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Proteins That Fuse and Fragment Mitochondria in Apoptosis: Con-Fissing a Deadly Con-Fusion?

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During apoptosis, mitochondria undergo multiple changes that culminate in the release of cytochrome *c* and other proapoptotic cofactors. Recently, a role for previously overlooked morphological changes, fission of the mitochondrial reticulum and remodeling of mitochondrial cristae, has been suggested in mammalian cells and in developmental apoptosis of *C. elegans*. Mitochondrial morphology is determined by fusion and fission processes, controlled by a growing set of "mitochondria-shaping" proteins, whose levels and function appear to regulate the mitochondrial pathways of cell death. Expression of pro-fusion proteins, as well as of inhibition of pro-fission molecules reduces apoptosis, suggesting a linear relationship between fragmentation and death. Mechanisms by which mitochondrial fragmentation promotes apoptosis and interactions between fragmentation and remodeling of the inner membrane are largely unclear. A tempting, unifying hypothesis suggests that fission is coupled to cristae remodeling to maximize cytochrome *c* release.

KEY WORDS: Mitochondria; fusion; fission; apoptosis; cytochrome *c*; dynamin-related protein 1; OPA1; mitofusin; BCL-2 family members.

Mitochondria are crucial organelles for life and death of the cell. They produce most of the ATP needed for endoergonic processes and are strategically located at the sites of greater energy demand. They modulate cytosolic Ca^{2+} transients, hence regulating complex signaling processes (Duchen, 2000; Orrenius *et al*., 2003; Rizzuto *et al*., 2000). The discovery by Korsmeyer and coworkers that the antiapoptotic oncogene BCL-2 targets its product to mitochondria (Hockenbery *et al*., 1990) suggested that they also play a crucial role in controlling cell death. This was subsequently substantiated by an enormous body of evidence showing that during apoptosis mitochondria integrate diverse stimuli by releasing protein cofactors needed for the efficient activation of effector caspases in the cytosol [for a review, see for example (Danial and Korsmeyer, 2004; Green and Reed, 1998; Wang, 2001)]. This release is either accompanied or preceded by mitochondrial dysfunction, an issue beyond the topic of this review, but exhaustively addressed by other papers in this issue and for example by (Bernardi *et al*., 2001; Green and Kroemer, 2004). Notwithstanding the recognition of a crucial involvement of mitochondria in apoptosis, the initial observation that mitochondrial structure was untouched during the process, as opposed to what happened during necrotic death, held true for several years and is still widely accepted. As a discriminating criterion between necrosis and apoptosis, Pathology textbooks often indicate the absence of mitochondrial structural changes in the latter. Alterations in mitochondrial morphology were occasionally reported during apoptosis (De Vos *et al*., 1998; Mancini *et al*., 1997; Mootha *et al*., 2001; Sanchez-Alcazar *et al*., 2001), but their functional role in programmed cell death was unclear and they were often considered as epiphenomena of the caspase-operated cell dismantling process. Our understanding of the functional role of mitochondrial structural changes during apoptosis greatly improved in the last years. This was mainly the consequence of a deeper knowledge of the molecular machinery of mitochondrial fusion and fission, and of the use of powerful techniques of electron tomography (ET) coupled to 3D image reconstruction to investigate mitochondrial structure during apoptosis. Here we will discuss in some details the mechanisms that could participate in

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the regulation of inner mitochondrial membrane remodeling during apoptosis; and the possible relationship between dynamics of the inner membrane and that of the mitochondrial reticulum.

THE MOLECULAR TOOLKIT FOR MITOCHONDRIAL FUSION AND FRAGMENTATION

Mitochondria are double-membrane bounded organelles. It is reasonable to think that fusion-fission processes are therefore highly complex, since they can act on the two membranes coordinately or separately. A further level of complexity is added by the organization of the mitochondrial inner membrane into cristae. Altogether, these considerations call for a precise regulation of mitochondrial dynamics. "Mitochondria-shaping" proteins, characterized originally in the budding yeast, include both pro-fusion and pro-fission members (Shaw and Nunnari, 2002). Several other mitochondria-shaping proteins have been identified in a screening of a yeast *petite* mutant library (Dimmer *et al*., 2002), further confirming how tightly regulated is this process.

Fusion and Fission in Budding Yeast

Among the many genes discovered to affect mitochondrial morphology in yeast, some encode for "master" proteins that are essential components of the import machinery (Meisinger *et al*., 2004) or are structural components of the lid of the proteasome (Rinaldi *et al*., 1998). Their effect on mitochondrial shape is therefore a secondary to their primary general function. Other proteins directly control the fusion–fission equilibrium and we will analyze them in deeper detail.

Fission of mitochondrial membranes is accomplished by the recruitment of the dynamin-related large GTPase Dnm1p to the outer membrane, where it complexes with the adapter Mdv1p and the integral outer membrane protein Fis1p (Bleazard *et al*., 1999; Mozdy *et al*., 2000; Sesaki and Jensen, 1999; Tieu *et al*., 2002). Another protein, Gag3p, interacts with Dnm1p in a yeast two-hybrid assay and might function as an essential component of the severing machinery (Fekkes *et al*., 2000). How inner mitochondrial membrane is severed is still unclear, but experimental evidences suggest that separate fission machinery exists. In *Caenorhabditis elegans*, expression of dominant negative mutants of DRP-1, the homologue of yeast Dnm1, results in fragmented matrix compartments, which are still connected each other by tubules of outer membrane (Labrousse *et al*., 1999). Thus, inner membrane fission persists even when outer membrane fission is blocked. A possible participant in inner membrane fission is Mdm33p, an inner mitochondrial membrane protein with predicted coiled-coil domains whose over expression leads to formation of inner membrane septa followed by fission of the mitochondrial reticulum (Messerschmitt *et al*., 2003).

Fusion process also involves proteins localized both at the outer and at the inner mitochondrial membrane. A crucial role has been ascribed to the GTPase Fzo1p (Hermann *et al*., 1998; Rapaport *et al*., 1998), which protrudes towards the cytosol, interplays with the adapter Ugo1p in the outer membrane (Sesaki and Jensen, 2001), and associates with the inner membrane dynamin-related protein Mgm1p to coordinate the fusion of the four membranes of two juxtaposed mitochondria (Sesaki *et al*., 2003b; Wong *et al*., 2000; Wong *et al*., 2003). Mgm1p is cleaved and presumably activated by Ugo2p/Pcp1p/Rbd1p, a rhomboid-like protease of the inner mitochondrial membrane (Herlan *et al*., 2003; McQuibban *et al*., 2003; Sesaki *et al*., 2003a). In vitro assays of reconstituted mitochondrial fusion indicate the requirement of mitochondrial membrane potential for inner membrane fusion.

Fusion and Fission in Mammalian Cells

Mammalian orthologues of *DNM1* and *FIS1*, called dynamin-related protein-1 (*Drp-1*) and *hFis1*, respectively, have been identified and shown to participate in mitochondrial fission (James *et al*., 2003a; Labrousse *et al*., 1999; Smirnova *et al*., 2001). Levels of DRP-1 control mitochondrial fission and DRP-1 is rapidly degraded, unless protected by sumoylation by SUMO1 (Harder *et al*., 2004). Endophilin B1, a fatty acyl transferase belonging to a family involved in endocytic vesicle formation, controls fission of mitochondrial outer membranes, probably downstream of DRP-1 (Karbowski *et al*., 2004b). No homologues of mdm33p have been identified so far, but silencing of mitofilin, a protein of the inner mitochondrial membrane, has been reported to cause the disappearance of regular cristae (John *et al*., 2005). The associated mitochondrial dysfunction questions its specificity and calls for deeper analysis.

Two *FZO1* homologues have been identified, mitofusin (*Mfn*) 1 and 2 (Rojo *et al*., 2002; Santel *et al*., 2003; Santel and Fuller, 2001). Both are required for embryonic development, as substantiated by their genetic ablation in the mouse (Chen *et al*., 2003), but it is unclear whether *Mfn* redundancy reflects functional differences between MFN1 and MFN2. MFN1 structure has been deeply investigated, identifying a heptad-repeat region that mediates

mitochondrial tethering by homotypic *trans* interaction prior to fusion (Koshiba *et al*., 2004). Several functional data recently emerged on MFN2: inactivating mutations have been associated with Charcot-Marie-Tooth type IIa axonal neuropathy (Kijima *et al*., 2005; Zuchner *et al*., 2004); levels of MFN2 are reduced in obese patients (Bach *et al*., 2003) and its overexpression can inhibit neointimal proliferation in rodent models of hypertension (Chen *et al*., 2004); ability of MFN2 to promote mitochondrial fusion is lower than MFN1, correlating with its GTPase activity (Ishihara *et al*., 2004). The mammalian orthologue of mgm1p is the inner membrane dynamin-related protein OPA1. Mutations in *Opa1* are associated with autosomal dominant optic atrophy (DOA), the leading cause of inherited blindness (Alexander *et al*., 2000; Delettre *et al*., 2000). Function of OPA1 is unclear as well as its interaction with other mitochondria shaping proteins. It has been proposed that OPA1 participates in a fission/fragmentation pathway (Griparic *et al*., 2004; Misaka *et al*., 2002; Satoh *et al*., 2003) or in the maintenance of the structural integrity of the mitochondrial reticulum (Olichon *et al*., 2003). We therefore took a genetic approach to investigate the role of OPA1 in shaping mitochondria. OPA1 promotes the formation of a branched network of tubular and interconnected mitochondria, which spans the entire volume of the cytoplasm, as substantiated by 3D-image reconstruction and volumetric rendering of stacks of mitochondrial images. Reduction of OPA1 levels by RNA interference (RNAi) results in globular and fragmented mitochondria as a consequence of reduced fusion, specifically measured by polyethylene glycol (PEG) fusion assays. On the other hand, expression of OPA1 increases mitochondrial fusion rates. A genetic dissection of the determinants of OPA1 function reveals an absolute requirement for MFN1 but not for MFN2. In complex, OPA1 impinges on MFN1-dependent fusion to regulate morphology of the mitochondrial reticulum (Cipolat *et al*., 2004). Our results raise the question of whether OPA1 functions primarily at the level of inner membrane, or it acts as a global controller of the mitochondrial reticulum. This question appears crucial, not only to understand the pathogenesis of dominant optic atrophy in which OPA1 is mutated, but also to clarify the relative role of fusion/fission of the mitochondrial reticulum vs. changes in the mitochondrial ultrastructure during apoptosis.

STRUCTURE OF MITOCHONDRIAL INNER MEMBRANE

The complexity of mitochondrial dynamics is further bamboozled by the ultrastructural organization of the inner membrane. Mitochondrial cristae, which according to the Palade's model were widely opened baffles of the inner membrane (Palade, 1952), have been identified as a separate compartment connected by narrow tubular junctions to the thin intermembrane space (Frey and Mannella, 2000). This structural organization raises two crucial questions: which molecules determine its formation, and which are its functional consequences.

To date, no single mitochondria-shaping protein has been identified to affect only cristae structure. In the budding yeast, mdm33p as well as mgm1p are required for normal cristae morphology and at the same time for an appropriate mitochondrial reticulum (Messerschmitt *et al*., 2003; Sesaki *et al*., 2003b; Shepard and Yaffe, 1999; Wong *et al*., 2000; Wong *et al*., 2003). In mammalian cells, levels of OPA1 regulate both mitochondrial ultrastructure and shape of the mitochondrial reticulum (Cipolat *et al*., 2004; Griparic *et al*., 2004; Olichon *et al*., 2003). Genetic tools will probably help us in dissecting the molecular machinery that regulates cristae morphology. For example, our recent discovery that OPA1 needs MFN1 to regulate dynamics of the mitochondrial reticulum (Cipolat *et al*., 2004), suggests that OPA1 could have a further function in regulating shape of the cristae, this time independently from outer membrane fzo1p homologues. Such a scenario would dissociate genetically and functionally the effects of OPA1 on the inner vs. those on the outer membrane.

It has been proposed that mdm38p, which is targeted to the inner mitochondrial membrane, plays a role in regulating its shape (Dimmer *et al*., 2002). This raised considerable interest, since its human homologue LETM1 is downregulated in Wolff–Hirschhorn syndrome, a complex genetic disease (Endele *et al*., 1999; Schlickum *et al*., 2004). Recent evidences point to a role for mdm38p in mitochondrial K^+ homeostasis rather than as a mitochondria-shaping protein (Nowikovsky *et al*., 2004). These findings leave mitochondrial inner membrane still without a specific shaping protein.

Functional consequences of the inner mitochondrial membrane compartmentalization have been revealed by both *in silico* and wet biology approaches. Computer-modeling studies suggested gradients of ions and small molecules along the narrow tubular junction of the cristae. (Mannella *et al*., 2001). These junctions are responsible for the segregation of cytochrome *c* in the cristae compartment (Bernardi and Azzone, 1981; Scorrano *et al*., 2002), where the majority of the respiratory chain complexes also localize (D'Herde *et al*., 2001; Perotti *et al*., 1983).

DYNAMIC OF MITOCHONDRIAL INNER MEMBRANE DURING APOPTOSIS

Intramitochondrial compartmentalization of cytochrome *c* raised the question of how its release can be fast and complete in the absence of mitochondrial swelling. We showed that "BH3-only" members of the BCL-2 family accomplished it by inducing a profound remodeling of the cristae, with fusion of the individual cristae and widening of the narrow tubular junction up to 70 nm. These changes increase the amount of free cytochrome *c* that redistributes to the intermembrane space, from where it can be released in the cytosol trough outer membrane pathways (Scorrano *et al*., 2002). A major dilemma is to understand the molecular mechanisms that regulate not only cristae formation, but also their remodeling during apoptosis. A natural candidate for this has been suggested in OPA1, which is located at the inner mitochondrial membrane. Down regulation of OPA1 levels causes mitochondrial fragmentation and dysfunction, with release of cytochrome *c* and apoptosis. These are accompanied by morphological changes of the cristae (Olichon *et al*., 2003), raising the possibility that OPA1 is required for the maintenance of their shape. Alternatively, cristae changes could be a consequence of the mitochondrial fragmentation induced by silencing of OPA1.

The relationship between fission and remodeling of the inner mitochondrial membrane is poorly understood, but a recent work by Shore and coworkers shed new light on this. In a set of elegant experiments, they showed that during apoptosis, mitochondrial Ca^{2+} uptake results in DRP-1 mediated mitochondrial fission and remodeling of the cristae (Germain *et al*., 2005). The latter is sensitive to cyclosporine A, which blocks the permeability transition pore, a large conductance, voltage dependent channel of the inner mitochondrial membrane (Bernardi, 1999) involved in cristae remodeling (Scorrano *et al*., 2002). These results raise the intriguing possibility that fission is coupled to cristae remodeling trough a yet unknown signal that propagates from DRP-1 to the inner mitochondrial membrane. They also call for additional evidence before concluding that OPA1 controls cristae morphology. An answer could come from overexpression experiments followed by analysis of the shape of the inner membrane in normal and apoptotic mitochondria. These experiments, coupled to analyses of intramitochondrial cytochrome *c* redistribution, should also answer to another unresolved question: how does OPA1 control the formation and maintenance of the narrow tubular junction? In analogy to the function of classic dynamins that tubulate membranes by forming a homopolymeric tangle of interconnected subunits around the lipid bundle, it has been proposed that OPA1 generates and maintains the narrow tubular junction of the cristae (Shaw and Nunnari, 2002). The general consensus is that OPA1 is located at the inner mitochondrial membrane, facing the intermembrane space (Olichon *et al*., 2002; Wong *et al*., 2000). A tempting hypothesis is that a ring of OPA1 molecules, inserted or attached to the neck of the cristae junction, works by pulling the membrane from inside. This model would be compatible with the existence of two pools of OPA1, one that regulates mitochondrial fusion together with MFN1 and is inserted in the inner membrane, the other only loosely attached to the membrane, perhaps localized at the neck of the cristae junction, where it controls the diameter of this structure. The double pool of OPA1 molecules could represent the mammalian counterpart of mgm1p processing by pcp1p in the yeast (Herlan *et al*., 2003; McQuibban *et al*., 2003), a protease belonging to the family of rhomboid intramembranous proteases that localizes to the inner mitochondrial membrane. To this end it will be essential to understand whether PARL, the mammalian homologue of pcp1p, processes OPA1.

MITOCHONDRIAL FISSION AND APOPTOSIS: WHICH ROLE FOR THE INNER MEMBRANE?

A set of evidences suggests that mitochondrial fission is an early step during apoptosis. Death by mitochondria-utilizing intrinsic stimuli is accompanied by mitochondrial fragmentation and blunted by dominant negative DRP-1 (Frank *et al*., 2001). Further analysis revealed that early in the course of cell death, MFN1 dependent mitochondrial fusion is largely inhibited (Karbowski *et al*., 2004a) and combined overexpression of MFN1 and MFN2 protects from death by intrinsic stimuli like etoposide and BID (Sugioka *et al*., 2004). Similarly, expression of hFis1 results in cytochrome *c* release and death (James *et al*., 2003b) and its downregulation by RNA interference prevents apoptosis to a greater extent than DRP-1 silencing (Lee *et al*., 2004). A crucial role for the fission machinery seems to be confirmed also by results obtained in the yeast, where DRP-1 homologue dnm1p mediates mitochondrial fragmentation and death following several stimuli, suggesting an old evolutionary role for mitochondrial fission in death (Fannjiang *et al*., 2004). In complex, these data seem to establish a linear correlation between fragmentation and apoptosis, but the picture is probably not so simple. First, not always fission promotes apoptosis, as confirmed by the ability of overexpressed DRP-1 to inhibit death by ceramide (Szabadkai *et al*., 2004). In this case, DRP-1 appears to protect by blunting the mitochondrial Ca^{2+} waves that transmit ceramide-mediated

apoptotic signal (Pacher and Hajnoczky, 2001). Second, a crucial recent finding by the group of G. Shore showed DRP-1 dependent remodeling of the inner membrane during apoptosis, establishing for the first time a relationship between changes in the morphology of the mitochondrial reticulum and remodelling of cristae structure. These experiments suggest for the first time a potential mechanism for the effect of mitochondria-shaping proteins on death: if fission is associated with cristae remodeling, fragmented mitochondria are prompted for maximal release of cytochrome *c*. Further analysis is required to support this possibility, but if it stands, nature would have marveled us once more, coupling fission of the outer membrane to fusion of the individual cristae.

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REFERENCES

Alexander, C., Votruba, M., Pesch, U. E., Thiselton, D. L., Mayer, S., Moore, A., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G., Bhattacharya, S. S., and Wissinger, B. (2000). *Nat. Genet.* **26**, 211–215.

Bach, D., Pich, S., Soriano, F. X., Vega, N., Baumgartner, B., Oriola, J., Daugaard, J. R., Lloberas, J., Camps, M., Zierath, J. R., Rabasa-Lhoret, R., Wallberg-Henriksson, H., Laville, M., Palacin, M., Vidal, H., Rivera, F., Brand, M., and Zorzano, A. (2003). *J. Biol. Chem.* **278**, 17190–17197.

Bernardi, P. (1999). *Physiol. Rev.* **79**, 1127–1155.

Bernardi, P., and Azzone, G. F. (1981). *J. Biol. Chem.* **256**, 7187–7192.

Bernardi, P., Petronilli, V., Di Lisa, F., and Forte, M. (2001). *Trends. Biochem. Sci.* **26**, 112–117.

Bleazard, W., McCaffery, J. M., King, E. J., Bale, S., Mozdy, A., Tieu, Q., Nunnari, J., and Shaw, J. M. (1999). *Nat. Cell Biol.* **1**, 298–304.

Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., and Chan, D. C. (2003). *J. Cell Biol.* **160**, 189–200.

Chen, K. H., Guo, X., Ma, D., Guo, Y., Li, Q., Yang, D., Li, P., Qiu, X., Wen, S., Xiao, R. P., and Tang, J. (2004). *Nat. Cell Biol.* **6**, 872–883.

Cipolat, S., de Brito, O. M., Dal Zilio, B., and Scorrano, L. (2004). *Proc. Natl. Acad. Sci.* U.S.A **101**, 15927–15932.

D'Herde, K., De Prest, B., Mussche, S., Schotte, P., Beyaert, R., Coster, R. V., and Roels, F. (2001). *Cell Death. Differ.* **7**, 331–337.

Danial, N. N., and Korsmeyer, S. J. (2004). *Cell* **116**, 205–219.

De Vos, K., Goossens, V., Boone, E., Vercammen, D., Vancompernolle, K., Vandenabeele, P., Haegeman, G., Fiers, W., and Grooten, J. (1998). *J. Biol. Chem.* **273**, 9673–9680.

Delettre, C., Lenaers, G., Griffoin, J. M., Gigarel, N., Lorenzo, C., Belenguer, P., Pelloquin, L., Grosgeorge, J., Turc-Carel, C., Perret, E., Astarie-Dequeker, C., Lasquellec, L., Arnaud, B., Ducommun, B., Kaplan, J., and Hamel, C. P. (2000). *Nat. Genet.* **26**, 207–210.

Dimmer, K. S., Fritz, S., Fuchs, F., Messerschmitt, M., Weinbach, N., Neupert, W., and Westermann, B. (2002). *Mol. Biol. Cell* **13**, 847–853.

Duchen, M. R. (2000). *J. Physiol.* 529 Pt **1**:57–68., 57–68.

Endele, S., Fuhry, M., Pak, S. J., Zabel, B. U., and Winterpacht, A. (1999). *Genomics* **60**, 218–225.

Fannjiang, Y., Cheng, W. C., Lee, S. J., Qi, B., Pevsner, J., McCaffery, J. M., Hill, R. B., Basanez, G., and Hardwick, J. M. (2004). *Genes Dev.* **18**, 2785–2797.

Fekkes, P., Shepard, K. A., and Yaffe, M. P. (2000). *J. Cell Biol.* **151**, 333–340.

Frank, S., Gaume, B., Bergmann-Leitner, E. S., Leitner, W. W., Robert, E. G., Catez, F., Smith, C. L., and Youle, R. J. (2001). *Dev. Cell* **1**, 515–525.

Frey, T. G., and Mannella, C. A. (2000). *Trends. Biochem. Sci.* **25**, 319–324.

Germain, M., Mathai, J. P., McBride, H. M., and Shore, G. C. (2005). *EMBO J.* **24**, 1546–1556.

Green, D. R., and Kroemer, G. (2004). *Science* **305**, 626–629.

Green, D. R., and Reed, J. C. (1998). *Science* **281**, 1309–1312.

Griparic, L., van der Wel, N. N., Orozco, I. J., Peters, P. J., and van der Bliek, A. M. (2004). *J. Biol. Chem.* **279**, 18792–18798.

Harder, Z., Zunino, R., and McBride, H. (2004). *Curr. Biol.* **14**, 340–345.

Herlan, M., Vogel, F., Bornhovd, C., Neupert, W., and Reichert, A. S. (2003). *J. Biol. Chem.* **278**, 27781–27788.

Hermann, G. J., Thatcher, J. W., Mills, J. P., Hales, K. G., Fuller, M. T., Nunnari, J., and Shaw, J. M. (1998). *J. Cell Biol.* **143**, 359–373.

Hockenbery, D. M., Nunez, G., Milliman, C., Schreiber, R. D., and Korsmeyer, S. J. (1990). *Nature* **348**, 334–336.

Ishihara, N., Eura, Y., and Mihara, K. (2004). *J. Cell Sci.* **117**, 6535–6546.

James, D. I., Parone, P. A., Mattenberger, Y., and Martinou, J. C. (2003a). *J. Biol. Chem.* **278**, 36373–36379.

James, D. I., Parone, P. A., Mattenberger, Y., and Martinou, J. C. (2003b). *J. Biol. Chem.* **278**, 36373–36379.

John, G. B., Shang, Y., Li, L., Renken, C., Mannella, C. A., Selker, J. M., Rangell, L., Bennett, M. J., and Zha, J. (2005). *Mol. Biol. Cell* **16**, 1543–1554.

Karbowski, M., Arnoult, D., Chen, H., Chan, D. C., Smith, C. L., and Youle, R. J. (2004a). *J. Cell Biol.* **164**, 493–499.

Karbowski, M., Jeong, S. Y., and Youle, R. J. (2004b). *J. Cell Biol.* **166**, 1027–1039.

Kijima, K., Numakura, C., Izumino, H., Umetsu, K., Nezu, A., Shiiki, T., Ogawa, M., Ishizaki, Y., Kitamura, T., Shozawa, Y., and Hayasaka, K. (2005). *Hum. Genet.* **116**, 23–27.

Koshiba, T., Detmer, S. A., Kaiser, J. T., Chen, H., McCaffery, J. M., and Chan, D. C. (2004). *Science* **305**, 858–862.

Labrousse, A. M., Zappaterra, M. D., Rube, D. A., and van der Bliek, A. M. (1999). *Mol. Cell* **4**, 815–826.

Lee, Y. J., Jeong, S. Y., Karbowski, M., Smith, C. L., and Youle, R. J. (2004). *Mol. Biol. Cell* **15**, 5001–5011.

Mancini, M., Anderson, B. O., Caldwell, E., Sedghinasab, M., Paty, P. B., and Hockenbery, D. M. (1997). *J. Cell Biol.* **138**, 449–469.

Mannella, C. A., Pfeiffer, D. R., Bradshaw, P. C., Moraru, I. I., Slepchenko, B., Loew, L. M., Hsieh, C. E., Buttle, K., and Marko, M. (2001). *IUBMB Life* **52**, 93–100.

McQuibban, G. A., Saurya, S., and Freeman, M. (2003). *Nature* **423**, 537–541.

Meisinger, C., Rissler, M., Chacinska, A., Szklarz, L. K., Milenkovic, D., Kozjak, V., Schonfisch, B., Lohaus, C., Meyer, H. E., Yaffe, M. P., Guiard, B., Wiedemann, N., and Pfanner, N. (2004). *Dev. Cell* **7**, 61–71.

Messerschmitt, M., Jakobs, S., Vogel, F., Fritz, S., Dimmer, K. S., Neupert, W., and Westermann, B. (2003). *J. Cell Biol.* **160**, 553–564.

Misaka, T., Miyashita, T., and Kubo, Y. (2002). *J. Biol. Chem.* **277**, 15834–15842.

Mootha, V. K., Wei, M. C., Buttle, K. F., Scorrano, L., Panoutsakopoulou, V., Mannella, C. A., and Korsmeyer, S. J. (2001). *EMBO J.* **20**, 661–671.

Mozdy, A. D., McCaffery, J. M., and Shaw, J. M. (2000). *J. Cell Biol.* **151**, 367–380.
Nowikovsky, K., Froschauer, E. M., Zsurka, G., Samaj, J., Reipert, S., Kolisek, M., Wiesenberger, G., and Schweyen, R. J. (2004). *J. Biol. Chem.* **279**, 30307–30315.
Olichon, A., Baricault, L., Gas, N., Guillou, E., Valette, A., Belenguer, P., and Lenaers, G. (2003). *J. Biol. Chem.* **278**, 7743–7746.
Olichon, A., Emorine, L. J., Descoins, E., Pelloquin, L., Brichese, L., Gas, N., Guillou, E., Delettre, C., Valette, A., Hamel, C. P., Ducommun, B., Lenaers, G., and Belenguer, P. (2002). *FEBS Lett.* **523**, 171–176.
Orrenius, S., Zhivotovsky, B., and Nicotera, P. (2003). *Nat. Rev. Mol. Cell Biol.* **4**, 552–565.
Pacher, P., and Hajnoczky, G. (2001). *EMBO J.* **20**, 4107–4121.
Palade, G. E. (1952). *Anat. Rec.* **114**, 427–451.
Perotti, M. E., Anderson, W. A., and Swift, H. (1983). *J. Histochem. Cytochem.* **31**, 351–365.
Rapaport, D., Brunner, M., Neupert, W., and Westermann, B. (1998). *J. Biol. Chem.* **273**, 20150–20155.
Rinaldi, T., Ricci, C., Porro, D., Bolotin-Fukuhara, M., and Frontali, L. (1998). *Mol. Biol. Cell* **9**, 2917–2931.
Rizzuto, R., Bernardi, P., and Pozzan, T. (2000). *J. Physiol.* 529 Pt **1**, 37–47.
Rojo, M., Legros, F., Chateau, D., and Lombes, A. (2002). *J. Cell Sci.* **115**, 1663–1674.
Sanchez-Alcazar, J. A., Schneider, E., Martinez, M. A., Carmona, P., Hernandez-Munoz, I., Siles, E., De La Torre, P., Ruiz-Cabello, J., Garcia, I., and Solis-Herruzo, J. A. (2001). *J. Biol. Chem.* **275**, 13353–13361.
Santel, A., Frank, S., Gaume, B., Herrler, M., Youle, R. J., and Fuller, M. T. (2003). *J. Cell Sci. Pt*,
Santel, A., and Fuller, M. T. (2001). *J. Cell Sci.* **114**, 867–874.
Satoh, M., Hamamoto, T., Seo, N., Kagawa, Y., and Endo, H. (2003). *Biochem. Biophys. Res. Commun.* **300**, 482–493.
Schlickum, S., Moghekar, A., Simpson, J. C., Steglich, C., O'Brien, R. J., Winterpacht, A., and Endele, S. U. (2004). *Genomics* **83**, 254–261.
Scorrano, L., Ashiya, M., Buttle, K., Weiler, S., Oakes, S. A., Mannella, C. A., and Korsmeyer, S. J. (2002). *Dev. Cell* **2**, 55–67.
Sesaki, H., and Jensen, R. E. (1999). *J. Cell Biol.* **147**, 699–706.
Sesaki, H., and Jensen, R. E. (2001). *J. Cell Biol.* **152**, 1123–1134.
Sesaki, H., Southard, S. M., Hobbs, A. E., and Jensen, R. E. (2003a). *Biochem. Biophys. Res. Commun.* **308**, 276–283.
Sesaki, H., Southard, S. M., Yaffe, M. P., and Jensen, R. E. (2003b). *Mol. Biol. Cell* **14**, 2342–2356.
Shaw, J. M., and Nunnari, J. (2002). *Trends Cell Biol.* **12**, 178–184.
Shepard, K. A., and Yaffe, M. P. (1999). *J. Cell Biol.* **144**, 711–720.
Smirnova, E., Griparic, L., Shurland, D. L., and van der Bliek, A. M. (2001). *Mol. Biol. Cell* **12**, 2245–2256.
Sugioka, R., Shimizu, S., and Tsujimoto, Y. (2004). *J. Biol. Chem.* **279**, 52726–52734.
Szabadkai, G., Simoni, A. M., Chami, M., Wieckowski, M. R., Youle, R. J., and Rizzuto, R. (2004). *Mol. Cell* **16**, 59–68.
Tieu, Q., Okreglak, V., Naylor, K., and Nunnari, J. (2002). *J. Cell Biol.* **158**, 445–452.
Wang, X. (2001). *Genes Dev.* **15**, 2922–2933.
Wong, E. D., Wagner, J. A., Gorsich, S. W., McCaffery, J. M., Shaw, J. M., and Nunnari, J. (2000). *J. Cell Biol.* **151**, 341–352.
Wong, E. D., Wagner, J. A., Scott, S. V., Okreglak, V., Holewinske, T. J., Cassidy-Stone, A., and Nunnari, J. (2003). *J. Cell Biol.* **160**, 303–311.
Zuchner, S., Mersiyanova, I. V., Muglia, M., Bissar-Tadmouri, N., Rochelle, J., Dadali, E. L., Zappia, M., Nelis, E., Patitucci, A., Senderek, J., Parman, Y., Evgrafov, O., Jonghe, P. D., Takahashi, Y., Tsuji, S., Pericak-Vance, M. A., Quattrone, A., Battologlu, E., Polyakov, A. V., Timmerman, V., Schroder, J. M., and Vance, J. M. (2004). *Nat. Genet.* **36**, 449–451.