High-level mitochondriology at high altitude

Workshop on mitochondrial (dys-)function

Robert N. Lightowlers & Roland Lill^{1,+}

Department of Neurology, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK and ¹Institut für Zytobiologie und Zytopathologie der Philipps-Universität Marburg, Robert-Koch-Strasse 5, D-35033 Marburg, Germany

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(dys-)function held in the impressive Alpine venue of the Paul Langevin Conference Centre in Aussois, France. The authors have chosen a selection of the presentations to reflect the scientific flavour of the workshop.

Mitochondria—the shape of things to come

Technical innovations in light and electron microscopy are being used to re-evaluate our understanding of mitochondrial structure. Using cryo-electron tomography (C. Mannella, Albany, NY) and 4Pi-confocal microscopy (a fluorescence technique for improving axial resolution by physical means; S. Jakobs, Göttingen, Germany), mitochondria in yeast and mammals are being visualized with impressive resolution in three dimensions. Although these techniques have only recently been applied to mitochondrial imaging, electron tomography in particular has already generated some remarkable and challenging data. Based on two-dimensional studies of electron micrographs, it had been accepted for many years that the structures called 'cristae' are merely infoldings of the mitochondrial inner membrane. Cryo-electron tomography has facilitated the construction of a three-dimensional image of mitochondria, revealing that cristae in mitochondria from various cell types are pleiomorphic, tubular or laminar structures, connected to the inner membrane via narrow tubular junctions (Frey and Mannella, 2000). Discrete structures have also been revealed at contact sites between the inner and outer membranes, and it is speculated that they attach to vesicles from the endoplasmic reticulum at the periphery of the outer membrane. Hopefully, studies of the kinetics of fusion and budding of mitochondrial networks will be possible soon with these advances in imaging.

The mechanisms governing mitochondrial fusion and fission processes are now beginning to be resolved at a molecular level (Labrousse *et al.*, 1999; Mozdy *et al.*, 2000; Tieu and Nunnari, 2000; Fritz *et al.*, 2001; Sesaki and Jensen, 2001). J. Nunnari

Introduction

Mitochondria are ubiquitous organelles that, apart from a few notable exceptions (amitochondriate protists), are present in virtually all eukaryotic cells. Although we know that they contain their own genome, they house key enzymatic components for oxidative metabolism and the production of ATP and they harbour factors central for regulating apoptosis, we remain largely ignorant of many fundamental issues governing mitochondrial function and homeostasis. For example, to what extent is mitochondrial morphology linked to the biochemical functions of the organelle, how is the shape of mitochondria regulated, what molecular mechanisms mediate transmission of mitochondria during cell division, what is the contribution of mitochondria to apoptosis, how is nuclear and mitochondrial gene expression co-ordinated to guarantee cellular homeostasis, and are there any other crucial functions that are performed within the organelle? These and many other important questions formed the bedrock of a recent EMBO workshop on mitochondrial

⁺Corresponding author. Tel: +49 6421 286 6449; Fax: +49 6421 286 6414; E-mail: lill@mail.uni-marburg.de

(Davis, CA) discussed the interplay between several *Saccharomyces cerevisiae* proteins intimately involved in regulating mitochondrial morphology (Mgm1p, Fzo1p, Dnm1p, Mdv1p and Fis1p), a function shared by the human homologue of Fzo1p, which has been shown to induce mitochondrial fusion when overexpressed (M. Rojo, Paris, France; Santel and Fuller, 2001). The importance of the function of these proteins in mammals was further described by P. Belenguer (Toulouse, France), who extended recent observations to show that the human homologue of the *S. cerevisiae* MGM1 gene (*OPA1*, encoding a member of the dynamin family) is often mutated in patients suffering from a dominantly inherited optic atrophy (Alexander *et al.*, 2000; Delettre *et al.*, 2000). Strikingly, the affected tissue in this disease is the retinal ganglion layer, as is the case for Leber's hereditary optic neuropathy (LHON), which is perhaps the most common disorder associated with mitochondrial DNA (mtDNA) mutations.

Transmission of mitochondria and mtDNA

The segregation of mitochondria and mtDNA has been studied for many years (see, e.g., Berger and Yaffe, 2000), but at a molecular level it is still poorly understood. In yeast, numerous mutations lead to the loss of all or segments of mtDNA to yield ρ^0 or ρ^- strains, respectively. A better understanding of the molecular basis of mtDNA maintenance was provided by work performed by R. Butow and colleagues (Dallas, TX). They identified some 30 proteins associated with nucleoids (the packaging unit of mtDNA; Kaufman *et al.*, 2000). Many of these proteins were already known to be essential for mtDNA maintenance (e.g. Abf2p, Rim1p, Rpo41p, Mgm101p and Ilv5p), but others (e.g. Aco1p, Atp1p, Hsp60p, Hsp10p and Ilv6p) had no previous connection to mtDNA maintenance and hence were more unexpected. Together, these proteins are thought to provide a complex scaffold that is necessary to tether the mtDNA to the inner membrane. Random mutagenesis of the *ILV5* gene allowed the separation of the two functions of the encoded protein, in amino-acid biogenesis and DNA maintenance, into two non-overlapping structural segments. *ILV5* hypermorphs, in which mtDNA loss is enhanced compared with that observed in the deletion strain Δ ilv5, harbour a mutant protein Ilv5p that accumulates at contact sites. These sites appear to localize the nucleoid within the matrix, in apposition to the mitochondrial outer-membrane protein Mmm1p, which has been described recently by R. Jensen's group to be of fundamental importance for mtDNA maintenance (Hobbs *et al.*, 2001).

Mitochondrial motility also plays an important role in inheritance during cell division (Boldogh *et al.*, 2001a). It appears that motility is due, at least in part, to interactions between mitochondrial polypeptides and the cytoskeleton. L. Pon (New York, NY) showed that, in budding yeast, the highly conserved Arp2/3p complex (which includes two actin-related proteins) is found in several intracellular locations including mitochondria and is essential for the motility of this organelle (Boldogh *et al.*, 2001b). Furthermore, M. Yaffe (San Diego, CA) identified a novel fission yeast protein that is necessary for mitochondrial transmission and that mediates the association of mitochondria with microtubular structures (Mmd1p).

In contrast to yeast mtDNA, replication and transmission of mammalian mtDNA is believed to be less strictly regulated. After a mtDNA mutation occurs, the mutated molecule can occasionally become amplified, resulting in two sub-populations of mtDNA coexisting in the same organism, a condition known as 'heteroplasmy'. Surprisingly, although different mtDNA genotypes segregate by random processes during oogenesis, it has been reported that two genotypes in heteroplasmic mice show a consistent tissue-specific and age-related segregation pattern (for a more detailed explanation, see Jenuth *et al.*, 1997). By a series of subspecies crosses, E. Shoubridge and colleagues (Montreal, Canada) have now been able to map the 'segregator' gene to chromosome 5. This is a tantalizing result and hopefully this important gene will be identified soon.

Import, export and assembly of mitochondrial polypeptides

Several groups have contributed to steady progress in analysing the insertion of inner-membrane proteins from the mitochondrial matrix (G. Dujardin, T. Fox, W. Neupert and R. Stuart) over the past 4 years. Currently, a surprisingly large number of proteins (Oxa1p, Pnt1p, Mss2p, Cox18p, Pet309p, Cbp1p and Mba1p) are known to directly or indirectly assist insertion of inner-membrane proteins, e.g. the export of N- and C-terminal domains of subunit 2 of cytochrome oxidase (Cox2p). T. Fox (Ithaca, NY) presented results to show that some proteins (such as Mss2p and Cox18p) support C-tail export. He and G. Dujardin (Gif-sur-Yvette, France) further pointed out the important role of the Afg3p/Rca1p (Yta10p/12p) protease in the assembly of inner-membrane proteins, possibly by providing a chaperone function during assembly of inner-membrane complexes (such as cytochrome oxidase). Having just identified these factors needed for membrane insertion, we are far from understanding their mechanistic function at a molecular level. Fox reported on a possible interaction between Pnt1p, Cox18p and Mss2p, suggesting that these proteins may form a heterooligomeric translocase. Nevertheless, these components seem to operate quite distinctly from the well studied TOM and TIM preprotein translocases, which are located at the outer and inner membranes, respectively. J. Herrmann (München, Germany) presented recent studies on Mba1p (an integral membrane protein initially identified in L. Grivell's group), showing it to be in close contact with nascent polypeptide chains of the Cox1p, Cox2p and Cox3p subunits. In later stages of the membrane insertion process, Mba1p may then hand over the chains to the assembly components discussed above.

Iron metabolism and Fe/S cluster assembly

Mitochondrial iron metabolism has been the focus of extensive research following the discovery in 1997 that cells harbouring mutants of yeast frataxin (Yfh1p) or the mitochondrial ABC transporter Atm1p/ABC7 accumulate high amounts of iron in mitochondria (Askwith and Kaplan, 1998). Although intensely studied, the molecular function of frataxin has remained enigmatic. F. Foury (Louvain, Belgium) has previously provided evidence for defects in mitochondrial Fe/S proteins upon deletion of *YFH1*. A key observation to further support this idea may

be the new finding that the iron-binding protein Isp1p interacts genetically with Yfh1p. Based on a series of yeast mutants, Foury proposed that the inner-membrane proteins Mrs4p and Mmt2p serve as low-specificity metal transporters that might contribute to iron uptake into the organelles. Despite extensive efforts to identify mitochondrial metal transporters, these components still remain ill defined (with the exception of Mrs2p; Bui *et al.*, 1999).

Mitochondrial iron homeostasis is of pivotal importance for the entire cell. One of the roles of mitochondria in this process is to utilize iron for the synthesis of Fe/S clusters, essential components of both mitochondrial and cytosolic enzyme complexes. One of the big surprises to arise from recent mitochondrial research is that mitochondria appear to be centres for Fe/S cluster synthesis, irrespective of the cluster's final cellular destination (Lill and Kispal, 2000). R. Lill (Marburg, Germany) summarized the current view of the biogenesis of cellular Fe/S proteins, a task that is thought to involve some 15 mitochondrial components. He introduced an essential protein that resides in the inter-membrane space (represented by Erv1p in yeast and ALR, 'augmenter of liver regeneration', in humans) as a novel component that is specifically required for maturation of extra-mitochondrial Fe/S proteins (Lange *et al.*, 2001). Although direct evidence is lacking, Erv1p might operate downstream of Atm1p, the first component that was shown to fulfil a specific function in Fe/S protein maturation in the cytosol.

How many polypeptides make a mitochondrion?

The number of mitochondrial proteins (the mitochondrial proteome) has been estimated to be between 600 and more than 2000. To increase our knowledge of identities, number and sub-mitochondrial localization of these proteins, L.-J. Sweetlove (Oxford, UK) has started to analyse the proteome of *Arabidopsis thaliana* mitochondria by two-dimensional gel electrophoresis followed by MALDI-TOF analysis (Millar *et al.*, 2001). So far, the group has identified almost 200 proteins, some of which have unknown functions. Sweetlove also pointed out some of the caveats that are associated with the technique. Highly purified mitochondria are needed, identification of the proteins may be hampered by post-translational modifications and hence gene products are not always correctly predicted from genomic sequences. Further, membrane proteins are notoriously problematic to resolve, and proteins of low abundance are difficult to detect. The group has now begun to tackle these problems by analysing sub-mitochondrial fractions such as the inter-membrane space, even though highly purified cellular sub-fractions are difficult to prepare. A similar proteome approach was also reported by C. Florentz (Strasbourg, France) as a method for identifying polypeptides that are differentially regulated in human cell lines carrying mutated mtDNA.

Model thinking and mitochondrial disease

The classic 'model' organisms in which to study mitochondrial function and biogenesis are budding yeast, *Neurospora crassa*, rat, mice and, to a lesser extent, the fruit fly. B. Lemire (Edmonton, Canada) introduced the worm *Caenorhabditis elegans* as a new experimental system that is genetically tractable. His

group has created mitochondrial mutants by targeted disruption of the complex I protein Nuo1 and the β -subunit of F_1F_0 -ATPase. Both deletions are lethal and lead to growth arrest after larval stage 3. Growth and development to this stage are supported by maternally inherited products, but maturation to the adult requires the synthesis of new mitochondrial proteins. Earlier observations made by N.G. Larsson and D. Clayton in 1998 had suggested that respiration may not be essential in early embryonic development of mice. Animals devoid of the mitochondrial transcription factor Tfam, a nucleus-encoded protein involved in mtDNA maintenance and gene expression, develop until days 8–10 of embryonic development, despite the lack of synthesis of mitochondria-encoded subunits of oxidative phosphorylation complexes (Larsson *et al.*, 1998). Larsson (Stockholm, Sweden) has recently extended this elegant work by producing a variety of tissue-specific Tfam-deficient mice (see, e.g., Silva *et al.*, 2000). The latest approach utilized the calcium-dependent calmodulin kinase II promoter to express cre-recombinase, thereby decreasing the level of Tfam (and consequently cellular respiration) in the forebrain. One striking observation was the relatively low level of reactive oxygen species (ROS) generated by the mtDNA-depleted neurones. This appears to be a tissue-selective difference, as ROS production was shown to increase substantially in the heart-specific knockout.

Perspectives

Finally, it is impossible to attend a meeting entitled 'mitochondrial (dys-)function' without asking what progress was being made towards a therapy for the many patients suffering from mitochondrial disease. Sadly, we must report that, although there has been important progress in the identification of numerous novel nuclear mutations that underlie mitochondrial disease (J. Smeitink, Nijmegen, The Netherlands, P. Rustin and A. Rötig, Paris, France), we are still unable to provide any effective treatment or therapy for the vast majority of patients with mitochondrial disease, irrespective of underlying mutations in nuclear or mitochondrial DNA. However, the networking of researchers involved in identifying disease-related genes and investigators dedicated to tackling the molecular mechanisms of mitochondrial function provoked invaluable discussions and created ideas for the future direction of investigations. Similar gatherings of classical mitochondrialists and scientists working on the central role of mitochondria in apoptosis (summarized in two talks by G. Kroemer and J.C. Martinou; see Martinou and Green, 2001) might be beneficial for the exchange of knowledge about these delicate organelles. Unquestionably, the high-altitude Alpine atmosphere of the meeting promoted the development of many concepts for novel experimental approaches that will lead eventually to the long-desired progress in the treatment of mitochondrial diseases.

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