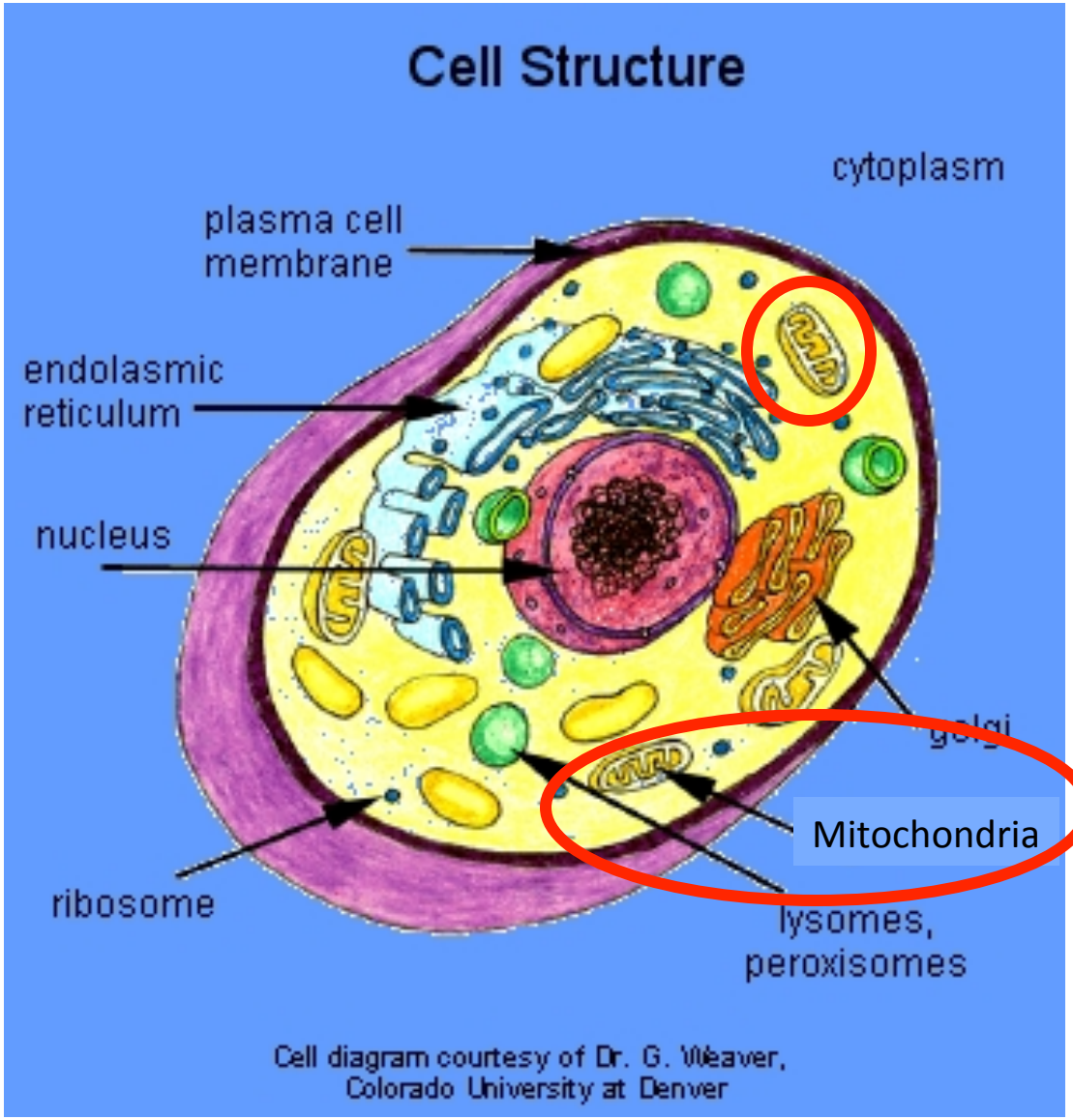
MitoSciences®

MITOCHONDRIA:more than energy production.

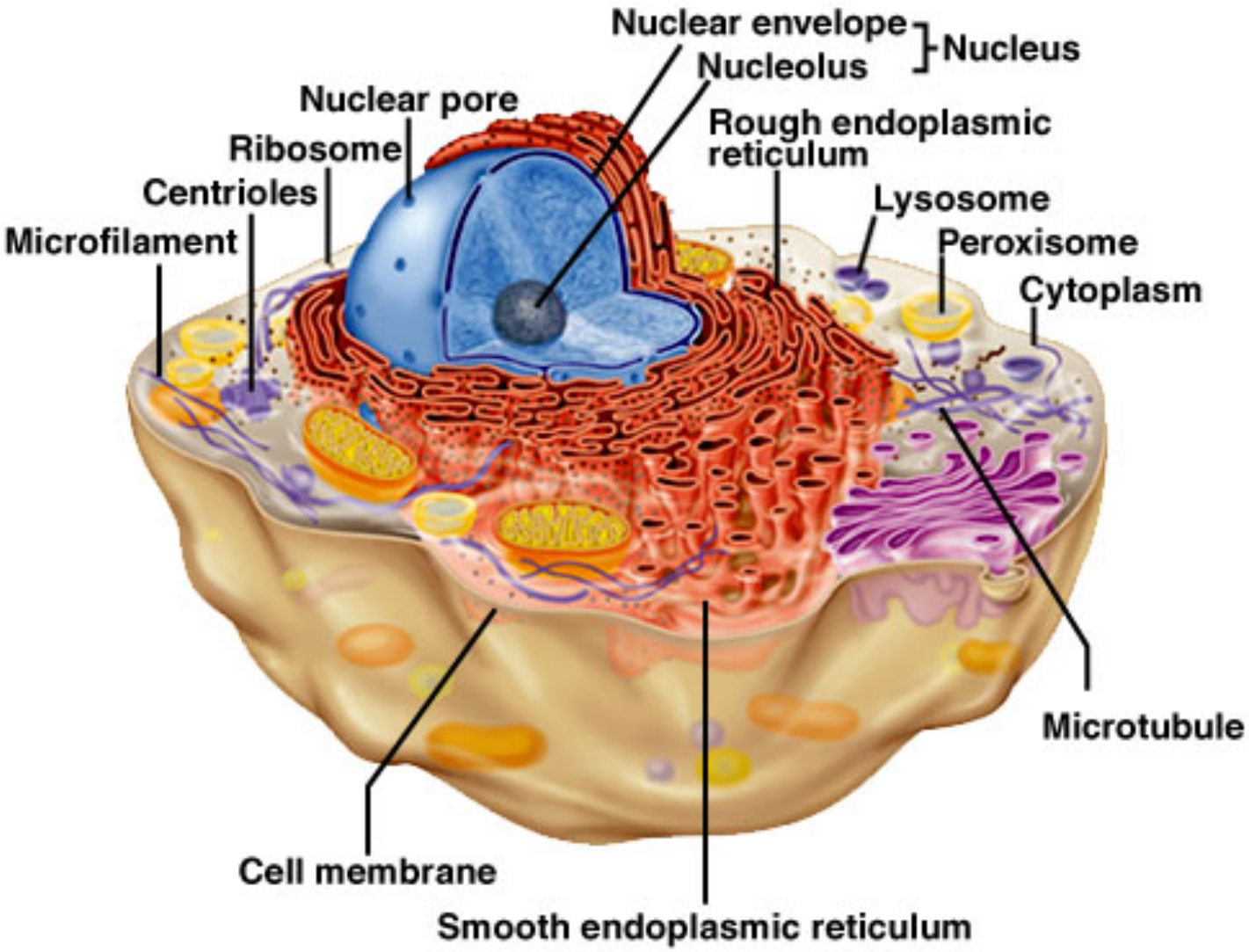
FUNCTIONS OF MITOCHONDRIA

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 text books


Cell Structure
cytoplasm
plasma cell membrane
endolasmic reticulum
nucleus
ribosome
lysomes, peroxisomes
Mitochondria
Cell diagram courtesy of Dr. G. Weaver, Colorado University at Denver

An animal cell



Mitochondria Structural Features

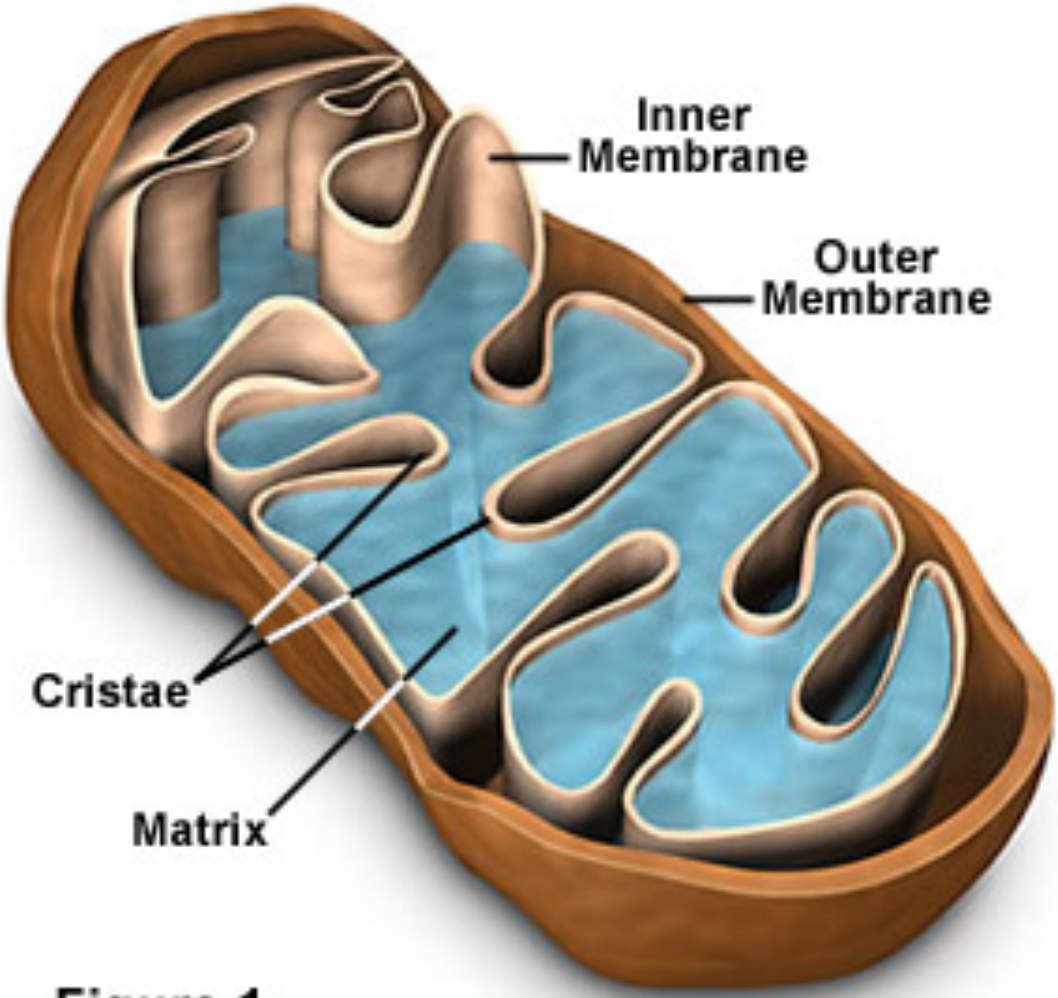
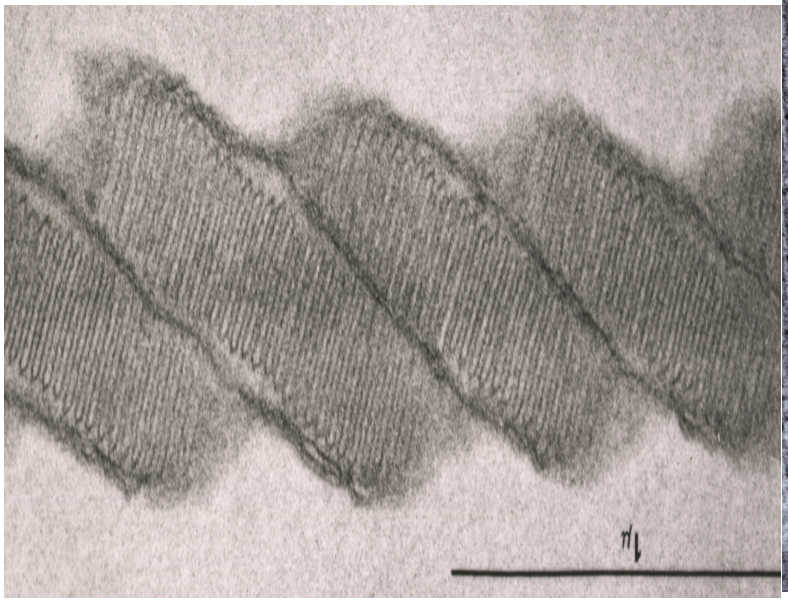
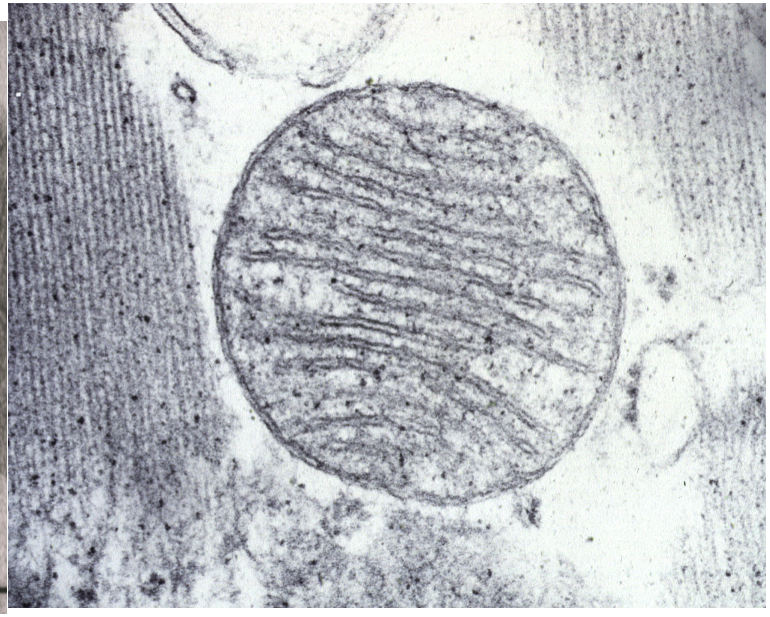
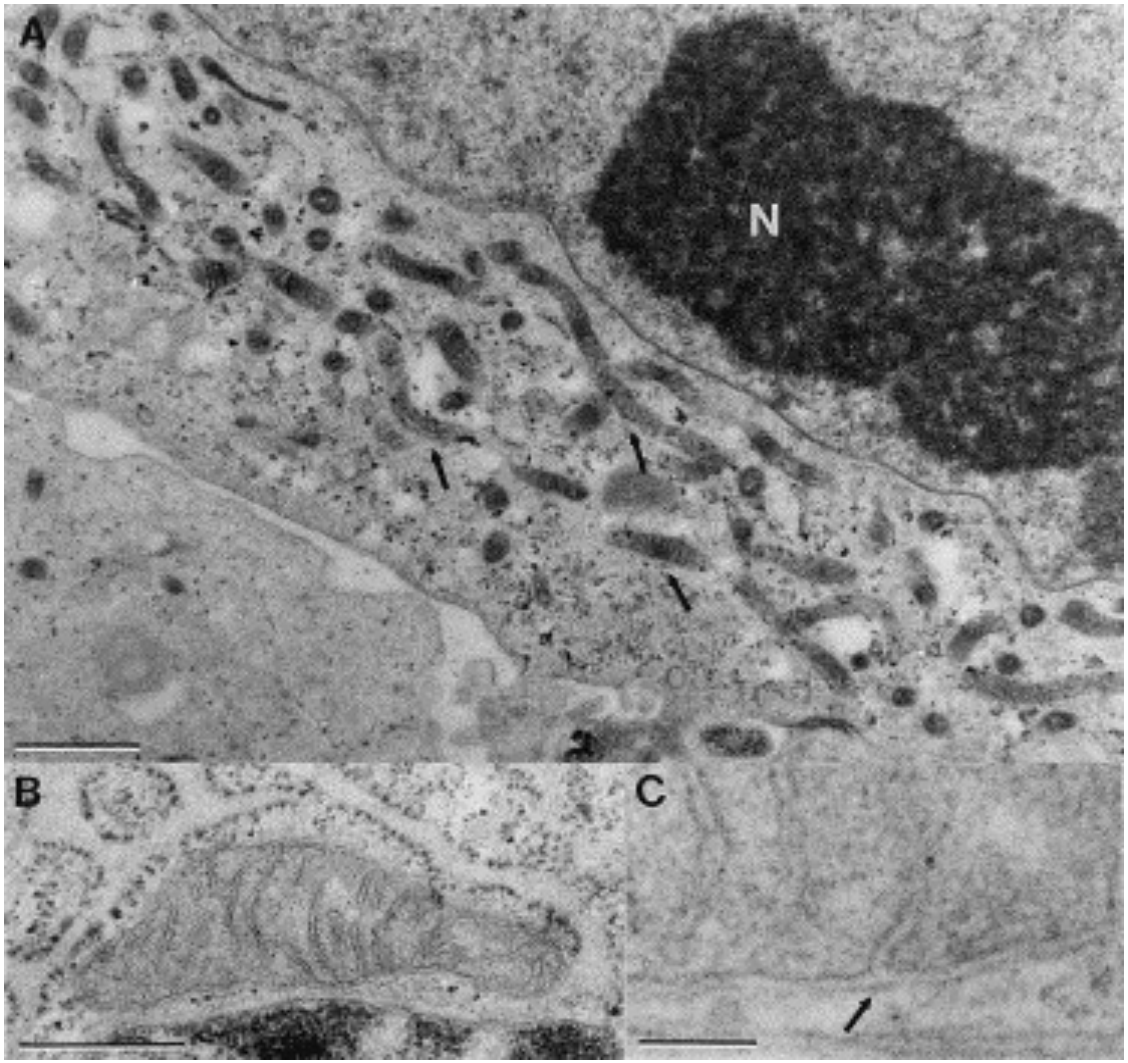


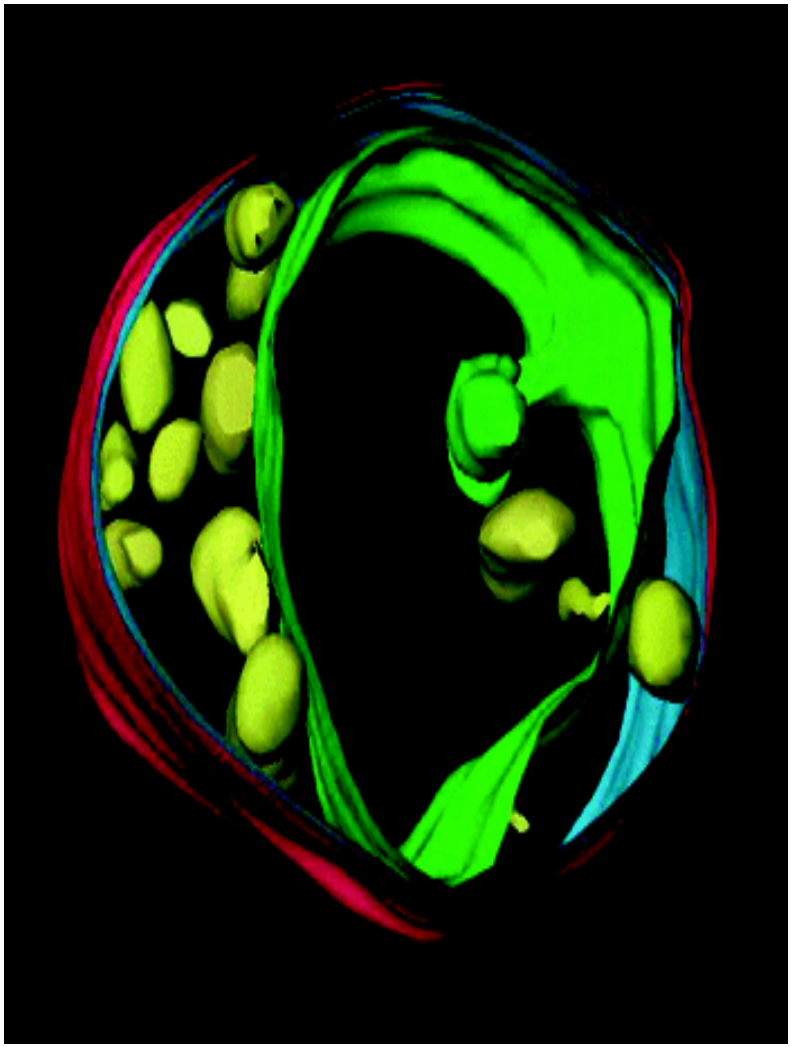
Figure 1

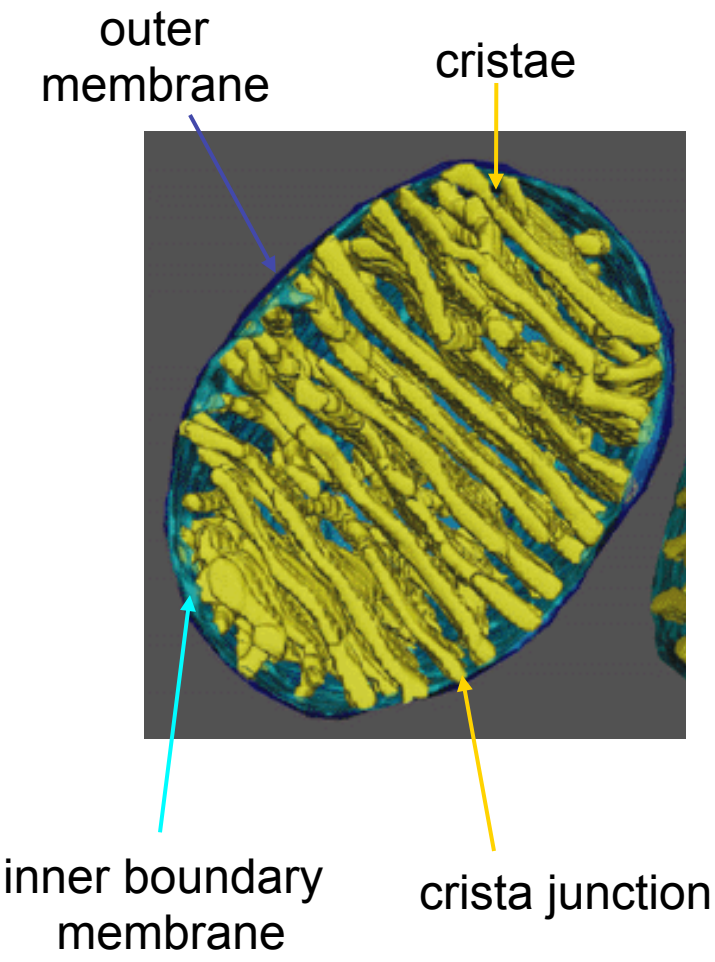

1μ




A
N
B
C

- 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

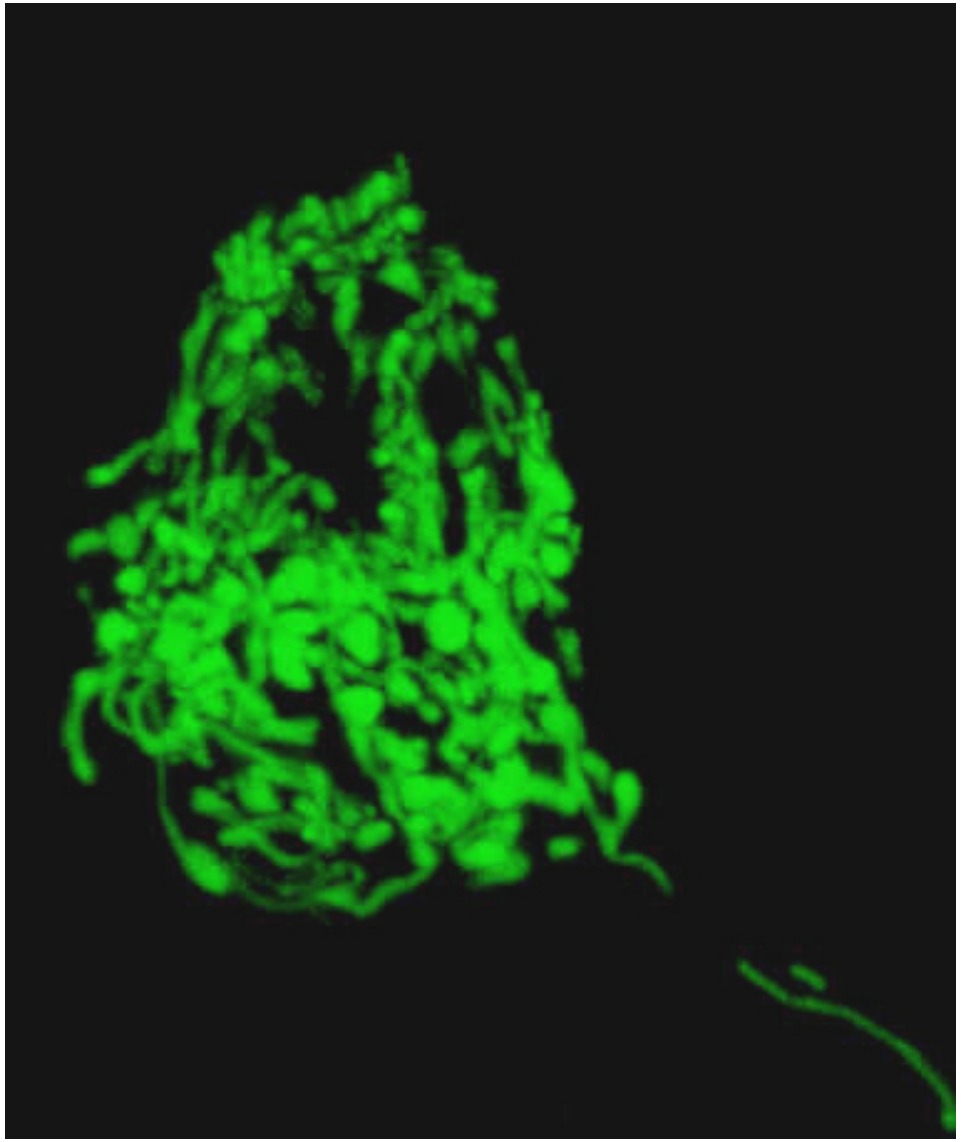
Change shape

Numbers

Composition

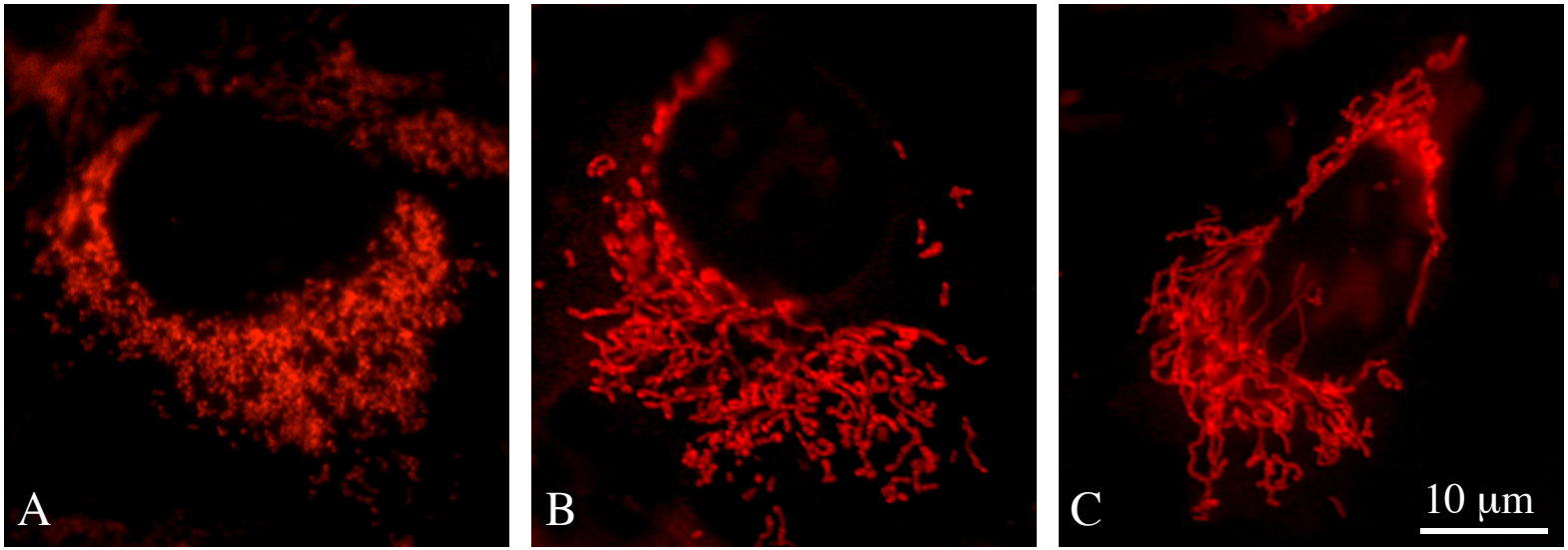
The mitochondrial mass can exist as a continuum or reticulum or as in a fragmented form. The switching Between the 2 forms i.e. fission and fusion involves sets of different proteins and is triggered by cell cycle. Fragmentation is an early and required step in Apoptosis.

3) 3D view of reticulum



143B osteosarcoma
GFP-pH transfected
Confocal microscope
3D projection

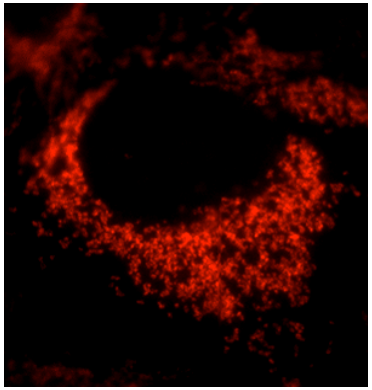
2) Different states (fragmented, intermediate, reticulum)



fragmented **intermediate** **reticular**

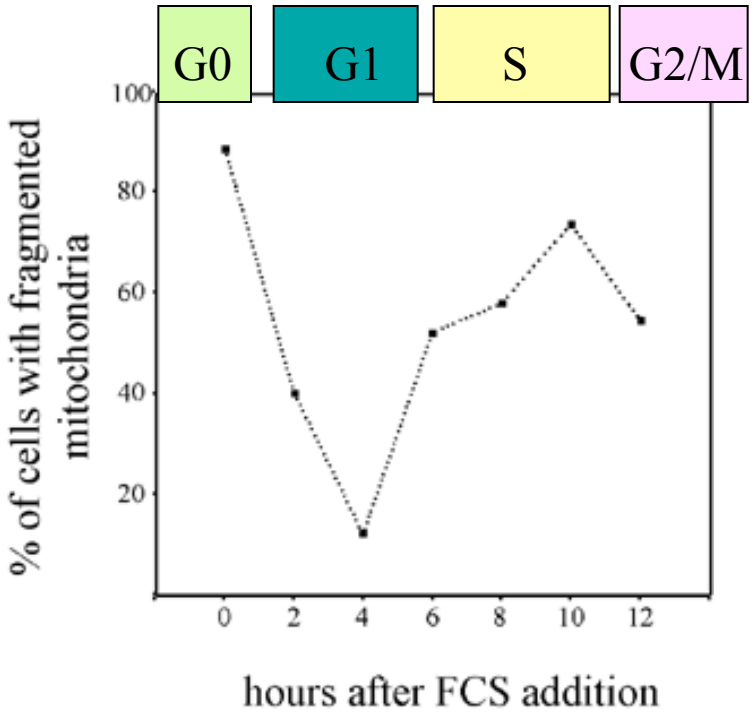
- MitoTracker Red staining
- osteosarcoma cells (143B)

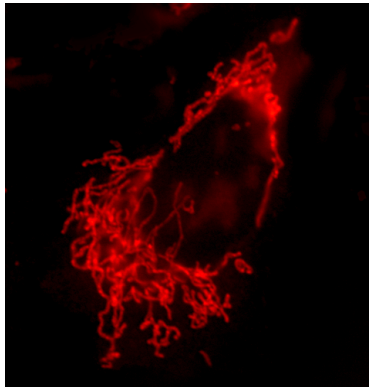
Cell cycle dependent changes of mitochondrial morphology



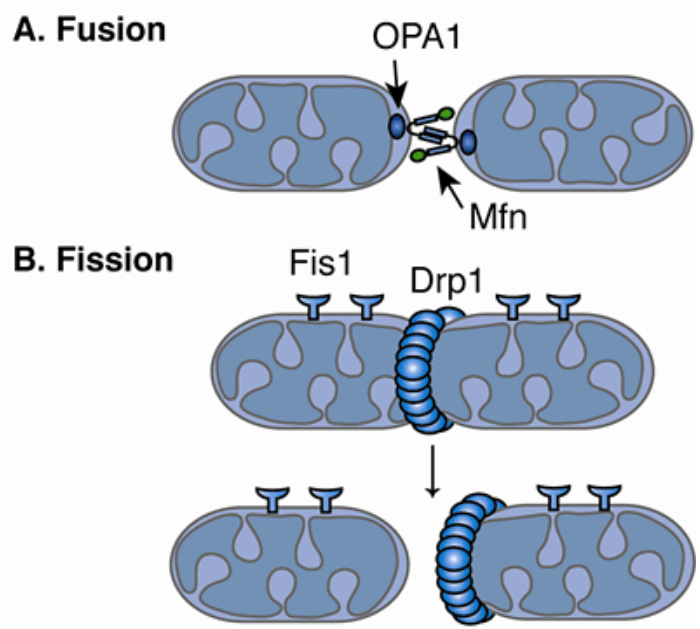
Fragmented

-**Osteosarcoma** cells were arrested in G0 by FCS starvation for 60-72 h
- Released by FCS addition
- Stained with MitoTracker Red

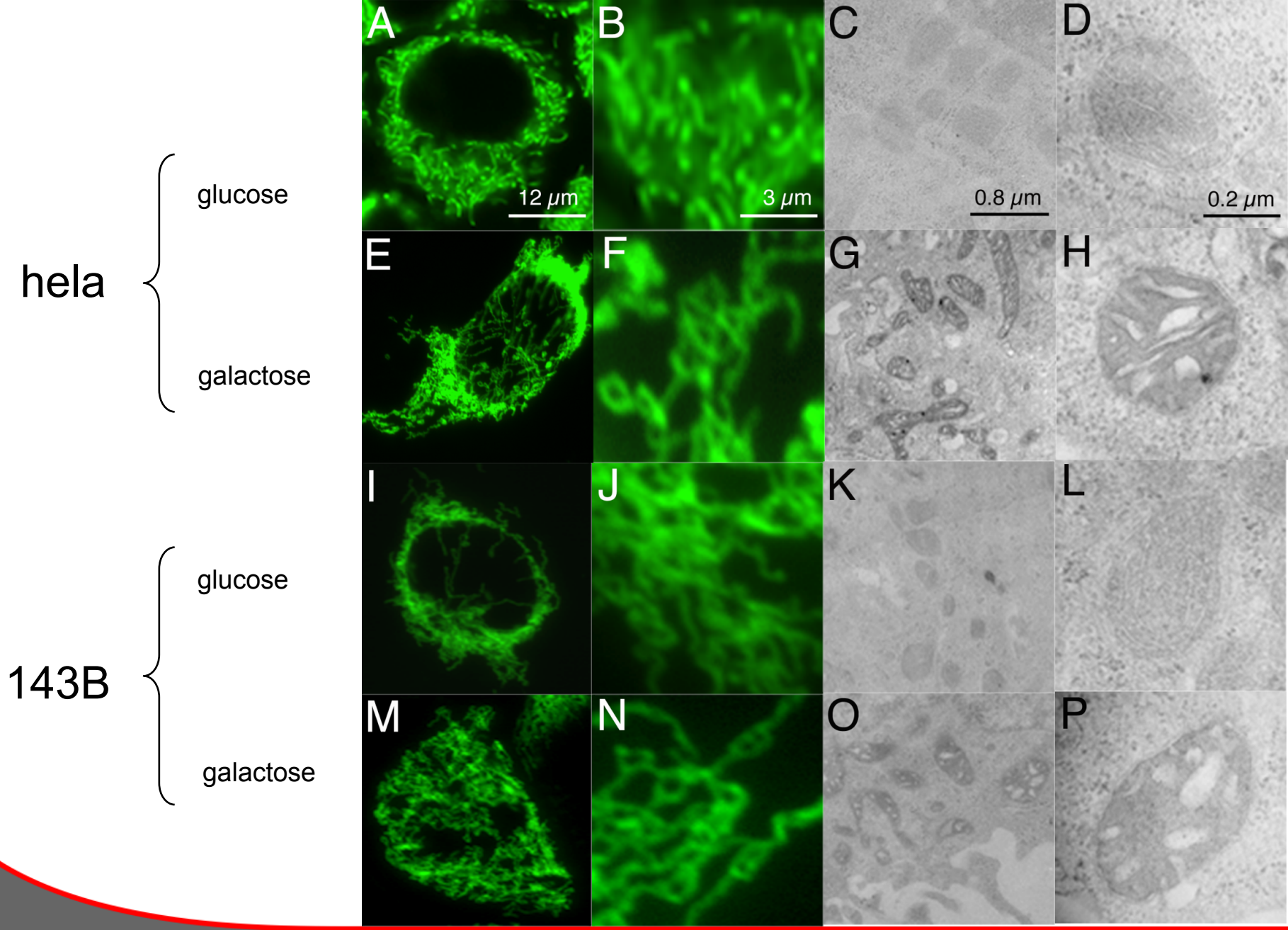


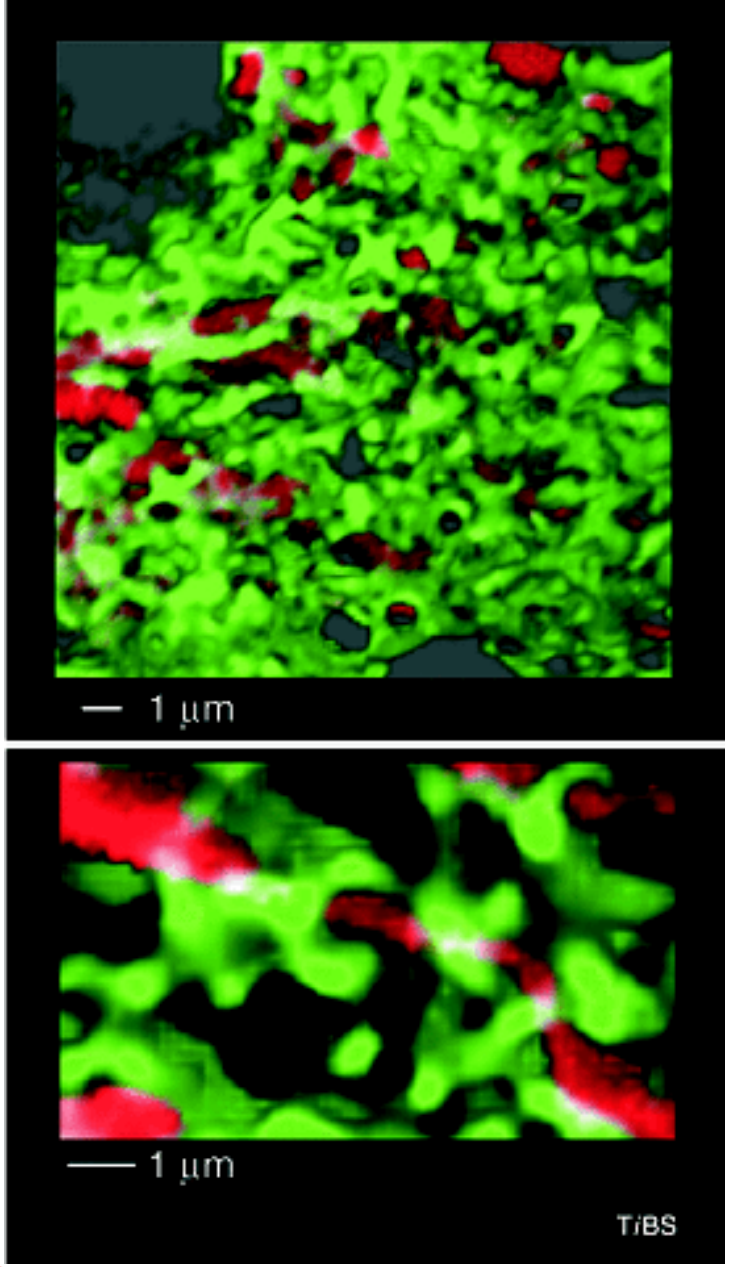


Reticular



FISSION AND FUSION IS ESSENTIAL FOR CELL SURVIVAL. It is orchestrated by special proteins several of which are altered in neurological diseases.


hela
glucose
galactose
143B
glucose
galactose
A
B
C
D
12 μm
3 μm
0.8 μm
0.2 μm
E
F
G
H
I
J
K
L
M
N
O
P



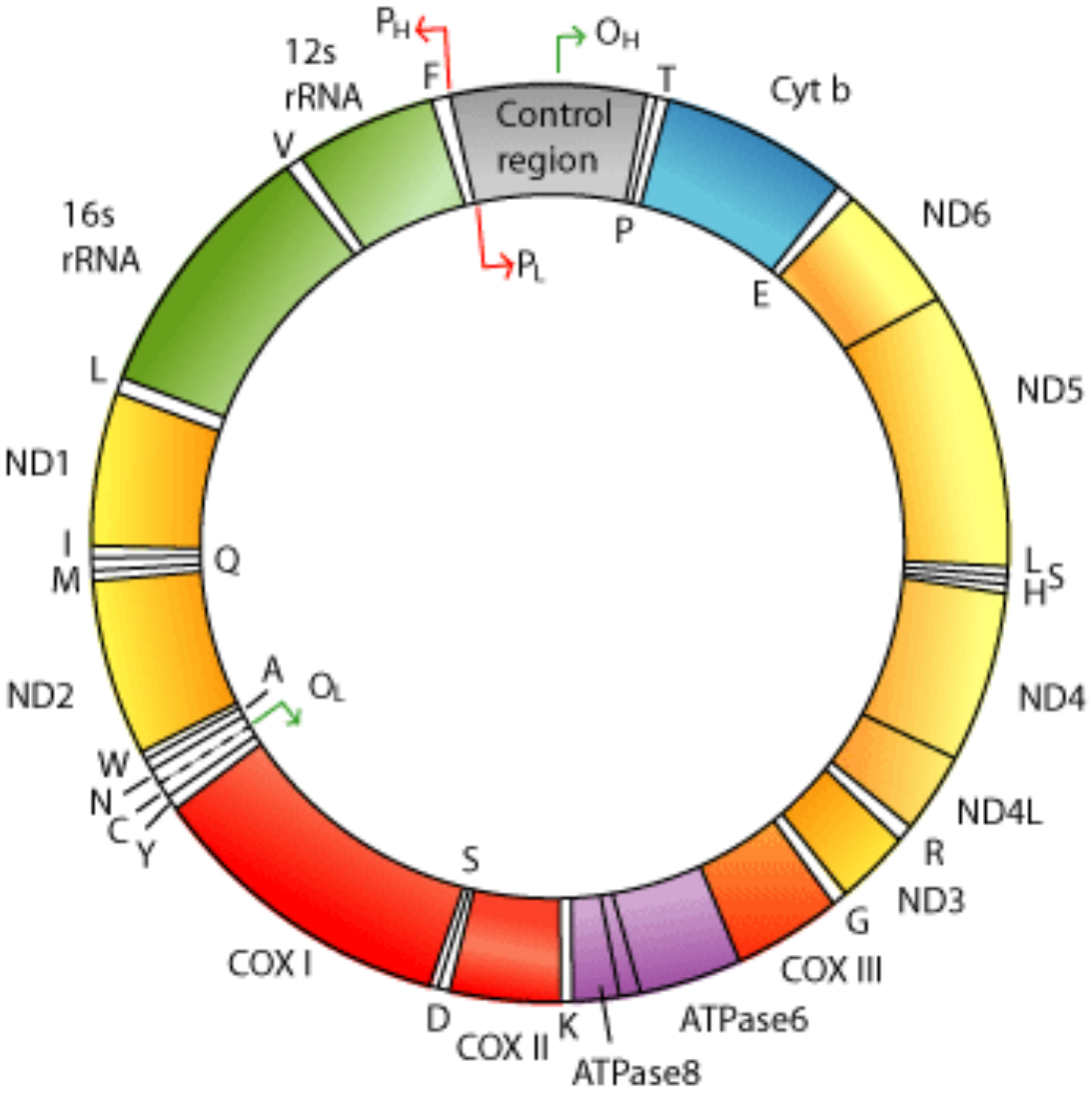
The mitochondrion (red) intercalates in and has specific connections With the ER (green)

THE BUILDING BLOCKS

MITOCHONDRIA ARE MADE FROM 2 GENOMES

mtDNA and nuclear genes

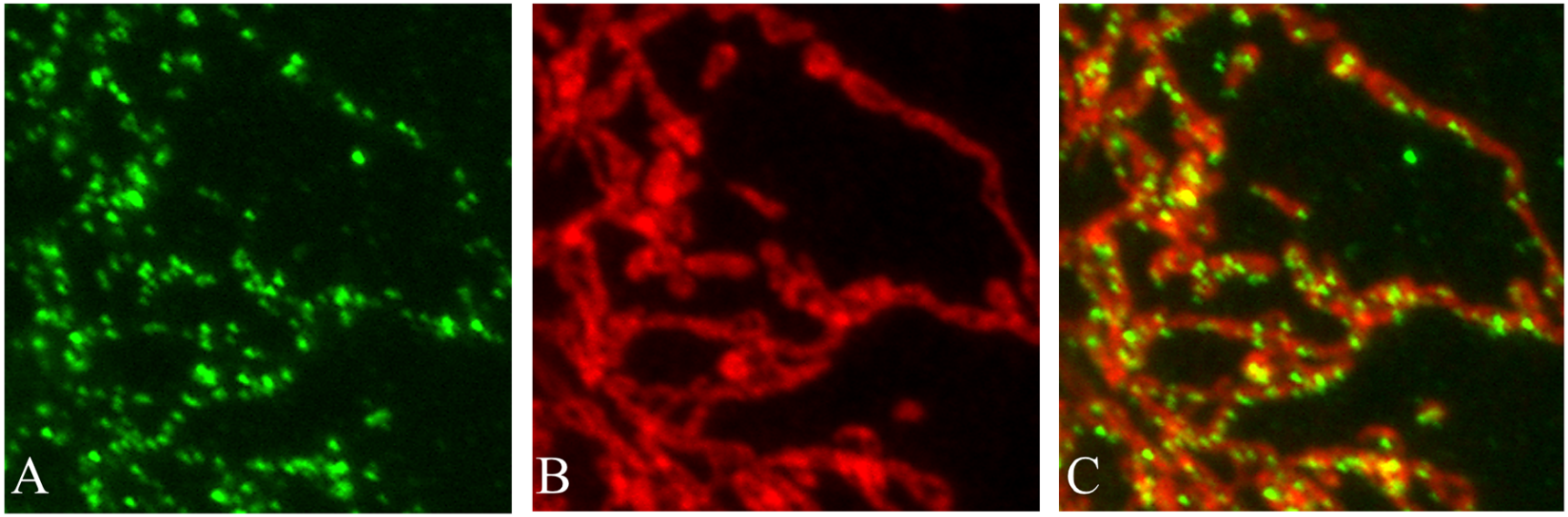
mtDNA is bacteria-like as is mt protein synthesis


P_H
O_H
12s
rRNA
F
Control
region
T
Cyt b
V
16s
rRNA
P
ND6
P_L
E
L
ND5
ND1
I
M
Q
L
H
S
A
O_L
ND2
ND4
W
N
C
Y
ND4L
R
S
ND3
G
COX I
COX III
D
COX II
K
ATPase6
ATPase8

Fluorescence In Situ Hybridization (FISH)

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

COX I - AF488 | MitoTracker Red | merged image



mtDNA content

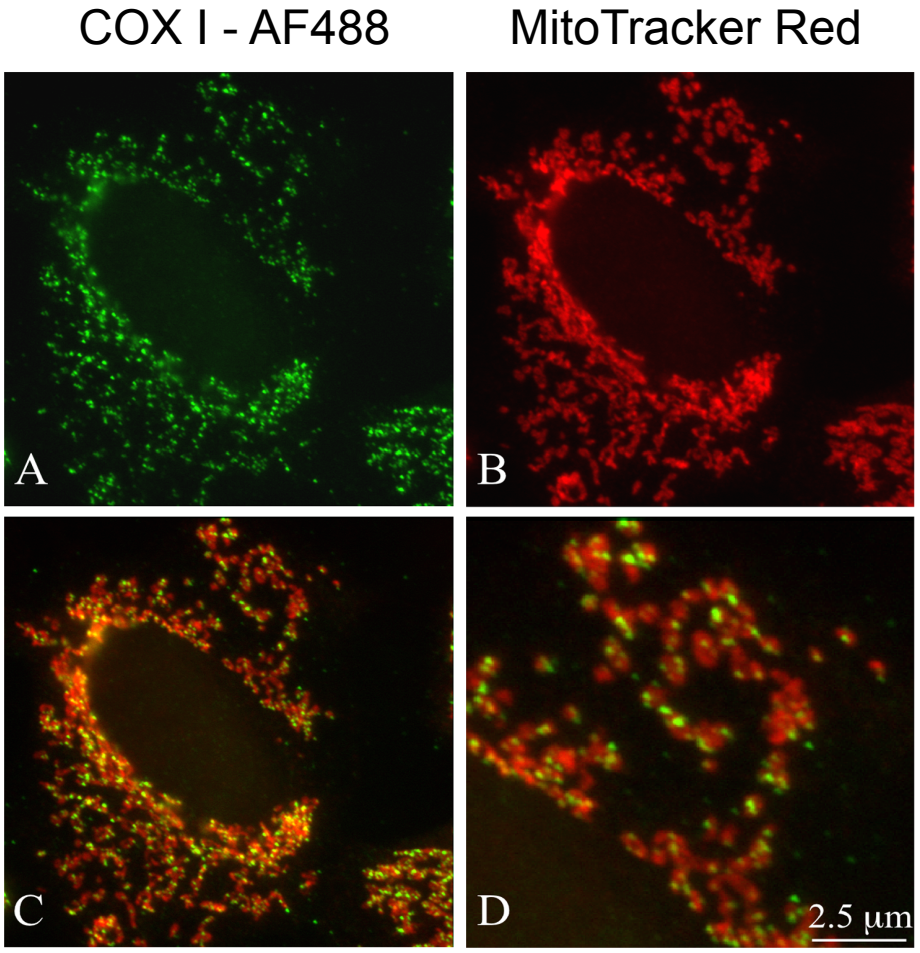
The number of copies of mtDNA varies by species, by tissue and by metabolic factors 100-5000 per cell.

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

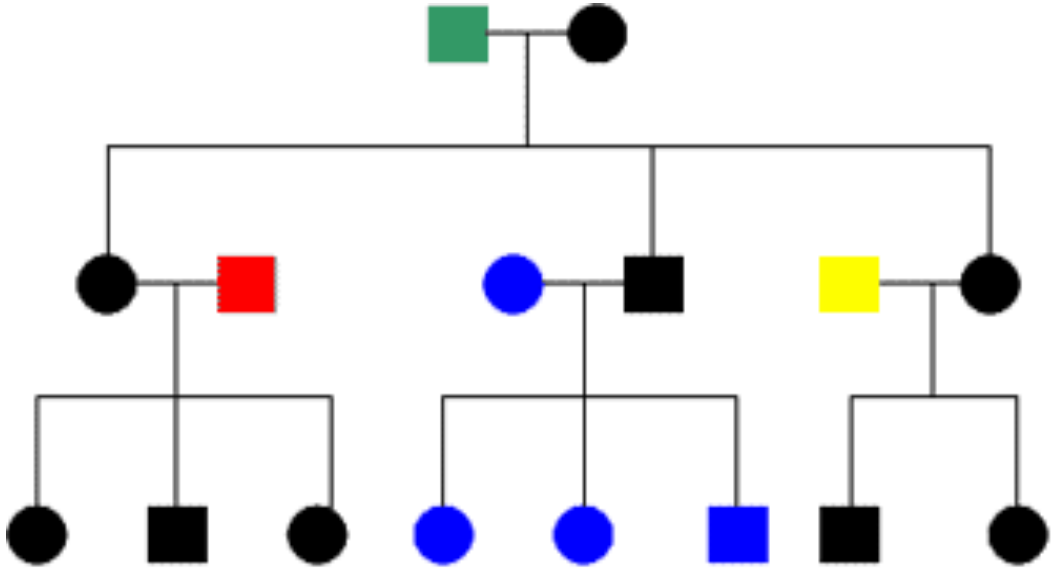
(1:15,000) Methylmalonicaciduria. Methylmalonyl-CoA mutase, cobalamin metabolism

(1:40,000) Aminoacidopathies

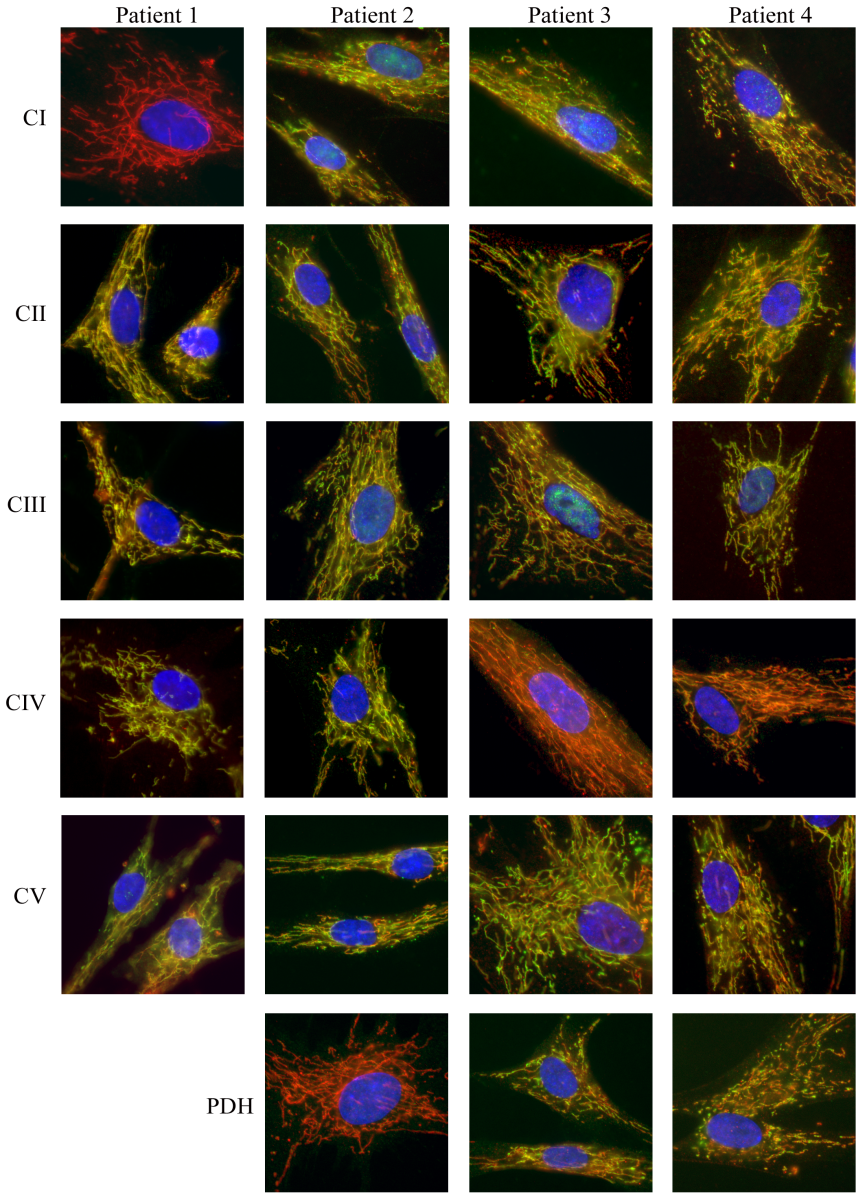
(1:50,000) Peroxisomal disorders; e.g., Zellweger syndrome, neonatal adrenoleukodystrophy, Refsum's disease)

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

MATERNAL INHERITANCE



*Colors reflect inheritance of the same mitochondrial genome

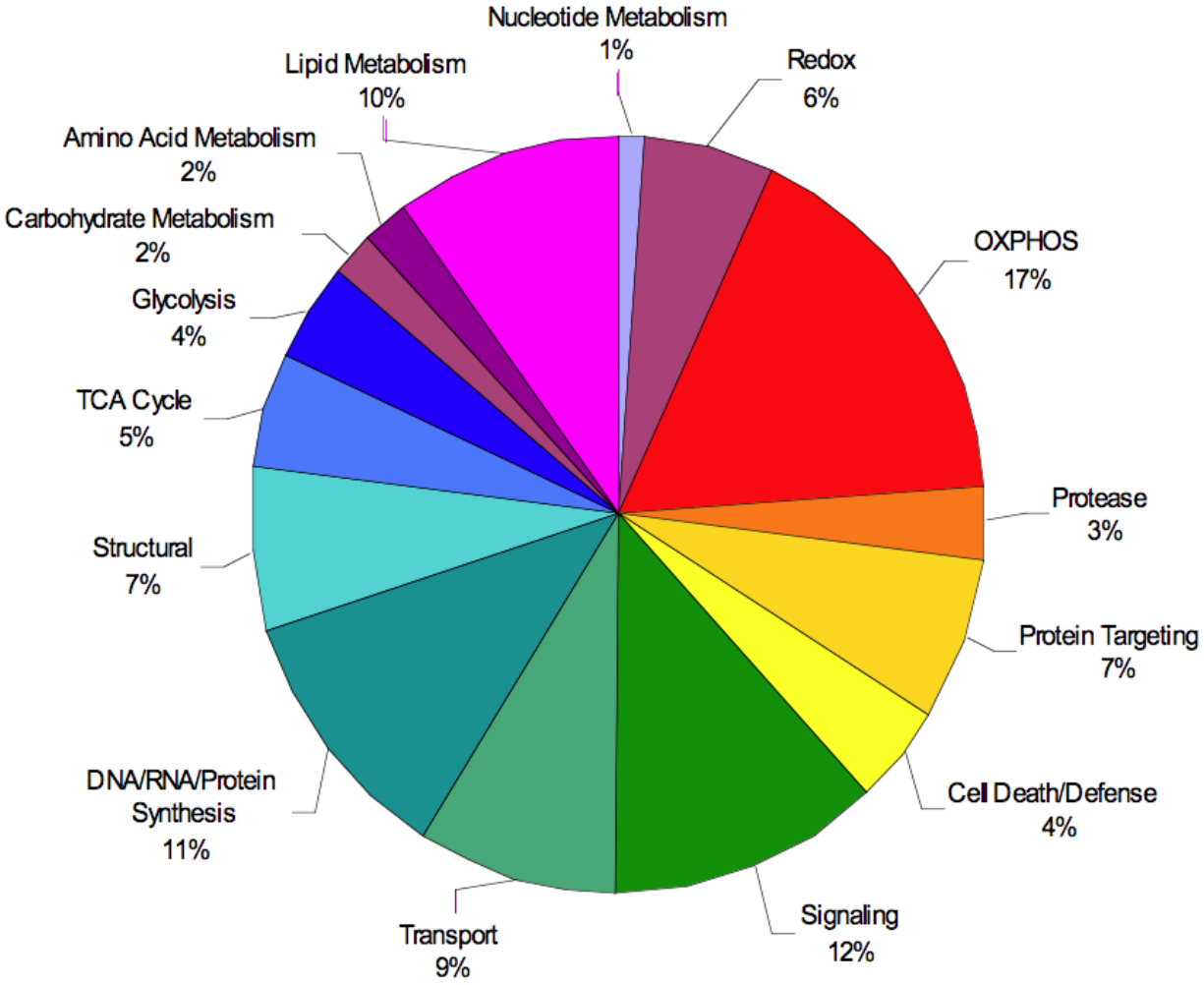

Patient 1
Patient 2
Patient 3
Patient 4
CI
CII
CIII
CIV
CV
PDH

MITOCHONDRIAL PROTEOME

Best estimate 2000 polypeptides.

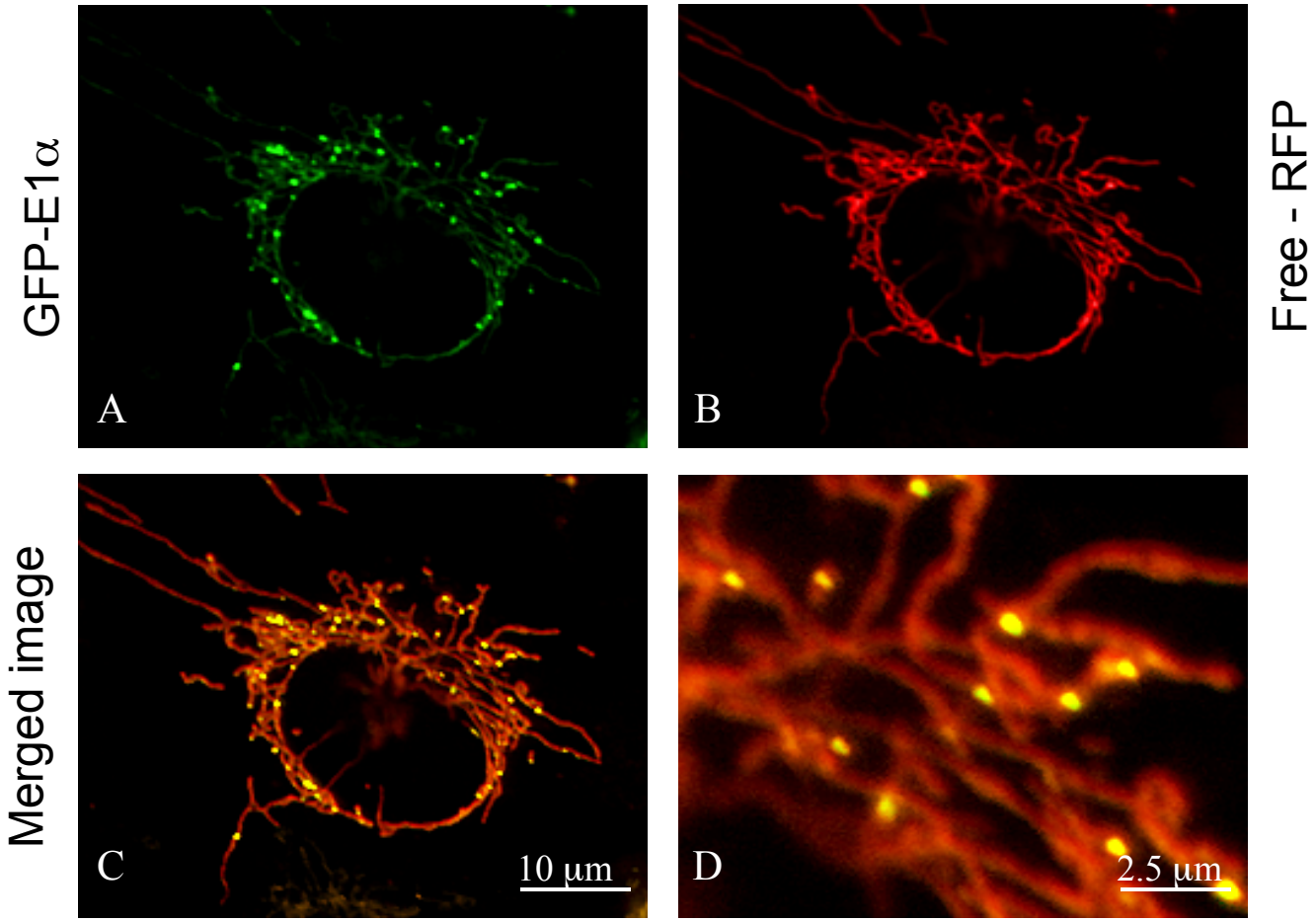
13 made in mitochondrion; rest nuclear.

Mitochondrial Proteome



PROTEIN DISTRIBUTION IS RELATIVELY FIXED TO OPTIMIZE METABOLIC PATHWAYS AND PROCESSES

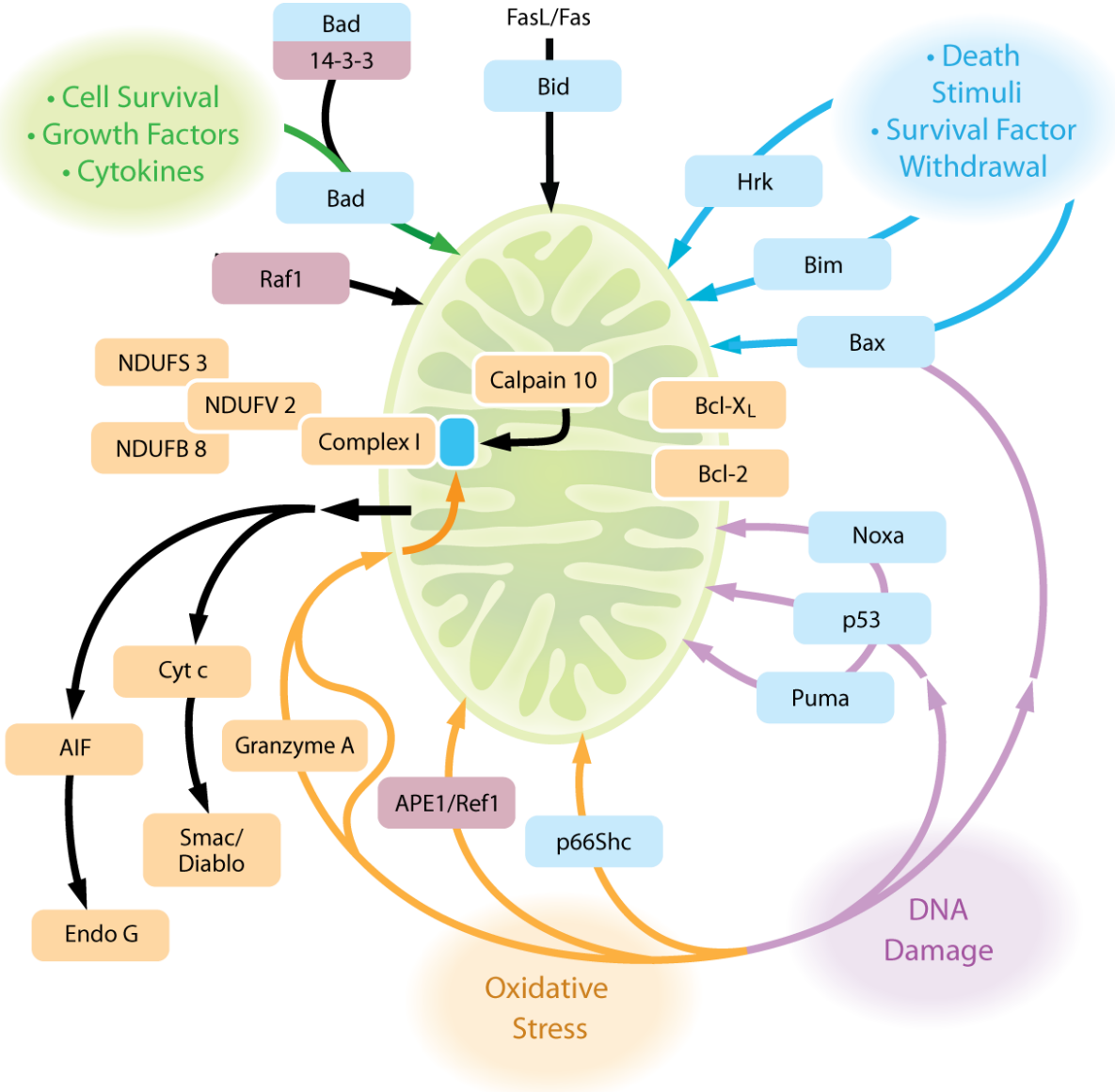
Distribution of GFP-E1a and RFP in co-transfected cells



24 hours post transfection

- THE PROTEIN COMPOSITION OF THE MITOCHONDRION IS NOT FIXED.

- THERE IS MOVEMENT OF PROTEINS ONTO, IN AND OUT OF THE ORGANELLE IN SEVERAL FUNCTIONS

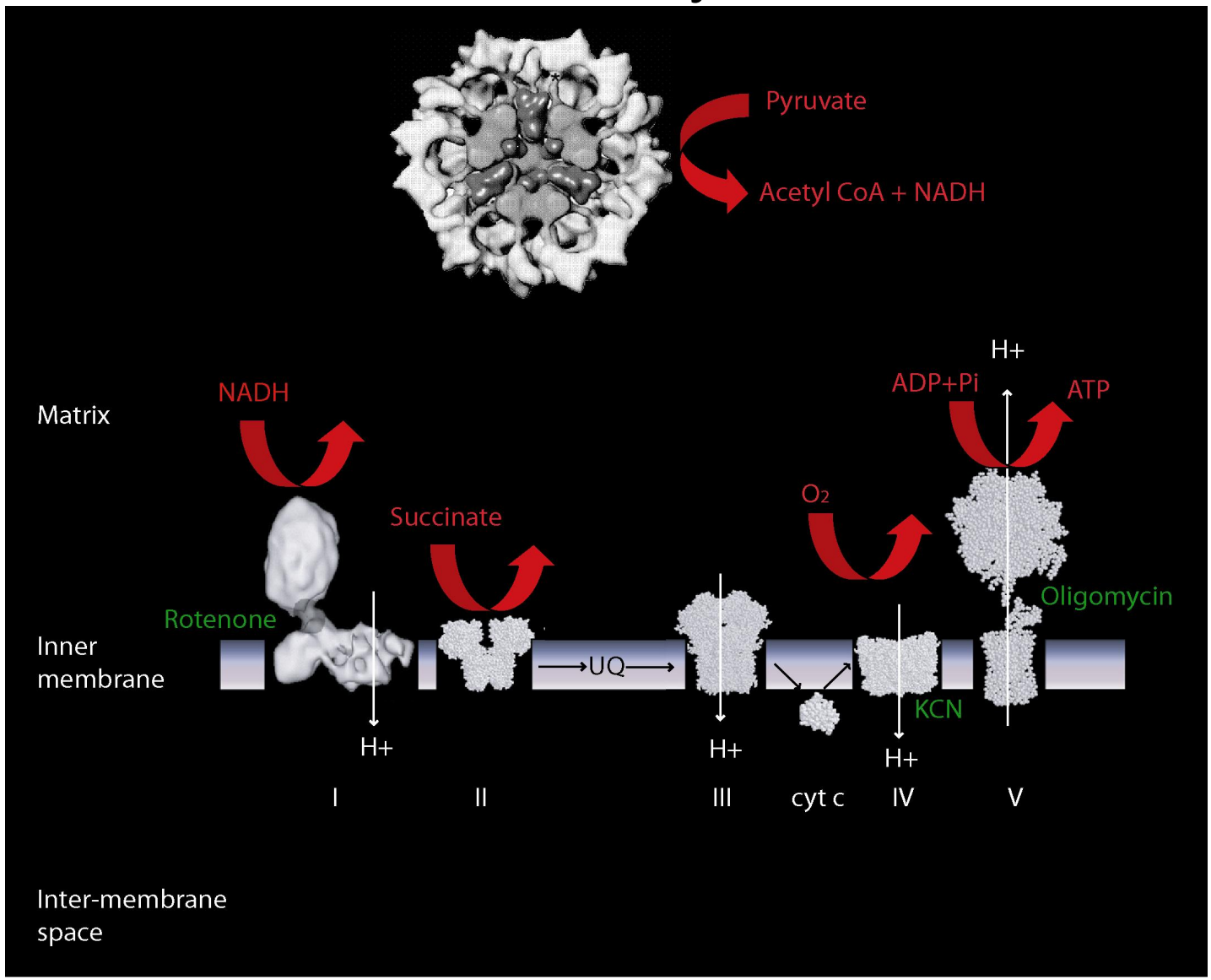

Bad
14-3-3
Bad
FasL/Fas
Bid
• Cell Survival
• Growth Factors
• Cytokines
• Death Stimuli
• Survival Factor Withdrawal
Hrk
Bim
Bax
Raf1
NDUFS 3
NDUFV 2
NDUFB 8
Complex I
Calpain 10
Bcl-X_L
Bcl-2
Noxa
p53
Puma
Cyt c
AIF
Granzyme A
Smac/ Diablo
Endo G
APE1/Ref1
p66Shc
DNA Damage
Oxidative Stress

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

OXPHOS System

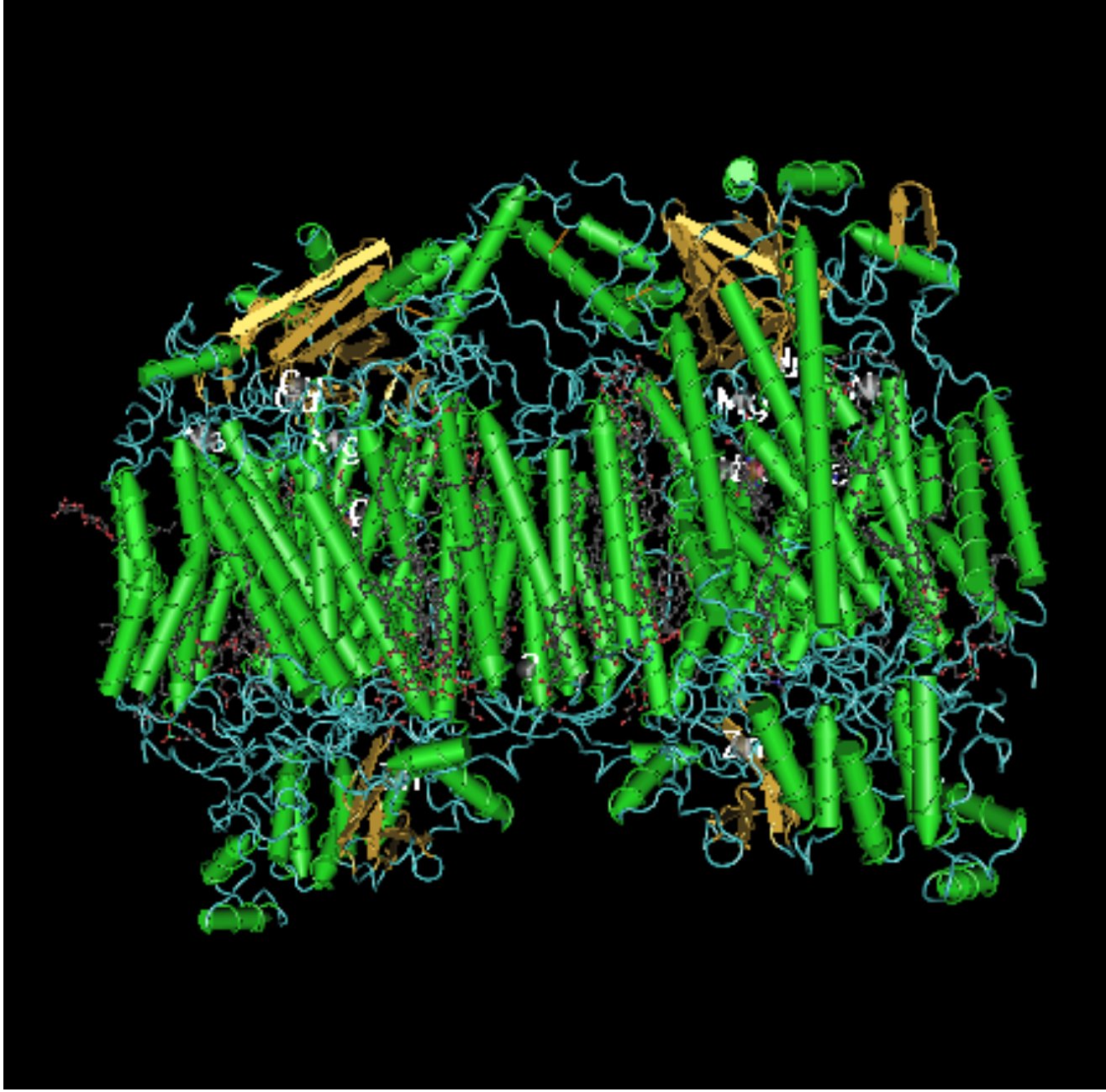


The high resolution structure of cytochrome c oxidase shows a complex of 13 different polypeptides some spanning the lipid bilayer, others located on the cytosol side where substrate cytochrome c binds. Segments that are within the bilayer are alpha helical. Hemes and copper prosthetic groups form an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

COX Model

gold=β-sheet

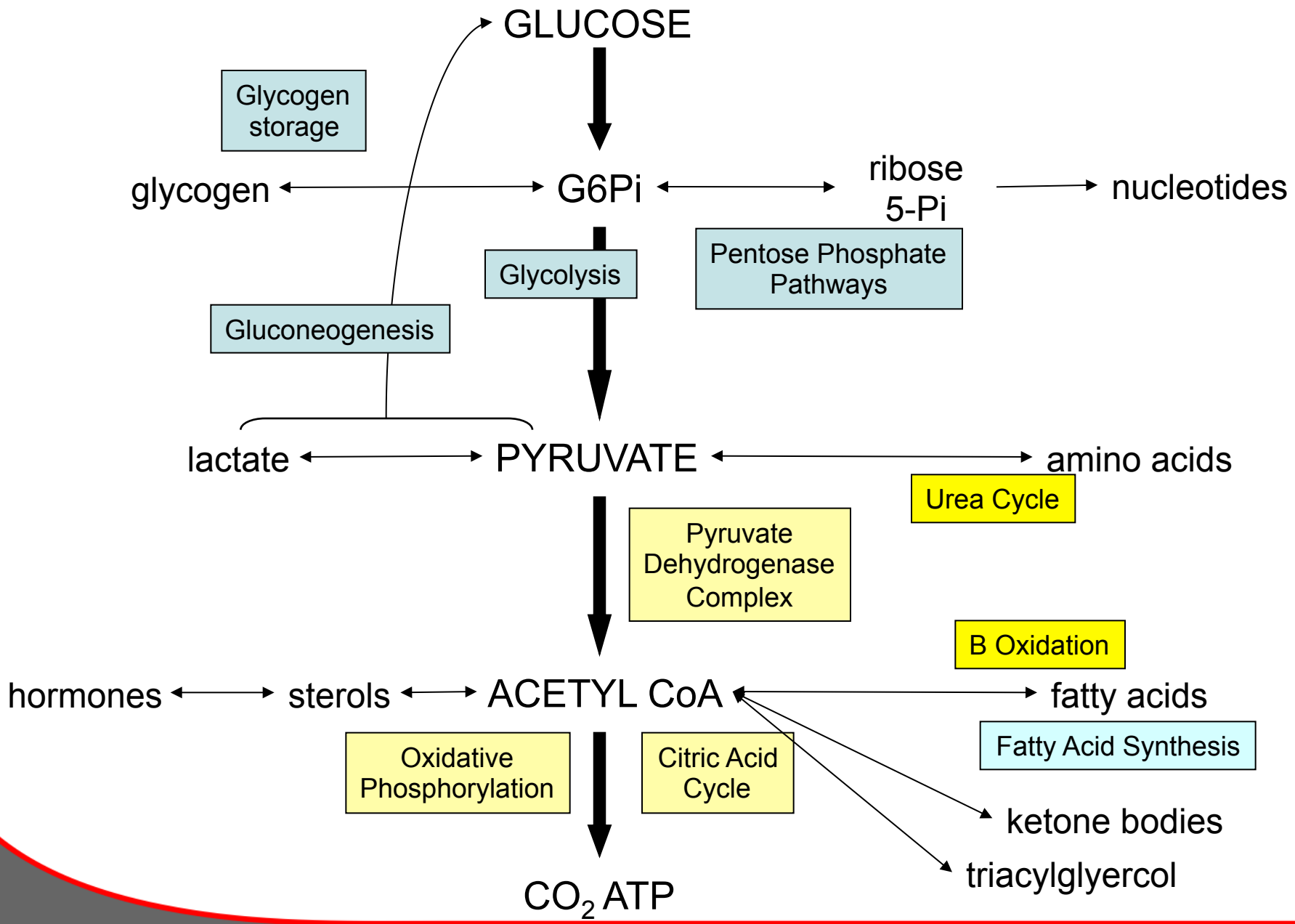
green=α-helix



- 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.


GLUCOSE
Glycogen storage
glycogen
G6Pi
ribose 5-Pi
nucleotides
Pentose Phosphate Pathways
Glycolysis
Gluconeogenesis
lactate
PYRUVATE
amino acids
Urea Cycle
Pyruvate Dehydrogenase Complex
B Oxidation
hormones
sterols
ACETYL CoA
fatty acids
Fatty Acid Synthesis
Oxidative Phosphorylation
Citric Acid Cycle
ketone bodies
triacylglyercol
CO_2 ATP

SHORT TERM REGULATION OR METABOLISM

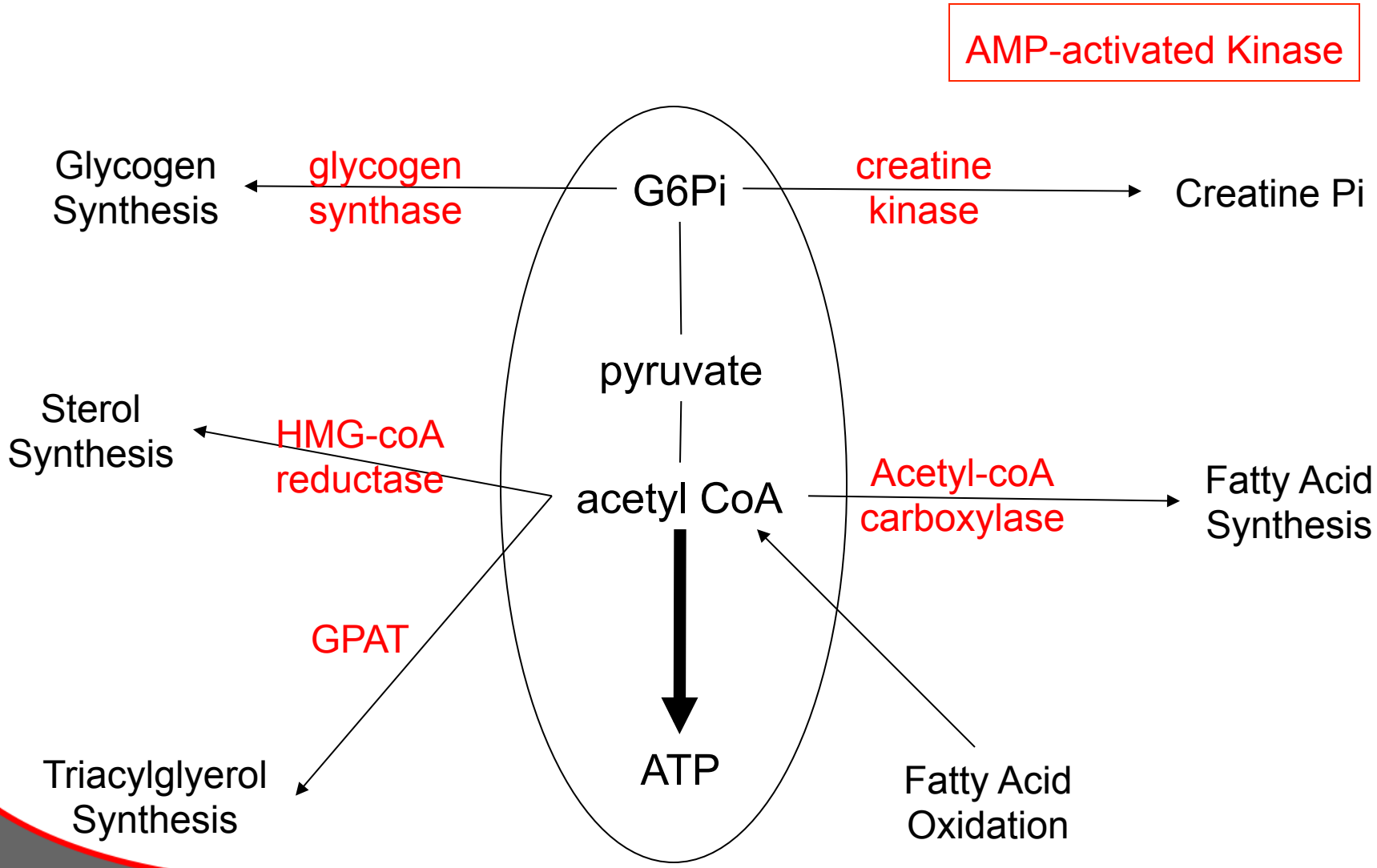
PHOSPHORYLATION

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



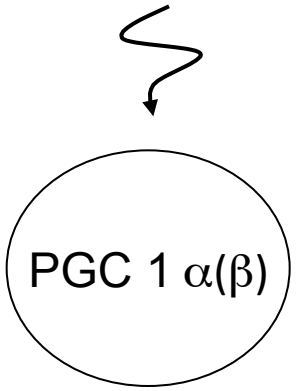
LONG TERM REGULATION

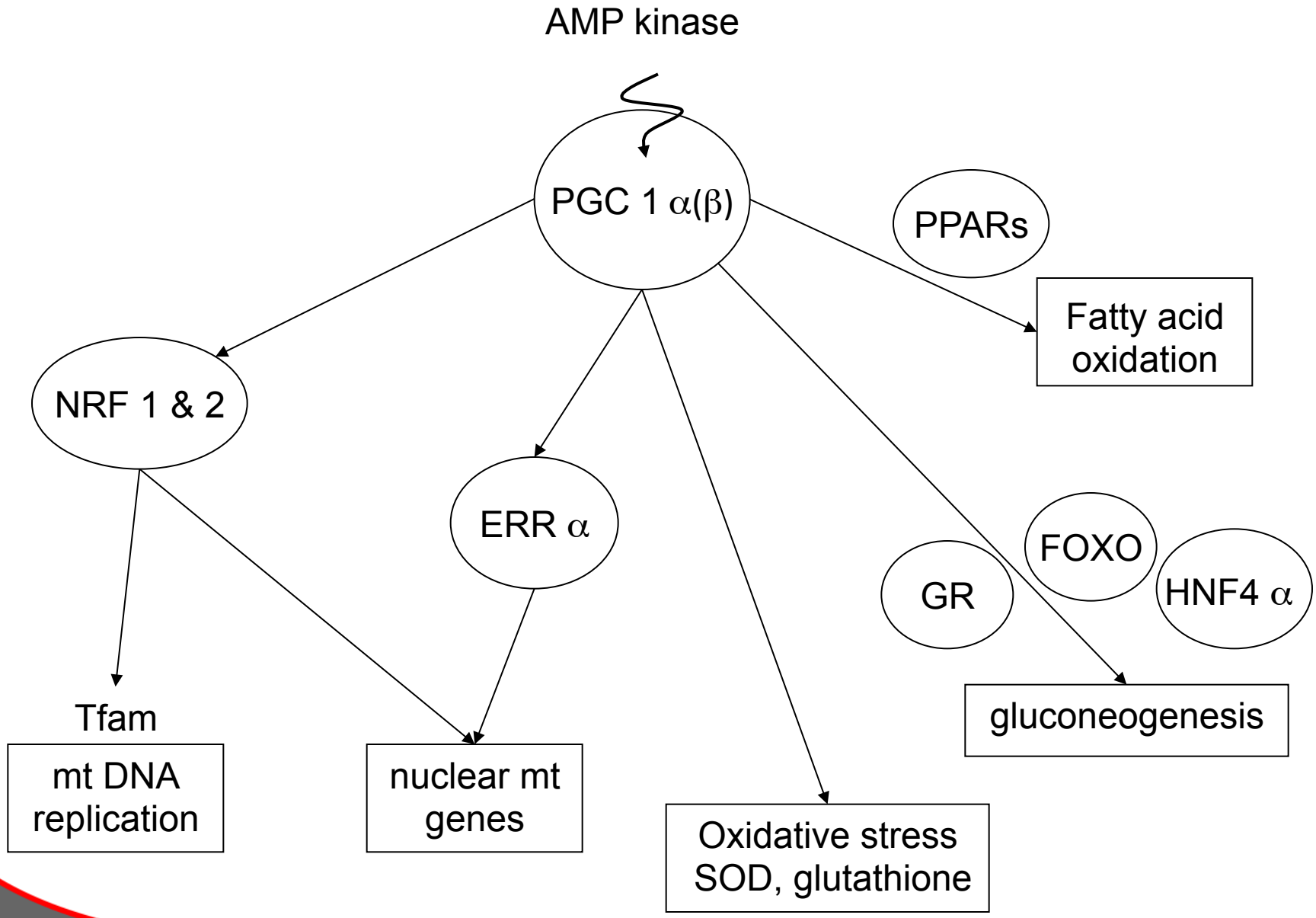
Transcription factors

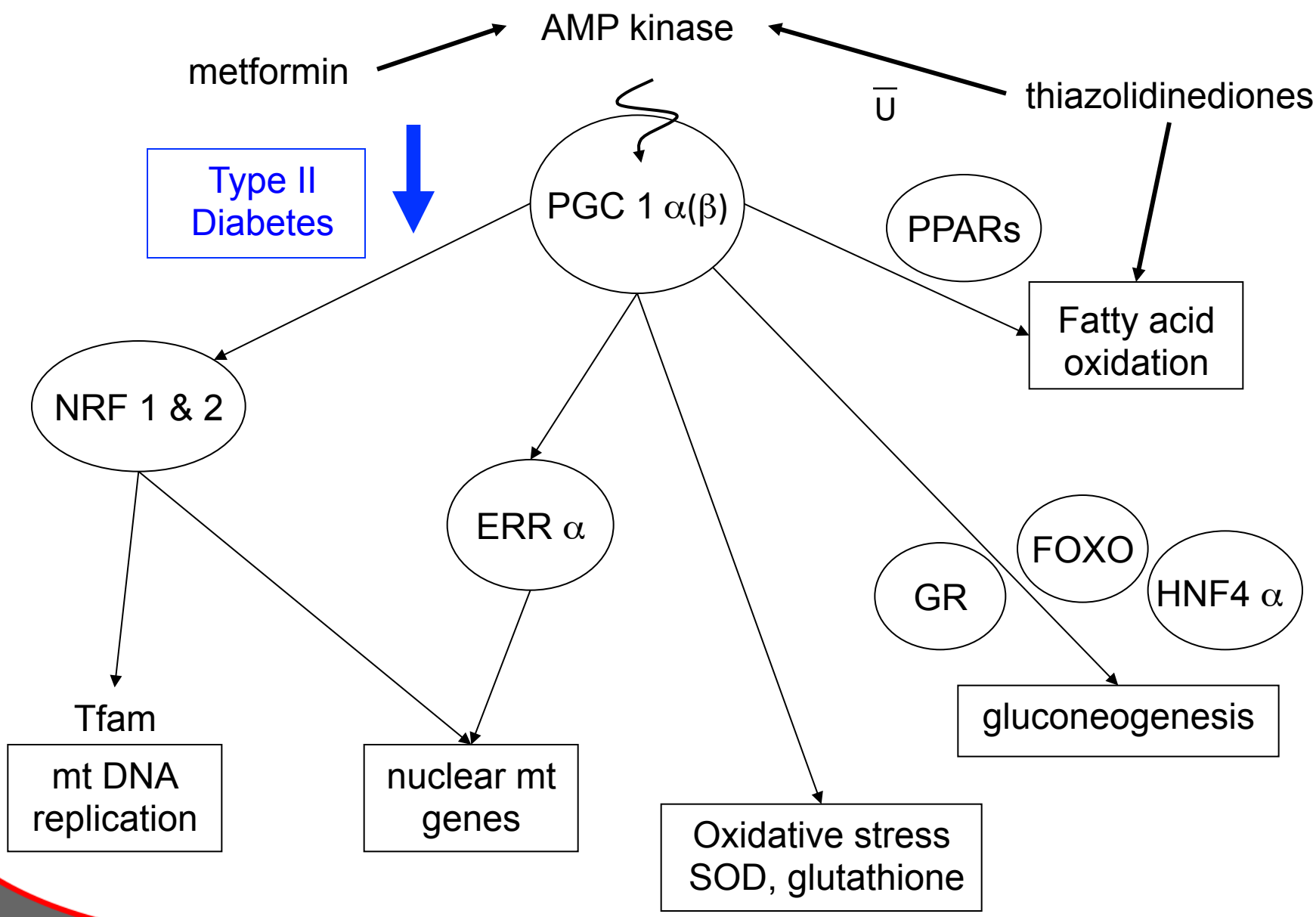
Transcription factor activators

Transcription factor activator activators (sirtuins!)

AMP kinase




AMP kinase
PGC 1 α(β)
PPARs
Fatty acid oxidation
NRF 1 & 2
ERR α
FOXO
GR
HNF4 α
Tfam
mt DNA replication
nuclear mt genes
Oxidative stress SOD, glutathione
gluconeogenesis


metformin
AMP kinase
thiazolidinediones
Type II Diabetes
PGC 1 α(β)
PPARs
Fatty acid oxidation
NRF 1 & 2
ERR α
FOXO
GR
HNF4 α
gluconeogenesis
Tfam
mt DNA replication
nuclear mt genes
Oxidative stress SOD, glutathione

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.