Novartis Foundation Symposium 287

MITOCHONDRIAL BIOLOGY: NEW PERSPECTIVES

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Published in 2007 by John Wiley & Sons Ltd,
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Jossey-Bass, 989 Market Street, San Francisco, CA 94103-1741, USA

Wiley-VCH Verlag GmbH, Boschstr. 12, D-69469 Weinheim, Germany

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Novartis Foundation Symposium 287

x + 244 pages, 32 figures, 2 tables

Anniversary Logo Design: Richard J Pacifico

British Library Cataloguing in Publication Data

A catalogue record for this book is available from the British Library

ISBN 978-0-470-06657-7

Typeset in 10½ on 12½ pt Garamond by SNP Best-set Typesetter Ltd., Hong Kong
Printed and bound in Great Britain by T. J. International Ltd, Padstow, Cornwall.
This book is printed on acid-free paper responsibly manufactured from sustainable forestry, in which at least two trees are planted for each one used for paper production.

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Symposium on New perspectives on mitochondrial biology, held at the Novartis Foundation, London, 28–30 November 2006

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Chair's introduction

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In this introduction I want to summarize where we are in the field and where we are going. What is the future of mitochondrial bioenergetics? A couple of weeks ago I had an idle moment, so I logged on to PubMed and entered the search term 'mitochondria' followed by the years 1950 and 2006, one after the other. The results were fascinating. The numbers of citations per year for mitochondria started off in the bioenergetic prehistory, going back almost 100 years to the first descriptions of mitochondria. For me, the single event that introduced the classic era of mitochondrial bioenergetics was the publication of papers by Chance and Williams in the mid 1950s which first described the oxygen electrode, and described the redox changes of the cytochromes. What happened then was an explosive growth over the next 10 or 15 years in the number of papers, which led to over 3000 papers per year by the time the next revolution came. This was Peter Mitchell's work in the late 1960s. This is interesting: whereas you would expect that the discovery of a mechanism would stimulate a lot of new research, what happened after the three or four years when Peter was publishing these fantastic papers is that the field stagnated for 20 years in terms of numbers of publications. Somehow, because bioenergetics had defined itself so narrowly in terms of understanding how the respiratory chain and ATP synthase works with a little bit of ion transport, this limited the field.

The next explosion of research came when our cell biology colleagues working on cell health and death discovered, sometimes to their discomfort, that mitochondria moved into the centre of the field. In the last 10 years the trend has been almost explosive in terms of the number of papers on mitochondrial bioenergetics. I didn't have time to do a statistical sampling of the different years, but my guess is that 80% of these papers come under the field of mitochondrial physiology: mitochondria in the context of the cell.

What is interesting is where we are going. To quote Donald Rumsfeld, it is the unknown unknowns that will define where the field moves in the next 10 years. In 1994, no one knew that they didn't know how cytochrome c was released, because it wasn't part of the vocabulary. What does this have to do with cell death? It is the things that we don't know that we don't know which are going to define the next 10 years or so.

What Michael Duchen has organized for this meeting is a series of four separate sessions. The first one is mitochondrial 'natural history', a phrase I like because it conjures up images of David Attenborough wandering through the cell and getting excited when he comes close to a mitochondrion. The three papers in this session deal with mitochondrial morphology, fission, fusion, replacement of proteins and shape. Some of the questions that occurred to me and which we will be dealing with are as follows. Do you repair a mitochondrion, or are they scrapped and replaced when they go wrong? How is a damaged mitochondrion recognized, and why doesn't this recognition work well in terms of mitochondrial genetic disease? Do we see more fission than fusion if there are more mitochondria being produced, and what is the relationship between fission/fusion and biogenesis?

The second session is mitochondria and oxidative stress. I'm fascinated by PGC1α, and equally fascinated by novel uncoupling proteins. Does PGC1α regulate gross mitochondrial bioenergetics? Does it regulate the specific induction of antioxidant pathways? What are the novel uncoupling proteins really doing? This is still surprisingly cloudy, some 10 years after the first description of these proteins.

The third session deals with signal transduction. Mitochondrial nitric oxide synthase is still controversial. Mitochondrial Ca^{2+} signalling and endoplasmic reticulum (ER) cross-talk is also a hot topic: what are the conditions where the ER and mitochondria talk to each other, and what are the conditions where they work on their own? Ion channels will be covered: one ion channel that is highly controversial is the mitochondrial K^{+}-ATP channel. What is the problem with this? Is it present or not? The permeability transition pore is another subject for discussion: when is it important and when is it not? What are the proven cases for its involvement and what are the more speculative cases? With regard to the regulation of cytochrome c release, do we have to look at this separately from the release of AIF and other proteins? Is there a single, holistic mechanism for releasing these proapoptotic proteins from mitochondria or is cytochrome c a special case? Mitochondria and neurodegeneration is an enormous topic: one of the central questions here is why does damage in these different diseases show such tissue specificity? Is it related to the cell or the mitochondrion itself?

The final session looks at mitochondrial mutations. Even with the Pol-g mutations, is the frequency of mitochondrial DNA mutations sufficient to account for the phenotype? That is, is there any disconnect between the proportion of mitochondria that possess the mutation and the dysfunctional phenotype? Why do the mechanisms of autophagocytosis not recognize some damaged mitochondria when there is heteroplasmic coexistence of normal and damaged mitochondria? This seems to work in the β cell; what goes wrong in some mitochondrial diseases where it goes the wrong way, and the mutated mitochondria seem to be dominant over

the wild-type ones? Also, why do we start off each generation with perfect mitochondria? What are the mechanisms that protect the germ cell or allow a Darwinian-style selection of perfect mitochondria in each succeeding generation? Finally, where will the next 10 years take us? We clearly don't know the unknown unknowns, but thinking of the known unknowns do we have a feel for where we are going? I feel that part of the future lies in understanding mitochondrial morphology: why are they shaped the way they are, and why do we have exactly the right number in a cell? Why do they appear to go where they are needed?

Outer mitochondrial membrane protein degradation by the proteasome

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Abstract. Protein turnover is used for regulatory processes and to eliminate superfluous, denatured or chemically inactivated polypeptides. Mitochondrial proteins may be particularly susceptible to damage induced by reactive oxygen species and several pathways of mitochondrial proteolysis have been illuminated. However, in contrast to matrix and inner mitochondrial membrane protein degradation, little is known about the turnover of integral outer mitochondrial membrane (OMM) proteins or the mechanisms involved. We have found that pheromone treatment of *Saccharomyces cerevisiae* induces the proteasome-dependent elimination of the OMM spanning protein, Fzo1, from the mitochondria and that Fzo1 is ubiquitylated while still associated with the membrane. These characteristic processing steps are similar to those of the endoplasmic reticulum (ER)-associated degradation (ERAD) pathway suggesting the term OMMAD, outer mitochondrial membrane-associated degradation, to describe the process. ERAD is dependent upon ER membrane spanning RING domain E3 ubiquitin ligases suggesting that certain E3 ligases in the OMM may also regulate OMMAD. This led us to clone and characterize all 54 predicted human gene products that contain both RING domains and predicted membrane spanning domains. A surprising number of these localize to mitochondria where some may control OMMAD. Some of these mitochondrial RING domain proteins also regulate mitochondrial morphology, indicating a critical role of ubiquitin signalling in the maintenance of mitochondrial homeostasis.

2007 Mitochondrial biology: new perspectives. Wiley, Chichester (Novartis Foundation Symposium 287) p 4–20

Regulated protein degradation is used to eliminate superfluous, denatured or chemically inactivated polypeptides and in signal transduction. Current evidence shows that in the mitochondria, protein degradation is controlled by pathways compartmentalized in the matrix and the inner mitochondrial membrane. For example, it has been demonstrated that Pim1/Lon proteases, of the AAA protease family homologous to bacterial proteases, degrade excess soluble matrix proteins whereas membrane embedded i- and m-AAA proteases remove integral membrane proteins in the inner mitochondrial membrane. Additional components of this

latter system include the chaperone prohibitin and a membrane channel to allow export of the peptide end-products from the matrix to the intermembrane space (Arnold & Langer 2002). Once within the intermembrane space, peptides smaller than 2 kDa would be expected to diffuse freely through the semipermeable outer mitochondrial membrane. In contrast to proteolysis of matrix and inner mitochondrial membrane proteins, little is known about the turnover of outer mitochondrial membrane (OMM) proteins or the molecular components involved.

During our exploration of the regulation of mitochondrial dynamics, we found that the large GTPase of the OMM required for mitochondrial fusion, Fzo1, is degraded during pheromone treatment of yeast cells (Neutzner & Youle 2005). The elimination of Fzo1 from the OMM during pheromone treatment correlates with a decrease in mitochondrial fusion and shorter overall length of mitochondrial tubules. Interestingly, the degradation of Fzo1 was suppressed by the proteasome inhibitor, MG132, indicating that the proteasome system plays a role in OMM protein turnover (Fig. 1). This conclusion has been extended by showing that pheromone-induced Fzo1 degradation depends upon ubiquitin, the proteolytic activity of the 20S proteasomal core particle (*pre1*) and the ATPase subunit (*cim5*) of the 19S regulatory complex of the proteasome (Escobar-Henriques et al 2006). Understanding how the cytosolic proteasomal system could degrade a membrane spanning protein in the OMM comes from other organelles.

In the endoplasmic reticulum (ER), transmembrane proteins are targeted for proteasomal degradation by the endoplasmic reticulum-associated degradation (ERAD) pathway (Meusser et al 2005). This pathway involves: (i) ubiquitylation of the target protein, typically followed by (ii) retrotranslocation of the ubiquitin-tagged protein out of the membrane by AAA ATPases, and (iii) degradation by proteasomes in the cytosol. As Fzo1 spans the OMM twice, proteasomal degradation would likely also require retrotranslocation from the OMM, and therefore participation of a heretofore unknown mitochondrial-associated degradation pathway. Since the OMM may have originated from the phagosomal membrane of a primitive eukaryotic cell that engulfed an endosymbiotic bacteria (yielding the

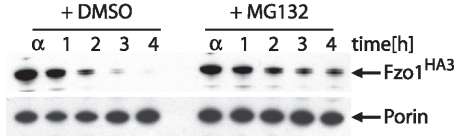

FIG. 1. Pheromone induced Fzo1 degradation is proteasome dependent. Cells of strain yAN018 (*FZO1HA3*Δ*bar1*) were arrested with mating pheromone for 2 h and treated either with 50 μM/ml proteasome inhibitor MG132 (Sigma) in DMSO (1% final concentration) or with DMSO alone as a control. Cells were harvested at 0, 1, 2, 3 and 4 h after MG132 treatment. Protein lysates were analysed by western blot using anti-HA and anti-porin antibodies.

inner mitochondrial membrane [IMM] and matrix of the mitochondrion) (van der Bliek 2000), it is possible that the protein degradation process occurring in the OMM descended from a eukaryotic process that utilizes ubiquitylation. To explore whether degradation of OMM-associated Fzo1 shares other features with ERAD we first determined whether Fzo1 is ubiquitinated.

To attain this, we purified ^{Myc3}Fzo1 by immunoprecipitation from cells with or without additional overexpression of ^{HA3}Ubiquitin. As shown in Fig. 2, immunopurified ^{Myc3}Fzo1 from ^{HA3}Ubiquitin overexpressing cells is modified with ^{HA3}Ubiquitin, while ^{Myc3}Fzo1 from control cells does not show this modification. We therefore conclude that Fzo1 can be tagged with ubiquitin *in vivo*. In order to determine whether Fzo1 is ubiquitylated while on the mitochondrial membrane, we overexpressed ^{Myc3}Fzo1 together with ^{HA3}Ubiquitin and performed subcellular fractionation followed by immunoprecipitation of ^{Myc3}Fzo1 from the heavy membrane fraction. Figure 3A shows that most of the ^{Myc3}Fzo1 sediments were in the heavy membrane fraction (12 K pellet). No Fzo1, however, was detectable in the high speed supernatant (100 K supernatant). Significantly, Fzo1 immunopurified from the heavy membrane fraction was modified with ^{HA3}Ubiquitin, indicating that ubiquitin conjugation of this protein occurs at the OMM. To determine the ubiquitylation site on Fzo1, we analysed several N-terminally truncated Fzo1 mutants for their

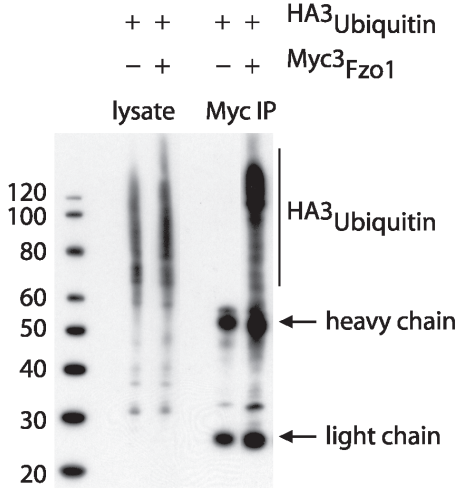

FIG. 2. Fzo1 is ubiquitylated *in vivo*. Cells of strain yAN186 (*GALHA3Ubiquitin*) and yAN251 (*GALHA3Ubiquitin GALMYC3FZO1*) were induced with 2% galactose for 2 h and protein lysates were prepared. Myc3Fzo1 was immunoprecipitated using anti-Myc antibodies (Roche) and then analysed by western blot using anti-HA antibodies to detect HA3Ubiquitin (Roche). The lysate from yAN186 cells served as immunoprecipitation control.

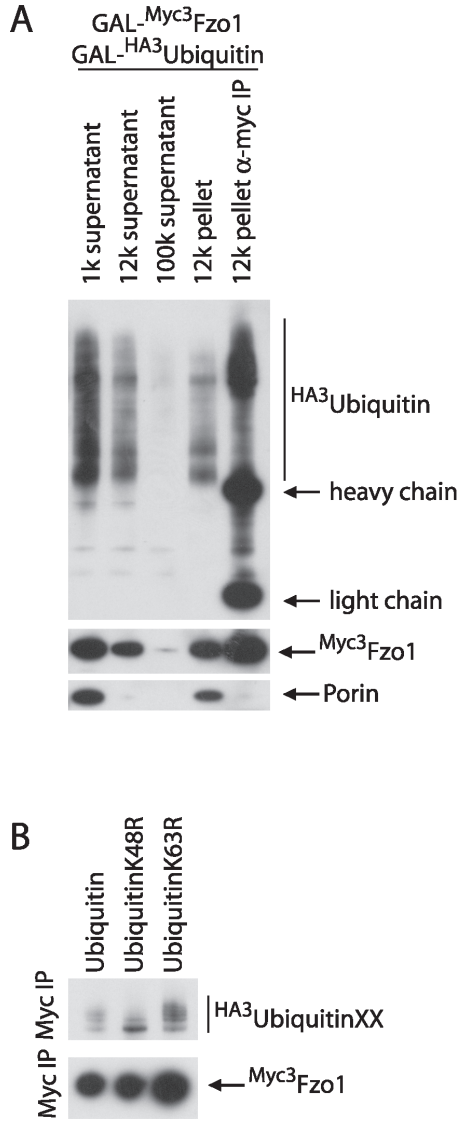

FIG. 3. (A) Fzo1 ubiquitylation takes place on the mitochondrial membrane. Cells of strain yAN251 (*GALHA3Ubiquitin GALMYC3FZO1*) were induced for two hours with 2% galactose to produce Myc3Fzo1 and HA3Ubiquitin. Protein lysates were prepared and fractionated using differential centrifugation at 1000 × g (1 K), 12 000 × g (12 K) and 100 000 × g (100 K). The heavy membrane fraction (12 K pellet) was solubilized and Myc3Fzo1 was immunoprecipitated. The samples were analysed by western blot using anti-HA antibodies to detect HA3Ubiquitin and anti-Myc antibodies to detect Myc3Fzo1. Detection of porin using anti-porin antibodies served as marker for the outer mitochondrial membrane. (B) Cells of strain yAN251 (*GALHA3Ubiquitin GALMyc3FZO1*), yAN301 (*GALHA3Ubiquitin GALMyc3FZO1K48R*) and yAN307 (*GALHA3Ubiquitin GALMyc3FZO1K63R*) were induced with 2% galactose and protein lysates were prepared. Ubiquitylation of Myc3Fzo1 was analysed by immunoprecipitation followed by western blot analysis with anti-HA and anti-Myc antibodies.

susceptibility as substrates for ubiquitylation. To this end we overexpressed ^{Myc3}Fzo1 or ^{Myc3}Fzo1 mutants together with ^{HA3}Ubiquitin and performed subcellular fractionation experiments. ^{Myc3}Fzo1 and ^{Myc3}Fzo1 mutant proteins were then immunoprecipitated from the heavy membrane pellet. As shown in Fig. 4, while the deletion

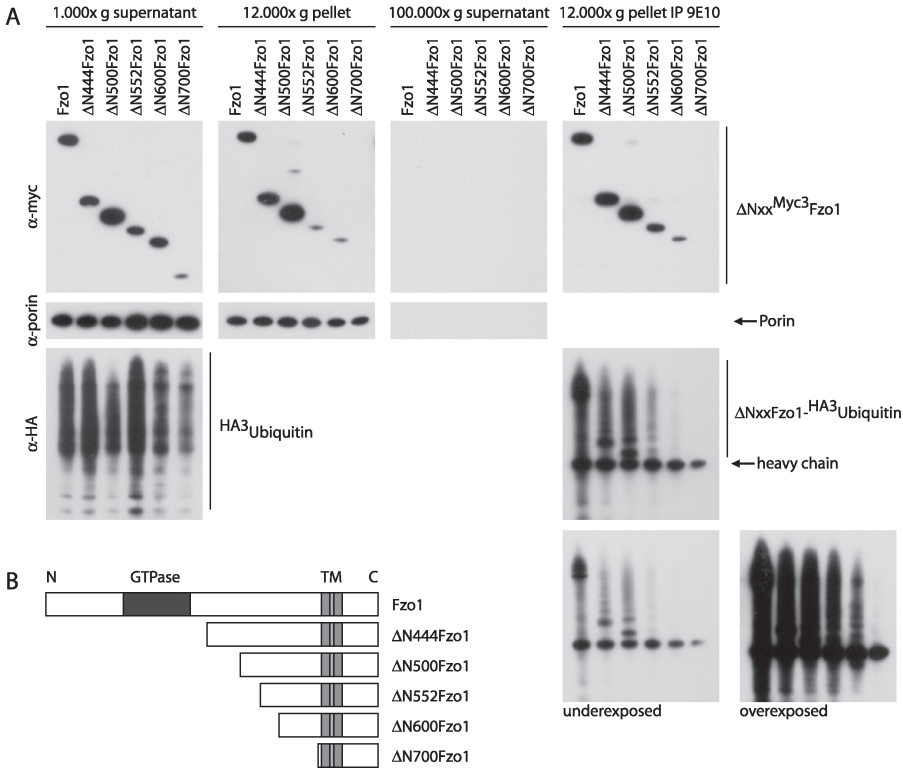

FIG. 4. Analysis of the ubiquitylation of Fzo1 truncation mutants. (A) Cells of strains yAN251 (*GALHA3Ubiquitin GALMyc3FZO1*), yAN231 (*GALHA3Ubiquitin GALMyc3*Δ*N444FZO1*), yAN227 (*GALHA3Ubiquitin GALMyc3*Δ*N500FZO1*), yAN216 (*GALHA3Ubiquitin GALMyc3*Δ*N552FZO1*), yAN294 (*GALHA3Ubiquitin GALMyc3*Δ*N600FZO1*) and yAN248 (*GALHA3Ubiquitin GALMyc3*Δ*N700FZO1*) were induced for 2 h with 2% galactose and protein lysates were prepared for subcellular fractionation by differential centrifugation. The heavy membrane fraction (12 K pellet) was solubilized and subjected to immunoprecipitation using anti-Myc antibodies in order to purify Fzo1 truncation mutants. Ubiquitylation of Fzo1 was analysed by western blot using anti-HA antibodies in order to detect HA3Ubiquitin. The detection of porin served as marker for the mitochondrial outer membrane and as a control for the subcellular fractionation. In order to show the different ubiquitylation levels of the Fzo1 mutants three different exposures ('underexposed' and 'overexposed') are shown. (B) Fzo1 constructs used in Figure 4A. GTPase marks the GTPase region of Fzo1. TM marks the two transmembrane regions which anchor Fzo1 in the outer mitochondrial membrane. ΔNxxx stands for an N-terminal deletion of xxx amino acids.

of amino acids 1 to 500 did not impair the localization of Fzo1 to the heavy membrane fraction, any further deletion greatly diminished the mitochondrial localization of Fzo1. Deletion of the first 552 and 600 amino acids still allowed mitochondrial import to a small extent whereas the deletion of amino acids 1–700 significantly inhibited protein expression and localization to heavy membranes. The analysis of heavy membrane localized Fzo1 truncation mutants by immunoprecipitation showed that even the deletion of the first 600 amino acids of Fzo1 still allowed ubiquitylation to occur. These results show a number of potential ubiquitylation sites exist on Fzo1 consistent with a role of ubiquitylation machinery that specifically targets substrates in the outer mitochondrial membrane.

Targeting proteins for proteosomal degradation involves the formation of a ubiquitin chain formed by lysine 48-linked ubiquitin moieties, rather than lysine 63-linked ubiquitin moieties that are formed during regulatory processes. To address whether Fzo1 is modified with lysine 48 or lysine 63 ubiquitin chains, we overexpressed ^{Myc3}Fzo1 together with ^{HA3}UbiquitinK48R or ^{HA3}UbiquitinK63R (Fig. 3B). Analysis of ^{Myc3}Fzo1 ubiquitylation by immunoprecipitation showed that overexpression of ^{HA3}UbiquitinK48R greatly inhibited the formation of ubiquitin chains while overexpression of ^{HA3}UbiquitinK63R did not diminish ubiquitin chain formation compared to ^{HA3}Ubiquitin. This suggests that Fzo1 is modified with lysine 48-linked ubiquitin chains, consistent with a role of the proteasome in degradation of Fzo1. Together, these data show that turnover of Fzo1 might be initiated by ubiquitylation at the OMM followed by proteasomal degradation in the cytosol. The molecular mechanism that mediates retrotranslocation of Fzo1 from the OMM to the proteasome as well as the nature of the E3 ligase for Fzo1 ubiquitylation on mitochondrial membranes remain elusive. As discussed above degradation of Fzo1 in *S. cerevisiae* at the OMM can include (i) ubiquitylation of Fzo1 and (ii) proteasome-dependent elimination of this membrane spanning protein. These processing steps are similar to those of ERAD, suggesting that an analogous pathway, which we term outer mitochondrial membrane associated degradation (OMMAD), may participate in protein quality control of the OMM.

One key step in elimination of ERAD substrates is the ubiquitylation of the target proteins by ER transmembrane E3 ligases, including Hrd1 and Doa10 in yeast (Carvalho et al 2006) and gp78 in mammals (Song et al 2005). To investigate potential E3 ligase components involved in OMMAD we first attempted to determine whether any RING-finger E3 ubiquitin ligases localize to mitochondria. We turned to mammalian systems where ERAD components have been extensively studied. The human genome encodes about 345 RING domain proteins based on NCBI gene and Genbank searches using several C3HC4 RING motifs of known E3-ubiquitin ligases related to $CX_2CX_{[9,39]}CX_{[1,3]}HX_{[2,3]}CX_{[2]}C_{[4,48]}CX_{[2]}C$ as reference sequences. Of these 345 candidates, 54 different cDNAs have predicted membrane-spanning domains as revealed by the transmembrane helix prediction program TMHMM (*http://www.cbs.dtu.dk/services/TMHMM*). We cloned

the cDNAs of these 54 genes from a human brain cDNA library or commercially available clones assuming that some of them may localize to mitochondria and participate in OMMAD. The domain organization of proteins cloned in our screen is shown in Fig. 5. A remarkable array of transmembrane domain organizations

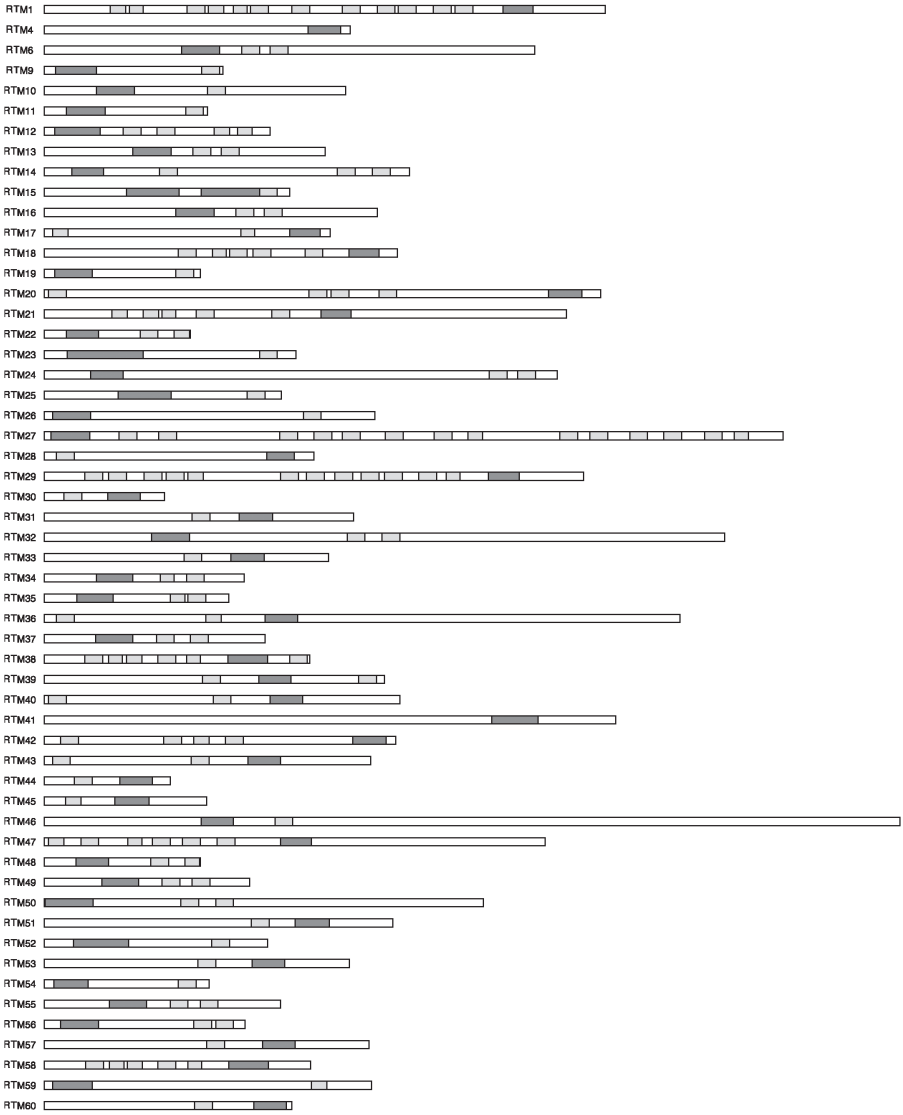

FIG. 5. Domain organization of 55 human RING domain containing proteins. A dark grey box marks the RING domains and lighter grey boxes mark putative transmembrane regions.

appears with predicted membrane spanning regions ranging from a single C-terminal anchor to 14 membrane spanning domains suggesting a wide diversity of membrane events that may control protein stability through the ubiquitin pathway.

To analyse the subcellular localizations of our candidate membrane-associated E3 ubiquitin ligases, cDNAs of all 54 genes were subcloned into mammalian expression vectors in frame with yellow fluorescent protein (pYFP), which we used as a fluorescent reporter in protein localization studies. To avoid potential subcellular localization artefacts due to fusion with the 27 kDa YFP, we constructed both N-and C-terminal chimeras (resulting in: 'YFP-protein of interest' and 'protein of interest-YFP' orientations). Figure 6 presents an overview of the subcellular localizations of these RING domain proteins. Importantly, we found that of the 54 integral membrane RING domain proteins, nine localized to mitochondria. Six of these mitochondrial proteins had the RING domain N-terminal to the membrane spanning regions and three displayed C-terminal RING domains, presumably orienting the RING domains toward the ubiquitination machinery in the cytosol. None of the nine mitochondrial-localized RING domain proteins displayed a mitochondrial import consensus sequence based on the PSORTII prediction program (*http://psort.nibb.ac.jp/form2.html*) consistent with OMM localizations. The number of membrane spanning segments ranged from 1 to 5. One of these mitochondrial associated proteins contains a RING IBR RING motif found in a subfamily of RING proteins that includes Parkin, an E3 ligase required for

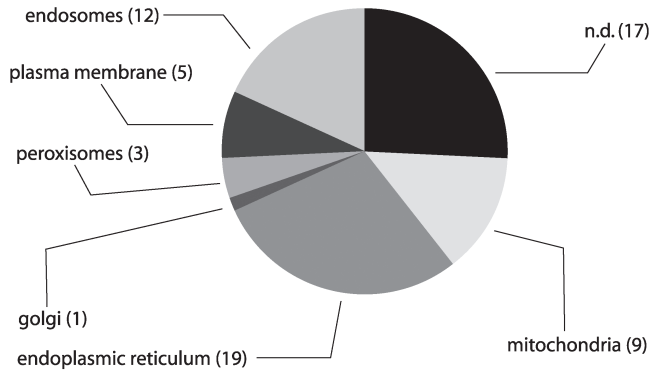

FIG. 6. Cellular distribution of membrane anchored RING domain proteins. Fifty-four open reading frames coding for proteins containing a RING domain as well as predicted transmembrane domains were fused to YFP and expressed in HeLa cells. The localization of these YFP fusion proteins was analysed by fluorescence microscopy and confirmed by co-staining with standard organelle markers. An overview of the various membrane organelles which harbour transmembrane RING domain proteins revealed by our screen is shown. Localization 'not determined (n.d.)' refers to work in progress.

mitochondrial maintenance that is mutated in certain familial cases of Parkinson's disease. To explore the potential role of these RING domain proteins in mitochondrial protein turnover, we mutated conserved histidines in the RING domain, already shown to be critical for this domain function, in all nine proteins to inactivate their potential E3 ligase activity. Current studies will determine if these mitochondrial RING domain proteins participate in the degradation of the human homologues of Fzo1, Mfn1 and Mfn2, or other integral OMM proteins. As discussed below, one of the nine proteins found by us to localize to mitochondria has been recently shown to participate in ubiquitin- and proteasome-mediated self-degradation, two characteristics predicted for OMMAD.

Interestingly, of the nine mitochondrial RING proteins identified in our screen, only MARCH5 was associated with this organelle in a previous proteomic study (Taylor et al 2003). The absence of prior identification of eight mitochondrial RING domain proteins (Mootha et al 2003, Taylor et al 2003) may reflect the poor recovery of the OMM in highly purified mitochondrial preparations, relative to the abundance of the inner mitochondrial membrane, or the difficulty in identifying proteins with several transmembrane domains by mass spectrometry procedures. It is also possible that a low abundance or tissue specific expression of the mitochondrial RING domain proteins could complicate their detection in proteomic studies. Recently, two groups have identified a role of MARCH5 in ubiquitylation of OMM substrates, including Fis1 and Drp1, two proteins vital for mitochondrial fission (Nakamura et al 2006, Yonashiro et al 2006). Interestingly, one of these proteins, the large GTPase Drp1, co-immunoprecipitates with MARCH5 only in an ubiquitylated form. These data also support a role of MARCH5 in the mitochondrial ubiquitin signalling. In addition, MARCH5 seems to be itself ubiquitylated and degraded by the proteasome, sharing two characteristics with another OMMAD substrate, Fzo1.

Discussion

Mitochondria produce reactive oxygen species that can be very damaging for mitochondrial proteins, lipids and DNA. Thus, selective removal and degradation of denatured and oxidized proteins is likely to be an important process in mitochondrial quality control. In contrast to the matrix and inner mitochondrial membrane, where systems of proteolysis are well characterized, protein turnover mechanisms in the OMM are not known. We propose, based upon examples of integral OMM protein ubiquitylation and proteasome degradation, that a process similar to ERAD regulates protein turnover in the OMM and call this outer mitochondrial membrane associated degradation or OMMAD.

To explore the mechanism of OMM degradation we cloned and localized all the membrane spanning RING domains identified in the human genome. Confocal

localization of YFP fusion proteins identified nine membrane spanning RING domain proteins on mitochondria. We will determine whether these are involved in OMM protein degradation. In addition to these candidate E3 ligases, E3 ligases not integrally spanning membranes may also regulate OMM protein stability. *MDM30* has been found in yeast to be required for mitochondrial fusion (Fritz et al 2003). Interestingly, *MDM30* contains an F-box domain frequently found in proteins complexed with the cytosolic SCF family of E3 ligases. Although not required for pheromone induced Fzo1 degradation, *MDM30* is involved in steady state degradation of Fzo1 and thereby controls the process of mitochondrial fusion but surprisingly through a proteasome independent process (Escobar-Henriques et al 2006). Mfb1, another F box protein, also regulates mitochondrial morphology (Durr et al 2006). Other examples of cytosolic E3 ligase regulation of mitochondria include Rsp5 and Parkin. The soluble HECT domain E3 ligase, Rsp5, is required for mitochondrial distribution and morphology in yeast although the substrate for this ubiquitin ligase activity involved in mitochondrial regulation remains unknown (Fisk & Yaffe 1999). The RING IBR RING domain protein, Parkin, not predicted to have membrane spanning domains is localized in the cytosol and associated with a variety of organelles (Kubo et al 2001) including mitochondria (Kuroda et al 2006) and is involved in mitochondrial responses to oxidative damage in metazoans. Importantly, Parkin is mutated in certain cases of early onset, familial Parkinson's disease (Kitada et al 1998) and may disrupt the normal participation of OMMAD of damaged mitochondrial proteins. The most likely mitochondrial substrates of soluble E3 ligases would reside either embedded in the OMM or bound to proteins on the OMM. However, analogous to ERAD extraction of soluble ER luminal proteins, inter-membrane space proteins in mitochondria are also candidate substrates for proteosomal degradation, particularly via the transmembrane OMM E3 ligases defined in this work.

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DISCUSSION

Nicholls: There seems to be a relationship between the excision of these proteins and the morphological changes. What is the link that triggers the morphological change as the result of protein excision?

Youle: From what we know now, I'd say there are two different links. One is that in yeast the fusion protein Fzo1 (Mfn in mammals) is excised and degraded by the proteasome, and this promotes fission because the mitochondria can't fuse. We have seen this physiologically in yeast during mating. It makes sense that they might want to have less interconnected mitochondria when the cells mate, because cells of different mating types fuse, and then undergo meiosis and may profit from a nice mixture of their mitochondria in the daughter cells. What we are now seeing is that other E3 ligases in mammals (and this is more speculative) function in a regulatory process of mono-ubiquitination that appears not to function by inducing proteasomal degradation. The ubiquitin can be added and taken off by de-ubiquitinylating enzymes in a regulatory capacity. We are looking to see whether Drp1 dynamics on the membrane are modulated by a monoubiquitination process. We know very little of what regulates Drp1 accumulation at mitochondrial scission sites and how it winds around mitochondria, and how it cycles off these helices.