Scale in the biological world

- **Atoms**: 0.2 nm
  - Minimum resolvable by electron microscope
- **Molecules**: 20 nm
  - Minimum resolvable by light microscope
- **Organelles**: 2 μm
  - Minimum resolvable by unaided eye
- **Cells**: 0.2 mm (200 μm)

1 μm = 10³ nm
= 10⁵ μm
= 10⁶ nm
A cell seen by TEM
From living cells to atoms
Compartmentalisation in the cell: internal membranes and the cytosol

(A) mitochrondion

(B) cytosol

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The Origin of mitochondria:
The endosymbion hypothesis
The cytosol: more than just H2O

Proteins

Ribosomes

RNAs
Living cells obey the laws of thermodynamics

Living cells are NOT isolated systems:

Cells take energy from the environment (chemicals ie foodstuffs or photons ie sunlight) to generate order ie assemblies

In doing so, they discharge HEAT to the environment

This increases the total ENTROPY

Energy conversion is vital for the cell

Mitochondria biogenesis and function
The Eukaryotic Cell

- Microvillus
- Microfilament
- Centriole
- Nucleus
- Ribosomes
- Smooth endoplasmic reticulum
- Mitochondrion
- Rough endoplasmic reticulum
- Golgi apparatus
- Plasma membrane
- Lysosome
Different ways to target proteins in the cell

Diagram showing the different ways proteins can be targeted within a cell, including pathways through the cytosol, nucleus, peroxisome, mitochondria, endoplasmic reticulum, Golgi, lysosome, endosome, secretory vesicles, and the cell surface. The diagram includes arrows indicating the flow of protein targeting, with a key explaining the color coding: red for gated transport, blue for transmembrane transport, and green for vesicular transport.
Mitochondria are essential for life
Energy Conversion: Mitochondria and Chloroplasts

- Membrane-bounded
- Occupy a major fraction of cell volume
- Large amount of internal membrane
- Common pathway for energy conversion: Chemiosmotic coupling
Chemiosmotic coupling
The Mitochondrion

- **1. Substantial portion of cell volume**
  - About 20% of the volume of a eukaryotic cell
  - Mitochondrial IM is 1/3 of total cell membrane

- **2. Mitochondrial function supplies 30**
  - ATP molecules (only 2 ATP from anaerobic (cytosolic) glycolysis

- **3. Mobile, shape-changing, fusion/separation**
EM view of a Mitochondrion

3D Reconstruction

mobile, shape-changing
Large GTPases control mitochondrial

fusion (Fzo1, OM)

fission (Dnm1, OM)

and

Inner membrane remodelling (Mgm1, IMS)
Mitochondrial Structure

- **Outer Membrane (semipermeable)**
  - 6% of total mit.protein
  - lipid metabolism enzymes, porin

- **IMS**
  - 6% of total mit.protein
  - enzymes that use ATP to phosphorylate other nucleotides

- **Inner Membrane (impermeable)**
  - 21% of total mit.protein
  - ATP synthase, respiratory chain enzymes, transport proteins

- **Matrix**
  - 67% of total mit.protein
  - Hundreds of enzymes, DNA, ribosomes, tRNAs
The Electrochemical Proton Gradient

\[ \text{140 mV} \]

\[ \text{60 mV (-1 pH unit)} \]

\[ \text{TOTAL} \]

\[ \text{200 mV} \]

Active transport processes are driven by the electrochemical proton gradient.
The Respiratory chain consists of 3 large membrane-embedded enzyme complexes.
ATP Synthase is a reversible coupling device: It interconverts the energies of the electrochemical proton gradient and chemical bonds.
**Functional Complexity**

- Respiration and ATP Synthesis
- Synthesis of heme, lipids, amino acids and nucleotides
- Intracellular homeostasis of inorganic ions

**Structural Complexity**

- 5-15% of total cell protein
- 20% volume of eukaryotic cell
- IM is 1/3 of total cell membrane
- About 1000 different polypeptides (600 in yeast)
- Only a dozen encoded by mtDNA
Protein import is the major mechanism of mitochondria biogenesis
Identification of components of the Mitochondrial
Protein Import System

- Genetic analyses (fungal genetics)
- In vitro import assay system with isolated functional mitochondria
  - Chemical Crosslinking
  - Biochemical reconstitution
IMPORT INTO YEAST MITOCHONDRIA

Proteins with N-terminal cleavable presequences:
Matrix proteins

Proteins with internal targeting signals:
AAC

OM

TOM

IMS

IM

Matrix

TIM23

TIM10

Hsp70

Mge1

TIM22

ΔΨ+
PROTEIN WITH PRESEQUENCE

OM

IMS

IM

Matrix

Hsp70

Hsp70

TIM23

TIM10

TOM

PROTEIN WITH INTERNAL SIGNALS

e.g. AAC

TIM22

MPP
Import into the matrix - 1

- Depends on a matrix-targeting signal: The presequence
  - Cleavable, usually located at the N-terminus
  - usually 12-15 residues long
  - amphiphilic, with positively charged residues on one side of an α-helix
Presequence binding to Tom20

Endo and Kohda
Import into the matrix - 2

- This is a multistep process
- Interactions with chaperones in the cytosol keep the precursor in an unfolded conformation (“import-competent”)
- Different import complexes in the OM (TOM complex) and the IM (TIM complex).
- Electrostatic interactions between the positive presequence and negative patches of receptors along the import pathway: The Acid chain hypothesis- Gradation of affinities leads the presequence along the import pathway
- The electrophoretic function of the potential across the IM draws the precursor across the IM
- The pulling force of the translocation motor mHsp70/Tim44 actively draws the precursor to complete translocation
Import into the matrix - 3

- Energy requirements:
  - **ATP Hydrolysis**
    - In the cytosol (function of ATPase chaperones)
    - In the mitochondrial matrix (Hsp70 translocation motor)
  - **Electrochemical potential across the inner membrane**
Components

**TOM Complex:**
- Receptors: Tom70, Tom20, Tom37, Tom22
- Channel-forming: Tom40, Tom5
- Channel modulating: Tom6, Tom7

**TIM Complex:**
- Receptor: Tim23
- Channel-forming: Tim23, Tim17
- Translocation motor: Tim44, Hsp70, GrpE (co-chaperone)
Summary of Protein Import into the Mitochondrial Matrix

Import into the IMS

A. Variation of the matrix targeting pathway
   - example: cytochrome b2

B. Distinct pathway involving a specific IMS targeting signal
   - example: mitochondrial heme lyases
The Signal:

- Bipartite nature: matrix targeting signal followed by an IMS sorting signal
- IMS sorting signal contains mainly uncharged residues
- Cleaved by specific IMS protease
- NOT very highly conserved
Energetics:
- Electrochemical potential absolutely essential but ATP hydrolysis NOT required

Components:
- Known Subunits of the TOM complex
- TIM23 complex
- IMS sorting peptidase

Mechanism:
Stop-transfer mechanism: the hydrophobic sorting signal is stuck at the TIM23 complex and laterally diffuses out into the lipid bilayer of the IM
Import into the IMS

- A. Variation of the matrix targeting pathway
  - example: cytochrome b2

- B. Distinct pathway involving a specific IMS targeting signal
  - example: mitochondrial heme lyases
**Heme Lyase IMS targeting - 1**

**The Signal:**

- Internal
- about 60 residues long
- highly conserved sequence
- highly hydrophilic (30% charged residues, similar number of + and - charged residues, distributed throughout the sequence)
- mainly a-helical, but NOT amphiphilic

The Mitochondrial Carrier Family

- Function as metabolite transporters

- 37 proteins in yeast
  (10-15% of the total mitochondrial protein)

- 30-35 kDa

- Common topology: 3 similar repeated motifs
  (3x2 helix model)