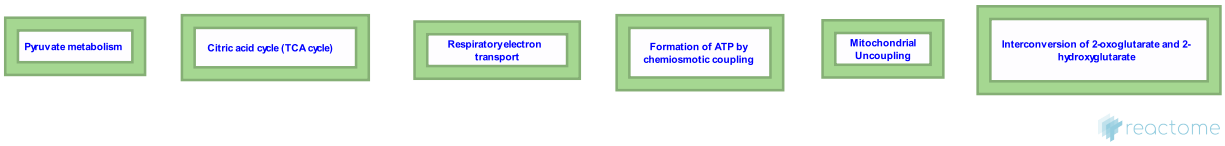
Aerobic respiration and respiratory electron transport



Birney, E., Brand, MD., D'Eustachio, P., Esteves, TC., Ferguson, SJ., Hill, DP., Jassal, B., Rush, MG., Schmidt, EE., Stephan, R.

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Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.
The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references

Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics, 18*, 142. ↗

Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics, 33*, 3461-3467. ↗

Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res, 46*, D649-D655. ↗

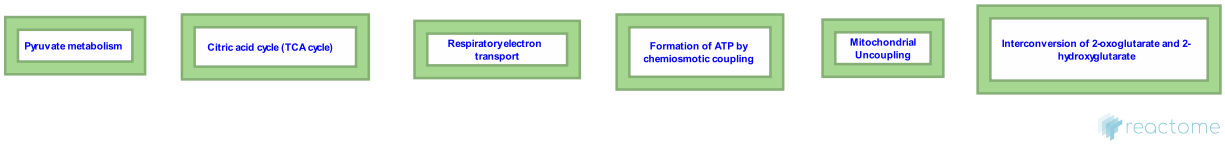
Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology, 14*, e1005968. ↗

Reactome database release: 89

This document contains 7 pathways (see Table of Contents)

Aerobic respiration and respiratory electron transport ↗

Stable identifier: R-HSA-1428517



Pyruvate metabolism and the citric acid (TCA) cycle together link the processes of energy metabolism in a human cell with one another and with key biosynthetic reactions. Pyruvate, derived from the reversible oxidation of lactate or transamination of alanine, can be converted to acetyl CoA. Other sources of acetyl CoA include breakdown of free fatty acids and ketone bodies in the fasting state. Acetyl CoA can enter the citric acid cycle, a major source of reducing equivalents. These reducing equivalents are re-oxidized back to NAD+ in the electron transport chain (ETC), coupling this process with the export of protons across the inner mitochondrial membrane. The chemiosmotic gradient created is used to drive ATP synthesis.

In addition to its role in energy generation, the citric acid cycle is a source of carbon skeletons for amino acid metabolism and other biosynthetic processes. One such process included here is the interconversion of 2-hydroxyglutarate, probably derived from porphyrin and amino acid metabolism, and 2-oxoglutarate (alpha-ketoglutarate), a citric acid cycle intermediate.

Editions

2003-11-03	Authored	Schmidt, EE., Birney, E., D'Eustachio, P.
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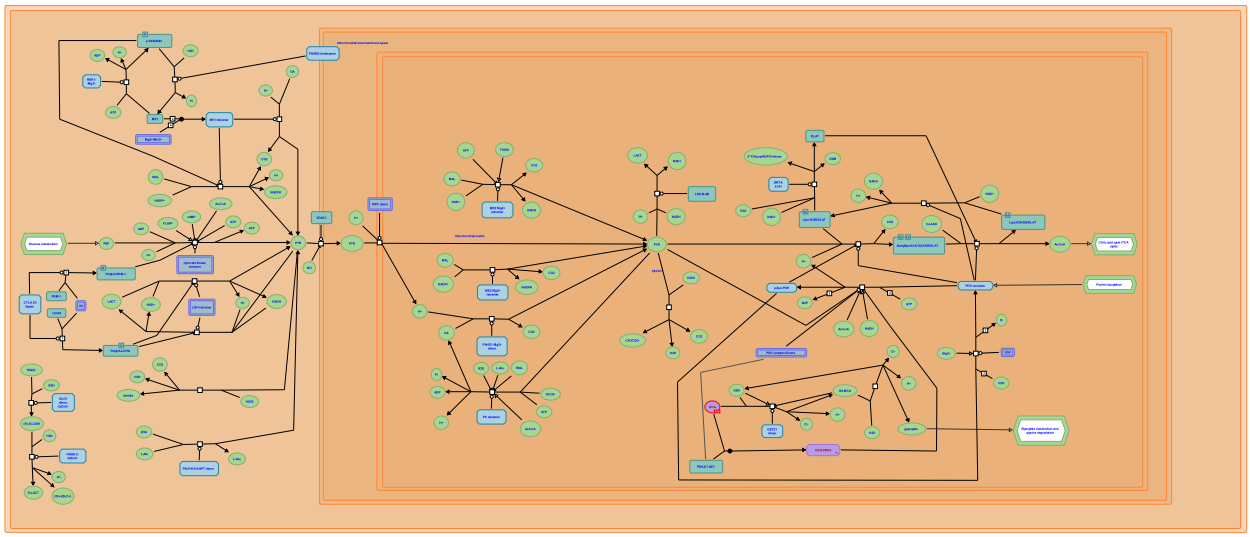
Pyruvate metabolism ↗

Location: Aerobic respiration and respiratory electron transport

Stable identifier: R-HSA-70268

Compartments: cytosol, mitochondrial matrix, mitochondrial intermembrane space



Pyruvate sits at an intersection of key pathways of energy metabolism. It is the end product of glycolysis and the starting point for gluconeogenesis and can be generated by the transamination of alanine. The pyruvate dehydrogenase complex can convert it to acetyl CoA (Reed and Hackert 1990), which can enter the TCA cycle or serve as the starting point for the syntheses of long-chain fatty acids, steroids, and ketone bodies depending on the tissue and metabolic state in which it is formed. It also plays a central role in balancing the energy needs of various tissues in the body. Under conditions in which oxygen supply is limiting, e.g., in exercising muscle, or in the absence of mitochondria, e.g., in red blood cells, re-oxidation of NADH produced by glycolysis cannot be coupled to the generation of ATP. Instead, re-oxidation is coupled to the reduction of pyruvate to lactate. This lactate is released into the blood and taken up primarily by the liver, where it is oxidized to pyruvate and can be used for gluconeogenesis (Cori 1981). For a recent review, see Prochownik & Wang, 2021.

Literature references

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Reed, LJ., Hackert, ML. (1990). Structure-function relationships in dihydrolipoamide acyltransferases. *J Biol Chem, 265*, 8971-4. ↗

Prochownik, EV., Wang, H. (2021). The Metabolic Fates of Pyruvate in Normal and Neoplastic Cells. *Cells, 10*. ↗

Editions

2009-12-18	Revised	D'Eustachio, P.
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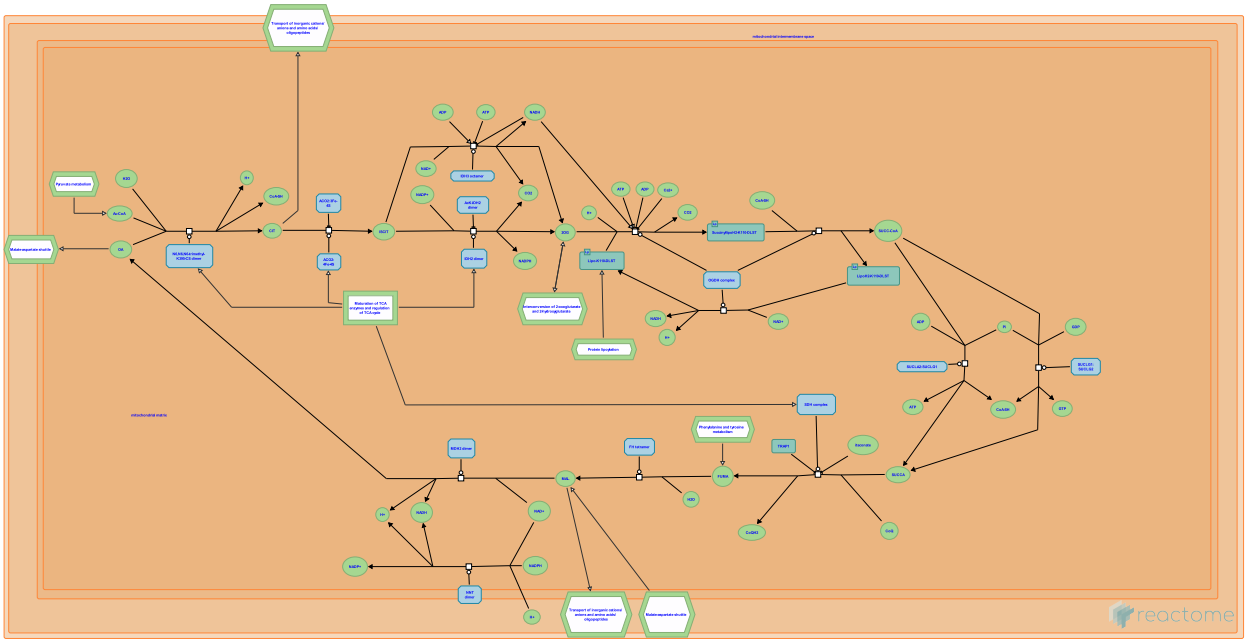
2024-02-21	Edited	Stephan, R.
2024-03-01	Reviewed	Hill, DP.

Citric acid cycle (TCA cycle) ↗

Location: Aerobic respiration and respiratory electron transport

Stable identifier: R-HSA-71403

Compartments: mitochondrion



In the citric acid or tricarboxylic acid (TCA) cycle, the acetyl group of acetyl CoA (derived primarily from oxidative decarboxylation of pyruvate, beta-oxidation of long-chain fatty acids, and catabolism of ketone bodies and several amino acids) can be completely oxidized to CO2 in reactions that also yield one high-energy phosphate bond (as GTP or ATP) and four reducing equivalents (three NADH + H+, and one FADH2). Then, the electron transport chain oxidizes NADH and FADH2 to yield nine more high-energy phosphate bonds (as ATP). All reactions of the citric acid cycle take place in the mitochondrion.

Eight canonical reactions mediate the synthesis of citrate from acetyl-CoA and oxaloacetate and the metabolism of citrate to re-form oxaloacetate. Three reactions are reversible: the interconversions of citrate and isocitrate, of fumarate and malate, and of malate and oxaloacetate. The reverse reactions are irrelevant under normal physiological conditions but appear to have a role in glucose- and glutamine-stimulated insulin secretion (Zhang et al., 2020) and cancer metabolism (e.g., Jiang et al., 2016). Succinate synthesis from succinyl-CoA can be coupled to the phosphorylation of either GDP (the canonical reaction) or ADP; we annotate both reactions. Two mitochondrial isocitrate dehydrogenase isozymes catalyze the oxidative decarboxylation of isocitrate to form alpha-ketoglutarate (2-oxoglutarate): IDH3 catalyzes the canonical reaction coupled to the reduction of NAD+, while IDH2 catalyzes the same reaction coupled to the reduction of NADP+, a reaction whose normal physiological function is unclear. Both reactions are annotated.

The cyclical nature of the reactions responsible for the oxidation of acetate was first suggested by Hans Krebs from biochemical studies of pigeon breast muscle (Krebs et al., 1938; Krebs and Eggleston, 1940). Ochoa and colleagues studied many molecular details of individual reactions, mainly by studying enzymes purified from pig hearts (Ochoa, 1980). While the human homologs of these enzymes have all been identified, their biochemical characterization has, in general, been limited, and many molecular details of the human reactions are inferred from those worked out in studies of the model systems. Studies examining the impact of elevated citric acid cycle intermediates such as succinate and fumarate led to the recognition of the role of metabolites in driving cancer progression ('oncometabolites') (Pollard et al., 2005; reviewed in Hayashi et al., 2018). The role of TCA enzymes in disease was reviewed by Kang et al., 2021.

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Gray, SM., Lu, D., Wang, Y., Jensen, MV., Zhang, GF., El, K. et al. (2021). Reductive TCA cycle metabolism fuels glutamine- and glucose-stimulated insulin secretion. *Cell Metab, 33*, 804-817.e5. ↗

Wortham, NC., Griffiths, JR., Moat, SJ., Pollard, PJ., Barclay, E., Barwell, J. et al. (2005). Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. *Hum Mol Genet, 14*, 2231-9. ↗

Editions

2003-01-28	Authored	Birney, E.
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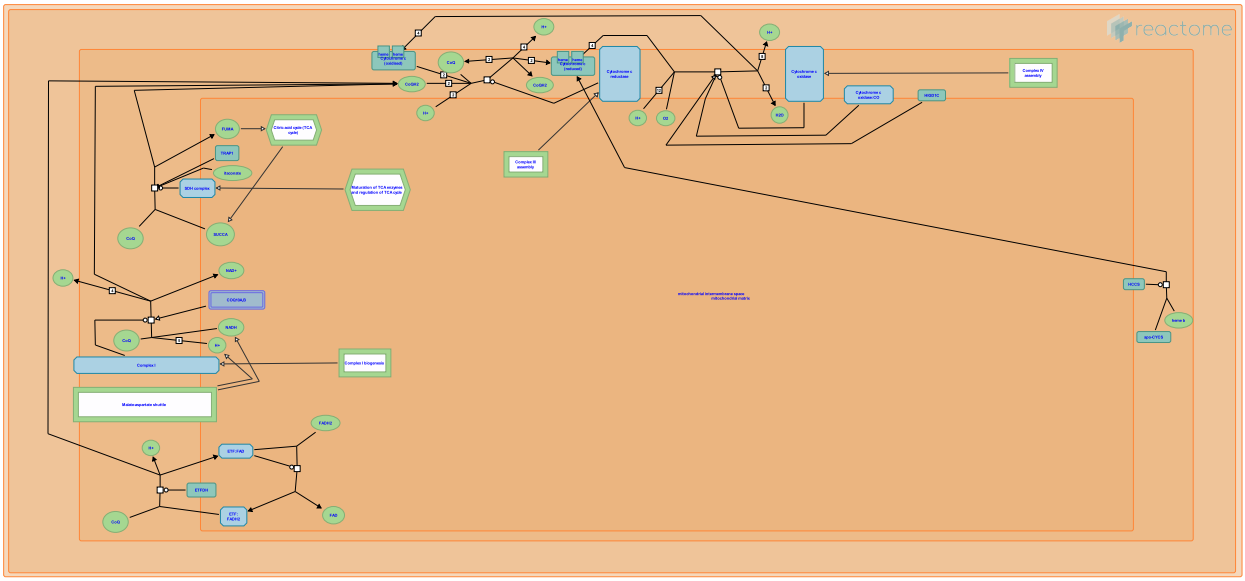
Respiratory electron transport ↗

Location: Aerobic respiration and respiratory electron transport

Stable identifier: R-HSA-611105



Mitochondria are often described as the "powerhouse" of a cell as it is here that energy is largely released from the oxidation of food. Reducing equivalents generated from beta-oxidation of fatty acids and from the Krebs cycle enter the electron transport chain (also called the respiratory chain). During a series of redox reactions, electrons travel down the chain releasing their energy in controlled steps. These reactions drive the active transport of protons from the mitochondrial matrix , through the inner membrane to the intermembrane space. The respiratory chain consists of five main types of carrier; flavins, iron-sulfur centres, quinones, cytochromes (heme proteins) and copper. The two main reducing equivalents entering the respiratory chain are NADH and FADH2. NADH is linked through the NADH-specific dehydrogenase whereas FADH2 is reoxidised within succinate dehydrogenase and a ubiquinone reductase of the fatty acid oxidation pathway. Oxygen is the final acceptor of electrons and with protons, is converted to form water, the end product of aerobic cellular respiration. A proton electrochemical gradient (often called protonmotive force) is established across the inner membrane, with positive charge in the intermembrane space relative to the matrix. Protons driven by the proton-motive force, can enter ATP synthase thus returning to the mitochondrial matrix. ATP synthases use this exergonic flow to form ATP in the matrix, a process called chemiosmotic coupling. A by-product of this process is heat generation.

An antiport, ATP-ADP translocase, preferentially exports ATP from the matrix thereby maintaining a high ADP:ATP ratio in the matrix. The tight coupling of electron flow to ATP synthesis means oxygen consumption is dependent on ADP availability (termed respiratory control). High ADP (low ATP) increases electron flow thereby increasing oxygen consumption and low ADP (high ATP) decreases electron flow and thereby decreases oxygen consumption. There are many inhibitors of mitochondrial ATP synthesis. Most act by either blocking the flow of electrons (eg cyanide, carbon monoxide, rotenone) or uncoupling electron flow from ATP synthesis (eg dinitrophenol). Thermogenin is a natural protein found in brown fat. Newborn babies have a large amount of brown fat and the heat generated by thermogenin is an alternative to ATP synthesis (and thus electron flow only produces heat) and allows the maintenance of body temperature in newborns.

The electron transport chain is located in the inner mitochondrial membrane and comprises some 80 proteins organized in four enzymatic complexes (I-IV). Complex V generates ATP but has no electron transfer activity. In addition to these 5 complexes, there are also two electron shuttle molecules; Coenzyme Q (also known as ubiquinone, CoQ) and Cytochrome c (Cytc). These two molecules shuttle electrons between the large complexes in the chain.

How many ATPs are generated by this process? Theoretically, for each glucose molecule, 32 ATPs can be produced. As electrons drop from NADH to oxygen in the chain, the number of protons pumped out and returning through ATP synthase can produce 2.5 ATPs per electron pair. For each pair donated by FADH2, only 1.5 ATPs can be formed. Twelve pairs of electrons are removed from each glucose molecule;

10 by NAD+ = 25 ATPs
2 by FADH2 = 3 ATPs.

Making a total of 28 ATPs. However, 2 ATPs are formed during the Krebs' cycle and 2 ATPs formed during glycolysis for each glucose molecule therefore making a total ATP yield of 32 ATPs. In reality, the energy from the respiratory chain is used for other processes (such as active transport of important ions and molecules) so under conditions of normal respiration, the actual ATP yield probably does not reach 32 ATPs.

The reducing equivalents that fuel the electron transport chain, namely NADH and FADH2, are produced by the Krebs cycle (TCA cycle) and the beta-oxidation of fatty acids. At three steps in the Krebs cycle (isocitrate conversion to oxoglutarate; oxoglutarate conversion to succinyl-CoA; Malate conversion to oxaloacetate), a pair of electrons (2e-) are removed and transferred to NAD+, forming NADH and H+. At a single step, a pair of electrons are removed from succinate, reducing FAD to FADH2. From the beta-oxidation of fatty acids, one step in the process forms NADH and H+ and another step forms FADH2.

Cytoplasmic NADH, generated from glycolysis, has to be oxidized to reform NAD+, essential for glycolysis, otherwise glycolysis would cease to function. There is no carrier that transports NADH directly into the mitochondrial matrix and the inner mitochondrial membrane is impermeable to NADH so the cell uses two shuttle systems to move reducing equivalents into the mitochondrion and regenerate cytosolic NAD+.
The first is the glycerol phosphate shuttle, which uses electrons from cytosolic NADH to produce FADH2 within the inner membrane. These electrons then flow to Coenzyme Q. Complex I is bypassed so only 1.5 ATPs can be formed per NADH via this route. The overall balanced equation, summing all the reactions in this system, is

NADH (cytosol) + H+ (cytosol) + NAD+ (mito.) = NAD+ (cytosol) + NADH (mito.) + H+ (mito.)

The malate-aspartate shuttle uses the oxidation of malate to generate NADH in the mitochondrial matrix. This NADH can then be fed directly to complex I and thus can form 3 ATPs via the respiratory chain. The overall balanced equation is

NADH (cytosol) + H+ (cytosol) + FAD (inner memb.) = NAD+ (cytosol) + FADH2 (inner memb.)

Both of these shuttle systems regenerate cytosolic NAD+.

The entry point for NADH is complex I (NADH dehydrogenase) and the entry point for FADH2 is Coenzyme Q. The input of electrons from fatty acid oxidation via ubiquinone is complicated and not shown in the diagram.

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Editions

2005-04-21	Authored	Jassal, B.
2005-05-12	Reviewed	Ferguson, SJ.
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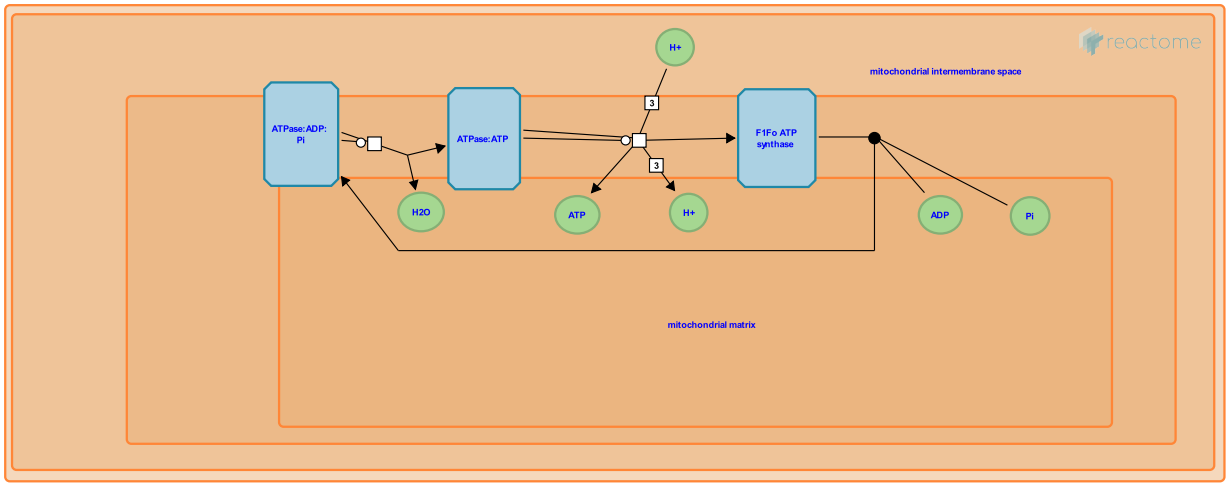
2024-02-15	Reviewed	Hill, DP.

Formation of ATP by chemiosmotic coupling ↗

Location: Aerobic respiration and respiratory electron transport

Stable identifier: R-HSA-163210



The re-entry of protons into the mitochondrial matrix through Complex V causes conformational changes which result in ATP synthesis. Complex V (ATP synthase) is composed of 3 parts; an F1 catalytic core (approx 5 subunits), an F0 membrane proton channel (approx 9 subunits) and two stalks linking F1 to F0. F1 contains three alpha subunits, three beta subunits, and one each of gamma, delta, and epsilon subunits. Each beta subunit contains an active site for ATP synthesis. F0 has at least 9 subunits (a-g, A6L and F6; see Lai et al., 2023; reviewed in Jonckheere et al., 2011).
The mechanism of ATP synthesis by Complex V was predicted by Boyer et al in 1973: ADP and Pi bind to the enzyme resulting in a conformational change. ATP is then synthesized, still bound to the enzyme. Another change in the active site results in the release of free ATP into the matrix. The overall reaction is:
ADP + Pi + H+ + nH+ (intermemb. space) = ATP + H2O + nH+ (matrix)

Mutations in several ATP synthase subunits can lead to different types of mitochondrial complex V deficiency (MC5D; reviewed in Garone et al., 2022; Del Dotto et al., 2024).

Literature references

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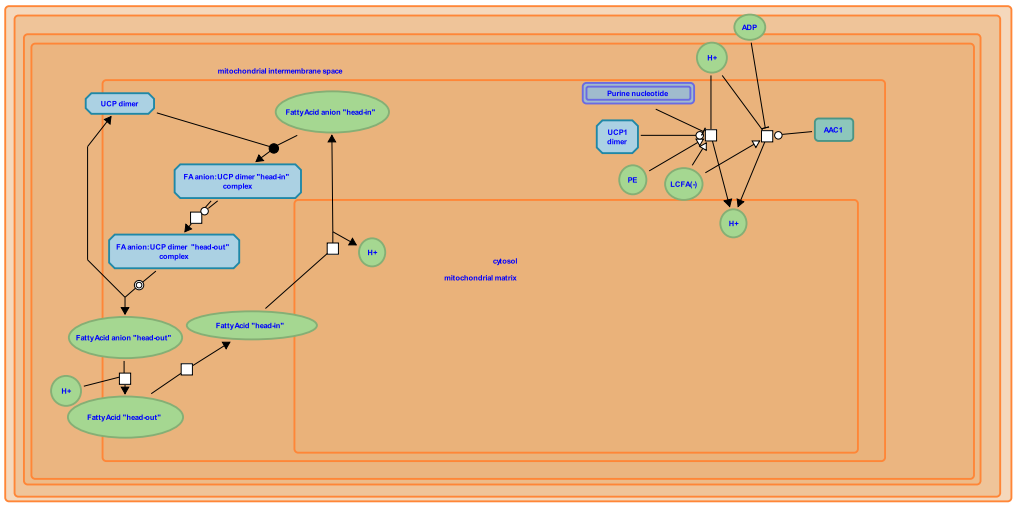
Editions

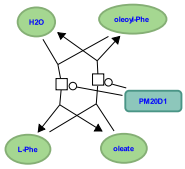
2005-06-29	Authored	Jassal, B.

Mitochondrial Uncoupling ↗

Location: Aerobic respiration and respiratory electron transport

Stable identifier: R-HSA-166187





The protonmotive force across the inner mitochondrial membrane built up by respiratory electron transport does not go entirely into ATP production. Transport proteins of the SLC25 type utilize the gradient to symport small molecules with protons into the matrix, simultaneously generating heat ("thermogenesis"). UCP1 and AAC1 have been shown to import protons exclusively and are responsible for most heat generated in brown fat and other tissue, respectively (reviewed in Bertholet & Kirichok, 2022).

Uncoupling proteins (UCPs) are members of the mitochondrial transport carrier family. The crystal structure of one member of the family, the adenine nucleotide translocase, is known, and UCPs can be successfully folded into this structure to indicate their probable 3D arrangement (Pebay-Peyroula et al. 2003, Kunji 2004, Esteves & Brand 2005).

The most studied member of the family, UCP1, catalyzes adaptive thermogenesis (i.e., heat generation) in mammalian brown adipose tissue. It does so by promoting a leak of protons through the mitochondrial inner membrane, which uncouples ATP production from substrate oxidation, leading to fast oxygen consumption and ultimately to heat production. The thermogenic activity of UCP1 in brown adipose tissue plays an important role when the organism needs extra heat, e.g., during cold weather conditions (for small rodents), the cold stress of birth, or arousal from hibernation. UCP1 homologs have been found in lower vertebrates such as fish, where their role is unclear (Cannon & Nedergaard 2004, Jastroch et al. 2005).

The proton conductance of UCP1 in brown adipose tissue is tightly controlled. It is strongly inhibited by physiological concentrations of purine nucleotides. This inhibition is overcome by fatty acids released from intracellular triacylglycerol stores following adrenergic activation in response to cold or overfeeding. Other activators include superoxide, retinoic acid, the retinoid 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetra-methyl-2-naphtalenyl)-1-propenyl]benzoic acid (TTNPB) and reactive alkenals, such as hydroxynonenal.

There is strong evidence that the regulated uncoupling caused by these proteins attenuates mitochondrial reactive oxygen species production, protects against cellular damage, and (in beta-cells) diminishes insulin secretion. There are also untested suggestions that their transport of fatty acids may be physiologically important (Brand & Esteves 2005, Esteves & Brand 2005, Krauss et al. 2005).

Several models have been proposed for the molecular mechanism by which fatty acids lead to increased proton conductance by UCP1 in brown adipose tissue mitochondria and presumably by the other UCPs. We have depicted the most likely model, the "fatty acid cycling" model, in this pathway .

Studies of mouse models and cultured human cells have suggested that oleoyl-phenylalanine, synthesized by extracellular PM20D1, may play a role in uncoupling independent of the action of UCPs (Long et al., 2016). Its synthesis and hydrolysis are annotated here.

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Kahn, R., Dahout-Gonzalez, C., Brandolin, G., Lauquin, GJ., Trezeguet, V., Pebay-Peyroula, E. (2003). Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. *Nature, 426*, 39-44. ↗

Kirichok, Y., Bertholet, AM. (2022). Mitochondrial H⁺ Leak and Thermogenesis. *Annu Rev Physiol, 84*, 381-407. ↗

Editions

2005-11-09	Authored	Jassal, B., Esteves, TC., Brand, MD.
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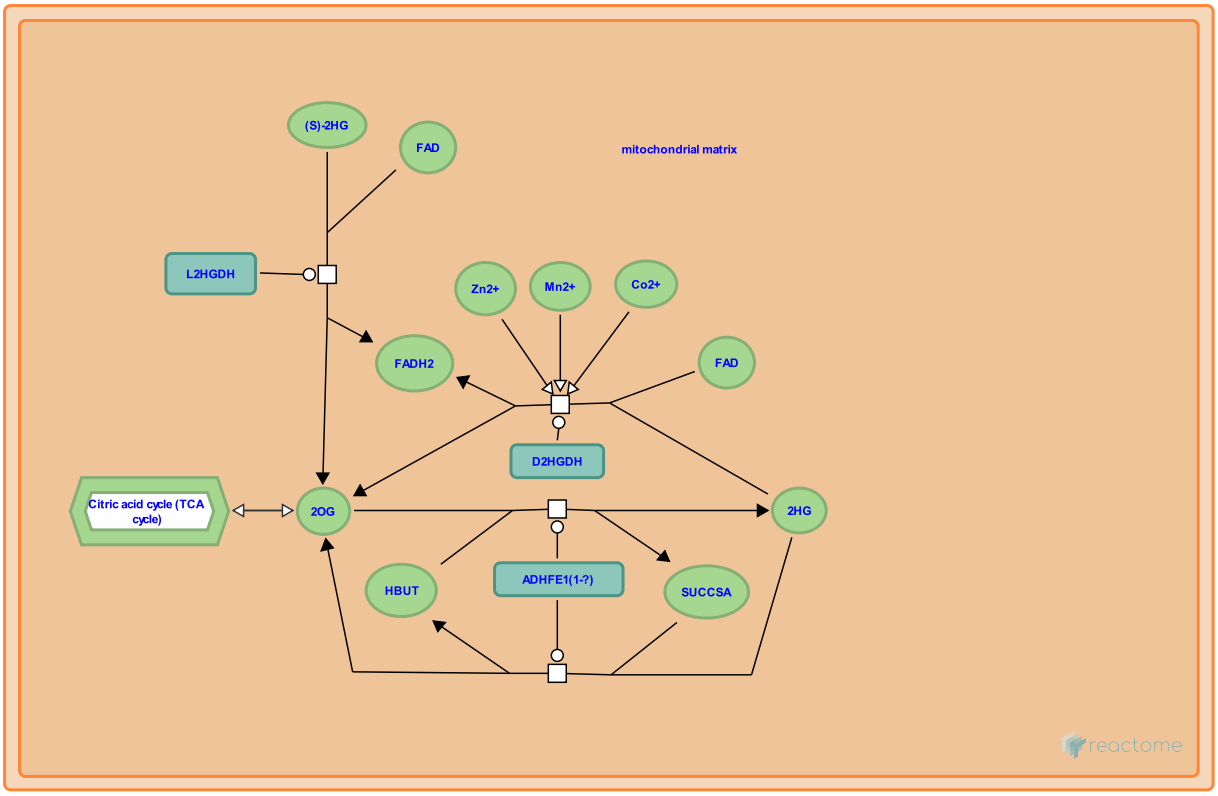
Interconversion of 2-oxoglutarate and 2-hydroxyglutarate ↗

Location: Aerobic respiration and respiratory electron transport

Stable identifier: R-HSA-880009



The two stereoisomers of 2-hydroxyglutarate are normally converted to 2-oxoglutarate in the mitochondrial matrix, and can then be metabolized by the citric acid cycle. The physiological sources of 2-hydroxyglutarate have not been established although plausible hypotheses are that it is generated by lysine breakdown or as a byproduct of delta-aminolevulinate metabolism. The stereoisomers are oxidized to 2-oxoglutarate in FAD-dependent reactions catalyzed by the enzymes D2HGDH (specific for R(-)-2-hydroxyglutarate) and L2HGDH (specific for S(-)-2-hydroxyglutarate). An inherited deficiency in either enzyme is associated with accumulation of 2-hydroxyglutarate and variable neurological symptoms. R(-)-2-hydroxyglutarate also reacts reversibly with succinate semialdehyde to form 4-hydroxybutyrate and 2-oxoglutarate, catalyzed by ADHFE1. No deficiencies of this enzyme have been found in patients with elevated 2-hydroxyglutarate levels (Struys 2006).

Literature references

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Bigner, DD., McLendon, R., Gallia, GL., Strausberg, RL., Karchin, R., Mankoo, P. et al. (2008). An integrated genomic analysis of human glioblastoma multiforme. *Science, 321*, 1807-12. ↗

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Editions

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2010-11-09	Reviewed	Jassal, B.
2011-01-31	Reviewed	Rush, MG.

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