

MITOCHONDRIA, THEIR STRUCTURE, FUNCTIONS, AND DISEASES LINKED TO THE ORGANELLE.

- **Introduction.**
- **Part 1. The structure of mitochondria.**
 - A). **Morphology and organelle interactions.**
 - B). **The fusion fission cycle of mitochondria.**
 - C). **Internal structure.**
 - D). **MtDNA, structure and packaging.**
 - E). **Import of proteins into mitochondria.**
- **Part 2. Mitochondrial functioning in intermediary metabolism.**
 - A). **Post-translational modifications control of Mitochondrial metabolism.**
 - B). **Pyruvate dehydrogenase.**
 - C). **Succinate dehydrogenase.**
 - D). **The ATP synthase.**
- **Part 3. Mitochondria in apoptosis.**
- **Part 4. Control of mitochondrial levels: production versus destruction.**
 - A). **Biogenesis.**
 - B). **Mitophagy.**
- **Part 5. Mitochondria in disease.**
 - A) **Oxidative phosphorylation deficiencies.**
 - B). **Fatty acid oxidation disorders.**
 - C). **Mitochondria in the innate immune response.**
 - D). **Mitochondria and cancer.**
 - E). **Mitochondria and neurodegeneration. Parkinsons disease, Alzheimers disease, ALS, Huntingtons disease).**

INTRODUCTION

Mitochondria are now known to be more than the hub of energy metabolism. They are the central executioner of cells, and control cellular homeostasis through involvement in nearly all aspects of metabolism. As our understanding of mitochondria has expanded it has become clear that the structure, function and pathology of the

organelle are so intimately connected that it is difficult, if not impossible, to study any one area without context of the others.

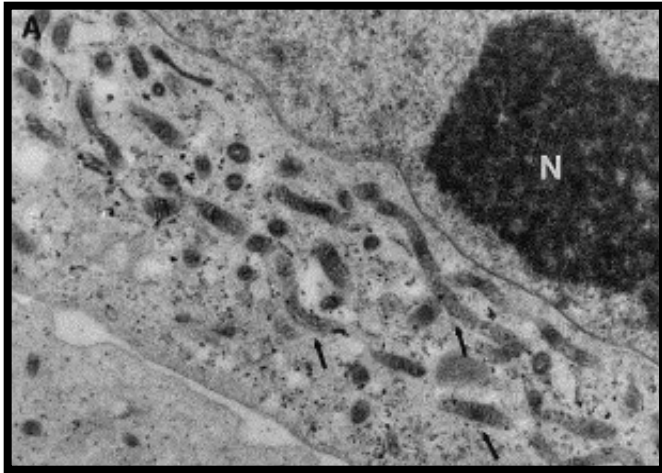
Here is presented a relatively brief overview of the diverse areas of mitochondrial research to provide the background and stimulate the broad thinking that is needed to understand the role that mitochondria play in some of the more common and devastating human conditions including genetic mitochondrial cytopathies and neuropathies, Parkinsons and Alzheimers disease, diabetes and cancer. To facilitate easy reading, the number of citations is limited with the idea that readers can obtain details of specific areas through the key recent reviews listed.

PART 1 THE STRUCTURE OF MITOCHONDRIA.

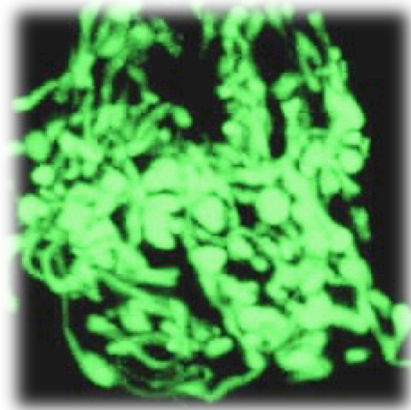
A) MORPHOLOGY AND ORGANELLE INTERACTIONS

The classic picture of cellular mitochondria based on low-resolution electron micrographs is of a set of relatively small bean shaped particles scattered around the cytosol. However, our understanding of the morphology of the organelle has changed with the advent of higher resolution electron microscopes and cryopreservation of samples.

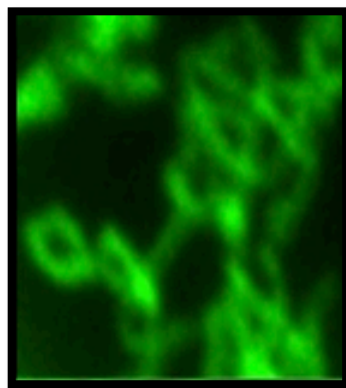
Foremost, mitochondria are now known to be highly dynamic and can be punctate as previously proposed, but can also be organized as a continuum or reticulum under some cell conditions. Further, the organelle moves within the cell in the punctate state or as a reticular unit to provide foci of energy production such as at the nucleus during cell division, or to synapses in neuronal cells at times of high information transfer. This movement is along microtubules in one direction and along actin filaments in the other.



Electron micrograph showing reticulum "tubes" sectioned as they extend through the cytosol. From Gilkerson et al. FEBS Lett. 474 1-4 2000



3D reconstruction of the mitochondrial reticulum fluorescently stained by matrix-localized GFP



High resolution of the GFP labeled mitochondria showing the looping of the reticulum throughout its length.

Several important studies have established that the switching between punctate and reticulum forms is physiologically important. For example it is cell cycle dependent, being reticular in the G1 phase but then converting to the punctate form for cell division. The

transitioning of mitochondria between punctate and reticulum states through alternating fission and fusion is now known to be critical to maintaining mitochondrial quality control. Fission allows separation of the healthy mitochondrial segments from the defective ones. Not surprising then, failure to transition between the different morphological forms is thought to contribute to several diseases e.g. Parkinsons disease: see later.

Another aspect of mitochondrial organization that is now known to be important is the interaction between this and other cellular organelles including, endoplasmic reticulum, lysosomes and peroxisomes. These interactions are labile and occur through contact sites involving proteins of the two organelles. Among key functions is the movement of Ca⁺⁺ between mitochondria and ER and the co-ordination of the unfolded protein response by these two organelles.

B). THE FUSION FISSION CYCLE OF MITOCHONDRIA

Mitochondrial fusion can be divided into two processes, the fusion of the mitochondrial outer membrane followed by that of the inner membrane. The outer membrane fusion requires proteins known as mitofusins, (Mfn1 and Mfn2). Inner membrane fusion mainly involves an inner membrane-localized protein (Opa1).

The mitofusins are transmembrane dynamin-related GTPases, which induce the joining of 2 mitochondrial “fragments” by forming dimers across the interface. The tethering of mitochondria together is followed by GTP hydrolysis, which induces conformation changes to cause mitochondrial fusion. The activity of mitofusins is regulated by ubiquitination, which causes their degradation in response to stress. The control of this degradation involves several proteins (PINK1, Parkin, E3 ligase Huwe1, MULAN and Bcl-2 family members).

The inner membrane protein Opa1 is named based on its identification as a mutated gene in optic atrophy. It is a dynamin-related GTPase that interacts with cardiolipin, a mitochondrial inner membrane lipid, and is mostly found in cristae, consistent with its role in maintaining cristae morphology. Opa 1 is also found in the cytosol in lipid droplets.

Opa1 activity involves the coordinated action of a long isoform of protein and its cleaved short isoform. The long isoform is located in

the mitochondrial cristae membrane, while the short isoform is soluble in the intermembrane space. Opa1 proteolytic cleavage is regulated by the mitochondrial membrane potential, apoptosis, ATP level and mtDNA stability.

Mitochondrial fission involves another GTPase called Drp1. On translocation from cytosol to mitochondria, this protein oligomerizes into an X-shaped dimer on mitochondrial outer membranes. The binding sites for Drp1 association include endoplasmic reticulum (ER)-mitochondria contact points. When localized to the mitochondrial outer membrane, Drp1 rims the mitochondria in multimeric spirals at the constriction site, with the GTPase domain pointing away from the membrane. Formation of a complete spiral is thought to activate the GTPase domain causing GTP hydrolysis leading to a spiral constriction.

In apoptosis, Drp1 is also involved in Bax oligomerization on the outer membrane and in cytochrome c release. Functioning of Drp1 is controlled by post-translational modifications including phosphorylation by a signaling kinase (GSK3) and the post-translational modifications S-nitrosylation, ubiquitination, SUMOylation and O-linked-N-acetyl-glucosamine glycosylation.

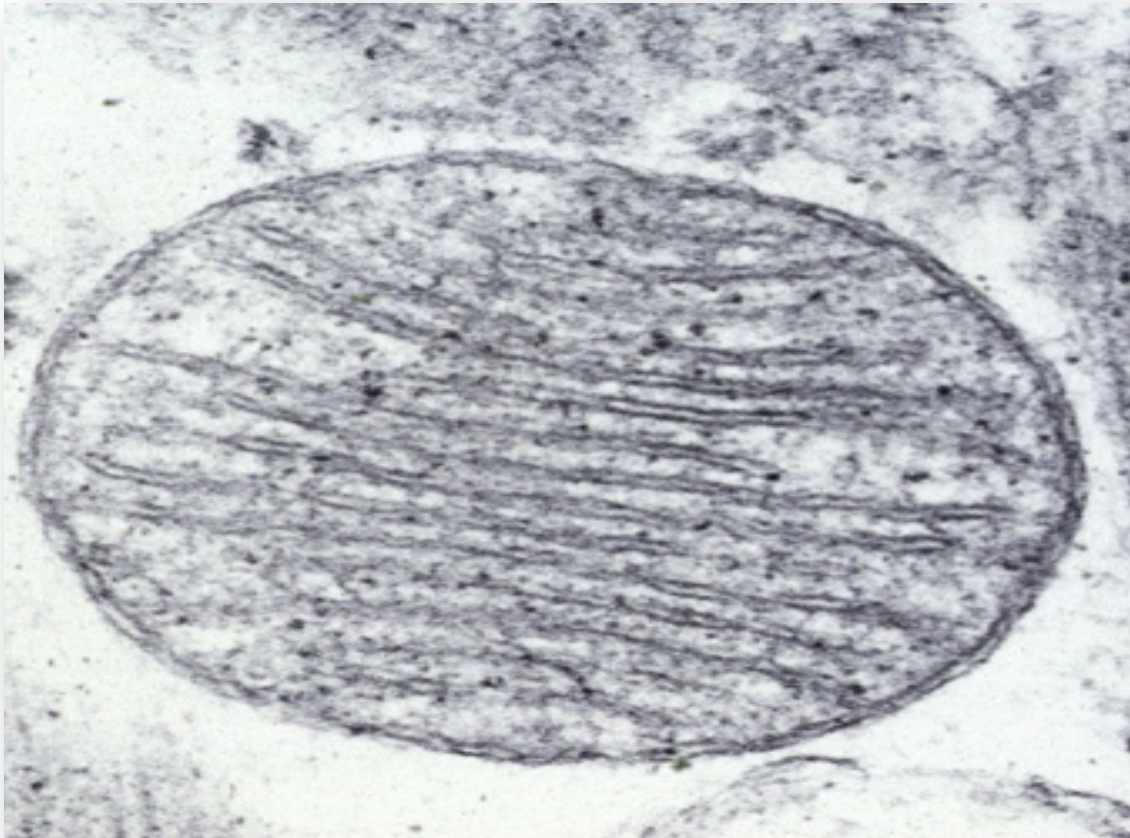
A second protein important for mitochondrial fission is human fission protein 1 homologue (Fis1). This protein binds to the mitochondrial outer membrane with the help of C-terminal trans-membrane domain. Unlike Drp1, Fis1 is distributed evenly on the outer mitochondrial membrane surface, and appears as punctate complexes. Functionally it is involved in binding of Drp1 (at least in yeast). Fis1 overexpression amplifies mitochondrial fragmentation, suggesting that it is a limiting factor for mitochondrial fission. Its expression is regulated via ubiquitination by a mitochondrial ubiquitin ligase (Mitol). Several other proteins have been identified as important to the fission process (Mff, trap1, MiD49 and Mid51). Interestingly, the levels of one of these (trap1) are related to ROS production, suggesting that this protein signals the mitochondrial stress that initiates fission for subsequent mitophagy of damaged organelle segments. Sensing of ATP levels (by the AMP kinase) can activate fission by phosphorylating the protein Mff to recruit the fission apparatus.

Note that two of the proteins involved in maintaining the

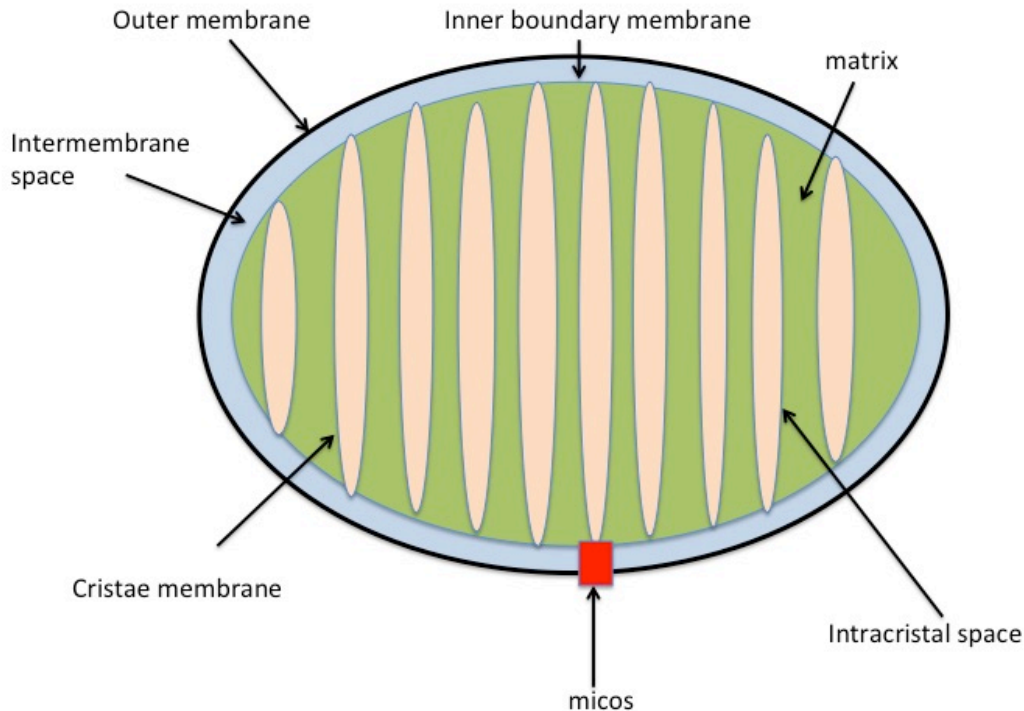
mitochondrial reticulum are Pink 1 and Parkin. Mutations of either of these two proteins along with DRP1 each cause early onset Parkinsons disease, hence the great interest in mitochondrial dynamics of those interested in neurological diseases.

C). INTERNAL STRUCTURE

A typical low-resolution electron micrograph of bovine heart mitochondria is shown below. Such images led to a model in which there was a distinct outer membrane and a convoluted inner membrane surrounding the matrix space. These convolutions were called cristae. The space between the inner and outer membranes was called the intracristal space. Improved electron microscopy techniques have provided a more complex picture.



SCHEMATIC SHOWING THE MITOCHONDRIAL COMPARTMENTS.



There are 3 distinct membranes, the outer membrane, an inner boundary membrane and cristae membrane(s) which are attached to the inner boundary membrane by a specific structure now called MICOS. This means that a mitochondrion contains three separated spaces each with different protein content, the inter-membrane space, the intracristal space and the matrix space.

The protein composition of the outer membrane, boundary membrane and cristae membrane are very different. The cristae membrane is the major seat of the OXPHOS complexes, the inner membrane contains the majority of the translocases such as the ATP/ADP transporter and ion transport proteins, while the outer membrane houses the key proteins involved in apoptosis.

In addition to the morphology changes discussed already, there are other physiologically relevant changes in mitochondrial structure. The relative volume of the intracristal space and matrix space changes with organelle functioning. Under conditions of OXPHOS the volume of the intracristal space is greatly increased to generate what has been called the orthodox state of the mitochondrion. With

reduced levels of OXPHOS, as when cells are using glycolysis for the bulk of their ATP, the cristae are much thinner, more regular and this is called the condensed state.

A result of these volume changes is that the protein concentration within the matrix in the orthodox state is very high to the point that it is essentially a thick gel. The implication is that under such conditions free diffusion of proteins is seriously limited, arguing that proteins of a specific pathway e.g. Krebs cycle enzymes are arranged in complexes so that the product of each reaction is passed directly as substrate to the next protein in the pathway.

Protein analysis has identified recently the structural complex that links the outer membrane, inner boundary membrane, and this is now called MICOS for mitochondrial contact site and cristae organizing system. This complex contains a number of different subunits (including Mic10 and Mic60 also called mitofilin, and Fcj1 respectively), along with three further subunits, which are all integral inner membrane proteins (Mic12, Mic26, and Mic27). Also involved is a peripheral membrane protein (Mic19) that is an important organizing factor. In addition, an inner membrane protein (called Aim24) is required for the integrity of MICOS and several recent studies add other proteins (apolipoprotein O, ApoO-like and QIL1 proteins) to the MICOS complex.

The MICOS complex also interacts with several protein complexes of the outer membrane (TOM and SAM complexes), with the outer membrane channel protein porin (VDAC), and with a component of the mitochondrial fusion machinery. It is evident that the MICOS complex participates in protein translocation into mitochondria although the precise role remains to be worked out.

D). MtDNA, STRUCTURE AND PACKAGING.

It came as a surprise in the 1960s to find that mitochondria have their own DNA (mtDNA). In humans mtDNA is 16kb and encodes 13 protein-encoding genes (all components of the OXPHOS complexes) along with information for several tRNAs. Other organisms have mtDNAs with as few as two protein-coding genes, and as big as that of the mitochondrial genome of *Saccharomyces cerevisiae* with 85,779 bp and encoding two rRNAs and 30 proteins. The mtDNA encoded proteins are made on ribosomes within the organelle that have many of the characteristics of those found in bacteria, and are

different from the cytosolic ribosomes that convert the nuclear genes to proteins.

The DNA strands in mitochondria are not well protected e.g. by chromatin as in the nucleus, but instead are bundled with several DNA-binding proteins into so-called nucleoids. These nucleoids are irregular ellipsoidal in shape and typically contain a single copy of mtDNA encased in the mitochondrial transcription factor TFAM. The average nucleoid diameter is around 220 nm in HepG2 cells. On fragmentation of the mitochondrial reticulum there is one nucleoid per minimal sized fragment. The crystal structure of TFAM shows that it bends mtDNA in a sharp U-turn. Several other proteins are a part, often transiently, of the nucleoid including prohibitin, single-stranded DNA-binding protein, mtSSB, twinkle, pol G, ATAD3 and Lon.

There is very limited repair of mtDNA with the result that mutations readily accumulate. Of considerable importance, unlike the nuclear genome which consists of a paternal and a maternal copy of each, there are anywhere from 20 to several thousand copies of mtDNA in mammalian cells, all maternal in origin. This leads to the concept of heteroplasmy, which typifies many diseases caused by mutations in mtDNA. Heteroplasmy can arise from de-novo mutations of the mtDNA but is much more often inherited by the cells receiving a mixture of normal and mutant mtDNA copies from the egg. Penetrance as it applies to inheritance of the mitochondrial genome thus depends on the copy number of "wild type" mtDNA required to make the needed mitochondrially -encoded proteins for adequate functioning. This threshold is different in different cells depending on their energetic need and extent of reliance on oxidative phosphorylation. Note that damaged mtDNA copies can and are removed as part of the process of mitophagy, the process in which cells remove mitochondria using the autophagic pathway.

Recent studies show that the ERMES complex, which links mitochondria to the ER, localizes with a subset of actively replicating mitochondrial nucleoids and that mitochondrial division is spatially linked to nucleoids. The implication is that ER-associated division serves to link the distribution of mitochondria and mitochondrial nucleoids in cells. Both twinkle and ATAD3 are attached at or close to the mitochondria-ER contact sites with interaction with mitochondrial cholesterol.

E) IMPORT OF PROTEINS INTO MITOCHONDRIA.

The biogenesis of mitochondria involves the coordinated transcription and translation of two genomes: one inside mitochondria and the other the nuclear genome. The vast majority of mitochondrial proteins are encoded by nuclear genes and synthesized on cytosolic ribosomes. These must be targeted to the organelle and then taken up and sorted to the correct compartment. Proteins destined for mitochondria are made as precursors that have both a targeting signal to direct them to receptors on the mitochondrial surface and intramitochondrial sorting signals.

At present five major classes of precursor proteins have been identified, each of which uses a different pathway of translocation into mitochondria. The protein translocase of the outer membrane (TOM) is the major mitochondrial entry site. Around 60% of all mitochondrial precursor proteins are synthesized with amino-terminal presequences that form positively charged amphipathic α -helices. These are translocated through an outer membrane channel formed by Tom40 and are transferred to the pre-sequence translocase of the inner membrane (TIM23 complex). Hydrophilic preproteins are imported into the matrix with the help of the presequence translocase-associated motor (PAM). Preproteins destined for the inner membrane have a hydrophobic sorting signal behind the positively charged matrix-targeting signal and are released into the lipid phase of the inner membrane. Once properly located the targeting signal is cleaved off by the mitochondrial processing peptidase (MPP). Some multi-spanning inner membrane proteins have an internal targeting signal rather than the N-terminal one, which is not removed during import, but remain part of the mature mitochondrial protein. Such proteins are initially translocated by the TOM complex and bind to soluble TIM chaperones present in the intermembrane space. These guide them to the carrier translocase of the inner membrane (TIM22 complex) that inserts them driven by the membrane potential. Proteins destined for the intermembrane space have their own import machinery. They are recognized by the TOM apparatus and then handed to the so-called mitochondrial intermembrane space import and assembly apparatus (MIA), which can recognize a cysteine containing signal and insert disulphide bonds into the protein.

The mitochondrial outer membrane contains two types of transmembrane proteins, beta barrel proteins and those with alpha-helical transmembrane segments. The precursors of beta-barrel proteins are initially recognized and translocated by the TOM complex before being transferred to the intermembrane space side where they bind to the small TIM chaperones. The insertion of proteins into the outer membrane is mediated by a sorting and assembly machinery called SAM. As yet the insertion of the alpha helical transmembrane proteins is only poorly understood but also appears to involved the SAM complex.

As polypeptides as delivered to their residence sites in the different mitochondrial compartments, many associate with partner polypeptides and with prosthetic groups to produce complexes. In the case of the respiratory chain complexes and ATP there is the added complication in that these are made up not only of nuclear encoded proteins but ones coded on mtDNA and made on mitochondrial ribosomes. The mitochondrially-made polypeptides are for the most part transferred into the cristae membrane by the cytochrome oxidase activity associated translocase (OXA). The assembly of complexes I,III and IV as well as the ATP synthase is orchestrated by multiple assembly factors that are specific for each complex. These maintain polypeptides in partly folded states during insertion of prosthetic group and stabilize partly assembled complexes to ensure correct protein-protein interactions. Form follows function for mitochondria.

SELECTED READING.

Shaping the dynamic mitochondrial network. Lackner LL. 2014 BMC Biol.12:12-35

Mitochondrial dynamics and inheritance during cell division, development and disease. Mishra P, Chan DC Nat Rev Mol Cell Biol. 2014 10. 634-46.

Transporting mitochondria in neurons. Course M.M Wang X. (2016) F1000Res. 1735 eCollection

Mitochondrial fusion and fission in cell life and death. Westermann B. Nat.Rev.Mol.Cell.Biol. 2010; 11:872-84.

Linking mitochondrial dynamics to mitochondrial quality control.

Haroon S. Vemuist M. Curr Opin Genet Dev 2016. 38. 38-74.

The emerging network of mitochondria-organelle contacts. Murley A, Nunnari J. Mol Cell. 2016 .61. 648-53

The ERMES complex and ER –mitochondria connections. Michel AH. Kornmann B. Biochem. Soc. Trans. (2012); 40: 445–450

Mitochondrial Dynamics and Metabolic Regulation. Wai T, Langer T. Trends Endocrinol Metab. 2016. 2):105-17.

The internal structure of mitochondria. Frey TG, Mannella CA. Trends Biochem Sci. 2000 Jul;25(7):319-24

Making proteins in the powerhouse. Hailberg BM. Larsson NG. Cell Metab. 2014; 20: 226-240

Mitochondrial nucleoids maintain genetic autonomy but allow for functional complementation. Gilkerson RW. Schon EA, Hernandez M. Davidson MM. J Cell Biol 2008;181, 1117

Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. Sato M. Bichim. Biophys. Acta 2013;1833: 1979-84

Role of MINOS in mitochondrial membrane architecture and biogenesis. van der Laan M, Bohnert M, Wiedemann N, Pfanner N. Trends Cell Biol. 2012. 22. 185-92.

PART2: MITOCHONDRIAL FUNCTIONING IN INTERMEDIARY METABOLISM.

A). POST-TRANSLATIONAL CONTROL OF MITOCHONDRIAL METABOLISM.

Mitochondria are integral in the metabolism of carbohydrates, fats and amino acids in that they house all of the components of oxidative phosphorylation, Krebs cycle, urea cycle, fatty acid oxidation and key proteins of ketogenesis, triacylglycerol synthesis and gluconeogenesis. The basic enzymology of all of these pathways is now well understood and can be found in standard textbooks. Issues

that are the focus of more recent study relate to the detailed mechanisms of several specific proteins in the pathways, particularly in oxidative phosphorylation, and to the regulation of intermediary metabolism in response to cellular events.

Intermediary metabolism as a whole is integrated to match the overall energy production and cell constituent synthesis with the prevailing cell conditions such as substrate availability, hormonal action and stress events. This integration involves feedback by metabolites and control by a set of signaling pathways that change the levels of specific proteins and/or induce post-translational modifications such as phosphorylation/dephosphorylation and acetylation/deacetylation.

ATP, NAD and acetyl CoA each can stimulate or inhibit cellular processes either by direct effect on an enzyme of a particular pathway, or by modulating signaling pathways. For example the levels of ATP are constantly monitored by the AMP kinase, which in turn regulates glucose utilization and energy dependent processes via the AKT/mTor signaling pathway. All three metabolites also directly affect the levels of metabolic enzymes and components of other cellular processes by inducing epigenetic effects in the nucleus.

Many proteins in mitochondria are subject to phosphorylation/dephosphorylation reactions at Thr, Ser and Tyr residues via mitochondrial kinases and phosphatases. The phosphoproteome of the organelle in humans has been extensively characterized. Such modifications are particularly important in the control of oxidative phosphorylation as well as in lipid metabolism. The acetylation of lysine residues by several acetylases in the organelle and deacetylation by a set of proteins, collectively called sirtuins, also regulates oxidative phosphorylation, as well as controlling the enzymes of the urea cycle, fatty acid oxidation and antioxidant proteins. At least 3 of the 7 well-defined sirtuins, i.e. 3, 4 and 5, are mitochondrial in location. These deacetylases has drawn widespread attention because of suggestions that their modulation may alter longevity. For example there is considerable work on the natural product resveratrol, an inhibitor of sirtuins, as an anti-aging compound.

The brief descriptions below of selected metabolic enzymes of mitochondria shows both the complexity and yet the progress made

recently toward understanding structure function relationships.

B). Pyruvate dehydrogenase complex (PDH) is central to glucose metabolism, converting pyruvate to acetylCoA (from a 3 carbon to two carbon compound) with release of CO₂ and production of NADH. This large 8KDa complex links and regulates the flow of energy in cells by determining when pyruvate should be used for oxidative phosphorylation versus "neutralized" to lactic acid to allow continued glycolysis. At the same time the control of acetyl Co-A directly influences fatty acid oxidation and production of ketone bodies.

The PDH complex is an example of the way that enzymes of a metabolic pathway are often organized in mitochondria. In this case the 3 enzymes required to complete the conversion of pyruvate to acetyl coA are together in a tight complex. These enzymes, pyruvate dehydrogenase, dihydrolipoyl transacetylase and dihydrolipoyl dehydrogenase respectively, work in sequence within the complex so that the product of the first reaction is handed off as substrate of the second etc. There is evidence of tight association of enzymes in other metabolic pathways as tight complexes. This includes the Krebs cycle enzymes the urea cycle enzymes, the enzymes of fatty acid oxidation and even the 4 respiratory chain complexes, which are now thought to exist in the mitochondrial inner membrane as supercomplexes.

PDH also provides an example of the control of intermediary metabolism by multiple post-translational modifications. The enzyme undergoes reversible phosphorylation by PDH kinases (PDK). There are four known isoforms of PDKs that are distributed differently in tissues. Their expressions are regulated differently by factors such as starvation, hypoxia, glucose utilization and oxidation of fatty acids in various tissues. In addition, transcription regulators such as PGC-1 α , retinoic acid and the glucocorticoid receptor are involved.

De-phosphorylation to restore the activity of PDH is catalyzed by PDH phosphatases (PDP). There are two known isoforms of PDPs, which are expressed differently in various tissues; PDP1 is present in high levels in skeletal muscle and PDP2 in liver and adipocytes. PDH is also acetylated by an as yet unknown acetylase and deacetylated by sirtuin 3 as part of the control of reactivity. This acetylation/deacetylation is different in different tissues, further optimizing pyruvate conversion to acetyl CoA in response to cell conditions.

Finally, PDH is one of most often causes of so-called mitochondrial diseases. It is an X-linked disease. Most of the mutations in PDH detected to date prevent proper assembly of the complex, and present as encephalopathies or myopathies with lactic acidosis.

C). Succinate dehydrogenase (SDH), complex II of the respiratory chain and a component enzyme of the Krebs cycle, shows similar features to PDH in terms of associations with other proteins (Krebs cycle enzymes) to facilitate substrate/product interchange, in terms of control of functioning by post –translational modifications, and in terms of mutations of the enzyme causing disease. In addition, studies of SDH have provided important insight into the role of energy metabolites in causing cell transformation. SDH deficiencies along with those of fumarase, both Krebs cycle enzymes, leading to increased concentrations of succinate or fumarate, cause paragangliomas and adrenal or extra-adrenal pheochromocytomas. The mechanism of this cell transformation is now understood. Accumulation of fumarate and succinate (so-called oncometabolites) in mitochondria and in the cytosol impairs the enzymatic activity of several alpha ketoglutarate-dependent dioxygenases. These include JMJD3, which regulates chromatin structure; PHD3 which is involved in promoting neuronal apoptosis in response to NGF withdrawal; and PHD2 which primarily regulates HIF α stability

D). The ATP synthase is ubiquitous to all organisms. It is in the plasma membrane of prokaryotes along with respiratory chain proteins. In eukaryotes it is located predominantly in the mitochondrial cristae (but in some circumstances it is also found in the plasma membrane as discussed later). This enzyme can be seen in electron micrographs and more recently in X ray structural studies to be organized in 3 parts, and F1 part in the matrix of mitochondria attached to an F0 part in the cristae membrane by a stalk region now known to contain 2 distinct stalks. In all forms of the enzyme, the F1 part contains 5 different subunits, alpha3, beta3, gamma, delta and epsilon. The F0 part contains 3 different subunits a, b2 and c 12 respectively in mammals. There are 3 catalytic sites per F1part, at the interfaces of the 3alpha/beta pairs respectively. The c subunits are arranged as a ring. The F1 part and F0 parts are connected by two stalks, one (the rotor includes gamma and epsilon, the other the stator contains the b subunit pair). The eukaryote ATP synthase is

more complex than that of prokaryotes. The mammalian enzyme has the additional subunits F6, d, e, A6L, f, g and OSCP with most of these in the so-called stalk region of the complex. The full X ray structure of the yeast ATP synthase has been obtained, while the structure of most of the individual segments of the ATP synthase of mammals have been determined and modeled into the unit complex.

The structure of the ATP synthase is an important clue to the workings of this complex as first recognized by Boyer in his alternating site hypothesis, and made clearer as the detailed X-ray data emerged. First the three catalytic sites are in three different conformations. These are specified by the different interaction of the alpha-beta pairs with the central gamma subunit. Remarkably, the gamma subunit rotates through 120 degree steps driven by ATP hydrolysis in one direction and by proton translocation in the reverse direction i.e. during ATP synthesis. This rotation has been shown elegantly in real time by the studies of Yoshida, Kinosita and their groups. The delta subunit and additional subunits in mammalian ATP synthase act as a stator or second stalk to facilitate this rotation.

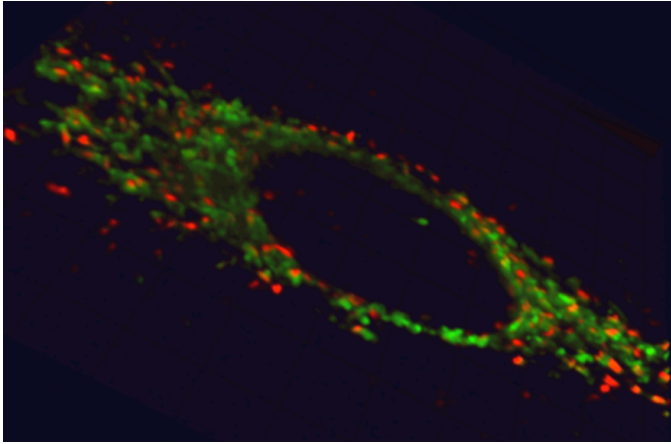
There are additional features of the ATP synthase that have changed thinking about mitochondrial functioning and broader cell homeostasis. First it is now clear that this enzyme is involved in programmed cell death or apoptosis as the mitochondrial permeability transition pore. At one time the MPT was considered to be complex of the VDAC, adenylate kinase in the intermembrane space, the peripheral benzodiazepine receptor, cyclophilin D and the adenine nucleotide translocator. It now seems that the MPT is the dimeric form of the ATP synthase. The evidence comes from reconstitution experiments with purified ATP synthase (free of the other components above). In lipid bilayers, and on addition of a benzodiazepine derivative known to induce MPT, the enzyme dimer forms a channel with many of the same features seen on MPT opening in vitro including Ca⁺⁺ sensitivity. Additionally, it has been shown that the c subunit ring of the complex alone forms a channel that is Ca⁺⁺ sensitive and partly blocked by the beta subunit of the F1 part.

As would be expected, the functioning of the mitochondrial ATP synthase is under tight control. An inhibitor protein, IF1, controls

whether the enzyme acts as an ATP synthase or an ATP hydrolase. IF1 binds to the F1 part of the ATP synthase. It is a small, predominantly alpha helical, polypeptide that interacts at the interface of an alpha and a beta subunit by associations with 5 subunits of the enzyme in total. IF1 is a dimer at pH below 6.5 when it binds to the ATP synthase to inhibit ATP hydrolysis. At higher pH the inhibitor progressively aggregates into a tetramer, which can no longer bind to the enzyme. In this way IF1 responds to the electron transfer-derived proton gradient that turns the matrix space acidic when OXPHOS is favored, thereby preventing wasteful ATP hydrolysis and indirectly promoting glycolysis. Interestingly, IF1 is highly expressed in cancer cells, a feature that is now considered a part of the Warburg effect. In forming a dimer, IF1 helps to stabilize the ATP synthase dimer in the cristae membranes. Down regulation of IF1 levels by RNAi induces increased apoptosis in response to cell stressors.

One further and surprising finding related to the ATP synthase is that it is not exclusive to mitochondria but can be found under some conditions on the plasma membrane of cells. It may be that other mitochondrial enzymes can occupy the plasma membrane e.g the respiratory chain complexes but the ectopic location of the ATP synthase is the most studied example.

The presence of the “mitochondrial” ATP synthase on the plasma membrane raises the interesting, and as yet unanswered question, of how it gets there. Assembly of a functional complex requires subunits encoded on mtDNA and synthesized in mitochondria. The most likely way is that there is fusion of mitochondria with the cell membrane and some re-sorting of proteins during this process.



Ectopic ATP synthase, shown for HepG2 cells. Green fluorescence shows labeled mitochondria, red fluorescence is surface F1F0 reacted with a labeled antibody to the enzymes beta subunit.

The ectopic ATP synthase, has been shown to have several interesting functions that go beyond its enzymatic activity to control of ATP and ADP in the cellular milieu.

It is the receptor for HDL and induces uptake of these particles into liver, an important role in cholesterol control.

It acts to control pH in the cytosol allowing cancer cells which have high levels of the enzyme on the plasma membrane to survive as the milieu gets more acid.

It is involved in blood pressure control. One of the subunits, CF1 is released by sheer stress to affect prostaglandin functioning.

Evidence has been presented that the ectopic ATP synthase binds beta amyloid protein.

There is data to indicate that it is a receptor for HIV virus.

SELECTED READING.

The mitochondrial side of epigenetics. Castegna A1, Iacobazzi V2, Infantino V3 Physiol Genomics. 2015. 47. 299-307.

Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. Tretter L, Patocs A, Chinopoulos C. Biochim Biophys Acta. 2016. 857.1086-101

A quantitative map of the liver mitochondrial phosphoproteome

reveals posttranslational control of ketogenesis. Grimsrud PA, Carson JJ, Hebert AS, Hubler SL, Niemi NM, Bailey DJ, Jochem A, Stapleton DS, Keller MP, Westphall MS, Yandell BS, Attie AD, Coon JJ, Pagliarini DJ. *Cell Metab.* 2012 Nov 7;16(5):672-83.

Mitochondrial sirtuins in the regulation of mitochondrial activity and metabolic adaptation. Lombard DB, Tishkoff DX, Bao J. *Handb Exp Pharmacol.* 2011;206:163-88

Mitochondrial protein acetylation as a cell-intrinsic, evolutionary driver of fat storage: chemical and metabolic logic of acetyl-lysine modifications. Ghanta S, Grossman RE, Brenner C. *Crit Rev Biochem Mol Biol.* 2013 ; 48: 561–5741)

Unearthing the secrets of mitochondrial ROS and glutathione in bioenergetics. Mailloux RJ, McBride SL, Harper ME. *Trends Biochem Sci.* 2013;38:592-602.

The Yin and Yang of redox regulation. Olsen LF, Issinger OG, Guerra B. *Redox Rep.* 2013;18:245-52.

The pyruvate dehydrogenase complexes: structure-based function and regulation. Patel MS, Nemeria NS, Furey W, Jordan F. *J Biol Chem.* 2014. 289. 16615-23.

Ecto-F₁-ATPase: a moonlighting protein complex and an unexpected apoA-I receptor. Vantourout P1, Radojkovic C, Lichtenstein L, Pons V, Champagne E, Martinez LO. *World J Gastroenterol.* 2010;16:5925-35

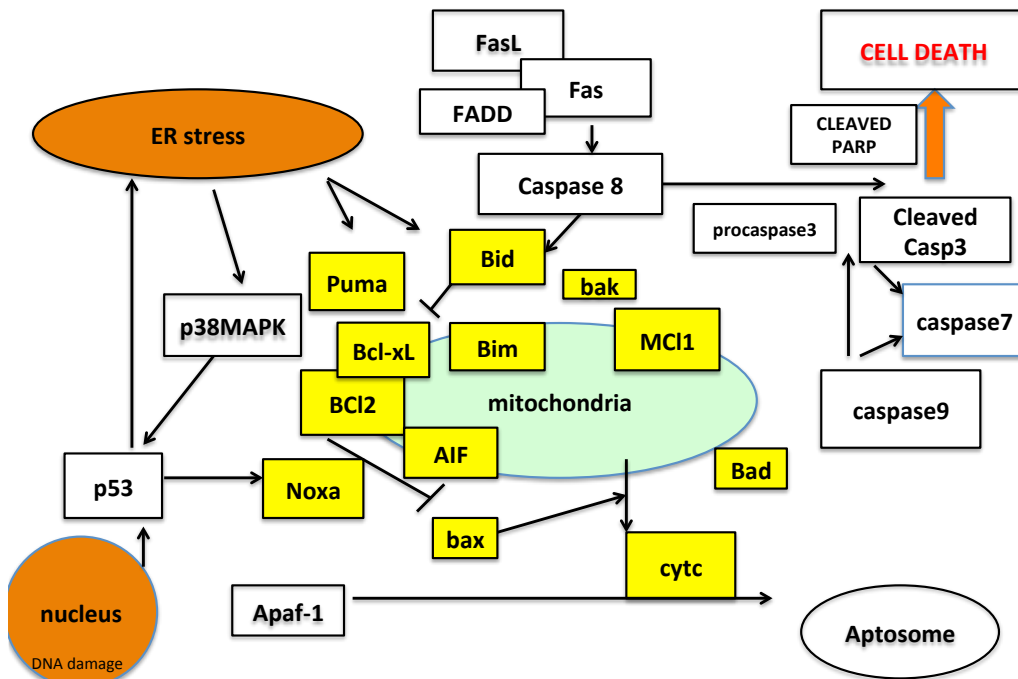
Dimers of mitochondrial ATP synthase form the permeability transition pore. Giorgio.V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick G, Petronilli P, Zoratti, M. Szabó I Lippe G, Bernardi P. *Proc Natl Acad Sci U S A.* 2013; 110: 5887–5892.

Understanding structure, function, and mutations in the mitochondrial ATP synthase. Xu T, Pagadala V, Mueller DM. *Microb Cell.* 2015. 2. 105-125.

PART 3) MITOCHONDRIA IN APOPTOSIS

As well as acting in intermediary metabolism, mitochondria have a second key cellular function: they are the central executioners in the process of programmed cell death or apoptosis.

Apoptosis can be signaled from outside the cell by signaling molecules and growth factors as in development of tissues and organs (extrinsic), or from within the cell in response to various cell stress events (intrinsic). Intrinsic apoptosis can be a response to ER stress (as shown in the figure) or from mitochondrial dysfunction, DNA modifications, loss of energy or other substrate molecules.



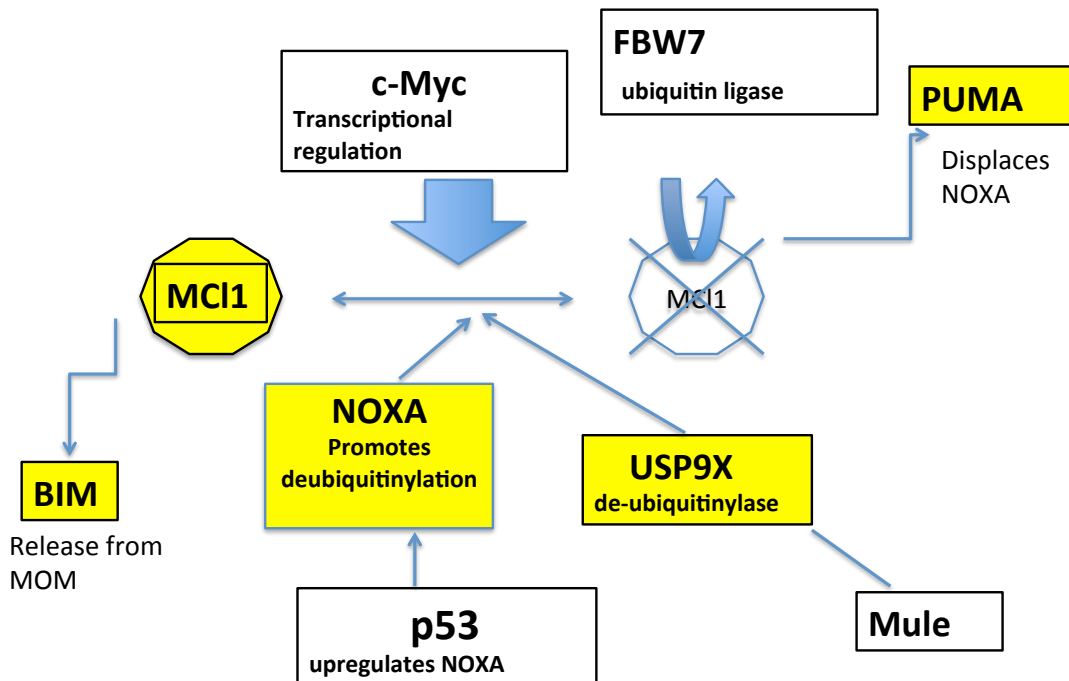
Key proteins in apoptosis. Those in yellow are mitochondrial proteins.

Mitochondria play an important role in the cell death process when initiated externally or internally. The key is that in healthy cells the mitochondrial outer membrane contains or has attached a set of anti-apoptotic proteins that protect the cell from death. During apoptosis these are neutralized via altered interactions and/or proteolytic digestion by a set of pro-apoptotic proteins. The outcome of this is release of molecules from mitochondria, specifically cytochrome c,

AIF, endonuclease G, Smac and OMI/HtrA2. The release of cytochrome c induces formation of the apoptosome, which in turn activates a caspase cascade that leads to cleavage of proteins and DNA and further degradation of these fragments for uptake into macrophages and other inflammatory cells.

The key proteins orchestrating apoptosis belong to the Bcl-2 family. The prototype member of this family, Bcl-2 itself, was initially identified in a common form of B-cell lymphoma, where a chromosome translocation causes overproduction of the Bcl-2 protein. The high levels of Bcl-2 promote cancer by inhibiting apoptosis, thereby prolonging cell survival.

More than 20 members of the Bcl-2 family have been identified, some pro-apoptotic and some anti-apoptotic, all defined by the presence of one to four Bcl-2 homology (BH) domains. The proapoptotic Bcl-2 proteins can be further subdivided into two subfamilies based on the sharing of BH domains. BH multidomain proteins, such as Bax and Bak, are the triggers of apoptosis, most likely as a result of their ability to form pores in the outer mitochondrial membrane. The other subfamily, the BH3-only proteins shown in the figure, which contain only the BH3 domain, act as upstream regulators by controlling the allosteric activation of the gatekeepers Bax and Bak. In healthy cells, BH3-only proteins are either not expressed or are inactive, until rapidly activated following exposure to cellular stresses. Different types of stresses activate distinct sets of BH3-only proteins, suggesting that BH3-only proteins act as essential sensors of different death stimuli.



Apoptosis: regulation of the levels of the anti-apoptotic protein Mcl-1.

The apoptotic degradation of Mcl1, as well as its turnover in non-apoptotic cells, is regulated by the counteracting activities of the Ub ligase ARF-BP1/Mule and the deubiquitinase Usp9x. Expression levels of ARF- BP1/Mule and Usp9x appears to be critical for the maintenance of proper cellular balance of anti- and pro-apoptotic proteins, and contributes to cell sensitivity to apoptosis. Turnover of other mitochondria-associated Bcl-2 family proteins, including Bax and Bcl-2 is also under Ub/proteasome control. Bax, a pro- apoptotic Bcl-2 family protein, is mainly localized in the cytosol in an apoptotically inactive form, but moves to mitochondria upon pro-apoptotic trigger- induced change in its conformation. Proteasome-dependent degradation of Bax occurs specifically on mitochondria, suggesting that the apoptotic conformation of Bax might be recognized by the Ub conjugation machinery, and serve as a degradation signal preventing the accumulation of potentially dangerous apoptotically-active Bax in healthy cell mitochondria. The protein Omi/HtrA2, once released from mitochondria along with

cytochrome c, promote apoptosis by counteracting the inhibitor-of-apoptosis proteins (IAPs), which comprise a family of endogenous caspase inhibitors. Other proteins released from mitochondria, i.e. apoptosis-inducing factor (AIF) and endonuclease G promote cell death in a caspase-independent manner by inducing chromatin condensation and DNA degradation. Thus, if for some reason cells do not activate caspases after MOMP, these mediators might still ensure that cell death proceeds. Phagocytic uptake of apoptotic cells, the last step of apoptosis, is identified by a phospholipid asymmetry and externalization of phosphatidylserine on the surface of apoptotic cells.

SELECTED READING.

Mitochondria-Judges and Executioners of Cell Death Sentences.

Bhola PD, Letai A. Mol Cell. 2016 . 3. 695-704.

Mitochondria and apoptosis: emerging concepts.

Li MX, Dewson G.

F1000Prime Rep. 2015. 7:42. eCollection

How do BCL-2 proteins induce mitochondrial outer membrane permeabilization?

Chipuk JE, Green DR. Trends Cell Biol. 2008.18. 157-6.

Ubiquitylation in apoptosis: a post-translational modification at the edge of life and death.

Vucic D, Dixit VM, Wertz IE.

Nat Rev Mol Cell Biol. 2011, 12. 439-52.

Parkin sensitizes toward apoptosis induced by mitochondrial depolarization through promoting degradation of Mcl-1.

Carroll RG, Hollville E, Martin SJ. Cell Rep. 2014. 9. 1538-53.

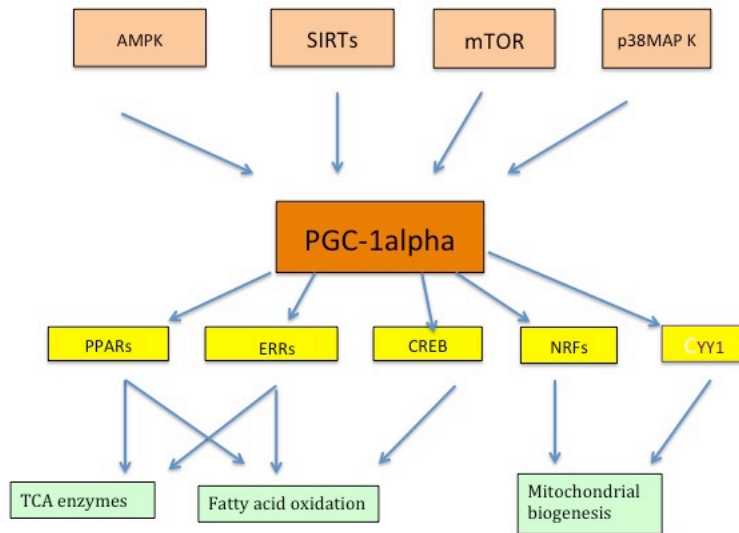
PART4. CONTROL OF MITOCHONDRIAL LEVELS: PRODUCTION VERSUS DESTRUCTION.

The total level of mitochondria in a cell is continually balanced by competing biosynthesis and mitophagy. Addition of proteins and lipids during biosynthesis uses existing mitochondria as a template.

A). BIOGENESIS.

Multiple intrinsic cellular signaling pathways monitor mitochondrial functioning and trigger organelle biogenesis. These include the AMPkinase pathway which monitors ATP levels, the calcium/calmodulin-dependent kinase and p38 mitogen-activated kinase which monitors Ca⁺⁺ homeostasis, mTOR a moderator of overall cell homeostasis, the cAMP/PKA pathway, ROS signaling and NO and CO₂ levels. These stimuli activate the synthesis of mitochondrial genes, both on mtDNA and nuclear DNA through a series of transcription factors mostly under control of a family of co-activators. (PGC-1alpha, PGC-1beta and PRC). Signaling through PGC-1 α and PGC-1 β is sufficient to increase total mitochondrial mass, reactive oxygen species scavenging enzymes, oxidative phosphorylation components, mitochondrial metabolic pathways, protein import complexes, proteins involved in fission and fusion, and the levels of mitochondrial sirtuins. The key transcription factors involved are NRFs, ERRs, and YY1.

NRF-1 controls the expression of a significant number of the proteins that make up the five respiratory complexes, as well as proteins integral to mitochondrial import and heme biosynthesis. NRF-1 is also able to integrate nuclear control of the transcriptional and replicative activity of the mitochondrial genome through the direct modulation of transcription factor A mitochondrial (TFAM) and transcription factor B proteins (TFBs) gene expression



ERRs = estrogen receptor related proteins

Control of mitochondrial biogenesis by PGC-1alpha.

NRF-2 is also able to regulate the expression of proteins in the electron transport chain. The differential regulation of NRF1 and NRF2 is not completely understood but phosphorylation of these factors can alter their transcriptional activities.

Thyroid hormone receptors (THRs) are another set of factors that promote mitochondrial biogenesis in relation to tissue-specific function. This includes mitochondrial-driven thermogenesis that occurs in brown fat during the adaptation to lower temperatures. In some instances, THRs directly drive the transcription of nuclear-encoded genes, whereas, in others, the effects can occur indirectly through the thyroid hormone-mediated up-regulation of NRF-1.

Also involved are estrogen-related receptors ERR- α , ERR- β , and ERR- γ . These receptors have no known endogenous ligands and are primarily expressed in tissues with high oxidative metabolism capacities. They are involved in transcriptional control of enzymes of oxidative phosphorylation, fatty acid oxidation, TCA cycle, and factors regulating mitochondrial fusion/fission.

Two other transcription factors that orchestrate mitochondrial biogenesis are CREB, a cAMP activated transcription factor that can promote the expression of several mitochondrial genes, including those for complex IV and enzymes involved in the β -oxidation pathway.

Finally the overall process is regulated by a set of NAD⁺-dependent protein deacylases, the sirtuins. Mammals have 7 sirtuins, SIRT1, SIRT6, and SIRT7 are nuclear proteins, SIRT3, SIRT4, and SIRT5 are imported into mitochondria, and SIRT2 is principally cytoplasmic. Through their deacylation activities, SIRT1, SIRT3, SIRT4, and SIRT5 have profound effects on mitochondrial function. SIRT1 deacetylates several key transcription factors that result in the up-regulation of numerous genes involved in mitochondrial respiration. In addition, as discussed already, sirtuins deacetylate numerous metabolic enzymes to govern their specific activity. Changes in SIRT3 activity have been shown to be an important determinant in the acetylation state of mitochondrial in response availability

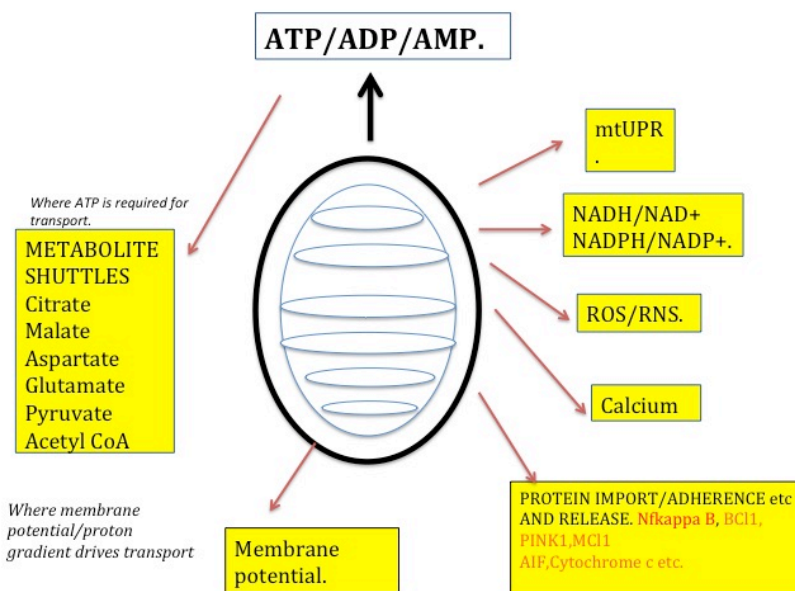
B). MITOCHONDRIAL DESTRUCTION: MITOPHAGY.

Mitochondrial quality control is essential for the health of a cell and functioning is constantly being evaluated. Organellar dysfunction can take many forms and result from multiple causes including genetic defects, stress events due to lack of substrate, and toxic insults such as the production of ROS by the mitochondrial electron transfer chain. Altered protein import and failure of proteins to fold properly within the organelle is another stress-related event.. The defective functioning of mitochondria is signaled to the cytosol through an altered ratio of ATP to ADP, Ca⁺⁺ accumulation inside the mitochondria, the excess ROS production, altered metabolite shuttling e.g. of citrate, malate etc. and the loss of membrane potential This signaling from mitochondria to alter metabolism more globally is called the retrograde signaling response.

The response to mitochondrial dysfunction can take the form of organelle repair such as occurs when there is a build up of unfolded proteins, in the same way as when there is an accumulation of unfolded proteins in the ER. The key protein in the mitochondrial unfolded protein response is the molecular chaperone HSP60, the

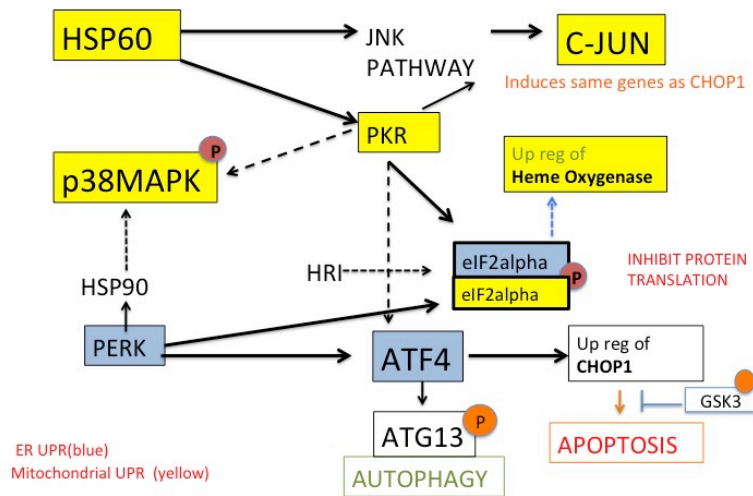
levels of which signal to the JNK pathway and PKR. In the event of a build up of unfolded proteins, a response is mounted in which protein synthesis is temporarily suspended and the unfolded proteins are removed by mitochondrial AAA proteases including Lon. A second cleansing mechanism involves vesicular transport of defective mitochondrial proteins to the lysosome for degradation.

THE MITOCHONDRIAL RETROGRADE SIGNALING



Finally, whole mitochondria (fragmented form) can be removed. This degradation of mitochondria by a process called mitophagy is of particular importance in the removal of male sperm mitochondria on fertilization of an egg. Mitophagy is also involved in the generation of erythrocytes, which lose their mitochondria before maturation.

CELLULAR UNFOLDED PROTEIN RESPONSE



Mitophagy can be triggered by a receptor response involving several outer membrane receptor proteins including NIX/BNIP3L, BNIP3 and FUNDC1 in mammalian systems. These proteins have a classic motif to bind directly to LC3 and initiate mitophagy. This receptor-mediated mitophagy is regulated by reversible protein phosphorylation. Thus phosphorylation of FUNDC1 by Src kinase, ULK1, and CK2 prevent mitophagy. In hypoxia, and with loss of membrane potential, the mitochondrial phosphatase PGAM5 dephosphorylates FUNDC1 to activate the process.

Mitophagy can also be initiated by the PINK1/parkin reaction in two different ways. PINK1 is imported to the inner mitochondrial membrane where the TIM complex-associated protease, mitochondrial MPP, cleaves off the mitochondrial targeting sequence. Thereafter PINK1 is also cleaved by the inner membrane presenilin-associated rhomboid-like protease PARL and ultimately proteolytically degraded. Loss of membrane potential in damaged mitochondria prevents the import of PINK1 leading to the accumulation of unprocessed PINK1 on the outer membrane surface where it associates with the TOM complex, and recruits cytosolic Parkin. This interaction promotes mitophagy. Parkin, presumably through its ubiquitin–ligase activity, causes the degradation of Miro and Mitofusin. Loss of these two proteins, quarantines damaged mitochondria and promote their autophagosomal engulfment.

Parkin- mediated hyper-ubiquitination of the mitochondrial outer membrane provides a second approach to initiating mitophagy. Thus parkin is recognized by a set of ubiquitin-binding adaptors, such as p62, HDAC6, that may recruit damaged mitochondria by direct interaction with autophagosomal protein L3.

BCL-2 and BCL-XL two anti-apoptotic also regulate mitophagy. These two proteins bind the essential autophagy protein BECLIN-1 to prevent its activation, Disruption and dissociation of BCL allows BECLIN-1 activation by AMBRA1. In order to facilitate phagophore formation, AMBRA1 translocates to the mitochondria and ER after initiation of mitophagy.

SELECTED READING.

Regulation of mitochondrial biogenesis through TFAM-mitochondrial DNA interactions: Useful insights from aging and calorie restriction studies. Picca A, Lezza AM. Mitochondrion. 2015.25:67-75.

Regulation of PGC-1 α , a nodal regulator of mitochondrial biogenesis. Fernandez-Marcos PJ, Auwerx J. Am J Clin Nutr. 2011. 93. 884S-90.

Transcriptional integration of mitochondrial biogenesis. Scarpulla RC, Vega RB, Kelly DP. Trends Endocrinol Metab. 2012(23).459-66.

Dual control of mitochondrial biogenesis by sirtuin 1 and sirtuin 3. Brenmoehl J, Hoeflich A. Mitochondrion. 2013. 6. 755-61.

Mitophagy plays a central role in mitochondrial ageing. Diot A, Morten K, Poulton J. Mamm Genome. 2016. 27. 381-95.

Mitochondrial remodeling: Rearranging, recycling, and reprogramming. Gottlieb RA, Bernstein D. Cell Calcium. 2016. 60. 88-101

The three 'P's of mitophagy: PARKIN, PINK1, and post-translational modifications. Durcan TM, Fon EA. Genes Dev. 2015. 29. 989-99.

PART 5. MITOCHONDRIA IN DISEASE

A). OXIDATIVE PHOSPHORYLATION DEFICIENCIES.

Mitochondrial disorders due to OXPHOS deficiencies are the most common inborn errors of metabolism with an incidence of 1 in 5,000. This compares with an incidence of 1 in 10,000 for fatty acid oxidation disorders and 1 in 15,000 for phenylketourea. The first biochemical evidence of a mitochondrial dysfunction: specifically a finding of loose coupling of oxidation and phosphorylation in a patient, was reported in 1962 by Luft and colleagues. Since then the number of the genotypes and breadth of phenotypes that are associated with OXPHOS disorders has expanded greatly. The genotype of electron transfer complexes I,III,IV and the ATP synthase is unique in that it can involve mutations in either mtDNA encoded or nuclear encoded genes. Complex I has 7 subunits encoded on mtDNA and 38 subunits encoded in the nucleus. Complex III has one mtDNA-encoded subunit and 10 nuclear encoded subunits, cytochrome c oxidase has three and 10 respectively and the ATP synthase has 2 and 14. Each of the OXPHOS complexes is assembled at the mitochondrial inner membrane by a set of nuclear encoded proteins acting as assembly factors through their role in inserting subunits in a specific order and adding prosthetic groups as the assembly is completed. Mutations in these assembly factors represent a significant proportion of the cases of OXPHOS defects.

Other mutations in mtDNA that cause OXPHOS deficiency include ones in the tRNAs encoded on mtDNA, in proteins responsible for mtDNA replication and translation, for protein folding and transport into the organelle, in lipid biosynthesis and specifically in a protein called tafazzin (which encodes an enzyme involved in cardiolipin synthesis and produces Barth syndrome).

The common biochemical phenotype induced by all of these different mutations is reduced ATP synthesis, increased oxidative stress, and often, uncoupling of the membrane potential. The physiological phenotype is much more varied. Among the conditions described are Lebers hereditary optic neuropathy (LHON), mitochondrial encephalopathy, lactic acidosis and stroke like episodes (MELAS),

Kearn-Sayre syndrome, Leighs disease, progressive external ophthalmoplegia, and neuropathy, ataxia and retinitis pigmentosa (NARP). Some mutations induce cardiomyopathy and diabetes. It is not uncommon for the same mutation to cause different conditions in different patients. Even when the same condition is diagnosed there is variability of phenotype. A recent study of over 300 patients with the mtDNA mutation 8344A>G gave a diverse clinical picture with myoclonus, muscle weakness and ataxia in around 40% of patients; generalized seizures and hearing loss in around 30%; cognitive impairment, multiple lipomatosis, neuropathy, and exercise intolerance in 20%; and increased creatine kinase levels, ptosis/ophthalmoparesis, optic atrophy, cardiomyopathy, muscle wasting, respiratory impairment, diabetes, muscle pain, tremor, migraine in about 10%.

There are 2 key reasons for this diversity of physiological phenotype. First is the combination of maternal inheritance of mtDNA (mitochondria from the sperm are destroyed in the fertilized egg as discussed already) along with the so-called bottleneck in which the number of copies of mtDNA is dramatically reduced and then segregated stochastically during embryogenesis. In this way the distribution of mutant mDNA in different tissues is random and depends on the position of normal and mutant copies with respect to cleavage patterns in the embryo. The second consideration is the threshold for dysfunction. The phenotype is dependent on the level of oxidative phosphorylation needed for a particular tissue. Thus the percent mutation mtDNA at which cells are unable to generate sufficient energy is a determinant of cell maintainance.

SELECTED READINGS.

*Mutations causing **mitochondrial disease**: What is new and what challenges remain? Lightowlers RN, Taylor RW, Turnbull DM. Science. 2015. 349. 1494-9.*

Preventing the transmission of mitochondrial DNA disorders using prenatal or preimplantation genetic diagnosis. Smeets HJ, Sallevelt SC, Dreesen JC, de Die-Smulders CE, de Coo IF. Ann N Y Acad Sci. 2015. 1350:29-36.

Human mitochondrial DNA replication machinery and disease.

Young MJ, Copeland WC. *Curr Opin Genet Dev.* 2016.38. 52-62.

Mitochondrial cytochrome c oxidase deficiency.

Rak M, Bénit P, Chrétien D, Bouchereau J, Schiff M, El-Khoury R, Tzagoloff A, Rustin P. *Clin Sci (Lond).* 2016. 130. 393-407

B). FATTY ACID OXIDATION DISORDERS

The degradation of fatty acids by mitochondrial fatty acid β -oxidation (FAO) is a key metabolic pathway for energy homeostasis in organs such as the liver, heart and skeletal muscle. During fasting, when glucose supply becomes limited, FAO is of particular importance. Under this condition, most tissues, except the brain, can use fatty acids directly to generate energy. Furthermore, the liver converts fatty acids into ketone bodies, a process for which FAO is indispensable. Ketone bodies serve as an additional energy source that is used by all tissues including the brain.

Beta-oxidation defects are potentially fatal disorders. Symptoms are usually seen during fasting or prolonged exercising. Often, but not always, the patients have hypoketotic hypoglycaemia along with alterations in liver, heart, muscular and nervous systems. There are three different presentations. First is the hepatic presentation, which is a severe, often lethal, disease in infancy or the neonatal period with hypoketotic hypoglycaemia and Reye-like syndrome.

During infancy, patients may also present with cardiac symptoms such as dilated or hypertrophic cardiomyopathy and/or arrhythmias. Alternatively, FAO defects might present as a milder, later ('adult') onset disease. This form is characterised by exercise-induced myopathy and rhabdomyolysis..

To date, FAO defects have been found in glutaric aciduria type 2, primary carnitine deficiency and deficiencies of CPT1a, CACT, CPT2, VLCAD, MTP (including isolated LCHAD or thiolase), MCAD, M/SCHAD, SCAD and 2,4-dienoyl CoA reductase (DECR).

SELECTED READINGS.

Fatty Acid Beta-Oxidation Disorders: A Brief Review. Vishwanath VA. *Ann Neurosci.* 2016 1. 51-5

The enzymology of mitochondrial fatty acid beta-oxidation and its application to follow-up analysis of positive neonatal screening results. Wanders RJ, Ruiten JP, IJLst L, Waterham HR, Houten SM. J Inherit Metab Dis. 2010 33. 479-94.

C). MITOCHONDRIA AND CANCER.

As the cells of various tissues transform into cancer they undergo a multitude of changes that have been elegantly summarized as the 6 hallmarks of cancer. These include sustaining proliferative signaling, evading growth suppression, inducing angiogenesis, enabling replicative immortality, activating invasion and metastasis and preventing cell death. Added to these changes, cancer cells alter energy metabolism to use glycolysis more readily and utilize substrates other than glucose for oxidative phosphorylation, and they have mechanisms to avoid immune detection.

The altered metabolism of cancer cells was first noted by Warburg and is now called the Warburg Effect. Warburg found that cancer cells tend to make the energy for their growth and replication by glycolysis in the cytosol rather than oxidative phosphorylation even when oxygen is plentiful. More recent studies have established that this is an oversimplification as some cancer cells use oxidative phosphorylation as well as glycolysis. It is now evident that cancer cannot be defined by a single effect on energy metabolism but is heterogeneous depending on tissue, stage of the cancer growth and environmental conditions under which the transformation is occurring. There is considerable variation within solid tumors as well as between them. Nevertheless there are sufficient differences between the energy metabolism of cancerous tissue and normal tissue to believe that practical treatments that attack these differences can be effective cancer treatments. Of particular importance, in transformation the stabilizing of HIF1alpha induces switching of isoforms of several of the glycolytic enzymes including transporters (GLUT1, GLUT3) and enzymes (HKI, HKII, PFK-L, ALD-A, ALD-C, PGK1, ENO-alpha, PYK-M2, LDH-A, PFKFB-3). These are now being exploited as targets for drug therapy

More specific to transformed cells than the mode of energy

generation is the substrates used. Amino acids and fatty acids are a major source of both energy production and generation of intermediates for biosynthesis of cell components. Cancer cells are particularly adept at using glutamine and also glycine and serine as sources of carbon and nitrogen. The oncogene, Cmyc, facilitates this through the coordinate enhanced expression of the genes responsible for amino acid catabolism. Thereby, glutaminolysis not only serves for ATP production in some situations, but also provides the needed metabolites such as glucose-6-phosphate, ammonia and aspartate for the synthesis of purine and pyrimidine nucleotides.

Another significant feature of cancer metabolism is the high level of free radicals produced in mitochondria mainly generated by complexes I and III of the respiratory chain, but also produced by cytosolic NADH oxidase. At low levels, free radicals such as superoxide act as signaling molecules, but at high levels they are toxic. Mitochondria of cancer cells show high levels of damage from oxidative stress both in mtDNA and in the organellar proteins. This has led to the proposal that mtDNA damage is oncogenic.

The strongest evidence of mitochondrial dysfunction leading directly to transformation is for the defects in the Krebs cycle enzymes succinate dehydrogenase and fumarase discussed already, along with mutations in isocitrate dehydrogenase, which causes glial tumors. These defects generate what are now called oncometabolites.

Also, given that mitochondria are the central executioners of the cell, it is not surprising that many of the mitochondrial proteins involved in apoptosis and in mitophagy are oncogenes. The dysfunction of such proteins, e.g Bcl2 proteins, can affect induction of the mitochondrial permeability pore. Additionally these proteins are required for fission and fragmentation, necessary steps in the cell death process, which if blocked, lead to cell proliferation and cancer induction.

SELECTED READINGS.

Mitochondria and Cancer. Vyas S, Zaganjor E, Haigis MC. Cell. 2016. 166. 555-66.

Targeting Cancer Metabolism - Revisiting the Warburg Effects. Tran Q, Lee H, Park J, Kim SH, Park J. Toxicol Res. 2016. 32. 177-93

Bioenergetic pathways in tumor mitochondria as targets for cancer therapy and the importance of the ROS-induced apoptotic trigger. Ralph SJ, Rodríguez-Enríquez S, Neuzil J, Moreno-Sánchez R. *Mol Aspects Med.* 2010. 31. 29-59.

Mitochondrial gateways to cancer. Galluzzi L, Morselli E, Kepp O, Vitale I, Rigoni A, Vacchelli E, Michaud M, Zischka H, Castedo M, Kroemer G. *Mol Aspects Med.* 2010. 31. 1-20.

The Warburg effect in tumor progression: mitochondrial oxidative metabolism as an anti-metastasis mechanism. Lu J, Tan M, Cai Q. *Cancer Lett.* 2015. 28;356(2 Pt A):156-64.

Defects in mitochondrial metabolism and cancer. Gaude E, Frezza C. *Cancer metab.* 2014; 2. 10-20

D). MITOCHONDRIA IN THE INNATE IMMUNE RESPONSE.

Mitochondria are now known to play a critical role in the fight against viral infections. So called innate immunity against RNA viral infection involves the activation of multiple signaling steps that culminate in the rapid production of type I interferons, such as IFN- α and - β , and other pro-inflammatory cytokines. Two distinct pathways initiate signal transduction; one involves the endosomal Toll-like receptor 3 which targets RNA viruses entering the cell by endocytosis; the other is prompted by retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), which recognize cytoplasmic viral-derived double-stranded (ds)RNA. RIG-1 and a second RNA helicase, MDA-5, recognize distinct types of cytosolic RNA species and are recruited to the mitochondrial outer membrane where they interact with the protein, mitochondrial antiviral signaling protein (MAVS): also called IPS-1, VISA and CARDIF. This protein of 56Kd contains an N-terminal caspase activation and recruitment domain (CARD) comprising six helices. The same structure is found in both RIG-1 and MDA-5. MAVS also contains a transmembrane domain that anchors it to the mitochondrial outer membrane. Activation of immunity. MAVS leads to

downstream translational activation of NF kappaB and/or IRF3/7, inducing rapid production of the interferons and cytokines. It also activates autophagy or apoptosis to rid the cell of viral elements or remove the infected cell from tissue.

Other mitochondrial outer membrane proteins are involved in the anti-viral response e.g. TOM70 which acts as a viral receptor including for the hepatitis C virus. HIV infection leads to induction of TOM70 expression, which in turn induces resistance to tumor necrosis factor-alpha (TNF- α)-mediated apoptosis but not to Fas-induced apoptosis in HepG2 cells. TOM70 was found to be induced by the HCV non-structural protein (NS)3/4A protein, and silencing of TOM70 decreased the levels of the NS3 and Mcl-1 proteins.

While much is known about the upregulation of MAVS, less is known about its degradation after viral infection. However two proteins have been shown to play such a role recently. These are the poly c-binding proteins (PCBP-1 and PCBP-2). Overexpression of PCBP1 impairs MAVS-mediated antiviral response while knockdown of this protein has the opposite effect. PCBP1 is abundantly expressed while PCBP2 shows low basal expression but rapid induction after infection. Several recent studies show how viruses can get the better of a cell. For example, murine gamma herpes virus 68 subverts cytokine production by modifying upstream signaling to MAVS. Further, HIV tat protein can react with mitochondria to induce permeabilization of the organelle and to inactivate cytochrome c.

SELECTED READINGS.

Mitochondria in innate immune response. WEST AP, SHADEL GS & GOSH S. Proc. Natl. Acad Sci USA 2011.108. 14590-5.

[Mitochondrial anti-viral immunity.](#) Zemirli N, Arnoult D. Int J Biochem Cell Biol. 2012. 44. 1473-6.

Murine gamma herpes virus 68 hijacks MAVS and IKKb to abrogate NFkappaB activation and antiviral cytokine production. DONG X & FENG P. Plos Pathog 2011 7. e1002336

E). MITOCHONDRIA AND NEURODEGENERATION.

It has long been thought that mitochondria play a critical role in a variety of diseases characterized by neuro-degeneration. Early on the focus was on oxidative stress and the effect this had on energy production. More recently emphasis has shifted to disease-causing alterations in mitochondrial trafficking and/or removal of defective organelle by mitophagy, although as yet there is no definitive evidence in any of the diseases below.

*The proposal that mitochondrial dysfunction played a role in **Parkinsons disease** originated with the observation that the Complex I inhibitors rotenone and MPTP caused Parkinsonian symptoms. More recent work has identified Complex I protein changes in patients with the disease. Proteomic studies showed that complex I of brains from Parkinsons patients had an average decrease of 34% in the 8 kDa subunit, and contained 47% more protein carbonyls in catalytic subunits coded for by mitochondrial and nuclear genomes. Further, NADH-driven electron transfer rates through complex I inversely correlate with complex I protein subunit modifications. Similar patterns were observed when the mitochondria from brains of control subjects were incubated with NADH in the presence of rotenone, but not with exogenous oxidant, indicating that the oxidative damage is induced from within the complex and not by exogenous free radicals.*

The damage caused by Complex I dysfunction and consequent superoxide production is broader than just in this complex, and is found in DNA, lipids and proteins of PD brains, particularly in the substantia nigra which has low concentrations of anti-oxidant proteins. Oxidative damage is also seen in peripheral tissues. Importantly, these broad oxidative effects are observed in animals treated with rotenone, confirming that the initial free radical generator is Complex I.

One way in which reduced respiratory chain activity and build up of oxidative damage can cause death of neurons in PD is through reduced ATP production. Alternatively, based on recent work, it is the failure to identify and excise damaged mitochondria by mitophagy that is central to the disease. Several genes have been linked to early onset Parkinsons including Pink 1, Parkin, DJ1, LRRK2 and alpha synuclein. Structure and function studies of pink1 and parkin proteins, two essential components of mitophagy signaling in neurons, have

provided the link between the disease and mitochondria. As discussed already, pink1 and parkin are direct participants in the process of mitophagy.

The link between mitochondrial dysfunction and PD is more tenuous with respect to forms caused by mutation of DJ1. DJ1 functions to protect cells against oxidative stress. It protects neurons from oxidative stress by scavenging H₂O₂ from the neuronal environment and thus it also protects mitochondrial integrity in these cells.

The role of LRRK2 is less clear. This ubiquitous protein has GTPase and kinase activity. Similarly the role of alpha synuclein in cells is not well known. The link with mitochondria for both proteins is their association with the organelle. Both associate with microtubules. Thus they may affect mitochondrial transport and/or the fusion/fission process when defective in PD.

Amiotrophic lateral sclerosis or ALS has been shown to involve the misfolding of the predominantly cytosolic antioxidant protein superoxide dismutase (SOD1). Mitochondria also contain SOD1 as well as a second form of this enzyme SOD2, which is not affected by the disease. Wild type SOD1, and a copper chaperone for SOD1 (CCS), are localized to the intermembrane space (IMS) in normal mitochondria. It has been proposed that the nascent SOD1 polypeptide with no metal ion bound can efficiently enter mitochondria and that the maturation of SOD1 including metal ion binding and intra-molecular disulfide bond formation inside mitochondria and the subsequent retention in IMS involve the SOD1-CCS interaction. The ALS-related mutant SOD1 proteins have also been found in the IMS, but also in the matrix and outer membrane of mitochondria. Once associated with mitochondria, the mutant SOD1 is seen to cause impaired respiratory complexes, disrupted redox homeostasis and decreased ATP production. However, the primary effect could be altered mitochondrial cell transport

As in Parkinsons disease, the reason that mitochondrial dysfunction is observed predominantly in neurons may relate to altered mitochondrial cell transport in these extended cells. Thus it has been shown that primary neurons isolated from G93A SOD1 transgenic mice and cortical neurons transfected with G93A SOD1, have reduced antegrade mitochondrial transport.

*In **Alzheimers disease (AD)**, as in PD, membrane-associated oxidative stress, increased free radical production, and perturbed Ca²⁺ homeostasis have been observed. Increased mitochondrial permeability and cyt c release, which is promoted by A β oligomerization and polymerization, is thought to trigger the opening of MPTP leading to apoptosis. Different from PD there is evidence of reduced cytochrome c oxidase activity. This is at least in part due to oxidative damage of mtDNA that is beyond that seen in normal age controls. Complex I down regulation is also seen in AD brains. As in PD, the primary insult leading to AD is not known. Most likely this is a heterogeneous disease, with altered mitochondrial function leading to reduced ATP production, increased free radical production, and increased apoptosis.*

***Huntingtons disease** is linked to the presence of an elongated polyglutamine (polyQ) stretch in huntingtin protein (Htt). This mutation in Htt correlates with neuronal dysfunction in the striatum and cerebral cortex and eventually leads to neuronal cell death. How this happens remains unclear but like PD and AD focus is now on anomalous mitochondrial dynamics, and trafficking along with disrupted mitophagy. In addition, deficiency in oxidative metabolism and defects in mitochondrial Ca²⁺ handling are considered essential contributing factors to neuronal dysfunction in HD*

SELECTED READINGS.

Evidence for a common biological pathway linking three Parkinson's disease-causing genes: parkin, PINK1 and DJ-1.

van der Merwe C, Jalali Sefid Dashti Z, Christoffels A, Loos B, Bardien S. Eur J Neurosci. 2015 May;41:1113- 24.

Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease.

Reddy PH, MF Beal MF. Trends. Mol. Med. 2008. 14. 45–53.

Understanding the susceptibility of dopamine neurons to mitochondrial stressors in Parkinson's disease. Haddad D, Nakamura

K. FEBS Lett. 2015. 21;589(24 Pt A).3702-13

The Parkinson Disease Mitochondrial Hypothesis: Where Are We at?
Franco-Iborra S, Vila M, Perier C. *Neuroscientist*. 2016. 22. 266-77

The role of mitochondrial disturbances in Alzheimer, Parkinson and Huntington diseases. Carvalho C, Correia SC, Cardoso S, Plácido AI, Candeias E, Duarte AI, Moreira PI. *Expert Rev Neurother*. 2015;1. 867-84.

Critical analysis of Alzheimer's amyloid-beta toxicity to mitochondria.
Kaminsky YG, Tikhonova LA, Kosenko EA. *Front Biosci* 2015. 20. 173-97

Mitochondria-associated ER membranes and Alzheimer disease.
Area-Gomez E, Schon EA. *Curr Opin Genet Dev*. 2016. 38:90-96.

Exploring new pathways of neurodegeneration in ALS: the role of mitochondria quality control. Palomo GM, Manfredi G. *Brain Res*. 2015
1607.36-46.

Mitochondria in neurodegeneration. Lezi E, Swerdlow RH. *Adv Exp Med Biol*. 2012. 942. 269-86

Mitochondrial dynamics and quality control in Huntington's disease.
Guedes-Dias P, Pinho BR, Soares TR, de Proença J, Duchen MR, Oliveira JM. *Neurobiol Dis*. 2016. 90.51-7.

PGC-1 α , mitochondrial dysfunction, and Huntington's disease.
Johri A, Chandra A, Beal MF. *Free Radic Biol Med*. 2013. 62. 37-46

