

## Mitochondrial Dynamics in Cell Life and Death

R Nandipati

### INTRODUCTION

The first workshop on "Mitochondrial Dynamics in Cell Life and Death," sponsored by the Federation of European Biochemical Societies and the International Union of Biochemistry and Molecular Biology, took place in August 2005 at the Venetian Institute of Molecular Medicine in Padova, Italy. The meeting was dedicated to the memory of Stanley J Korsmeyer, one of the leading scientists in apoptosis research, who died in March 2005. Meeting organizer Luca Scorrano and his scientific committee set up an interesting programme in which key researchers in the biology of mitochondrial dynamics presented novel insights and intriguing data. It was the first forum of this kind that allowed scientists to meet and discuss the molecular mechanisms that regulate mitochondrial morphology and their importance in pathophysiological processes. In this meeting report, I will summarize some of the contributions that dealt with aspects of mitochondrial dynamics that relate to degenerative processes like apoptosis and ageing.

### Dynamin-like Proteins Regulate Mitochondrial Dynamics

Mitochondria are dynamic organelles that move along elements of the cytoskeleton (for example, microtubules), undergo fusion and fission events, and form interconnected tubular networks. Although the basis of this behaviour is presently unclear, research in yeast and other model systems has led to the identification of crucial molecular components involved in the fission and fusion of mitochondria (1). This dynamic is regulated by several factors, including members of the family of large dynamin-like guanosine triphosphatases (GTPases) (2). One such protein, MGM1, was previously shown to be necessary for fusion of mitochondria in yeast (3, 4). Alexander van der Bliek (University of California, Los Angeles, USA) spoke on the characterization of the corresponding *Caenorhabditis elegans* gene, MGM1 *eat-3* mutant animals bear a mutation in MGM1 that leads, as in other organisms, to a marked fragmentation of mitochondria. Electron microscopy revealed that the mutant mitochondria contain septa, suggesting additional divisions of the inner mitochondrial membrane, and have a reduced number of

cristae as compared with wild-type mitochondria. On the organismic level, *eat-3* mutants grow more slowly than the wild type and have a reduced fecundity. Interestingly, they are long-lived relative to wild type. The phenotype can be partially suppressed in animals bearing mutations in two other genes encoding large dynamin-like GTPases, *DRP1* and *FZO1*. *DRP1* is involved in the fission of the outer mitochondrial membrane, whereas *FZO1* is needed for fusion of mitochondria. *FZO1* mutants are characterized by increased mitochondrial fragmentation, similar to the morphology displayed by *eat-3* animals. Therefore, the observed suppression of the *eat-3* phenotype was rather surprising. The mechanisms underlying these effects are under investigation by the van der Bliek group.

G Angus McQuibban (University of Toronto, Canada) gave a presentation on the regulation of MGM1 activity in the fruit fly *Drosophila melanogaster*. Previous work has shown that in yeast, MGM1 is regulated by the mitochondrial rhomboid protease Rbd1/Pcp1 (5, 6). Cleavage of the transmembrane domain of MGM1 leads to the release of this protein into the mitochondrial intermembrane space and stimulates mitochondrial fusion. To develop a genetic model of mitochondrial membrane fusion in higher eukaryotes, the role of the *Drosophila* rhomboid protease (Rhomboid-7) in the regulation of mitochondrial dynamics was investigated. *Rhomboid-7* mutants display mitochondrial fragmentation that is accompanied by severe defects of the neuromuscular system, such that the flies are not able to move properly, for example. Moreover, males are sterile. The life-span of the mutants is drastically reduced. In contrast to wild-type strains of *Drosophila* that exhibit a mean life-span of around 60 days, the mutants usually die after three days. These results demonstrate the impact of an abrogated control of mitochondrial dynamics on the organismic level.

### Mitochondrial Dynamics and Apoptosis

Several speakers discussed the role of the mammalian MGM1 homolog, *OPA1*, in apoptosis. Recent data show that programmed cell death is involved in mammalian ageing (7). Thus, elucidating the role that the machinery of mitochondrial dynamics plays in apoptosis might well give important insights into the ageing process.

Bart de Strooper (University of Leuven, Belgium) described mice lacking the rhomboid protease PARL, which is an ortholog of yeast Rbd1/Pcp1. Processing of the long

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From: Department of Biomedical Sciences, The University of Sheffield, Sheffield, UK.

Correspondence: Dr R Nandipati, Department of Biomedical Sciences, The University of Sheffield, Sheffield, UK, e-mail: ramesh.nandipati@gmail.com.

isoform of OPA1 is impaired in *Part<sup>-/-</sup>* mitochondria. The resulting lack of the small OPA1 isoform leads to a faster remodelling of mitochondrial cristae with increased mobilization and release of cytochrome *c*. This molecule, a component of the mitochondrial electron transport chain, is released into the cytoplasm after permeabilization of the mitochondrial outer membrane, an event that is triggered early in the process of apoptosis. In the cytoplasm, cytochrome *c* functions in the activation of several caspases, proteases that are central to the process of apoptosis. Consequently, *Part<sup>-/-</sup>* cells are much more sensitive to death stimuli than wild-type cells, although mitochondrial morphology is not altered. Knockout mice initially exhibit normal development, but from the fourth week on, progressive atrophy in multiple tissues of the animal leads eventually to the death of the organism. The observed atrophy is accompanied by increased apoptosis. These results are in line with observations supporting a protective role for OPA1 in programmed cell death. Luca Scorrano (Venetian Institute of Molecular Medicine) described evidence showing that OPA1 protects cells from apoptosis at the mitochondrial level by preventing the release of cytochrome *c*. For this function, OPA1 does not require the protein mitofusin-1, which is needed for fusion of the outer mitochondrial membrane. Moreover, it does not interfere with activation of the pro-apoptotic molecules BAX and BAK. Taken together, these results show that the small diffusible form of OPA1 regulates both remodelling of the cristae and mobilization of cytochrome *c* stores, and thus plays an important role in the apoptotic process.

In addition to OPA1, several other factors of the machinery involved in mitochondrial dynamics have a profound effect in the regulation of apoptosis (8). Yisang Yoon (University of Rochester, USA) discussed the regulation of mitochondrial fission and apoptosis by the mitochondrial outer membrane protein hFIS1. hFIS1 acts in mammals in conjunction with the dynamin-related protein DRP1 to divide mitochondria (9). Two regions of hFIS1 that are essential for mitochondrial scission have been identified. The tetratricopeptide (TPR) region is involved in the interaction with DRP1 or a DRP1-containing complex. The first alpha helix of hFIS1 ( $\alpha 1$ ) functions as a fission effector domain by regulating the interaction between TPR motifs and DRP1. If the  $\alpha 1$  region of hFIS1 is deleted, mitochondrial swelling and significantly delayed cell death are observed. Yoon speculated that the  $\alpha 1$  helix might function as a DRP1 releasing factor.

DRP1 co-localizes with the pro-apoptotic BAX molecule at future scission sites on the outer mitochondrial membrane, where these factors form a structure that circumscribes the mitochondrion. Richard Youle (National Institutes of Health, USA) spoke on the role of endophilin B1 (a fatty acyl transferase) in mitochondrial fission and apoptosis. Together with BAX, endophilin B1 translocates to focal regions of mitochondria during apoptosis. If expression of endophilin B1 is decreased, BAX translocation is inhibited and the

cellular sensitivity to apoptosis is consequently reduced. In contrast, down-regulation of DRP1 inhibits cytochrome *c* release and mitochondrial fission, whereas binding of BAX on the outer mitochondrial membrane is not negatively affected. These data suggest that endophilin B1 acts upstream of DRP1 in the apoptotic pathway.

Cell survival after mitochondrial outer membrane permeabilization during apoptosis was discussed by Douglas R Green (La Jolla Institute for Allergy and Immunology, USA). The well-known glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was identified as an important regulator of caspase-independent cell death. In this case, cells die because the permeabilization of the outer mitochondrial membrane still takes place, so that pro-apoptotic factors are released from the mitochondria into the cytosol. Although over-expression of GAPDH does not block release of cytochrome *c*, improved recovery into mitochondria of this proapoptotic factor is observed. Moreover, these cells regain their proliferative potential over time. These findings indicate that GAPDH is able to protect against apoptosis after cytochrome *c* release in the absence of caspase activation.

## CONCLUSION

Overall, the meeting succeeded in bringing together scientists working in the field of mitochondrial dynamics to discuss fascinating topics in this fast-growing field. It became clear that the elucidation of the molecular pathways controlling mitochondrial fusion and fission is crucial for fully understanding diverse physiological processes, including apoptosis and ageing. Given that apoptosis is a common characteristic of neurodegenerative diseases a better understanding of these pathways should be relevant to these diseases as well. At the end of the workshop, there was common agreement that this kind of meeting should be repeated in the future.

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