MITOCHONDRIA: more than energy production.
FUNCTIONS OF MITOCHONDRIA

- Oxidative phosphorylation
- Fatty acid oxidation
- Ca2+ homeostasis
- Urea cycle
- Steroid synthesis
- APOPTOSIS
- Krebs cycle
- And more
STRUCTURE: the mitochondrion is not the bean shaped organelle often pictured in textbooks.
Mitochondria
An animal cell
Mitochondria Structural Features

- Inner Membrane
- Outer Membrane
- Cristae
- Matrix

Figure 1
As shown by 3D tomographic EM studies, there is a discrete inner membrane and the cristae are not invaginations of this but distinct compartments linked to the inner membrane by narrow connections or pores.
The internal structure of mitochondria [Review]
Terrence G. Frey and Carmen A. Mannella
Trends in Biochemical Sciences, 2000, 25:7:319-324

3D reconstructed electrontomogram of mitochondria
Dr. Terry Frey; San Diego State University
MITOCHONDRIA ARE HIGHLY PLEOTROPIC

Change shape
Numbers
Composition
The mitochondrial mass can exist as a continuum or reticulum or as in a fragmented form. The switching Between the 2 forms i.e. fission and fusion involves sets of different proteins and is triggered by cell cycle. Fragmentation is an early and required step in Apoptosis.
3) 3D view of reticulum

143B osteosarcoma
GFP-pH transfected
Confocal microscope
3D projection
2) Different states (fragmented, intermediate, reticulum)

- MitoTracker Red staining
- osteosarcoma cells (143B)
- Osteosarcoma cells were arrested in G0 by FCS starvation for 60-72 h
- Released by FCS addition
- Stained with MitoTracker Red

Cell cycle dependent changes of mitochondrial morphology
FISSION AND FUSION IS ESSENTIAL FOR CELL SURVIVAL. It is orchestrated by special proteins several of which are altered in neurological diseases.
The mitochondrion (red) intercalates in and has specific connections with the ER (green).
THE BUILDING BLOCKS

MITOCHONDRIA ARE MADE FROM 2 GENOMES
mtDNA and nuclear genes

mtDNA is bacteria-like as is mt protein synthesis
Fluorescence In Situ Hybridization (FISH)

- FISH probes: amino-allyl dUTP conjugated to Alexa Fluor dyes

COX I - AF488
MitoTracker Red
merged image

A  B  C
mtDNA content

The number of copies of mtDNA varies by species, by tissue and by metabolic factors 100-5000 per cell.

Not all copies are the same based on genetic variations and accrued mutations = HETEROPLASMY
Heteroplasmy is the presence of multiple forms of mtDNA with small polymorphic variations in the sequence of open reading frames for tRNAs or the OXPHOS proteins. There are distinct differences in mtDNA sequence between different human populations called haplogroups. There is good evidence that different haplogroups respond differently to some drugs because of subtle changes in OXPHOS enzymes.
Distribution of mtDNA in fragmented mitochondria

Each mitochondrion contains mtDNA
Inborn Errors of Metabolism and Incidence

(1:8,000) Mitochondrial disorders; (e.g., mitochondrial DNA depletion syndromes)

(1:10,000) Fatty acid oxidation disorders inc. medium-chain acyl-CoA dehydrogenase deficiency (1:20,000)

(1:15,000) Phenylketonuria

(1:15,000) Methylmalonicaciduria. Methylmalonyl-CoA mutase, cobalamin metabolism

(1:40,000) Aminoacidopathies

(1:50,000) Peroxisomal disorders; e.g., Zellweger syndrome, neonatal adrenoleukodystrophy, Refsum's disease)

(1:150,000) Maple syrup urine disease (BCOAD)
MATERNAL INHERITANCE

*Colors reflect inheritance of the same mitochondrial genome*
MITOCHONDRIAL PROTEOME

Best estimate 2000 polypeptides.
13 made in mitochondrion; rest nuclear.
Mitochondrial Proteome

- OXPHOS: 17%
- Transport: 9%
- Signaling: 12%
- Cell Death/Defense: 4%
- DNA/RNA/Protein Synthesis: 11%
- Structural: 7%
- TCA Cycle: 5%
- Glycolysis: 4%
- Carbohydrate Metabolism: 2%
- Amino Acid Metabolism: 10%
- Lipid Metabolism: 1%
- Nucleotide Metabolism: 1%
- Redox: 6%
- Protease: 3%
- Protein Targeting: 7%
PROTEIN DISTRIBUTION IS RELATIVELY FIXED TO OPTIMIZE METABOLIC PATHWAYS AND PROCESSES
Distribution of GFP-E1α and RFP in co-transfected cells

A

B

C

D

GFP-E1α

Free - RFP

Merged image

24 hours post transfection
• THE PROTEIN COMPOSITION OF THE MITOCHONDRIUM IS NOT FIXED.

• THERE IS MOVEMENT OF PROTEINS ONTO, IN AND OUT OF THE ORGANELLE IN SEVERAL FUNCTIONS
In apoptosis there is movement of proteins such as Bax, BCl2 and other proapoptotic factors onto mitochondria. In response the organelle releases cytochrome c, SMAC and other proteins into the cytosol and into the nucleus.

During normal cellular events there are movements of kinases and transcription factors into and out of the organelle to co-ordinate nuclear and mitochondrial biogenesis
• FOCUS ON THE ROLE OF MITOCHONDRIA IN INTERMEDIARY METABOLISM.

• THE KEY ROLE OF ENERGY PRODUCTION
The high resolution structure of cytochrome c oxidase shows a complex of 13 different polypeptides some spanning the lipid bilayer, others located on the cytosol side where substrate cytochrome c binds. Segments that are within the bilayer are alpha helical. Hemes and copper prosthetic groups form an electron wire carrying electron to the oxygen binding site for reduction of O2 to H2O.
COX Model

gold=$\beta$-sheet

green=$\alpha$-helix
• INTERMEDIARY METABOLISM IS A HIGHLY INTEGRATED

• HIGHLY REGULATED

• MITOCHONDRIA-CENTRIC

• CELLULAR PROCESS.
It is important to consider mitochondrial OXPHOS in the context of overall glucose metabolism. As all metabolic pathways are interlinked by the levels of their intermediates that are used in multiple reactions.
SHORT TERM REGULATION OR METABOLISM

PHOSPHORYLATION
ACETYLATION
UBIQUITINYLATION
Kinases and phosphatases provide integrated regulation of multiple pathways as in the control of the utilization of glucose based on the cellular ATP/ADP ratio as measured by AMP Kinase. When ATP levels are low, phosphorylation of key enzymes in anabolic pathways are down regulated and those in catabolic pathways are upregulated.
Short term Cellular Control of Energy Metabolism

G6Pi → Creatine Pi

Glycogen Synthesis
- glycogen synthase

Sterol Synthesis
- HMG-coA reductase

Triacylglycerol Synthesis
- GPAT

pyruvate → Acetyl-CoA

- Acetyl-CoA carboxylase

- Fatty Acid Synthesis

acetyl CoA → ATP

- AMP-activated Kinase
- creatine kinase

Fatty Acid Oxidation

ATP
LONG TERM REGULATION

Transcription factors
Transcription factor activators
Transcription factor activator activators
(sirtuins!)
AMP kinase

PGC 1 $\alpha(\beta)$
AMP kinase

PGC 1 $\alpha(\beta)$

- NRF 1 & 2
  - Tfam
    - mt DNA replication
  - nuclear mt genes

- ERR $\alpha$
  - Oxidative stress
    - SOD, glutathione

- PPARs
  - Fatty acid oxidation

- FOXO
  - gluconeogenesis

- GR
  - HNF4 $\alpha$
  - gluconeogenesis

PPARs
Type II Diabetes

AMP kinase

PGC 1 $\alpha$($\beta$)

NRF 1 & 2

Tfam

mt DNA replication

ERR $\alpha$

nuclear mt genes

Fatty acid oxidation

PPARs

GR

FOXO

HNF4 $\alpha$

gluconeogenesis

Oxidative stress

SOD, glutathione

mt DNA replication

metformin

thiazolidinediones

Type II Diabetes

PPARs

Fatty acid oxidation

GR

FOXO

HNF4 $\alpha$

gluconeogenesis

Oxidative stress

SOD, glutathione

mt DNA replication
NOT SUPRISINGLY, THERE IS TISSUE VARIATION IN METABOLISM.

Expression of whole pathways are different between tissues e.g. urea cycle and gluconeogenesis are liver specific. Actin and myosin are muscle (inc. heart) specific.

Even pathways in common can be controlled differently through the presence of different isoforms of the same protein with different specificity, rate of turnover. Particularly true of substrate carriers and hormone receptors etc.