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Meeting Report

Mitochondrial dynamics in cell life and death

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FEBS-IUBMB Workshop: Mitochondrial Dynamics in Cell Life and Death:Venetian Institute of Molecular Medicine, Padova, Italy, 27–30 August 2005.

The International FEBS-IUBMB Workshop: 'Mitochondrial Dynamics in Cell Life and Death', 27–30 August 2005, was dedicated to the memory of Stanley J Korsmeyer (1950–2005). It was held at the Venetian Institute of Molecular Medicine in Padova, a charming city near Venice that hosts a respected university with a long-standing tradition for scientific studies. Galileo Galilei, one of its best-known professors, made some of his most significant discoveries during the 18 years (1592–1610) he spent in this city. This meeting was the very first specifically dedicated to mitochondrial dynamics, and reflecting the exciting state of this field, the program was densely packed with innovative talks. Roughly 100 scientists attended 33 talks by invited speakers and 10 talks selected from submitted abstracts. Some 50 posters were displayed throughout the duration of the meeting. Discussions were lively during the conferences, the poster sessions and the breaks on the beautiful terrace of the Institute. The members of the local organizing committee and the friendly personnel of the excellent catering service were rewarded with long applauses from all participants. We apologize for the other numerous excellent contributions that could not be included into this report owing to space limitations.

Deformation and division of biological membranes

In contrast to most organelles, mitochondria are enveloped by a double membrane, which appears to not participate in vesicular trafficking with other organelles. The available data show that some aspects of membrane dynamics, like fission, are partly mediated by molecules similar to those of other intracellular membranes while others, like fusion, appear to use mitochondria-specific machineries. In a series of three plenary lectures, Jenny E Hinshaw, Pietro De Camilli and Marc A. McNiven presented the features of molecules capable of modulating the morphology and dynamics of biological and artificial membranes, especially in relationship to membrane bending, constriction and fission.

In her presentation, Jenny Hinshaw (Bethesda/MD, USA) presented data on the properties of Dnm1p, a dynamin-related protein involved in yeast mitochondrial division. She reported that recombinant Dnm1p assembles into spirals, like classical dynamin. The diameter of Dnm1p spirals ($\sim$100 nm) is significantly larger than that of dynamin spirals, suggesting that the structure of Dnm1p is tailored to divide mitochondria ($\sim$400 nm diameter).

Marc McNiven (Rochester/MN, USA) reviewed the numerous members of the dynamin superfamily and the different cellular sites of their action. The mitochondrial dynamin-related proteins Dlp1/Drp1 lack the proline rich and pleckstrin homology domains common to dynamins 1, 2 and 3, indicating that the interactions of Dlp1/Drp1 with membranes and cytoskeleton are supported by other regions and mechanisms.

Pietro De Camilli (New Haven/CT, USA) discussed the curvature changes imposed on biological membranes during fusion and fission. The GTPase dynamin is closely linked to the fission reaction in endocytosis. Its recruitment to membranes is assisted by a variety of binding partners, many of which also have membrane-deforming properties via a BAR domain, a curvature generating and sensing module. Interestingly, members of a family of proteins known to function in actin nucleation have a homologous domain, termed F-BAR, which also has powerful membrane deforming properties.

Mitochondrial dynamics in model organisms

Many of the molecules involved in mitochondrial fusion and division were identified from genetic screens in yeast, and several talks impressively demonstrated that yeast continues

to be an ideal model organism to study mitochondrial dynamics.

To better understand interactions between the fission components, Janet Shaw's group (Salt Lake City/UT, USA) performed a genetic screen for suppressors of a temperature-sensitive *fis1* allele in which Dnm1p assembly on mitochondria was impaired. Second-site suppressors were found in Mdv1p (but not Dnm1p) that were able to restore fission complex assembly and mitochondrial division. Their results support a model in which recruitment of Dnm1p to mitochondria depends on Mdv1p interactions with Fis1p.

Complementing these findings, David Chan (Pasadena/CA, USA) found that Fis1p binds to not only Mdv1p, but also to a related protein called Caf4p. Caf4p was found to interact with all known components of the fission machinery – Fis1p, Mdv1p, and Dnm1p. His results indicate that Mdv1p and Caf4p are partially redundant proteins, either of which is sufficient for Dnm1p recruitment.

Recently, it has been noted that mutants in the mitochondrial import pathway can lead to mitochondrial morphology defects. This topic was discussed by Nikolaus Pfanner (Freiburg, Germany), who showed that defects in several import proteins lead to the formation of giant mitochondria that appear as very large spheres, a phenotype that resembles that of *mdm10* mutants. Mdm10p was found to be a component of the SAM (sorting and assembly machine) complex, which is needed for import of polytopic proteins to the mitochondrial outer membrane. These results point to a connection between mitochondrial import and morphology, and suggest that Mdm10p has two distinct functions.

Michael Yaffe (San Diego/CA, USA) used a genetic approach to understand the Mdm10/Mmm1/Mdm12 complex. These proteins assemble in a complex on the outer membrane of mitochondria (OMM), and loss of any of the components leads to giant, round mitochondria that fail to be inherited properly. These screens identified *SOI1/VPS13*, a gene previously implicated in vacuolar protein sorting and retention of proteins in the trans Golgi network.

Stephan Jacobs (Göttingen, Germany) used confocal live microscopy to investigate the intramitochondrial distribution of various inner membrane proteins in the giant mitochondria of *mdm10* mutant cells. He found that components of the respiratory chain and of the protein import machinery display different intramitochondrial distributions, the latter being more enriched in the mitochondrial boundary region. This suggests that, at least in these mutant cells, inner boundary and inner cristae membranes represent subcompartments with different protein compositions.

Jodi Nunnari (Davis/CA, USA) reported further results from the *in vitro* fusion assay established by her laboratory. In this assay, both Fzo1p and Mgm1p are required on opposing mitochondrial membranes for fusion. Interestingly, some temperature-sensitive *mgm1* alleles can complement each other, suggesting that Mgm1p self-associates. Importantly, *mgm1* mutants show OMM but not inner membrane (IMM) fusion both *in vitro* and *in vivo*.

Benedikt Westermann (Bayreuth, Germany) presented a new screen to identify essential genes in yeast that play roles in maintaining mitochondrial morphology. His screens identified a large set of genes that could be categorized into the following five broad functional categories: ergosterol synthesis, mitochondrial protein import, vesicular trafficking, ubiquitin-mediated protein degradation, and actin transport. Closer examination of these functional classes will lead to better understanding of how mitochondrial dynamics are integrated into cell physiology.

Andreas Reichert (Munich, Germany) discussed how processing of yeast Mgm1p by the rhomboid protease Pcp1p/Rbd1p regulates mitochondrial morphology. Generation of the proper ratio of processed Mgm1p is dependent on matrix ATP levels, and therefore Mgm1p-processing is proposed to regulate mitochondrial morphology in response to mitochondrial function. Mitochondria with low ATP levels would be unable to fuse and perhaps would be eliminated.

Alexander van der Bliek (Los Angeles/CA, USA) presented studies on the role of Mgm1 for mitochondrial function and dynamics in *Caenorhabditis elegans*. Ultrastructural analysis of Mgm1 mutant cells showed frequently occurring septae within mitochondria, as well as a reduction in the size and numbers of cristae. The presence of IMM septae may result from additional divisions of inner membranes, as suggested by van der Bliek, or from the inability of the IMM to fuse (as shown by Jodi Nunnari for yeast Mgm1).

Much of our current knowledge on the molecular mechanisms regulating programmed cell death is based on genetic studies of the nematode *C. elegans*. Aimed at elucidating the role of mitochondria in initiating the apoptosis program, Barbara Conradt (Hanover/NH, USA) undertook a genetic dissection of the cell death events occurring during *C. elegans* embryonic development. In *C. elegans*, cells destined to die show Drp1-dependent mitochondrial fragmentation. When mitochondrial fragmentation is inhibited, a fraction of these cells are rescued from death, suggesting that organelle fragmentation plays an important role in *C.elegans* developmental cell death.

Mitochondrial dynamics in mammalian cells

Numerous studies have depicted sites of close contact between OMM and IMM that have been involved in protein import and mitochondrial fusion. Carmen Mannella (Albany/NY, USA) showed that, in frozen-hydrated tissues and isolated mitochondria, where structure has not been affected by fixation and/or contrasting reagents, OMM and IMM are separated by an uninterrupted intermembrane space (IMS) of constant width. Instead of contact-sites, this IMS sometimes contains electron-dense particles that contact both OMM and IMM. He also identified electron-dense structures that 'touch' both the endoplasmic reticulum and the OMM at sites where both organelles are in close contact (5–15 nm apart).

It is now well accepted that mitochondria form a dynamic network where matrix contents are exchanged by fusion, but we ignore the kinetics and frequency of individual fusion events. Orian Shirihai (Boston/MA, USA) labeled all mitochondria with TMRE and individual mitochondria with photoactivatable GFP targeted to the mitochondrial matrix (paGFP-mito). Within a short time frame, the diffusion of paGFP-mito was often restricted to small regions of the

mitochondrial network, indicating that the matrices of apparently continuous mitochondria do not form a fused continuum. Manuel Rojo (Paris, France) used the cell fusion approach to investigate whether mitochondrial double membranes fuse in single or in separate reactions. He showed that the fusion of outer and inner membranes can proceed separately and that the dissipation of the inner membrane potential selectively inhibits IMM fusion while leaving OMM fusion unaffected. This suggests that mitochondria possess fusion machineries (on OMM and IMM) that can function separately.

Several laboratories have unambiguously demonstrated that mitochondrial fusion is mediated by transmembrane GTPases of the Fzo/Mfn-family in mammals, flies and yeast. Interestingly, the genomes of plants appear to be devoid of mitofusin-encoding genes, pointing to the existence of a Fzo/Mfn-independent fusion mechanism. Shin-Ichi Arimura (Tokyo, Japan) demonstrated mitochondrial fusion in plant cells using Kaede, a protein that changes from green to red fluorescence upon irradiation.

David Chan (Pasadena/CA, USA) showed that the formation of antiparallel coiled-coils between the HR2-domains of mitofusins mediates tethering of mitochondria. Interestingly, mitochondrial respiration is diminished in cells completely lacking mitochondrial fusion. However, mitochondrial dysfunction of Mfn-null cells was less severe than that of cells largely devoid of OPA1, the mammalian homolog of Mgm1. He further showed that mice lacking Mfn2 appear to have severe neuromuscular defects, including an inability to make coordinated movements, and die within 2 weeks of birth.

Antonio Zorzano (Barcelona, Spain) discussed the role of Mfn2 in the control of mitochondrial metabolism. Mfn2 reduction impairs pyruvate, fatty acid as well as glucose oxidation and decreases the mitochondrial membrane potential, whereas gain-of-function mutations apparently cause opposite effects. Interestingly, Mfn2 reduction also leads to alterations in IMM composition, particularly by repressing nuclear-encoded subunits of various OXPHOS complexes, with complex I activity being primarily affected.

Yisang Yoon (Rochester/NY, USA) described recent findings on the function of human Fis1, a small transmembrane protein of the OMM oriented towards the cytosol. While the inactivation of Fis1 leads to mitochondrial elongation, its overexpression provokes fragmentation, indicating that it is a limiting factor in mitochondrial fusion. Yoon showed through immunoprecipitation experiments that Fis1 can bind Drp1 and could contribute to its recruitment to mitochondria. The activation/inactivation of Fis1 also affects the sensitivity of cells to apoptotic stimuli.

Heidi McBride (Ottawa, Canada) extended her previously published findings on Drp1 SUMOylation, which protects Drp1 from degradation and results in mitochondrial fragmentation. Somewhat unexpectedly, she found that SenP5, a mitochondrial member of the SUMO1 protease family, regulates Drp1 levels, and thereby mitochondrial morphology, in a cell cycle-dependent manner. McBride also introduced the audience to a novel, DRP1-binding mitochondrial protein, which they called MARi-1 (for Mitochondrial Anchored Ring protein1). Expression of MARi-1 leads to partial mitochondrial fragmentation and to the emergence of 70–100 nm mitochondrially derived, double-membrane vesicles that retained membrane potential. This budding-like process, which proceeds in the absence of obvious organelle constriction, requires Drp1 and MARi-1's RING domain.

An interesting observation concerns the capacity of mitochondrial fission proteins (Dlp1/Drp1 and Fis1) to act on peroxisomes. Peroxisomes are enveloped by a single membrane, but resemble mitochondria in that they are thought to represent independent organelles with direct protein targeting and no (or little) connection to other organelles by vesicular traffic. Michael Schrader (Marburg, Germany) reported that mitochondrial fission proteins Drp1 and Fis1 are involved in peroxisomal fission.

In contrast to other dynamin family members, the Mgm1/OPA1 GTPase does not localize to the cytosol but to the IMS of mitochondria, where it is partly IMM-associated. OPA1 is ubiquitously expressed in humans, but heterozygous mutations of the OPA1 gene in humans lead to the selective atrophy of the optic nerve (ADOA: autosomal dominant optic atrophy), causing blindness. This tissue-specific defect is reminiscent of the selective dysfunction of peripheral nerves in CMT2A patients with underlying Mfn2 mutations.

In the first part of his lecture, Katsuyoshi Mihara (Fukuoka, Japan) reported on the mechanisms of targeting, processing and inner membrane anchoring of AIF (apoptosis inducing factor). The second part of his presentation was devoted to an analysis of OPA1 isoform generation by mechanisms of alternative splicing and protease processing. Earlier work from the Reichert and Freeman laboratories had shown that yeast Mgm1p is a substrate of the Pcp1p/Rbd1p rhomboid protease. Using yeast to identify the mitochondrial proteases involved in OPA1 processing, Katsuyoshi Mihara presented surprising data showing that human OPA1 was processed equally efficiently in wild-type and ΔPcp1/Rbd1p yeast, but was impaired in yeast cells lacking Yta10p/Yta12p, mitochondrial inner membrane metalloproteases of the AAA family unrelated to rhomboid proteases. As a close human homolog of Yta10p/Yta12p, the mitochondrial protein paraplegin appears to be a prime candidate for an OPA1-processing protease. Of note, in humans, paraplegin mutations are causally associated with hereditary spastic paraplegia (HSP), a progressive neurodegenerative condition characterized by predominantly distal spinal cord degeneration. Nicely complementing this thematic focus, Elena I. Rugarli (Naples, Italy) presented data on the characterization of paraplegin-deficient mice which, although viable and fertile, display first symptoms of HSP at approximately 4 months of age. Ultrastructural studies of affected axons in paraplegin-deficient mice demonstrated that, long before axonal degeneration, the distal regions of axons are filled with mitochondria of abnormal size and morphology. Given paraplegin's known role as a metalloprotease, this observation led her to propose that proteolytic mechanisms within the matrix may be involved in some aspect of mitochondrial membrane dynamics.

Luca Scorrano (Padova, Italy) discussed his latest results on the role of OPA1 in apoptosis. By preventing mitochondrial cytochrome *c* release and organelle dysfunction, OPA1-expression protects from cell death induced by various stimuli, including hydrogen peroxide, staurosporine or recombinant tBid treatment. The presented data suggest that, during apoptosis, processed (soluble) OPA1 regulates cristae

morphology and mobilization of cytochrome *c* from intracristae stores by controlling the width of cristae junctions.

Luca Pellegrini (Quebec, Canada) presented work on the presenilin-associated rhomboid-like protein, PARL. Previous work from the D'Adamio and Freeman laboratories had defined Rhomboids as intramembrane-cleaving proteases. Based on a phylogenetic analysis of the rhomboid family, Pellegrini's data indicate that the N-terminal domain of PARL emerged only recently during vertebrate evolution to become strictly conserved in mammals to likely serve novel function(s) absent in invertebrates. Following-up on his earlier observation of a complex, self-regulated proteolytic processing of this domain, Pellegrini now reported that this cleavage is governed by phosphorylation/dephosphorylation events. Although the underlying mechanisms remain unknown at present, his findings suggest the attractive possibility that phosphorylation switches may participate in the regulation of mitochondrial morphology in the mammalian system.

Bart de Strooper (Leuven, Belgium) reported on the characterization of PARL knockout mice. PARL is a seven-transmembrane protein expressed at the IMM, where it has been assumed to be involved in OPA1-processing. From the age of 4 weeks onwards PARL-deficient mice display progressive cachexia (weight loss associated with chronic disease), and finally succumb around the ninth week with signs of pronounced muscle wasting as well as massive apoptosis in lymphoid organs. Interestingly, PARL deficiency increases sensitivity towards various apoptotic stimuli, with PARL −/− cells undergoing faster cristae remodeling and a more rapid cytochrome *c* release during cell death. As the repertoire of OPA1 isoforms was not altered in PARL knockout cells, this somewhat surprising result potentially argues against the hypothesis that PARL is the primary protease mediating OPA1-processing.

Mitochondrial membrane dynamics and programmed cell death

Mitochondria are important participants in programmed cell death (PCD) via the release of cytochrome *c* and other apoptogenic factors from the IMS into the cytoplasm. Mitochondrial dysfunction may thus provoke and/or stimulate the liberation of apoptogenic factors and cell death. In addition, it is further possible that proteins governing mitochondrial membrane dynamics under normal conditions also participate in OMM permeabilization during apoptosis.

Two presentations focused on the link between mitochondrial dysfunction and ageing. Nils-Göran Larsson (Stockholm, Sweden) reported on the characterization of mice expressing an error-prone, mutated catalytic subunit of the mtDNA polymerase PolgA. These animals develop a 'mtDNA mutator' phenotype characterized by signs of premature ageing, such as weight loss, kyphosis, osteoporosis, and alopecia in conjunction with a significantly shortened lifespan. Earlier studies in mouse models had suggested that intermitochondrial exchange of mtDNA by fusion and fission might represent a protective mechanism. Quantifying mitochondrial fission and fusion activity in living human umbilical vein endothelial cells (HUVECs), Marina Jendrach *et al*. (Frankfurt, Germany) observed a significant, equal decrease of both fusion and fission events following treatment with hydrogen peroxide to induce ROS generation, and the same phenomenon was seen in old and postmitotic HUVECs. Therefore, potentially by keeping complementation effects low, reduced mitochondrial dynamics may significantly contribute to an age-dependent accumulation of mutated mtDNA, which by itself may accelerate the process of ageing.

The lecture of Paolo Bernardi (Padova, Italy) was dedicated to the permeability transition pore (PTP). He reminded the audience that the current models depicting the putative/presumed structure and composition of the PTP still await formal proof and experimental validation. He presented his data on mice devoid of two putative components of the PTP, outer membrane VDAC and matrix cyclophilin D, and described the role of both proteins in permeability transition and apoptosis.

Douglas R Green (San Diego/CA, USA) presented data showing that cytochrome *c* (cyt *c*) release during apoptosis occurs almost invariably as a single-step event within a few minutes, and prior to loss of the mitochondrial membrane potential. There was no evidence for a caspase-dependent amplification mechanism of cytochrome *c* release nor caspase-induced changes in the mitochondrial membrane potential before cytochrome *c* release. Unexpectedly, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) – an enzyme serving pleiotropic functions in glycolysis, transcription, membrane fusion and DNA-repair – was indentified as an inhibitor of cytochrome *c* release. It is interesting to note here that GAPDH is upregulated in various tumor entities. Whether or not GAPDH's protective effects (by inhibiting cytochrome *c* release in the absence of caspase activity) have implications for tumorigenesis remains to be established.

Richard Youle (Bethesda/MD, USA) reported on the apoptosis-specific interaction between Bax and Endophilin B1 (EndoB1), a BAR domain protein involved in membrane-curvature regulation. During cell death, in analogy to Drp1, both Bax and EndoB1 translocate to mitochondrial constriction sites. Under conditions of decreased EndoB1 expression, mitochondrial Bax recruitment is blocked, resulting in fused mitochondrial outer membranes and a decreased cellular sensitivity towards apoptosis. In addition, Youle reported EndoB1 knockout MEFs to be resistant to various apoptosis inducers. Because Drp1 depletion does not inhibit Bax translocation, endophilin B1 is proposed to act upstream in the cascade of apoptosis-associated events.

Philippe Parone presented the findings of Jean-Claude Martinou's group (Geneva, Switzerland). Investigating the role of Drp1 in the mitochondrial membrane changes related to apoptosis, they demonstrated that inhibition of mitochondrial fission by RNAi-mediated Drp1 knockdown not only alters mitochondrial respiratory function and cellular energy metabolism, but also has an impact on cell-cycle regulation.

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