Shaping the mitochondrion: mitochondrial biogenesis, dynamics and dysfunction

Conference on Mitochondrial Assembly and Dynamics in Health and Disease

Janet M. Shaw & Dennis R. Winge⁺

University of Utah Health Sciences Center, Salt Lake City, Utah, USA

The summer research conference on Mitochondrial Assembly and Dynamics in Health and Disease took place between 5 and 10 July 2009, in Carefree, Arizona, USA, and was organized by C. Koehler and T. Langer.

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See Glossary for abbreviations used in this article.

Introduction

Almost a century after being defined as the workhorses of cell metabolism, mitochondria are continuing to reveal new secrets. At this conference, the pathways that control mitochondrial biogenesis, function and dysfunction were discussed. The first part of the meeting focused on the molecular events required to build and shape the organelle, whereas the latter sessions explored how mitochondria influence and respond to changes in cell physiology and contribute to ageing and human disease. Many

elegant studies were presented, but here we will discuss only a few selected highlights.

Assembly of the mitochondrial respiratory complexes

A resurgence of mitochondrial research has focused on the biogenesis of the organelle, the definition of the molecules and mechanisms required to sort proteins into the various subcompartments, and the pathways that lead to respiratory complex assembly. Significant progress has been made in yeast towards clarifying the formation of the OXPHOS respiratory complexes through the characterization of OXPHOS-deficient mutants, which has enabled the identification of several assembly factors that function in respiratory complex biogenesis. As *Saccharomyces cerevisiae* lacks complex I (CI), the characterization of its biogenesis has been slower than that of other complexes, but genetic manipulations in *Yarrowia lipolytica* are being exploited to address CI biogenesis. CI is the most intricate respiratory complex in that it contains 45 subunits and eight distinct Fe/S clusters. CI is the remaining structural challenge among the OXPHOS complexes as only a cryoEM structure is available. CI is present in a supercomplex that also contains complexes III (bc_1 complex) and IV (CcO), a model of which is shown in Fig 1. U. Brandt (Frankfurt, Germany) reported that mutant *Y. lipolytica* cells that lack individual CI subunits form subassembly complexes. In one case, the depletion of a particular subunit resulted in the loss of 13 additional subunits that encompass the entire distal segment of the membrane arm of CI. The remnant complex retained residual proton pumping, indicating that the intact CI contains two proton-pumping centres. The stability of the subassembly complex also provides new opportunities for the structural clarification of the CI complex.

In the past five years, nine CI assembly factors have been identified. M. Ryan (Melbourne, Australia) characterized steps in CI biogenesis that require the CIA30 and B17.2L assembly factors (Lazarou *et al*, 2009), and R. Lill (Marburg, Germany) discussed an intriguing assembly factor: the candidate Fe/S scaffold protein Ind1 (Bych *et al*, 2008). The depletion of Ind1 in human cells by siRNA results in a selective CI impairment, the depletion of certain Fe/S-containing subunits, and the formation of a membrane subcomplex that could

Department of Biochemistry, University of Utah Health Sciences Center, Salt Lake City, Utah 84132, USA

⁺Corresponding author. Tel: +1 801 585 5103; Fax: +1 801 585 3432;

E-mail: dennis.winge@hsc.utah.edu

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Fig 1 | The OXPHOS respiratory supercomplex. The OXPHOS respiratory chain is organized in supercomplexes, one of which consists of complex I, dimeric complex III and complex IV. The arrangement of the complexes in the superstructure is depicted based on the known cryoEM images of the supercomplex (Schafer *et al*, 2006, 2007). The cofactors in each complex are shown. Although the only structure of mammalian complex I is a cryoEM image, a crystal structure of the peripheral arm of the *Thermus thermophilus* complex I is available (Sazanov & Hinchliffe, 2006). In the *T. thermophilus* enzyme, nine distinct Fe/S centres exist, whereas the mammalian enzyme seems to have only eight Fe/S centres. The dimeric complex III contains two haem b centres in the mitochondrially encoded CytB subunit, cytochrome c1 and the Rieske 2Fe/2S centre per subunit. Complex IV contains two haem a moieties and three copper ions as redox cofactors. Electrons are passed from complex I to complex III through coenzyme Q, and from complex III to complex IV through cytochrome c. bc₁, cytochrome c reductase; CcO, cytochrome c oxidase; CoQ, coenzyme Q; CryoEM, cryoelectron microscopy; Cyc, cytochrome c; Fe/S, iron/sulphur; OXPHOS, oxidative phosphorylation.

be an assembly intermediate that forms when Fe/S clusters are not inserted into CI. The elucidation of the assembly of the eight Fe/S centres in CI—which are depicted in the supercomplex model shown in Fig 1—will be an interesting challenge. Iron is an essential element in each of the four electron transfer OXPHOS complexes, either as a component in Fe/S centres or in haem (Fig 1). The main iron transporter in the mitochondrial inner membrane—mitoferrin 1—was shown by B. Paw (Boston, MA, USA) to interact with ABCB10, which is an ATP-binding cassette transporter. ABCB10 seems to modulate the stability of mitoferrin 1 and could therefore regulate mitochondrial iron import.

Two new assembly factors for the biogenesis of succinate dehydrogenase (SDH), also known as complex II (CII), were presented at this conference. SDH transfers electrons from the oxidation of succinate through a flavin and Fe/S centres to reduce coenzyme Q, which is oxidized by complex III (Fig 2). J. Rutter (Salt Lake City, UT, USA) reported the discovery of SDH5, which mediates the covalent flavination of a subunit of CII (Hao *et al*, 2009). In the absence of SDH5, the assembly of SDH is impaired and the complex is not assembled. Mutations in SDH5 cause a type of human neuroendocrine tumour known as hereditary paraganglioma. M. Zeviani (Milan, Italy)

reported the identification of a CII assembly factor—SDHAF1—from a CII-deficient patient cell line, although its function is unresolved (Ghezzi *et al*, 2009).

Although many assembly factors for cytochrome c oxidase are known, additional factors continue to be identified. D. Mick (Gottingen, Germany) reported the discovery of Coa3, which participates with Mss51 and Cox14 in an early stage of Cox1 maturation.

Mitochondrial translation

Specific activators mediate the translation of yeast mitochondrial gene products but whether such activators exist in mammals was unknown. E. Shoubbridge and W. Weraarpachai (Montreal, QC, Canada) have now identified two mammalian translation activators. TACO1 is necessary for the efficient translation of COX1, one of the mitochondrially encoded subunits of cytochrome c oxidase (Weraarpachai *et al*, 2009), and Pro1853 is implicated in CI formation as its depletion led to attenuated levels of two newly synthesized mitochondrial CI subunits. Human mitochondrial translation elongation and termination are also poorly understood. Two translation termination factors present in human mitochondria were discussed at the meeting: ICT1 was reported by Z. Chrzanowska-Lightowlers (Newcastle, UK) to exhibit a codon-independent peptidyl tRNA hydrolase activity that is potentially involved in kick-starting stalled mitoribosomes, and Shoubbridge showed that mutations in a second factor, C12ORF65, cause a pathogenic mitochondrial translation defect in two patients.

All mitochondrial translation occurs on mitoribosomes that are tethered to the inner membrane and the resulting products are inserted into the membrane by the Oxa1 translocase. R. Stuart (Milwaukee, WI, USA) reported the association of Oxa1 with Mrp20 and Mrp140 in the large subunit of the mitoribosome, near the exit channel of nascent chains. This interaction would position Oxa1 close to the emerging nascent chains for co-translational membrane insertion. Regulatory interconnections between respiratory complexes in yeast are an emerging area of study. I. Soto (Miami, FL, USA) reported that mutations in ATP synthase biogenesis attenuate Cox1 translation. A. Tzagoloff (New York, NY, USA) reported that the translation of the mitochondrially encoded Atp6 and Atp8 subunits of ATP synthase is dependent on the assembly of the α/β hexamer of the F1 complex of ATP synthase. Translation of the bicistronic *ATP8/ATP6* mRNA is directly activated by F1, which therefore leads to a balanced expression of the mitochondrial and nuclear gene products that constitute the ATP synthase complex.

Mitochondrial proteome

Most mitochondrial proteins are imported from the cytoplasm. Proteins destined for the inner membrane or matrix compartment typically have amino-terminal targeting sequences that are proteolytically removed by the mitochondrial processing protease (MPP). In a study that defines the cleavage specificity of the MPP, N. Pfanner (Freiburg, Germany) reported that, for many proteins, import signal processing is mediated by the MPP and a second peptidase, Icp55. MPP processing often generates N-terminal residues that are destabilizing according to the N-end rule of protein stability. Icp55 processing removes destabilizing residues to enhance protein stability. Future work will address whether Icp55 function is regulated, which would modulate protein stability.

Fig 2 | Cofactors involved in electron flux from complex II. The oxidation of succinate in the Krebs cycle by succinate dehydrogenase results in electron flux to coenzyme Q (CoQ) and subsequently to complex III, as depicted. Succinate dehydrogenase consists of four subunits with a covalently bound flavin in SDH1, three Fe/S centres in SDH2 and a haem b moiety bound between the two membrane subunits, SDH3 and SDH4 (Sun *et al*, 2005). bc_1 , cytochrome *c* reductase; CcO, cytochrome *c* oxidase; Cyc, cytochrome *c*; Fe/S, iron/sulphur; SDH, succinate dehydrogenase.

Mitochondrial membrane fission and fusion

Mitochondria can be discrete tubules or interconnected networks in living cells, depending on the activities of large GTPases that control fission and fusion of the organelle. A dynamin-related GTPase—known as Dnm1 in yeast and DRP1 in mammals—assembles into spirals that encircle and constrict the outer membrane before fission. The recruitment of this GTPase, its assembly and its activation at discrete sites on mitochondrial tubules remain the focus of intense study. Previous work has shown that Dnm1 binds to the predicted β -propeller domain of mitochondrial division 1 protein (Mdv1). J. Shaw (Salt Lake City, UT, USA) and colleagues provided insight into this interaction, revealing that Mdv1 dimerizes through an anti-parallel coiled-coil, thereby exposing two β -propeller ‘landing platforms’ for Dnm1 in the cytoplasm. Using a coupled assembly/GTP hydrolysis assay, J. Nunnari (Davis, CA, USA) showed that on binding to Dnm1-GTP, the Mdv1 β -propeller nucleates Dnm1 self-assembly (Lackner *et al*, 2009). *In vivo*, this process would promote the formation of Dnm1 spirals and the concomitant constriction of the mitochondrial tubule. Studies of DRP1 post-translational modifications, including phosphorylation and SUMOylation, have yielded insight into the cellular mechanisms that regulate mitochondrial fission in mammals. H. McBride (Ottawa, ON, Canada) and colleagues previously showed that several mitochondrial proteins, including DRP1, are reversibly SUMOylated by the E3 ligase MAPL and the SUMO protease (Harder *et al*, 2004; Zunino *et al*, 2007, 2009; Braschi *et al*, 2009). In a surprising new twist to this story, McBride reported that SenP5 moves from the nucleolus to the mitochondrial surface during the G2/M phase of the cell cycle, where its deSUMOylation activity promotes DRP1 assembly and mitochondrial fission (Zunino *et al*, 2009). SenP5 silencing causes cells to arrest in G2/M, which is consistent with the idea that deSUMOylation of DRP1 and other mitochondrial proteins is crucial for progression through mitosis.

Mgm1/OPA1 is a second dynamin-related GTPase that regulates inner mitochondrial membrane fusion in yeast and mammals,

Glossary

Bad	Bcl2-associated death promoter
Bak	Bcl2 homologous antagonist killer
Bax	Bcl2-associated X protein
Bcl2	B-cell lymphoma 2
Bcl-Xl	B-cell lymphoma extra large
BID	BH3-interacting domain death agonist
Cox	cytochrome <i>c</i> oxidase
cryoEM	cryoelectron microscopy
Dnm1	dynamin-related GTPase
Drp1	dynamin-related protein 1
ER	endoplasmic reticulum
Fe/S	iron/sulphur
MAPL	mitochondrial-anchored protein ligase
Mgm1	mitochondrial genome maintenance protein 1
OPA1	optic atrophy 1 (mammalian orthologue of Mgm1)
OXPHOS	oxidative phosphorylation
Pink1	PTEN-induced kinase
RNAi	RNA interference
Sdh5	succinate dehydrogenase assembly factor 5
SDHAF1	succinate dehydrogenase assembly factor 1
SenP5	SUMO/sentrin-specific peptidase 5
siRNA	small interfering RNA
SUMO	small ubiquitin-like modifier
TACO1	translational activator COX1
tBID	truncated Bid

respectively. A long isoform (l-Mgm1, anchored to the inner membrane facing the intermembranous space) and a short isoform (s-Mgm1, which lacks the inner membrane anchor) are expressed in approximately equivalent amounts *in vivo*. However, whether these isoforms carry out the same or distinct functions during inner membrane fusion was unclear. M. Zick (Munich, Germany) and A. Reichert (Frankfurt, Germany) showed that the two isoforms have distinct intramitochondrial distributions, with l-Mgm1 concentrated in cristae and s-Mgm1 concentrated at the inner boundary membrane (Zick *et al*, 2009). Interestingly, a functional GTPase domain is only required in s-Mgm1, consistent with the idea that the two isoforms carry out distinct functions during fusion. Reichert and colleagues propose that homotypic l-Mgm1 interactions anchor opposing inner membranes in such a way that facilitates their subsequent membrane fusion, which would be mediated by the GTP-dependent s-Mgm1 isoform.

Molecular events during mitophagy

Several pathways collaborate to generate a functional mitochondrion and mistakes can happen in the process. If these errors are allowed to accumulate, the resulting dysfunctional mitochondria can trigger cell death pathways. Fortunately, cells have developed surveillance systems in the form of intraorganellar proteases and mitochondrial autophagy—known as mitophagy—that prevent accumulated mistakes from becoming a serious problem (Tatsuta & Langer, 2008). The failure of these surveillance systems is linked to inherited neurodegenerative diseases. During the past few years, several groups have identified and characterized the intraorganellar proteases that remove misfolded or unassembled mitochondrial proteins. More recently, investigators have begun to identify some of the molecules required for mitophagy.

Fig 3 | Mitochondria–ER attachment and function. Tethering proteins generate contact sites that hold the ER membrane and mitochondrial outer membrane (MOM) in close proximity. These contact sites also contain proteins that mediate calcium transport/signalling and lipid metabolism. ER, endoplasmic reticulum; MIM, mitochondrial inner membrane; PtdSer, phosphatidylserine; PtdEtn, phosphatidylethanolamine; Ser, serine.

Mitochondrial dysfunction has been implicated in the neurodegeneration that is associated with Parkinson disease. Mutations in Parkin, which is an E3 ligase, and Pink1, a mitochondrial membrane-anchored kinase, are linked to several autosomal recessive forms of the disease. R. Youle's laboratory (Bethesda, MD, USA) previously showed that cytosolic Parkin selectively relocates to depolarized mitochondria before its elimination by mitophagy (Narendra *et al*, 2008). Although genetic studies in *Drosophila* indicate that Pink1 acts upstream from Parkin, the effect of Pink1 on the translocation of Parkin was not known. Youle's group has now shown that Pink1 expression, kinase activity and mitochondrial localization are required for the translocation of Parkin to mitochondria and the subsequent mitophagy. Significantly, mutations identified in several Parkinson patients also inhibit the mitochondrial translocation of Parkin, indicating that defects in the targeted elimination of damaged mitochondria might have a role in Parkinson disease.

Mitochondria, death and ER–mitochondrial contacts

During apoptosis, the insertion and oligomerization of Bax and/or Bak in the outer mitochondrial membrane leads to mitochondrial outer membrane permeabilization (MOMP) and the release of proapoptotic factors such as cytochrome *c* from the intermembranous space. The connection between DRP1 GTPase-mediated mitochondrial fission and MOMP has been explored extensively *in vivo*. In some cell types, mitochondria can undergo MOMP without fission. However, other experiments indicate that interfering with DRP1 activity—by loss-of-function mutations, dominant negative mutations or RNAi—delays mitochondrial fission, cytochrome *c* release and apoptosis. A more recent study that used a chemical inhibitor of DRP1 assembly indicated that this GTPase has a role in MOMP that is distinct from the induction of mitochondrial fission, but did not reveal the effect of DRP1 on its apoptotic targets (Cassidy-Stone *et al*, 2008). By using an *in vitro* assay, J.-C. Martinou (Geneva, Switzerland) and colleagues have now shown that DRP1 stimulates the tBID-induced oligomerization of Bax. This DRP1 activity requires a conserved Arg247 residue

downstream from the canonical G4 motif in the GTPase domain, both *in vitro* and *in vivo*. Surprisingly, Bax oligomerization in this system does not depend on the GTPase activity of DRP1 and is supported by both hydrolysable and nonhydrolysable adenine nucleotides. Ongoing studies are exploring how nucleotides are used during this process and the possibility that DRP1 also exerts a direct effect on membrane lipids during permeabilization.

Cell-free assays that recapitulate Bax/Bak-mediated MOMP in response to proapoptotic effectors such as BID and BIM are used routinely in the cell death field. These assays use mitochondrial-enriched fractions that also contain ER membranes of the ER–mitochondrial contact sites, which mediate lipid transfer and Ca^{2+} flux *in vivo*. D. Green (Memphis, TN, USA) reported that removing ER membranes from the assay markedly decreased the sensitivity of the remaining mitochondria to BID-induced and BIM-induced MOMP. Conversely, adding back ER fractions restored sensitivity. The purification of the ER-associated factor combined with reconstitution experiments revealed that a downstream product of sphingomyelin metabolism cooperates with the BID activator to promote Bax-mediated MOMP. Green and co-workers also showed that inhibition of the sphingomyelin pathway inhibits apoptosis *in vivo* and increases cell survival on exposure to a chemotherapeutic agent, which further validates sphingomyelin-derived mediators as drug targets for the treatment of cancer and other diseases.

ER–mitochondrial contact sites are known to mediate crucial cellular functions (Fig 3). J. Vance (Alberta, AB, Canada) discussed the importance of the ER for phosphatidylserine formation and its subsequent transfer to the mitochondrion for use and conversion to phosphatidylethanolamine (PE). The mitochondrial PE content is mainly made *in situ*, rather than being transferred from the ER. Calcium transport also occurs at ER–mitochondrial contact sites. Recent reports have described the components of ER–mitochondrial connections in budding yeast (Kornmann *et al*, 2009) and mammalian cells (de Brito & Scorrano, 2008; Merkwirth & Langer, 2008).

The BCL2 family of proteins has cellular functions that are independent of apoptosis. N. Danial (Boston, MA, USA) discussed the role of the proapoptotic BCL2 member BAD in regulating glucose metabolism in pancreatic β -cells (Danial, 2008). BAD phosphorylation prevents it from interacting with BCL-XL, thereby blocking apoptosis. In addition, the same phosphorylated form of BAD activates glucokinase and stimulates oxidative metabolism. This switch allows cells to coordinate the apoptotic activity of BAD with the availability of metabolites that promote cell growth and survival.

Summary

The conference on Mitochondrial Assembly and Dynamics in Health and Disease showcased the advances that have been made in various aspects of mitochondrial biogenesis and function. Significant progress has been achieved in understanding the biogenesis of respiratory complexes and the regulation of the assembly process. The combination of genetic studies with model systems and analyses of immortalized cells from patients with respiratory deficiencies has provided exciting advances in our understanding of the biogenesis process. The conference also highlighted the emergence of new topics, such as quality control pathways that maintain functional organelles and inter-organelle communication, as evidenced by the ER–mitochondrial links in apoptosis and lipid biosynthesis. The growing number of proteins with dual functions in apoptosis,

morphology and metabolism highlight the importance of mitochondria as the hubs that connect cellular energy status with cell survival. Given the significance of these findings and the new questions that they raise, we anticipate that the next meeting on this topic will be equally exciting.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Janet M. Shaw

Dennis R. Winge