanism by which cells can use different intracellular compartments to regulate metabolic processes.