Universidad de Chile

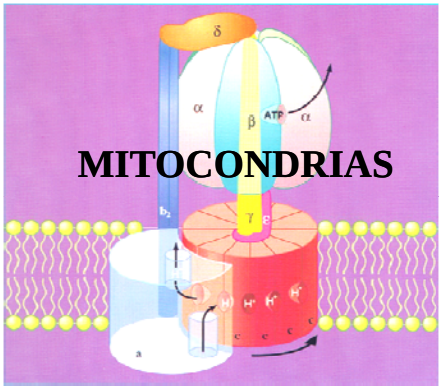
Programa Académico de Bachillerato

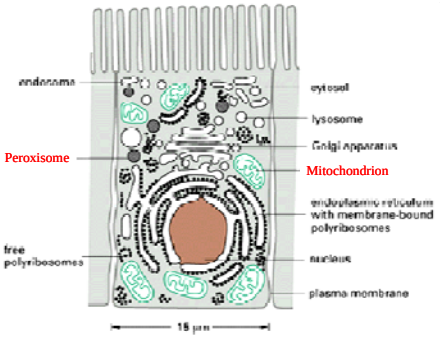
Introducción a la Biología Celular

2011

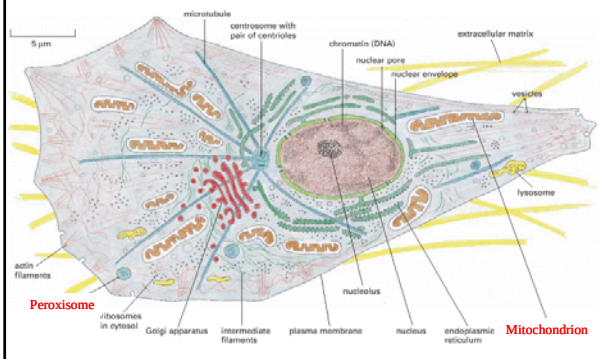
Eduardo Kessi C.
Departamento de Ciencias Biológicas Animales
Facultad de Ciencias Veterinarias y Pecuarias
Universidad de Chile
ekessi@uchile.cl

MITOCONDRIAS

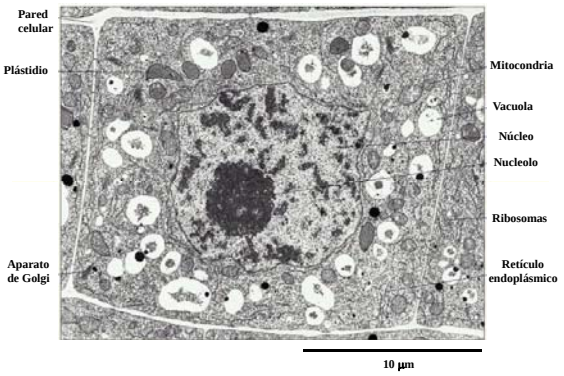


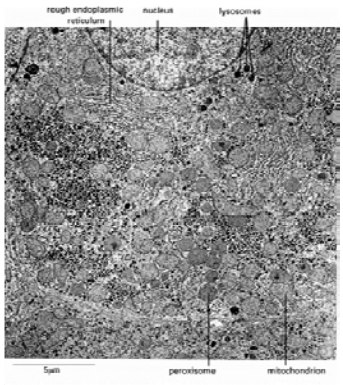


The major intracellular compartments of an animal cell. The cytosol (*gray*), endoplasmic reticulum, Golgi apparatus, nucleus, mitochondrion, endosome, lysosome, and peroxisome are distinct compartments isolated from the rest of the cell by at least one selectively permeable membrane.

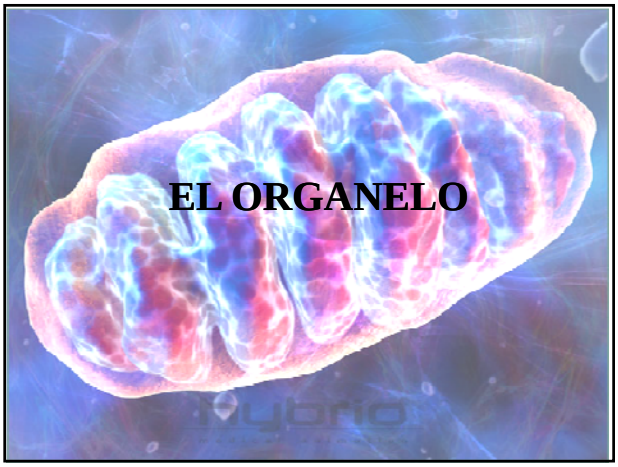


Todas las células tienen el mismo conjunto básico de organelos conformados por membranas





Electron micrograph of part of a liver cell seen in cross-section. Examples of most of the major intracellular compartments are indicated.



Kölliker (1850-1890) describió arreglos de gránulos en el sarcoplasma de músculo estriado, que fueron llamados posteriormente *sarcosomas* por Retzius (1890)

Fleming (1890) describió estructuras filamentosas en el citoplasma de muchos tipos celulares distintos.

Altman (1890) desarrolló una tinción específica para esas estructuras. Sugirió su autonomía (unidades vivas elementales) y notó su similitud con las bacterias (viven de manera independiente o en colonias en el citoplasma de la célula)

Benda (fines del siglo XIX) usa la palabra mitocondria que vino a reemplazar a los términos blefaroblastos, condriocontos, condriomitos, condrioplastos, condriosomas, condrioesferas, fila, cuerpos intersticiales, mitogel, cuerpos parabasales, esferoplastos, vermículos.

Table 12-1. Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)

INTRACELLULAR COMPARTMENT	PERCENTAGE OF TOTAL CELL VOLUME
Cytosol	54
Mitochondria	**22**
Rough ER cisternae	9
Smooth ER cisternae plus Golgi cisternae	6
Nucleus	6
Peroxisomes	**1**
Lysosomes	1
Endosomes	1

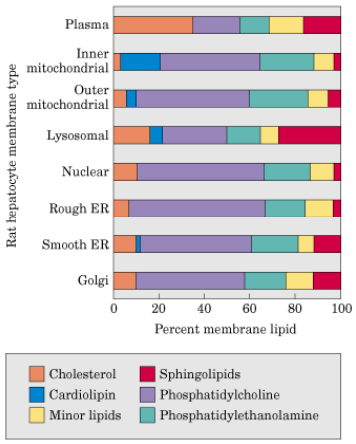
Table 12-2. Relative Amounts of Membrane Types in Two Kinds of Eucaryotic Cells

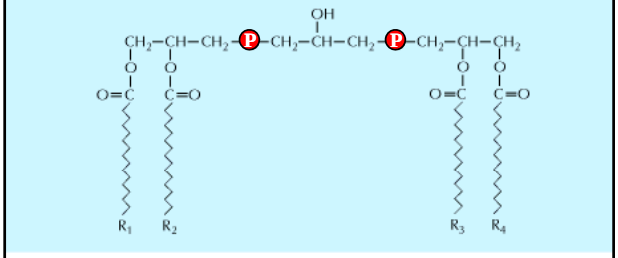
MEMBRANE TYPE	PERCENTAGE OF TOTAL CELL MEMBRANE	
	LIVER HEPATOCYTE*	PANCREATIC EXOCRINE CELL*
Plasma membrane	2	5
Rough ER membrane	35	60
Smooth ER membrane	16	<1
Golgi apparatus membrane	7	10
Mitochondria		
Outer membrane	7	4
Inner membrane	32	17
Nucleus		
Inner membrane	0.2	0.7
Secretory vesicle membrane	not determined	3
Lysosome membrane	0.4	not determined
Peroxisome membrane	0.4	not determined
Endosome membrane	0.4	not determined

Approximate Lipid Compositions of Different Cell Membranes

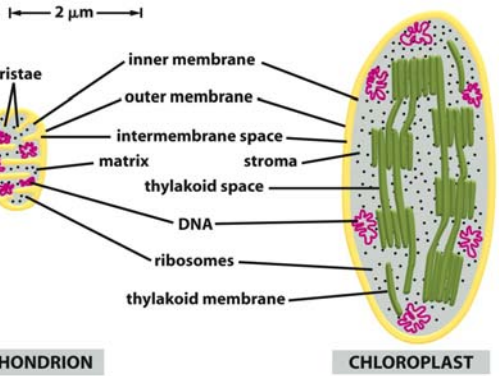
	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
LIPID	LIVER CELL*	RBC*	MYELIN	MIT**	ER	*E. coli*
Cholesterol	17	23	22	3	6	0
Phosphatidylethanolamine	7	18	15	25	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidylcholine	24	17	10	39	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	13	8	21	27	30

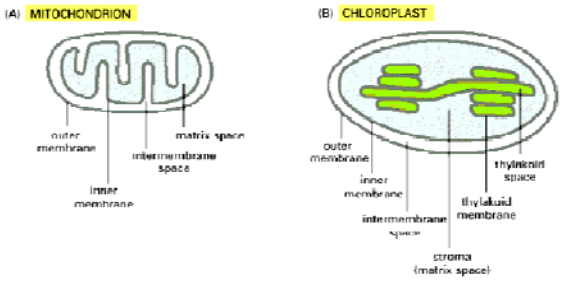
* Plasma membranes; ** Inner and Outer membranes



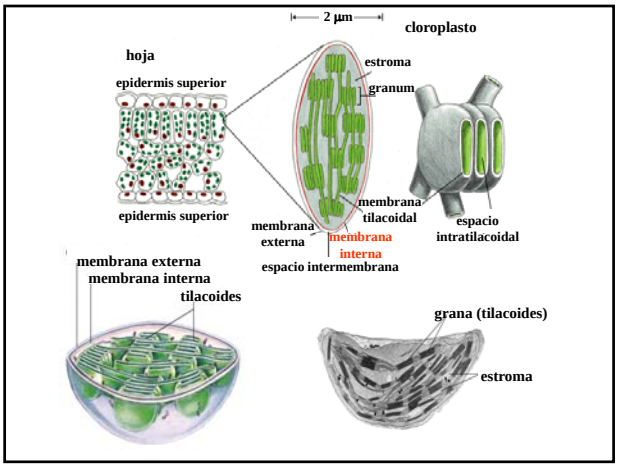


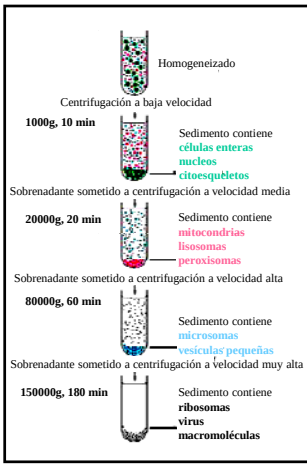
Structure of cardiolipin Cardiolipin is an unusual "double" phospholipid, containing four fatty acid chains, that is found primarily in the inner mitochondrial membrane.



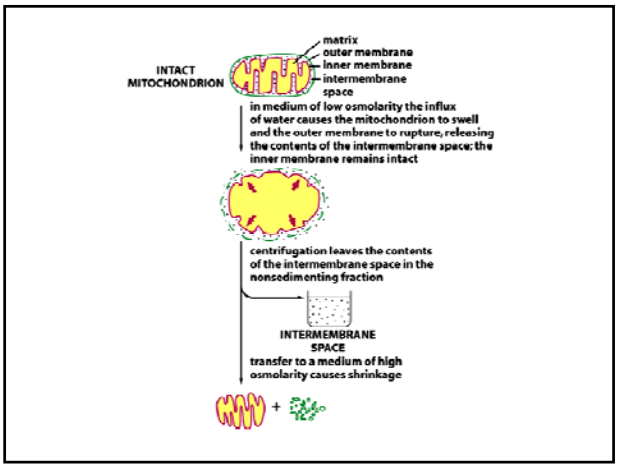


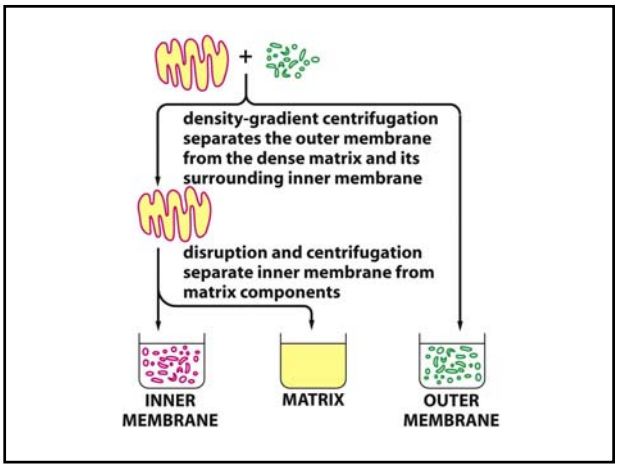
The subcompartments of mitochondria and chloroplasts. In contrast to the cristae of mitochondria (A), the thylakoids of chloroplasts (B) are not connected to the inner membrane and therefore form a compartment with a separate internal space

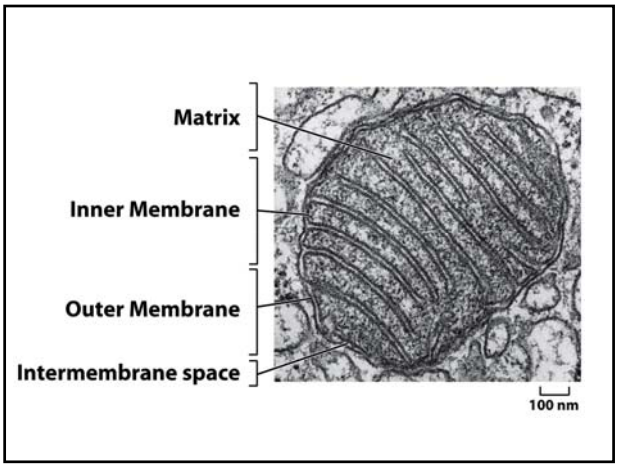


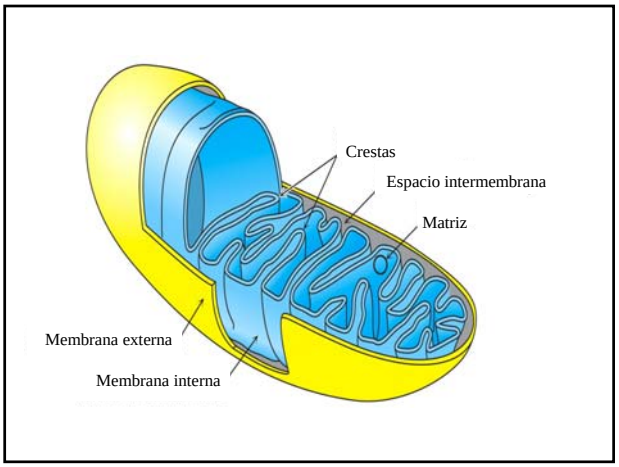


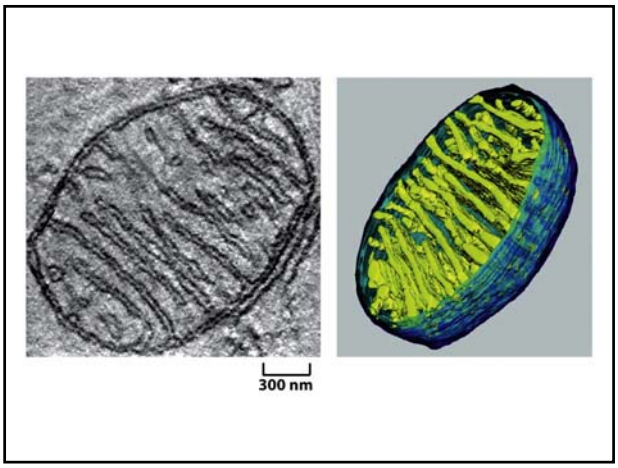
Fraccionamiento subcelular por centrifugación diferencial. Centrifugaciones repetidas a velocidades progresivamente mayores permiten fraccionar homogeneizados de células en sus componentes. En general, los componentes más pequeños requieren de valores mayores de fuerza centrífuga para sedimentarlos.

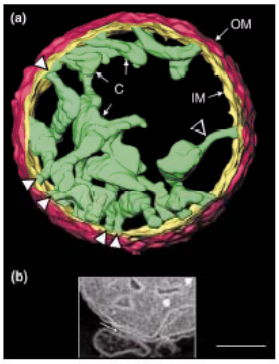




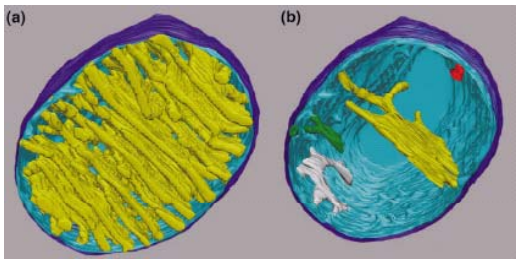




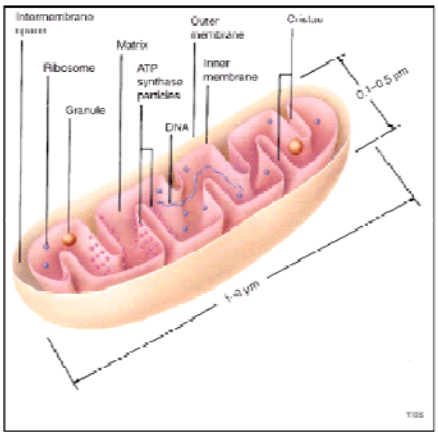


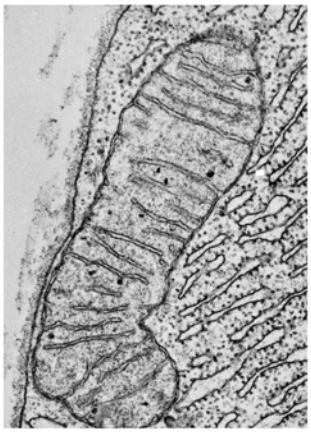


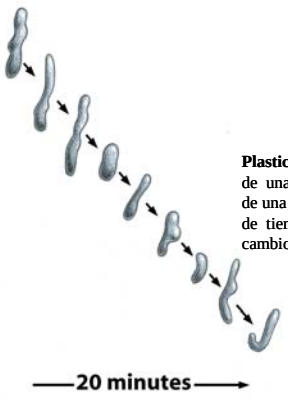
Tomography of an isolated rat-liver mitochondrion. (a) Surface-rendered 3D image of an isolated rat-liver mitochondrion. C, cristae; IM, inner boundary membrane; OM, outer membrane. Arrowheads point to tubular regions of cristae that connect them to IM and to each other. Reproduced, with permission, from Ref. 4. (b) Region of a 5-nm slice from the same tomogram showing numerous contact sites between OM and IM. Arrow points to particle bridging OM with attached vesicle of putative endoplasmic reticulum. Bar, 0.4 μm.(TIBS **25**:319, 2000)



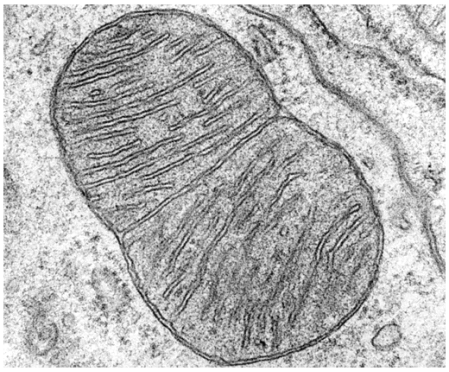
Computer models generated from segmented 3D tomograms of a mitochondrion in chick cerebellum. (a) The entire model showing all cristae in yellow, the inner boundary membrane in light blue, and the outer membrane in dark blue. (b) Outer membrane, inner boundary membrane and four representative cristae in different colors. (TIBS **25**:319, 2000)

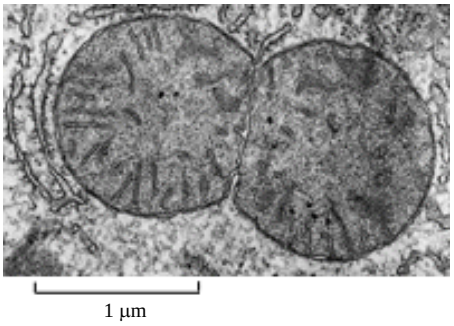


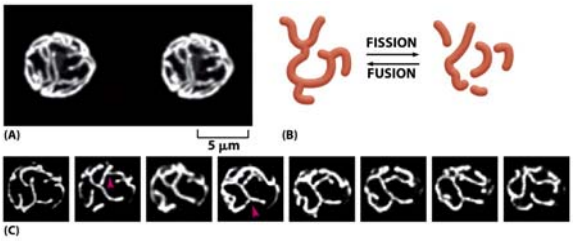




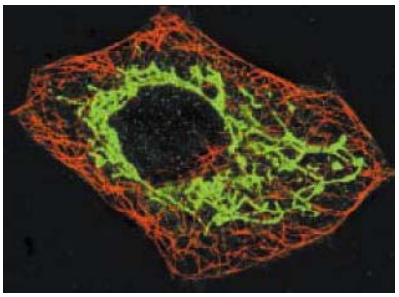
Plasticidad mitocondrial. La observación de una mitocondria individual, al interior de una célula viva durante un cierto período de tiempo, permite constatar que ocurren cambios rápidos en su morfología.



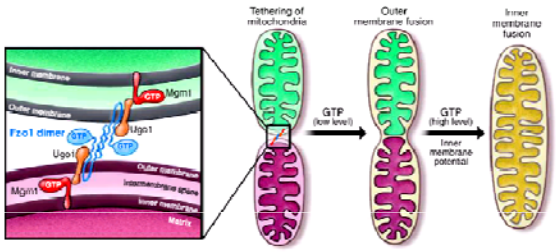




A dynamic mitochondrial reticulum. (A) In yeast cells, mitochondria form a continuos reticulum underlying the plasma membrane. (B) A balance between fission and fusion determines the arrangement of mitochondria in different cells. Time-lapse fluorescence microscopy shows the dynamic behaviour of the mitochondrial network in a yeast cell. In addititon to shape changes, fission and fusion constantly remodel the network (red arrows). The pictures were taken at 3-minute intervals



Mitochondria, Stained Green, Form a Network Inside a Fibroblast Cell. Mitochondria oxidize carbon fuels to form cellular energy. This transformation requires electron transfer through several large protein complexes some of which pump protons, forming a proton gradient that powers the synthesis of ATP.



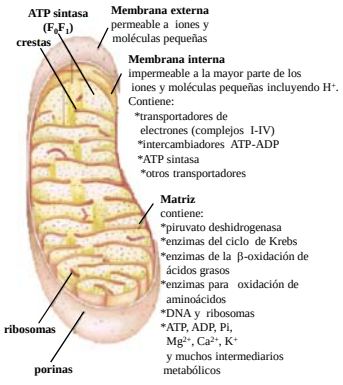
Mitochondrial fusion dissected. Fusion of mitochondria requires the sequential interaction of outer and inner membranes. Fusion of the outer membranes of two adjacent mitochondria requires low GTP levels, whereas the subsequent fusion of the inner membranes requires high GTP levels and the presence of an inner-membrane electrical potential. (Box) Three components of the mitochondrial fusion machinery are known: the outer-membrane GTPase (Fzo1), the intermembrane-space GTPase (Mgm1), and the outer-membrane protein Ugo1 that links both GTPases. Formation of Fzo1 homodimers between adjacent mitochondria promotes their initial tethering. Subsequent fusion of the two mitochondria requires the cooperation of Fzo1, Ugo1, Mgm1, and additional fusion proteins and regulatory factors. (*Science 305:1723-1724, 2004*)

Mitochondria are dynamic organelles whose morphologies are controlled by fusion and fission. Mitochondrial fusion and fission are essential for normal mitochondrial function, implying that mitochondria do not function well as autonomous organelles.

Chan D.C. 2006 Annu. Rev. Cell. Dev. Biol. 22:79-99

Mitochondrial dynamics plays important roles in vertebrate development and programmed cell death. Mutations in the mitochondrial fusion machinery lead to two human neurodegenerative disorders, Charcot-Marie-Tooth subtype 2A and autosomal dominant optic atrophy.

Chan D.C. 2006 Annu. Rev. Cell. Dev. Biol. 22:79-99

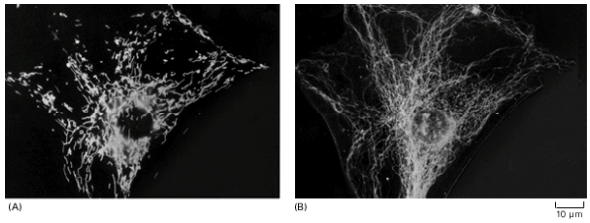


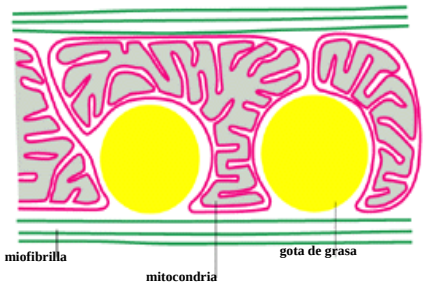
Membrana externa
permeable a iones y moléculas pequeñas

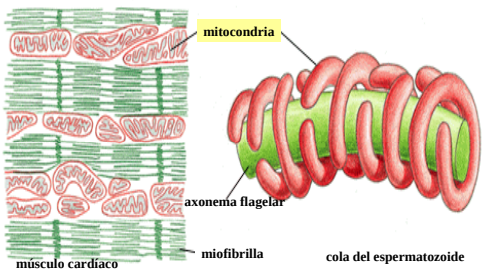
Membrana interna
impermeable a la mayor parte de los iones y moléculas pequeñas incluyendo H^+.
Contiene:
- *transportadores de electrones (complejos I-IV)
- *intercambiadores ATP-ADP
- *ATP sintasa
- *otros transportadores

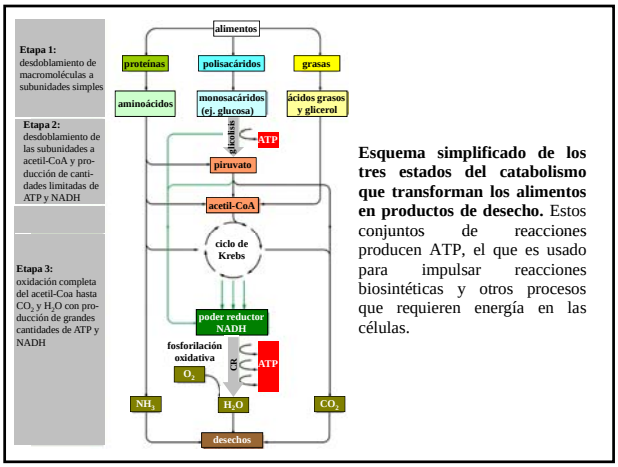
Matriz
contiene:
- *piruvato deshidrogenasa
- *enzimas del ciclo de Krebs
- *enzimas de la β-oxidación de ácidos grasos
- *enzimas para oxidación de aminoácidos
- *DNA y ribosomas
- *ATP, ADP, Pi, Mg^{2+}, Ca^{2+}, K^+ y muchos intermediarios metabólicos

mitocondrias microtúbulos

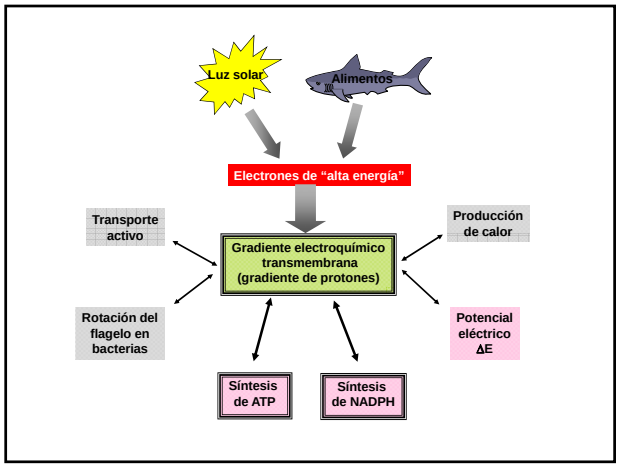


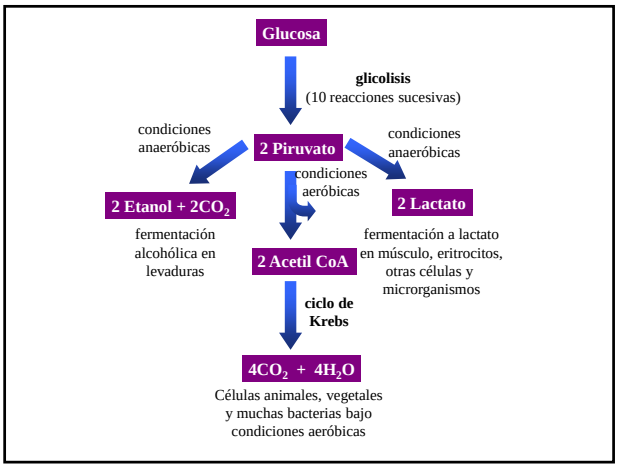


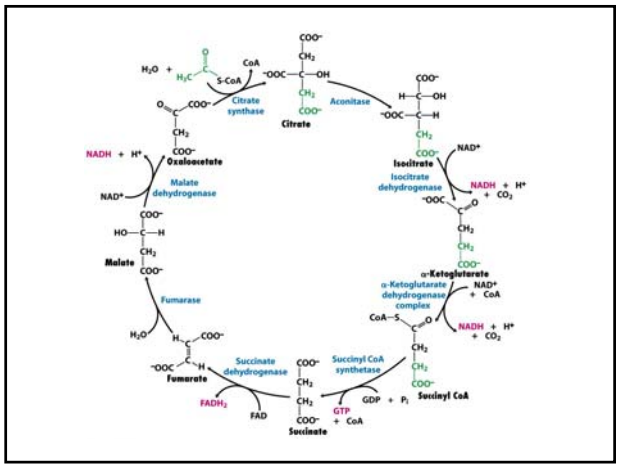




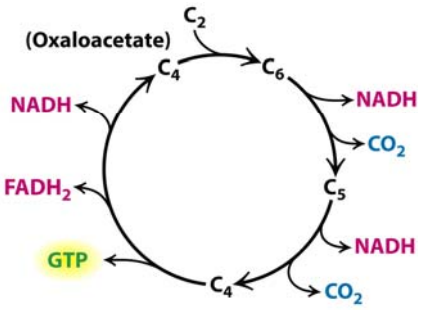
Esquema simplificado de los tres estados del catabolismo que transforman los alimentos en productos de desecho. Estos conjuntos de reacciones producen ATP, el que es usado para impulsar reacciones biosintéticas y otros procesos que requieren energía en las células.

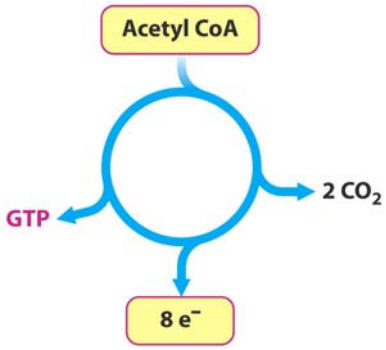


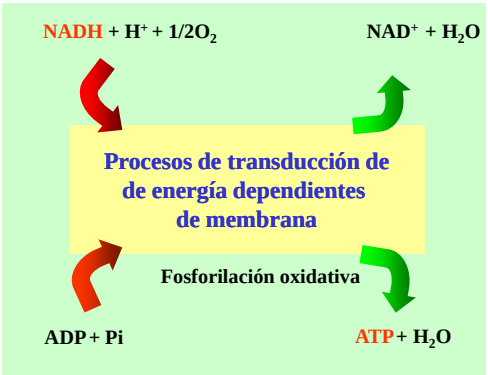


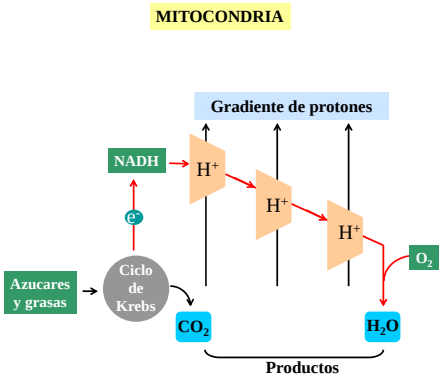


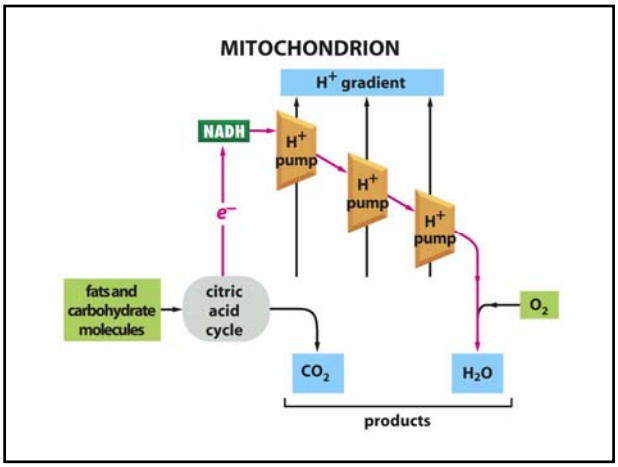
Ciclo de Krebs
(ciclo de los ácidos tricarboxílicos)
(ciclo del ácido cítrico)

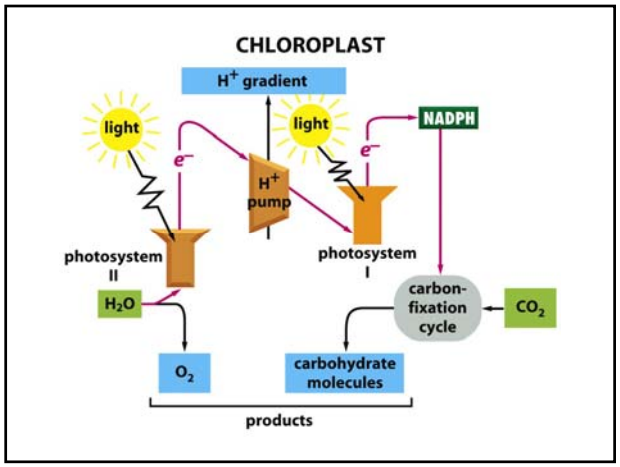


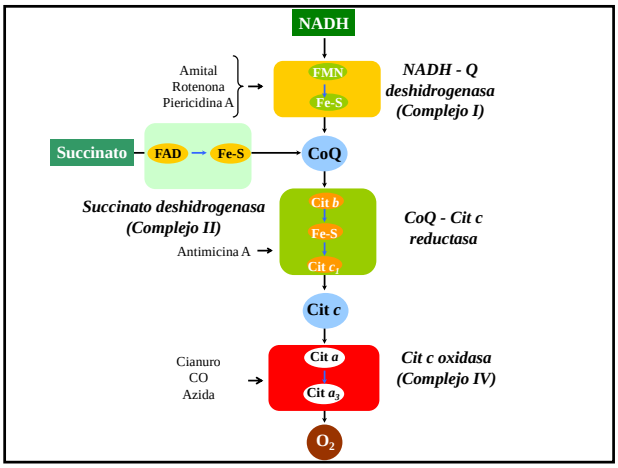


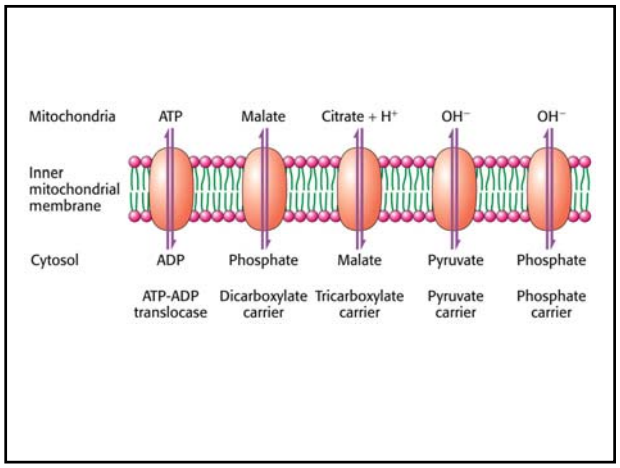


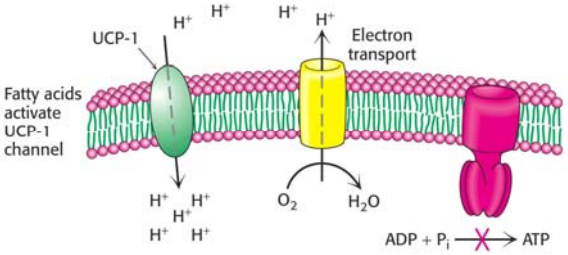


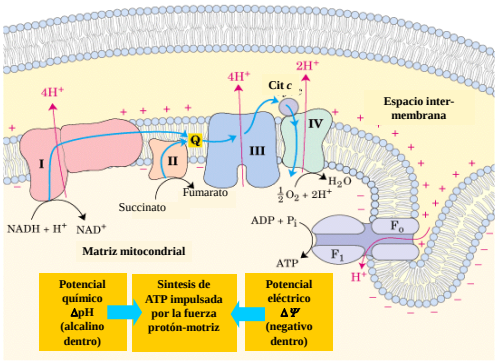

MITOCHONDRION
H+ gradient
NADH
H+ pump
H+ pump
H+ pump
e−
fats and carbohydrate molecules
citric acid cycle
O2
CO2
H2O
products


CHLOROPLAST
H+ gradient
light
light
e−
e−
NADPH
H+ pump
photosystem II
photosystem I
H2O
O2
carbon-fixation cycle
CO2
carbohydrate molecules
products


NADH
Amital
Rotenona
Piericidina A
FMN
Fe-S
NADH - Q deshidrogenasa (Complejo I)
Succinato
FAD
Fe-S
CoQ
Succinato deshidrogenasa (Complejo II)
Antimicina A
Cit b
Fe-S
Cit c1
CoQ - Cit c reductasa
Cit c
Cianuro
CO
Azida
Cit a
Cit a3
Cit c oxidasa (Complejo IV)
O2


Mitochondria
Inner mitochondrial membrane
Cytosol
ATP
ADP
Malate
Phosphate
Citrate + H+
Malate
OH−
Pyruvate
OH−
Phosphate
ATP-ADP translocase
Dicarboxylate carrier
Tricarboxylate carrier
Pyruvate carrier
Phosphate carrier





Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemiosmotic Type of Mechanism

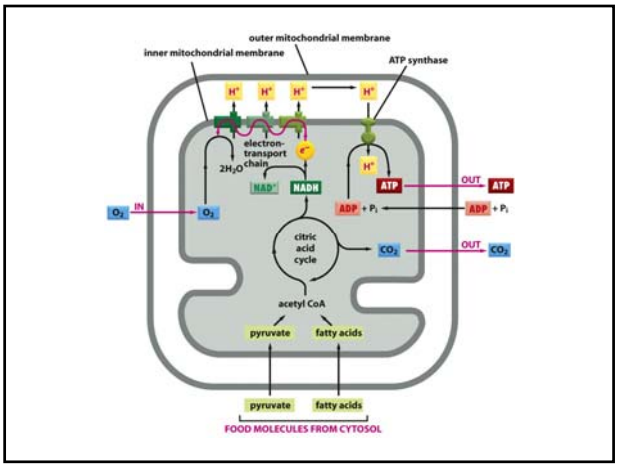
Peter Mitchell, University of Edinburgh, Edinburgh, Scotland

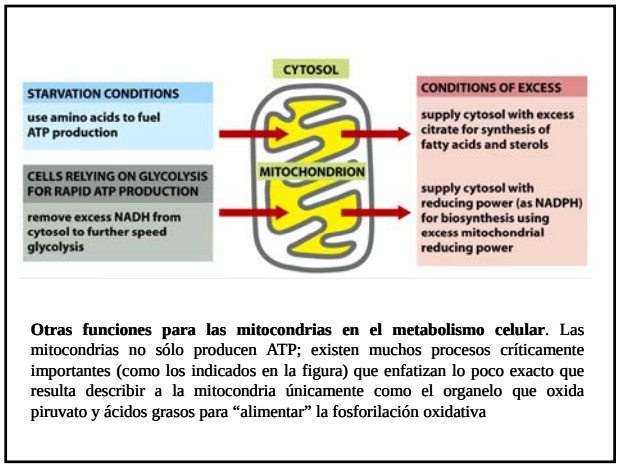
In the exact sciences, cause and effect are no more than events linked in sequence. Biochemists now generally accept the idea that metabolism is the cause of membrane transport. The underlying thesis of the hypothesis put forward here is that if the processes that we call metabolism and transport represent events in a sequence, not only can metabolism be the cause of transport, but also transport can be the cause of metabolism.

Nature, 1961, Volume 191, pages 144-148

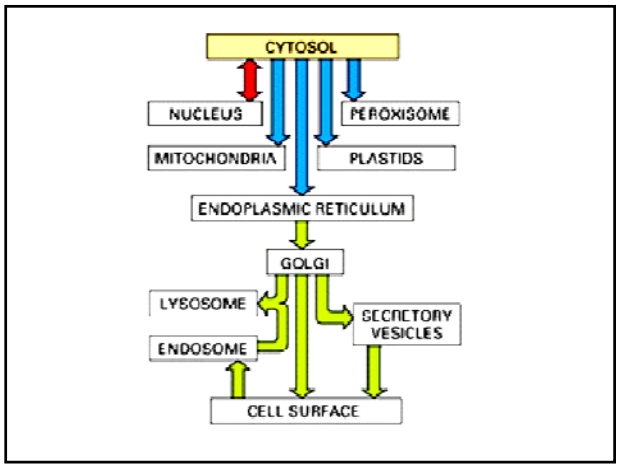
Mitchell's Nobel Prize Lecture, in 1978, began as follows:

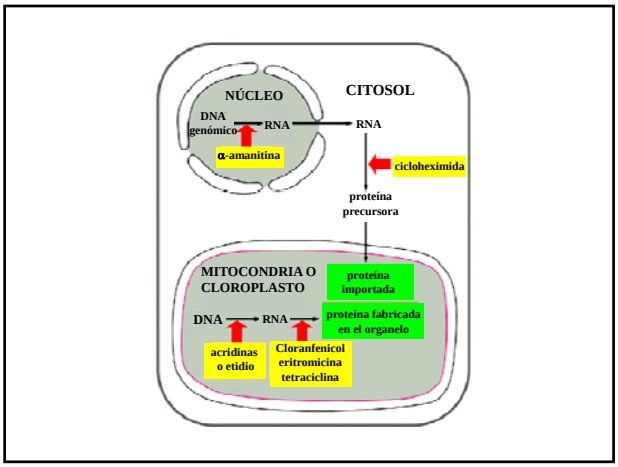
Although I had hoped that the chemiosmotic rationale of vectorial metabolism and biological energy transfer might one day come to be generally accepted, it would have been presumptuous of me to expect it to happen. Was it not Max Planck who remarked that a new scientific idea does not triumph by convincing its opponents, but rather because its opponents eventually die? The fact that what began as the chemiosmotic hypothesis has now been acclaimed as the chemiosmotic theory . . . has therefore both astonished and delighted me, particularly because those who were formerly my most capable opponents are still in the prime of their scientific lives.

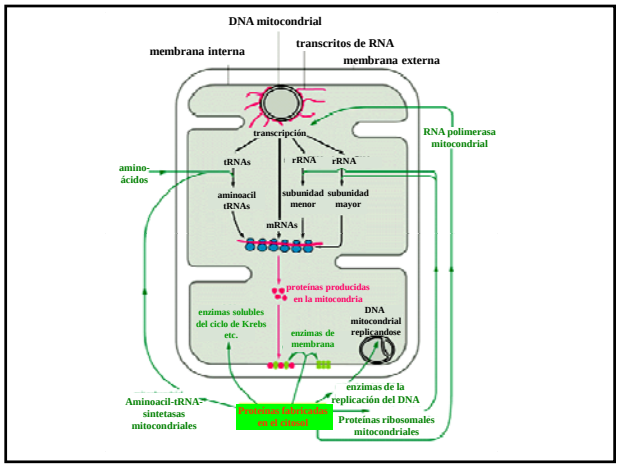




Otras funciones para las mitocondrias en el metabolismo celular. Las mitocondrias no sólo producen ATP; existen muchos procesos críticamente importantes (como los indicados en la figura) que enfatizan lo poco exacto que resulta describir a la mitocondria únicamente como el organelo que oxida piruvato y ácidos grasos para "alimentar" la fosforilación oxidativa

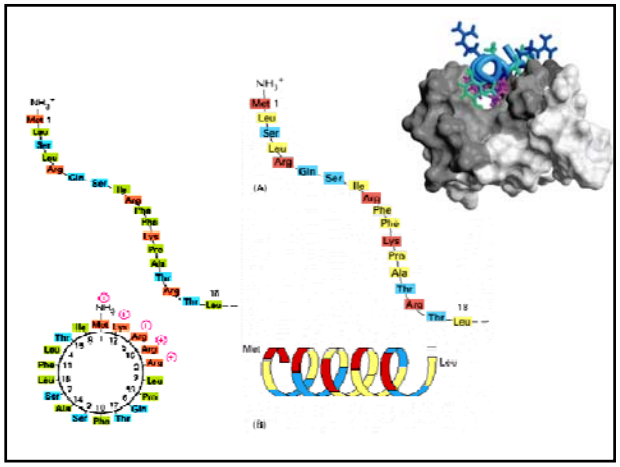


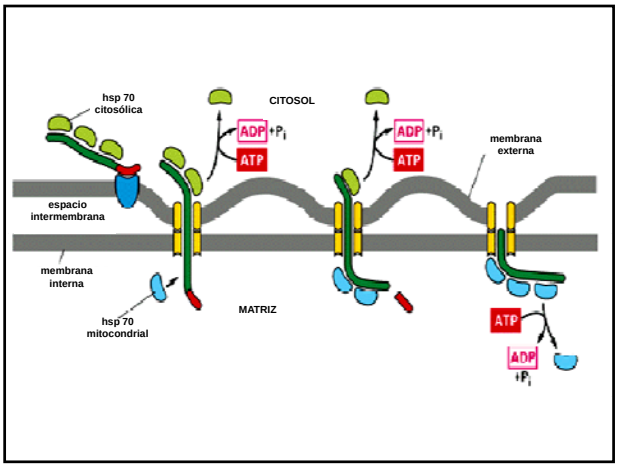


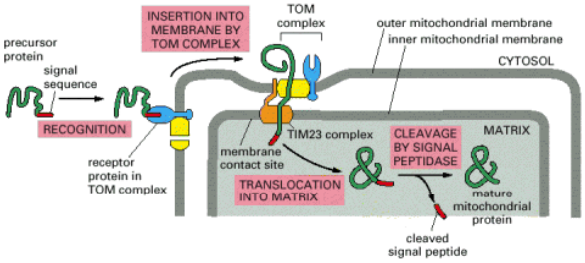


Function of Signal Peptide	Example of Signal Peptide
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln
Retain in lumen of ER	-Lys-Asp-Glu-Leu-COO^{-}
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-
Attach to membranes via the covalent linkage of a myristic acid to the amino terminus	$^{+}H_3N$-Gly-Ser-Ser-Lys-Ser-Lys-Pro-Lys-

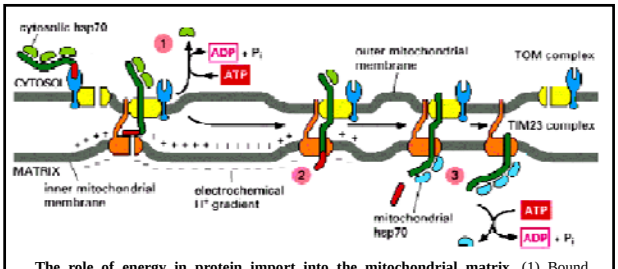
Positively charged amino acids are shown in *red* and negatively charged amino acids in *green*. An extended block of hydrophobic amino acids is enclosed in a *yellow* box. H_3N^{+} indicates the amino terminus of a protein; COO^{-} indicates the carboxyl terminus.



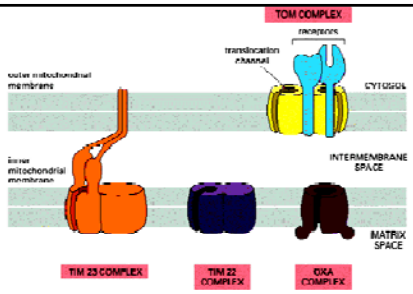




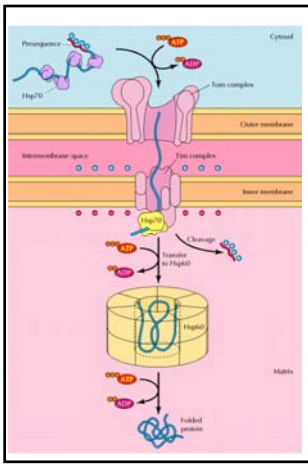
Protein import by mitochondria. The N-terminal signal sequence of the precursor protein is recognized by receptors of the TOM complex. The protein is thought to be translocated across both mitochondrial membranes at or near special contact sites. The signal sequence is cleaved off by a signal peptidase in the matrix to form the mature protein. The free signal sequence is then rapidly degraded (not shown).



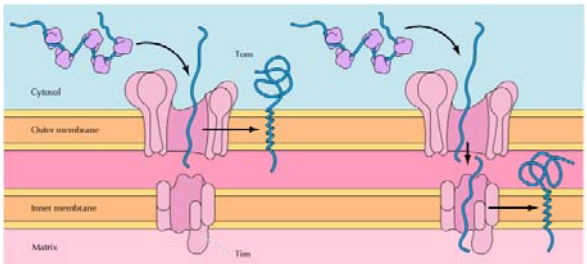
The role of energy in protein import into the mitochondrial matrix. (1) Bound *cytosolic hsp70* is released from the protein in a step that depends on ATP hydrolysis. After initial insertion of the signal sequence and of adjacent portions of the polypeptide chain into the TOM complex, the signal sequence interacts with a TIM complex. (2) The signal sequence is then translocated into the matrix in a process that requires an electrochemical H^+ gradient across the inner membrane, positioning the unfolded polypeptide chain so that it transiently spans both membranes. (3) *Mitochondrial hsp70* binds to regions of the polypeptide chain as they become exposed in the matrix, thereby "pulling" the protein into the matrix. ATP hydrolysis then removes the mitochondrial hsp70, allowing the imported protein to fold.



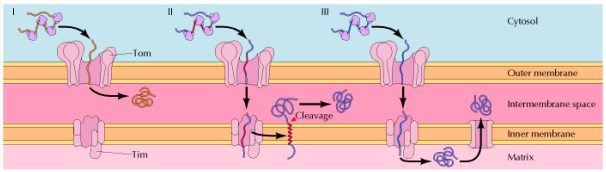
Three protein translocators in the mitochondrial membranes. The TOM and TIM complexes and the OXA complex are multimeric membrane protein assemblies that catalyze protein transport across mitochondrial membranes.. As indicated, one of the core components of the TIM23 complex contains a hydrophobic a-helical extension that is inserted into the outer mitochondrial membrane; the complex is therefore unusual in that it simultaneously spans two membranes



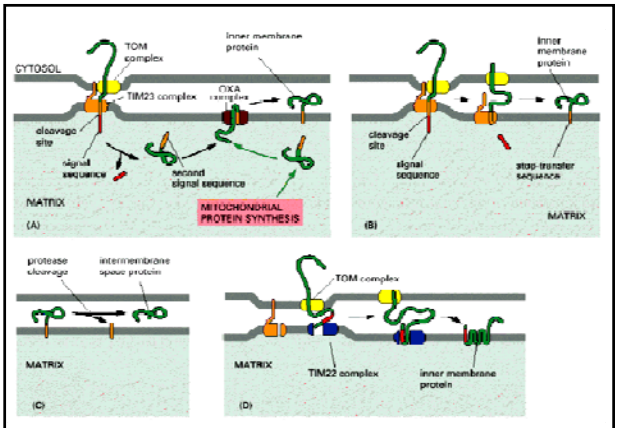
Import of proteins into mitochondria. Proteins are targeted for mitochondria by sequence containing positively charged amino acids. Proteins are maintained in a partially unfolded state by a cytosolic Hsp70 and are recognized by a receptor on the surface of mitochondria. The unfolded polypeptide chains are then translocated through the **Tom** complex in the outer membrane and transferred to the **Tim** complex in the inner membrane. The voltage component of the electrochemical gradient is required for translocation across the inner membrane. The presequence is cleaved by a matrix protease, and a mitochondrial Hsp70 binds the polypeptide chain as it crosses the inner membrane, driving further protein translocation. A mitochondrial Hsp60 then facilitates folding of the imported polypeptide within the matrix.

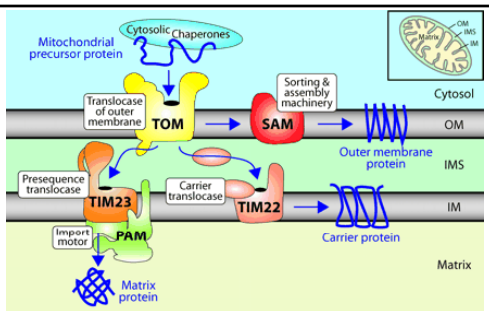


Insertion of mitochondrial membrane proteins. Proteins targeted for the mitochondrial membranes contain hydrophobic stop-transfer sequences that halt their translocation through the Tom or Tim complexes and lead to their incorporation into the outer or inner membranes, respectively.



Sorting proteins to the intermembrane space. Proteins can be targeted to the intermembrane space by several mechanisms. Some proteins (I) are translocated through the Tom complex and released into the intermembrane space. Other proteins (II) are transferred from the Tom complex to the Tim complex, but they contain hydrophobic stop-transfer sequences that halt translocation through the Tim complex. These stop-transfer sequences are then cleaved to release the proteins into the intermembrane space. Still other proteins (III) are imported to the matrix. Removal of the presequence within the matrix then exposes a hydrophobic signal sequence, which targets the protein back across the inner membrane to the intermembrane space.





Protein import pathways into mitochondria. Most mitochondrial proteins are synthesized in the cytosol. With the help of cytosolic chaperones, mitochondrial precursor proteins are transferred to the general entry gate of mitochondria, the TOM complex, from where they are subsequently sorted into one of the mitochondrial sub-compartments. The precursors of β -barrel outer membrane proteins require the SAM complex. Preproteins destined for the matrix depend on the presequence translocase (TIM23 complex) and its associated import motor (PAM complex) for their transport across the inner mitochondrial membrane. Carrier proteins are inserted into the inner membrane with the help of the carrier translocase (TIM22 complex). OM, outer membrane; IMS, intermembrane space; IM, inner membrane. (JBC **279**:14473, 2004)

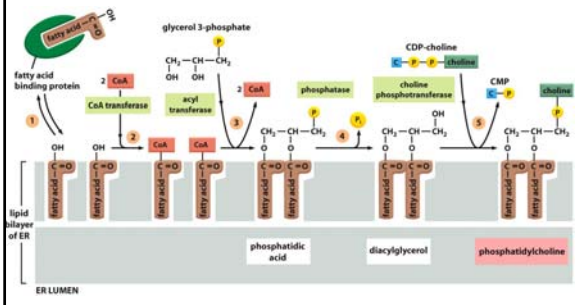
La translocación de proteínas a través de las membranas mitocondriales es mediada por complejos proteicos compuestos por múltiples subunidades que funcionan como translocadores de proteínas

El complejo TOM (Translocase of Outer Membrane) funciona a través de la membrana externa y el complejo TIM (Translocase of Inner Membrane) funciona a través de la membrana interna. Algunos componentes de estos complejos actúan como receptores de las proteínas a importar, y otros forman el canal de translocación

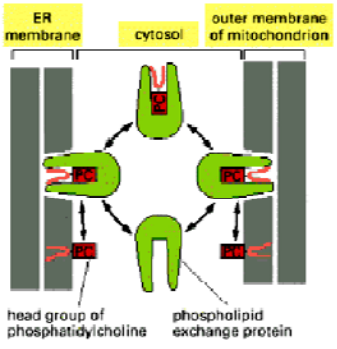
El complejo TOM se requiere para la importación de todas las proteínas codificadas en el genoma nuclear y ayuda a insertar proteínas transmembrana en la membrana externa. El complejo TIM23 transporta proteínas a la matriz mitocondrial y ayuda a insertar proteínas en la membrana interna

El complejo TIM22 media la inserción de una subclase de proteínas de la membrana interna que incluye una proteína que transporta ATP, ADP y fosfato

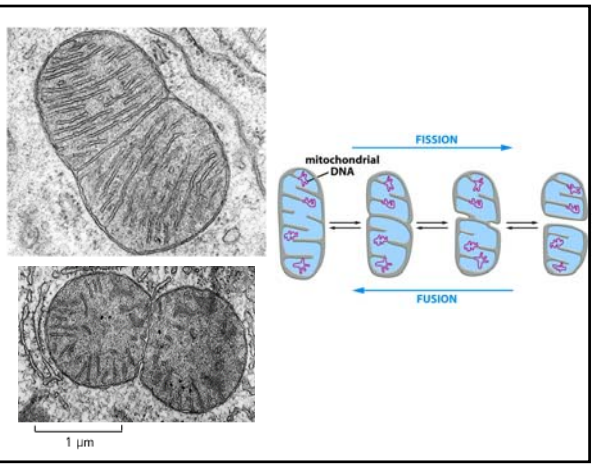
El complejo OXA, ubicado en la membrana interna, media la inserción de proteínas sintetizadas dentro de la mitocondria en la membrana interna. Además ayuda a insertar proteínas que han sido transportadas a la matriz previamente por los complejos TOM y TIM

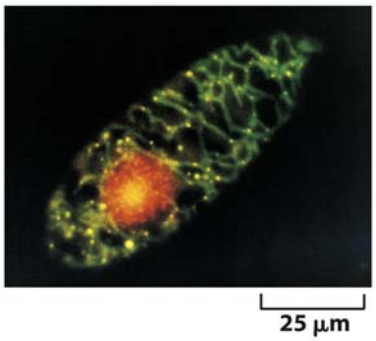


The synthesis of phosphatidylcholine. This phospholipid is synthesized from glycerol 3-phosphate, cytidine-bisphosphocholine (CDP-choline), and factty acids delivered to the ER by fatty acid binding protein.

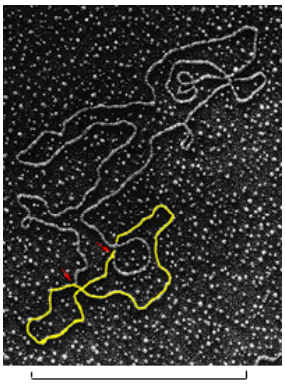


Phospholipid exchange proteins. Because phospholipids are insoluble in water, their passage between membranes requires carrier proteins. Phospholipid exchange proteins are water-soluble proteins that carry a single molecule of phospholipid at a time; they can pick up a lipid molecule from one membrane and release it at another, thereby redistributing phospholipids between membrane-enclosed compartments. The net transfer of phosphatidylcholine (PC) from the ER to mitochondria can occur without the input of additional energy, because the concentration of PC is high in the ER membrane (where it is made) and low in the outer mitochondrial membrane..





Mitochondrial and nuclear DNA stained with a fluorescent dye. The micrograph shows the distribution of the nuclear genome (red) and the multiple small mitochondrial genomes (bright yellow spots) in a *Euglena gracillis* cell



Tamaño del genoma de los organelos

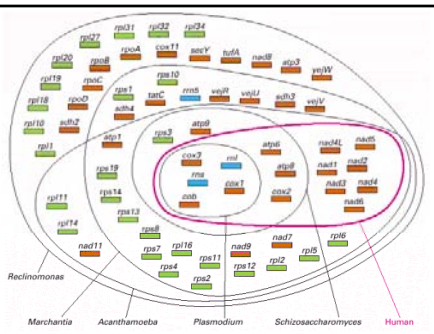
Tipo de DNA	Tamaño (kbp)
DNA de cloroplastos	
Plantas superiores	120-200
Chlamydomonas (alga verde)	180
DNA de mitocondrias	
Animales (gusanos planos,insectos, mamíferos)	16-19
Plantas superiores	150-2500
Hongos	
Schizosaccharomyces pombe	17
Aspergillus nidulans	32
Neurospora crassa	60
Saccharomyces cerevisiae	78
Chlamydomonas (alga verde)*	16
Protozoos	
Trypanosoma brucei	22
*Paramecium**	40

* moléculas lineales

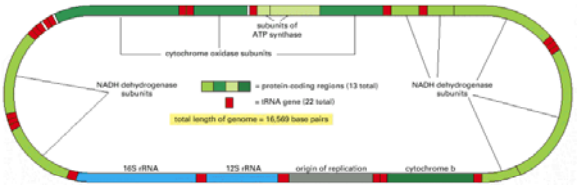
Cantidades relativas de DNA de organelos en algunos tejidos y tipos celulares

Organismo	Tejido o tipo celular	Moléculas de DNA por organelo	Número de organelos por célula	DNA del organelo como % del DNA total
DNA de mitocondrias				
Rata	hígado	5-10	1000	1
Levadura*	vegetativa	2-50	1-50	15
Xenopus laevis	oocito	5-10	10^7	99
DNA de cloroplastos				
Chlamydomonas	vegetativa	80	1	7
Maíz	hojas	20-40	20-40	15

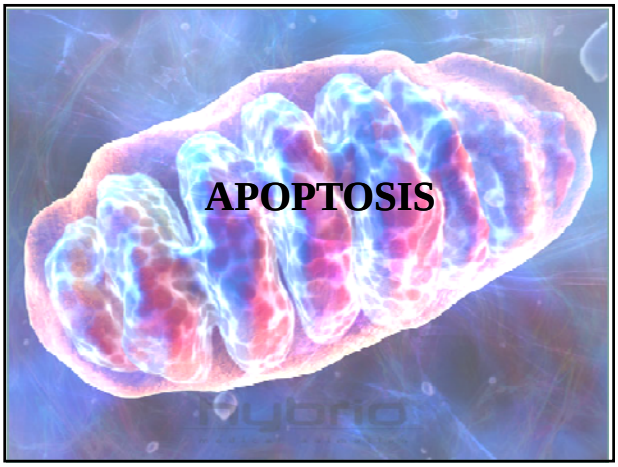
* la gran variación en el número y tamaño se debe a fragmentación y fusión mitocondrial

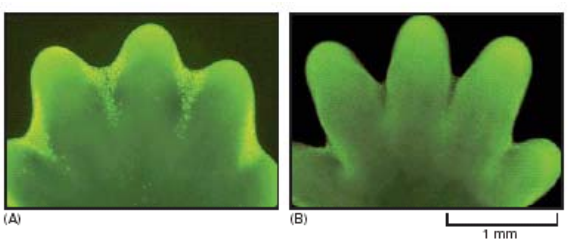


Comparison of mitochondrial genomes. Less complex mitochondrial genomes encode subsets of the proteins and ribosomal RNAs that are encoded by larger mitochondrial genomes. The five genes present in all known mitochondrial genomes encode ribosomal RNAs *(rns* and *rnl)*, cytochrome *b (cob)*, and two cytochrome oxidase subunits *(cox1* and *cox3)*. (Adapted from M.W. Gray et al., *Science* 283:1476–1481, 1999.)



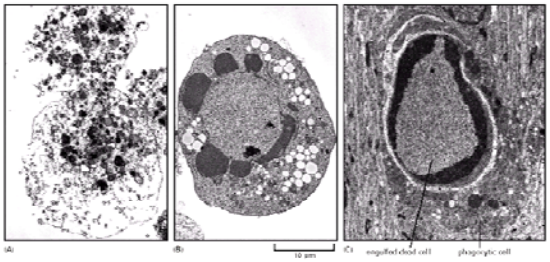
The organization of the human mitochondrial genome. The genome contains 2 rRNA genes, 22 tRNA genes, and 13 protein-coding sequences. The DNAs of many other animal mitochondrial genomes have also been completely sequenced. Most of these animal mitochondrial DNAs encode precisely the same genes as humans, with the gene order being identical for animals that range from mammals to fish



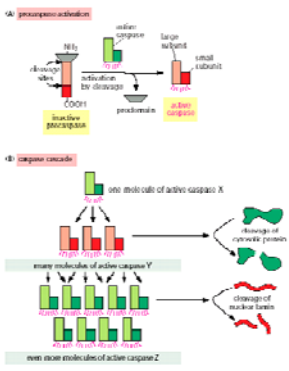


Sculpting the digits in the developing mouse paw by apoptosis. (A) The paw in this mouse embryo has been stained with a dye that specifically labels cells that have undergone apoptosis.The apoptotic cells appear as *bright green* dots between the developing digits. (B) This interdigital cell death eliminates the tissue between the developing digits, as seen one day later, when few, if any, apoptotic cells can be seen. (From W.Wood et al., *Development* 127:5245–5252, 2000. © The Company of Biologists.)

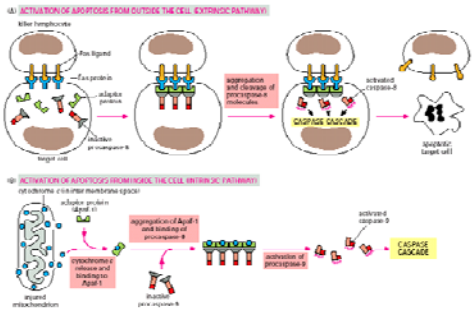
Apoptosis during the metamorphosis of a tadpole into a frog. As a tadpole changes into a frog, the cells in the tadpole tail are induced to undergo apoptosis; as a consequence, the tail is lost. All the changes that occur during metamorphosis, including the induction of apoptosis in the tail, are stimulated by an increase in thyroid hormone in the blood.



Cell death. These electron micrographs show cells that have died by (A) necrosis or (B and C) apoptosis.The cells in (A) and (B) died in a culture dish, whereas the cell in (C) died in a developing tissue and has been engulfed by a neighboring cell. Note that the cell in (A) seems to have exploded, whereas those in (B) and (C) have condensed but seem relatively intact. The large vacuoles visible in the cytoplasm of the cell in (B) are a variable feature of apoptosis.

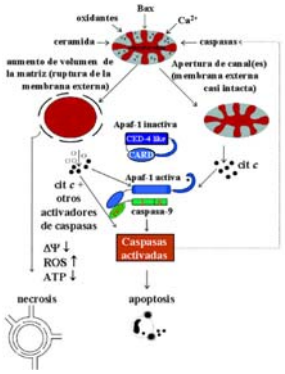


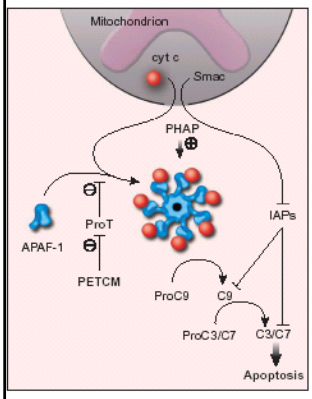
The caspase cascade involved in apoptosis. (A) Each suicide protease is made as an inactive proenzyme (procaspase), which is usually activated by proteolytic cleavage by another member of the caspase family. As indicated, two of the cleaved fragments associate to form the active site of the caspase.The active enzyme is thought to be a tetramer of two of these units (not shown). (B) Each activated caspase molecule can cleave many procaspase molecules, thereby activating them, and these can then activate even more procaspase molecules. In this way, an initial activation of a small number of procaspase molecules (called initiator caspases) can lead, via an amplifying chain reaction (a cascade), to the explosive activation of a large number of procaspase molecules. Some of the activated caspases (called effector caspases) then cleave a number of key proteins in the cell, including specific cytosolic proteins and nuclear lamins, leading to the controlled death of the cell.



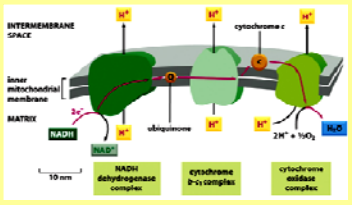
Induction of apoptosis by either extracellular or intracellular stimuli. (A) Extracellular activation. A killer lymphocyte carrying the Fas ligand binds and activates Fas proteins on the surface of the target cell. Adaptor proteins bind to the intracellular region of aggregated Fas proteins, causing the aggregation of procaspase-8 molecules. These then cleave one another to initiate the caspase cascade. (B) Intracellular activation. Mitochondria release cytochrome *c*, which binds to and causes the aggregation of the adaptor protein Apaf-1. Apaf-1 binds and aggregates procaspase-9 molecules, which leads to the cleavage of these molecules and the triggering of a caspase cascade. Other proteins that contribute to apoptosis are also released from the mitochondrial intermembrane space (not shown).

Señales que gatillan muerte celular programada





Controlling the caspase cascade. Formation of the heptameric "apoptosome" is a crucial step in the initiation of apoptosis. Selective mitochondrial proteins such as cytochrome c (cyt c) (red) are released from mitochondrial intermembrane space in response to apoptotic stimuli. Cytochrome c induces the assembly of Apaf-1 monomers (blue) into apoptosome, which recruits and activates caspase- 9 (C9), which in turn activates caspase-3 (C3) and -7 (C7). IAP proteins inhibit rogue caspases, but their inhibitory actions are blocked by antagonists (such as Smac/Diablo and Omi/HtrA2) that are coreleased with cytochrome c. Two additional tiers of apoptosome regulation have been identified (5): the oncoprotein proT, which retards assembly of the apoptosome complex, and tumor suppressor PHAP proteins, which stimulate the apoptosome's deadly activities. (Science **229**:214, 2003)

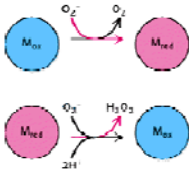


1 in every 2000 electrons goes directly from NADH to oxygen which results in production of superoxide anion

$$O_2 \xrightarrow{e^-} O_2^{\bullet -} \xrightarrow{e^-} O_2^{2-}$$

Anión superóxido — Peróxido

$$2O_2^{\cdot-} + 2H^+ \xrightarrow{\textit{Superoxide Dismutase}\ \textbf{(SOD)}} O_2 + H_2O_2$$



Superoxide Dismutase Mechanism. The oxidized form of superoxide dismutase (Mox) reacts with one superoxide ion to form O2 and generate the reduced form of the enzyme (Mred). The reduced form then reacts with a second superoxide and two protons to form hydrogen peroxide and regenerate the oxidized form of the enzyme.

Dismutation A reaction in which a single reactant is converted into two different products.

$$\mathbf{H_2O_2 + H_2O_2} \xrightarrow{\textit{Catalase}} \mathbf{O_2 + 2H_2O}$$

$$\mathbf{H_2O_2 + 2GSH} \xrightarrow{\textit{Glutathione peroxidase}} \mathbf{GSSG + 2H_2O}$$