Published in final edited form as:

Micron. 2013 April ; 47: 50–58. doi:10.1016/j.micron.2013.01.005.

Aquaporin-assisted and ER-mediated mitochondrial fission: A hypothesis

Jin-Sook Lee^{a,d,1}, Xia Hou^{a,1}, Nicole Bishop^c, Sunxi Wang^b, Amanda Flack^a, Won Jin Cho^a, Xuequn Chen^a, Guangzhao Mao^b, Douglas J. Taatjes^c, Fei Sun^a, Kezhong Zhang^d, and Bhanu P. Jena^{a,c,*}

^aDepartment of Physiology, School of Medicine, Wayne State University, Detroit, MI 48201, USA

^bDepartment of Chemical Engineering & Materials Science, College of Engineering, Wayne State University, MI 48202, USA

^cDepartment of Pathology, Microscopy Imaging Center, University of Vermont College of Medicine, Burlington, VT 05405, USA

^dCenter for Molecular Medicine and Genetics, School of Medicine, Wayne State University, Detroit, MI 48201, USA

Abstract

It is well established that the status of the endoplasmic reticulum (ER) and mitochondria, and the interactions between them, is critical to numerous cellular functions including apoptosis. Mitochondrial dynamics is greatly influenced by cell stress, and recent studies implicate ER in mitochondrial fission. Although a number of proteins have been identified to participate in ER-induced mitochondrial fission, the molecular mechanism of the process is little understood. In the current study, we confirm the involvement of ER in mitochondrial fission and hypothesize the involvement of water channels or aquaporins (AQP) in the process. Previous studies demonstrate the presence of AQP both in the ER and mitochondrial membranes. Mitochondrial swelling has been observed following mitochondrial calcium overload, and studies report that chelation of cytosolic calcium induces extensive mitochondrial division at ER contact sites. Based on this information, the involvement of ER in mitochondrial division, possibly via water channels, is hypothesized. Utilizing a multi-faceted imaging approach consisting of atomic force microscopy on aldehyde-fixed and semi-dry cells, transmission electron microscopy, and immunofluorescence microscopy on live cells, the physical interactions between the two organelles are demonstrated. Mitochondrial fission following ER stress was abrogated with exposure of cells to the AQP inhibitor mercuric chloride, suggesting the involvement of AQP(s) especially AQP8 and AQP9 known to be present in the mitochondrial membrane, in mitochondrial fission.

Keywords

ER-mitochondria interactions; Mitochondrial fission; Aquaporins; Atomic force microscopy; Electron microscopy

1. Introduction

Drp1 a dynamin-like GTPase in mammalian cells, self-assemble to form approximately 109 nm in diameter oligomeric helices capable of fission of both the outer and inner mitochondrial membrane, leading to division of the organelle. Although GTP hydrolysis is thought to provide the mechanical constrictive force to enable Drp1 to carry out mitochondrial fission, additional proteins called mitochondrial fission factors or Mff, and endoplasmic reticulum (ER)–mitochondria encounter structures (ERMES) have also been implicated in the process. In mammalian cells, ER tubules are typically 100–200 nm in diameter, whereas the mitochondria measure 0.5–1 μm in diameter. Since Drp1 oligomeric helices are significantly smaller than the diameter of the mitochondrion, it is hypothesized that ER tubules play a mechano-constrictive role in aiding Drp1 action.

In earlier investigations, based on co-isolation of ER particles with mitochondria in sedimentation experiments, followed by their observation using electron microscopy (EM), interactions between the two organelles have been suggested (Shore and Tata, 1977; Meier et al., 1981; Mannella et al., 1998). Moreover, the physical interaction between mitochondria and the ER via tethers has been reported in rat hepatocytes (Csordas et al., 2006). In the Csordas study using electron tomography, it was demonstrated that ER and mitochondria are linked by tethers that measure ~ 10 nm between smooth ER and mitochondria, and ~ 25 nm between rough ER and mitochondria. Although composition of the tethers remains to be determined, it was suggested in the report that they may be involved in Ca^{2+} signal propagation from ER to mitochondria, and that cell function and survival may depend on the proper spacing between the two organelles (Csordas et al., 2006). In a recent study, the involvement of ER in mitochondrial fission has also been reported (Friedman et al., 2011); this study suggests that the ER defines the site of mitochondrial division, thus regulating mitochondrial distribution and architecture in the cell. It is well known that the fusion and fission of mitochondria are critical for cellular function and survival. For example, the fusion of mitochondria is required for the distribution of energy in the cell, whereas the fission of mitochondria generating smaller organelles capable of being transported to different parts of a large cell such as a neuron could also fulfill the same requirement of energy distribution within the cell. Additionally, mitochondrial division is also implicated in the release of cytochrome c into the cytosol to trigger apoptosis, or for the destruction of damaged cellular organelles (Westermann, 2010).

Insights into the 3-D organization and function of intracellular structures at nanometer resolution, holds the key to our understanding of the molecular underpinnings of cellular structure–function. To achieve this understanding regarding ER-induced mitochondrial fission, the Friedman et al. (2011) study employed EM tomography. While EM tomography is no doubt a powerful approach, major hurdles hindering its more general use include the tedious procedures involved in tissue fixation, high-pressure freezing, staining, serial sectioning, imaging, and finally compiling the EM images to obtain a 3-D profile of subcellular structures. Although atomic force microscopy (AFM) has been of immense help in studying live cells, cellular organelles and biomolecules in 3-D at nanometer resolution and in real time, it has had limited success in contributing to our understanding of intracellular structure–function in situ. In the current study, using AFM on aldehyde-fixed and semi-dry mouse pancreatic acinar cells, together with conventional transmission EM and immunofluorescence microscopy, new insights into ER-mediated mitochondrial division in mammalian cells were obtained. Results from the study suggest that ER tubules (primarily rough ER), interact via tethers and wrap around mitochondria, aiding in mitochondrial fission. Tunicamycin-induced ER stress, or cellular stress induced by supramaximal stimulation, results in mitochondrial fission. Importantly, results from the

study hypothesize the involvement of water channels or aquaporins (AQP) in generating the constrictive force required for ER-induced mitochondrial fission.

2. Materials and methods

2.1. Isolation of acinar cells and lobules of the exocrine pancreas

Acinar cells and hemi-acini from the exocrine pancreas were isolated using minor modifications of published procedures (Schneider et al., 1997; Wang et al., 2012). ICR or Institute for Cancer Research mice, are general multipurpose mice for research application, obtained from Charles River Laboratories, Wilmington, MA, USA. Briefly an ICR mouse weighing 25–30 g was euthanized by CO₂ inhalation for each experiment, using animal procedures preapproved by the Institution Animal Care & Use Committee (IACUC). The pancreata was dissected out and placed in DMEM (Dulbecco's Modified Eagle Medium). Five milliliters of Solution D (5 ml DMEM, 12.5 mg bovine serum albumin (BSA), 0.5 mg collagenase P, 5 mg trypsin inhibitor) were injected into the pancreatic tissue, and the distended tissue with the remaining Solution D was then transferred to a 25 ml polycarbonated flask, infused with 5% CO₂/95% O₂ and incubated at 37 °C for 10 min in a 120 cycles/min shaking water bath. The tissue was then removed and incubated in 5 ml Solution D for another 40 min under the above conditions, and acini were gently separated by pipetting 6–12 times, using a pipette first with a 2–4 mm opening, followed by one with a 0.9 mm opening. The resultant suspension was filtered through a 224- μ m Spectra-Mesh (Spectrum Laboratory Products) polyethylene filter to remove large clumps of acini and undissociated tissue. The resultant acini were washed six times (10 ml per wash) in ice-cold Solution R (60 ml DMEM, 6 mg trypsin inhibitor, 600 mg BSA). The acini were resuspended in Solution C (60 ml DMEM, 6 mg trypsin inhibitor, 2.4 g BSA), gravity collected, and finally resuspended in Solution I (60 ml DMEM, 6 mg trypsin inhibitor). Prior to fixation using 4% glutaraldehyde and 2% paraformaldehyde in phosphate buffered saline (PBS) pH 7.4 for 24 h, cells were either stimulated using 1 μ M carbamylcholine for 20 min or unstimulated, followed by resuspension in pH 7.4 PBS. Similarly, pancreatic lobules from both mouse and rat (procedures approved by IACUC) were prepared according to published methods (Jena et al., 1994), and either stimulated using 1 μ M carbamylcholine for 20 min or unstimulated, followed by fixation using 4% glutaraldehyde and 2% paraformaldehyde in PBS pH 7.4 for 24 h, and similarly resuspended in PBS pH 7.4. Cells and tissue were stored at 4°C prior to use.

2.2. Atomic force microscopy

Intact aldehyde-fixed mouse pancreatic acinar cells, and cells broken following fixation to expose internal structures, were used for imaging using the AFM. Cells were placed on a cleaved mica surface, partially dehydrated during 20 min of air-drying, and imaged using a modification of our previously published procedure (Jena et al., 2003; Jeremic et al., 2003). A Nanoscope IIIa AFM from Digital Instruments (Santa Barbara, CA) was used for imaging. Images were obtained using “tapping” mode in air, using aluminum coated silicon tips with a spring constant of 40 Nm⁻¹, and an imaging force of <200 pN, line frequencies of 1–2 Hz, with 256 lines per image, and constant image gains. The topographical dimensions of cellular structures were analyzed using the Nanoscope IIIa.43r8 software supplied by Digital Instruments.

2.3. Induction of pancreatitis

Pancreatitis was induced using an established published procedure (Chen et al., 2010). Male Wistar rats (Harlan, Indianapolis, IN) weighing 150–200 g were used in the study. To induce pancreatitis with caerulein, rats were injected i.p. twice with 50 μ g/kg synthetic caerulein dissolved in saline (one at 0 h and the other at the 1 h). Control animals followed the same

time regime except they were injected with saline. Blood and pancreas were collected from both control and caerulein-injected rats. In each experimental group four to seven animals were used. Pancreatitis was characterized using both biochemical and morphometric analysis.

2.4. Electron microscopy

Electron microscopy on pancreatic tissue was performed as previously described in detail (Wang et al., 2012). Briefly, isolated mouse pancreatic acinar cells, and pancreatic lobules from both mouse and rat were fixed in 4% glutaraldehyde/2% paraformaldehyde in ice-cold PBS for 24 h, embedded in 2% SeaPrep agarose, followed by post-fixation with cells were 1% OsO₄ in 0.1 M cacodylate buffer for 1 h at 4°C. Finally, the samples were dehydrated in a graded series of ethanol, through propylene oxide, and infiltrated and embedded in Spurr's resin. Ultrathin sections were cut with a diamond knife, retrieved onto 200 mesh nickel thin-bar grids, and contrasted with alcoholic uranyl acetate and lead citrate. Grids were viewed with a JEOL 1400 transmission electron microscope (JEOL USA, Inc., Peabody, MA) operating at 60 or 80 kV, and digital images were acquired with an AMT-XR611 11 megapixel ccd camera (Advanced Microscopy Techniques, Danvers, MA).

2.5. Immunofluorescence microscopy

HEK293 cells were cultured in DMEM with 10% fetal bovine serum (FBS) and transfected with pDsRed2-Mito, a mammalian expression vector encoding a fusion of a red fluorescent protein containing the mitochondrial targeting sequence. Two days post-transfection, the cells were re-plated onto glass bottom dishes (MatTek). One day after re-plating, cells were either untreated or pretreated with 100 μ M HgCl₂, and or posttreated with 5 μ M Tunicamycin (Sigma–Aldrich, St. Louis, MO). To determine the position of the cell nucleus, cells were exposed to 50 μ l/ml of the nuclear stain NucBlue™ Live Cell Stain (Molecular Probes, Life Technologies, Carlsbad, CA). Cells were then mounted on a chamber maintained at 37 °C, and real-time imaging performed using an immunofluorescent FSX100 Olympus microscope.

3. Results and discussion

As previously described (Wang et al., 2012), electron micrographs of the fixed cells from mouse pancreas demonstrate them to be intact polarized pancreatic acinar cells, with their typical basolaterally located nucleus (N), elaborate network of mostly rough endoplasmic reticulum (ER), the presence of scattered mitochondria (M), apically located electron-dense secretory vesicles called zymogen granules (ZGs) measuring 0.1–1.2 μ m in diameter, and microvilli (MV) at the apical plasma membrane of the cell (Fig. 1a). The pancreatic acinar cell of the rat (Fig. 1b) has a similar polarized structure and sub-cellular distribution of organelles. Microvilli are present at the apical plasma membrane, and the apical compartment is predominantly occupied with ZGs. The nucleus, Golgi, and mitochondria, are primarily present at the lower half or basolateral part of the cell (Fig. 1b). In our study, both control and stimulated cells (near physiological stimulation of secretion) were used to demonstrate the absence of morphological changes in either mitochondria or ER following stimulation of cell secretion. Since acinar cells of the exocrine pancreas are secretory cells, even mild handling of the tissue during isolation may elicit a secretory response. Besides accumulation of some empty and partially empty ZGs following carbamylcholine stimulation, no noticeable morphological changes in either ER or mitochondria were observed (Fig. 2).

The rough ER is found to interact with mitochondria in both mouse and rat pancreatic acinar cells (Figs. 3–5), and most of the ER and mitochondria are present in the mid to basal

compartment of the cell (Fig. 1), as opposed to the apically located ZGs. AFM performed on intact semi-dried and aldehyde-fixed pancreatic acinar cells adhering to mica, as well as cells where the plasma membrane has been removed following mild homogenization after aldehyde fixation, exposing the cell interior, demonstrates the presence of tubular ER processes, some wrapped around elongated structures at the center that appear to be mitochondria (Fig. 3). This premise is confirmed by EM imaging of these cells (Fig. 4), further suggesting that mitochondrial fission is likely mediated in part by the physical interaction, and possibly mechano-constriction of the mitochondria by ER tubules as seen in both mouse (Fig. 4a–d) and rat (Fig. 4e–h) pancreatic acinar cells. EM analysis of mitochondrial fission in both mouse and rat pancreatic acinar cells suggests mitochondrial fission to occur laterally at a certain point on its longitudinal axis (Fig. 4); however in Fig. 4b, a mitochondrion appears to be undergoing fission by first pushing out a lateral outgrowth before this mitochondrial outgrowth is interacted by the ER to be fissioned off the parent structure. Alternately, the mitochondrion may be fusing laterally with another mitochondrion instead of end-to-end fusion. However, the presence of ER at the neck of the mitochondrion (Fig. 4b, red arrowheads) suggests that it may be undergoing fission rather than fusion. It is also suggested by the EM micrographs (Fig. 4a–h) that mitochondria may undergo fission at more than one location, potentially yielding 3 or 4 mitochondria from an individual parent organelle (Fig. 4d).

It has previously been reported that Drp1, a dynamin-like GTPase in mammalian cells, and Dnm1 in yeast, self-assemble to form oligomeric helices capable of assisting in the fission of both the outer and inner mitochondrial membranes (Lackner and Nunnari, 2009). Although hydrolysis of GTP is thought to provide the mechanical constrictive force to enable Drp1/Dnm1 to carry out mitochondrial fission (Ingelman et al., 2005), additional proteins (De Brito and Scorrano, 2008; Gandre-Babbe and van der Bliek, 2008; Kornmann et al., 2009) accumulate at ER-associated mitochondrial division (ERMD) contact microdomains such as Darp1 receptor and effector, mitochondrial fission factors (Mff) some facilitating interactions between mitochondria and the ER, prior to the recruitment of Drp1 (Friedman et al., 2011). In mammalian cells, where ER tubules typically measure 100–200 nm in diameter, mitochondria are between 0.5–1 μm in diameter and 1–2 μm in length. Since both Drp1 and Dnm1 oligomeric helices are significantly smaller, somewhere in the range of 109 nm in diameter rings (Friedman et al., 2011), it is suggested that ER tubes may play a mechano-constrictive role to enable Drp1/Dnm1 action on mitochondrial fission. Possibly, the physical constriction of the mitochondrion to a certain minimum diameter by ER tubules may initially be required for Drp1 or Dnm1 to form the appropriate oligomeric helical ring required for mitochondrial fission. Interestingly, close examination of the EM micrograph of mitochondria fission in rat exocrine pancreas presented in Fig. 3h reveals two ring-like structures (red arrowheads), each approximately 100 nm in diameter, which could potentially represent the GTPase Drp1 oligomeric helices.

To further understand the interactions between the ER and mitochondria, EM micrographs at higher magnification were closely examined for the presence of tethers linking both organelles (Csordas et al., 2006). Electronically magnified images of the mitochondria (Fig. 4a–o) from Fig. 3, suggests that they may be undergoing fission, and demonstrate their close association with the ER. Similar to the physical interaction between mitochondria and the ER via tethers in rat hepatocytes (Csordas et al., 2006), 8–10 nm thick and up to 100 nm in linear length tethers are found to connect ER and mitochondria undergoing fission in pancreatic acinar cells (Fig. 5a–o). Interestingly, $\sim$ 100 nm in diameter ring-like structures are also observed at mitochondria fission sites (red rings in Fig. 5). Some candidate proteins of the ER-mitochondria tethers in the EM micrographs may represent the recently described organelle-bridging proteins (Martins de Brito and Scorrano, 2010), as well as yet unknown mammalian homologues of the ER-mitochondrial encounter structure (ERMS) found in

yeast (Kornmann et al., 2009). Deletion of any of the ERMS subunits results in severe defects in mitochondrial morphology (Merz et al., 2007), further suggesting the possible importance of ERMES/tethers in mitochondrial division.

The other unknown issue concerns how constrictive forces are actually generated by the ER. Since various lengths of tethers are observed in the EM micrographs in our study, this may represent the various extents of tether coiling and supercoiling, enabling ER tubules to press tightly against the mitochondria. Although cytoskeletal proteins such as actin, vimentin and microtubules have been implicated in the morphology and organization of ER and mitochondria in cells (Tang et al., 2008; Friedman and Voeltz, 2011; Park and Blackstone, 2010), their presence at mitochondrion fission sites and their involvement in ER-mediated mitochondrial fission has not been observed. Therefore, what provides ER the force to constrict the mitochondria? It may reside in a rapid dilation of the ER tubes wrapped around the mitochondria targeted for fission. While water is pumped into the ER tube wrapping around the mitochondrion, the concomitant loss of water from the mitochondrion at the ER attachment sites may serve to constrict its diameter, enabling the establishment of Drp1 oligomeric helices for mitochondrial fission. This hypothesis is based on earlier studies that demonstrated the presence of water channels or aquaporins (AQP) both in the ER (Skach et al., 1994; Okada et al., 2008) and mitochondrial membranes (Calamita et al., 2005). It has been reported that volume homeostasis in mitochondria is critical to a number of housekeeping cellular functions (Kaasik et al., 2006). Mitochondrial matrix volume is critically influenced by the osmolarity of the cytosol. Mitochondrial swelling has been observed following mitochondrial calcium overload, mitochondrial depolarization, or opening of permeability transition pores (Kaasik et al., 2006). Furthermore, the Friedman et al. (2011) study reports that chelation of cytosolic calcium induces extensive mitochondrial division at ER contact sites. These studies therefore suggest that regulation of mitochondrial and ER volumes through water channels at contact sites between the two organelles is critical to ER-induced mitochondrial constriction and fission. Therefore, the entry of water into ER, and a concomitant loss of water from the mitochondria at the ER-mitochondria contact sites, would lead to constriction of the mitochondrial diameter at the location, enabling the establishment of Drp1 oligomeric helices, leading to mitochondrial fission (Fig. 6). In support of this hypothesis, it is to be noted that most of the ER at the mitochondria fission sites appear swollen (Fig. 5).

To physiologically perturb ER, and determine its effect on mitochondrial fission, we induced pancreatitis by supramaximal stimulation of the exocrine pancreas using the cholecystokinin analog caerulein. It has previously been reported that pancreatitis is known to induce profound biochemical changes in the ER, resulting in ER stress (Chen et al., 2010). This pancreatic ER stress model was therefore chosen to determine the effect of pancreatitis on the status of the mitochondria and their interactions with the ER. Results from these experiments (Fig. 6) demonstrate that 2 h following supramaximal stimulation of the exocrine pancreas using caerulein, the ER appear beaded and dissociate from each other, resulting in somewhat closer interactions with the mitochondria (Fig. 5d). Measurement of mitochondria lengths demonstrates that 2 h following caerulein exposure, there is significant ($*p < 0.001$) reduction of the mitochondria length in pancreatic acinar cell (Fig. 6c, d and e) from 1195 ± 78 nm in control to 762 ± 34 nm in experimental [mean $\pm$ SEM; $n = 40$ mitochondria randomly picked from electron micrographs of three control and three experimental tissue] (Fig. 6e). Furthermore, once ER stress sets in 2 h following caerulein administration, ER vesiculation occurs and the mitochondria appear more spherical in shape besides a significant reduction in size (Fig. 6d). These studies confirm the involvement of ER stress on mitochondrial fission in cells.

To determine the influence of water channel aquaporins (AQP) on mitochondrial fission, AQP inhibition by mercuric chloride (the only known AQP blocker) on ER stress-induced mitochondrial fission in HEK293 cells was carried out. HEK293 cells expressing a mitochondrial targeted red fluorescent protein, were used in the study to determine mitochondrial structure and dynamics following ER stress. Exposure of cells to tunicamycin, a known inducer of ER stress results in mitochondrial fission within 30 min of exposure (Fig. 7). In contrast however, cells pre-treated with mercuric chloride for 30 min, followed by exposure to tunicamycin, fail to respond to the ER stress, suggesting the potential involvement of water channels in mitochondrial fission. Although mercuric chloride is widely used as an AQP blocker, it is after all a nonspecific inhibitor. Since no specific water channel inhibitors are known, our next studies will involve ER and mitochondria-specific AQP knockdown in HEK293 cells expressing both ER-targeted GFP and mitochondria targeted red fluorescent proteins. These studies are currently in progress.

4. Conclusion

In the current study, both AFM and EM micrographs suggest that mitochondrial fission in both mouse and rat pancreatic acinar cells is mediated by the physical association and constriction of the mitochondrion by the 100–200 nm in diameter ER tubules. Although the exact molecular mechanism of this constriction remains to be established (Youle and van der Bliek, 2012; Hoppins and Nunnari, 2012), this study suggests for the first time that water channel-mediated swelling of ER wrapped around mitochondria may provide the required mechanical force for the process. Since such physical constriction of mitochondria by ER may be required for Drp1 function in mitochondrial fission, this suggests a more complex engagement of ER in mitochondrial fission. It is to be noted that besides accumulation of Drp1 receptor and effector and Mff at ERMD microdomains (Hoppins and Nunnari, 2012) and the redistribution of both mitochondrial and ER membrane proteins such as AQP's, would play a vital role in mitochondrial fission. However, the possible involvement of motor proteins cannot be discounted at this time. Simply identifying proteins at the ER–mitochondria contact sites may not be sufficient to elucidate the molecular underpinnings of mitochondrial fission. Electron-dense 8–10 nm thick and up to 100 nm in length lipid and calcium exchanges that may facilitate lipid effectors (Chipuk et al., 2012; tethers connect ER and mitochondria), and ~100 nm in diameter rings, which could possibly represent previously defined Drp1 oligomers were observed at mitochondria fission sites (Fig. 8). This study further demonstrates that cellular imaging using AFM on fixed and semi-dry cells in combination with traditional transmission EM, and immunofluorescence light microscopy is a powerful approach to study cellular structure–function relationships and interactions. Moreover, the involvement of water channel aquaporins in ER-mediated mitochondrial fission is suggested from the study. Future work will therefore involve confirmation of the involvement of water channels using aquaporin knock-out mice, and identification of the biochemical composition of ER–mitochondria tethers and the ~100 nm rings at the junction of fission sites. Immunoelectron microscopy using colloidal gold and Drp1 and Mff antibodies in Drp1 and Mff overexpressed and knockout cell lines is also planned to address this most important cell biological process.

Acknowledgments

Supported by grants from NIH and NSF (BPJ), WSU startup (XC), NIH (FS), and NSF MRI grants (GM). Contribution: BPJ designed the research and wrote the paper. SW performed the AFM and prepared the AFM micrographs. JSL prepared tissue for EM, performed detailed analysis of the AFM and EM images, and participated with WJC in the preparation of all micrographs for the manuscript, and formatted the paper for submission. AF performed studies involving caerulein-induced pancreatitis. DJT and NB performed the EM studies, and XH and FS developed the HEK293 cell expression of the mitochondrial targeted red fluorescent protein and the fluorescent study on mitochondrial fission. XC isolated both mouse and rat pancreatic acinar cells and lobules for AFM and EM. BPJ, DJT, GM, and XC critically analyzed results and proof read the manuscript.

References

- Calamita G, Ferri D, Gena P, Liquori GE, Cavalier A, Thomas D, Svelto M. The inner mitochondrial membrane has aquaporin-8 water channels and is highly permeable to water. *Journal of Biological Chemistry*. 2005; 280:17149–17153. [PubMed: 15749715]
- Chen X, Sans D, Strahler JR, Michailidis G, Ernst SA, Andrews PC, Williams JA. Quantitative proteomics analysis of rough endoplasmic reticulum from normal and acute pancreatitis rat pancreas. *Journal of Proteome Research*. 2010; 9:885–896. [PubMed: 19954227]
- Chipuk JE, McStay GP, Bharti A, Kuwana T, Clarke CJ, Siskind LJ, Obeid LM, Green DR. Spingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. *Cell*. 2012; 148:988–1000. [PubMed: 22385963]
- Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnoczky G. Structural and functional features and significance of the physical linkage between ER and mitochondria. *Journal of Cell Biology*. 2006; 174:915–921. [PubMed: 16982799]
- De Brito OM, Scorrano L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature*. 2008; 456:605–610. [PubMed: 19052620]
- Friedman JR, Voeltz GK. The ER in 3D: a multifunctional dynamic membrane network. *Trends in Cell Biology*. 2011; 21:709–717. [PubMed: 21900009]
- Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK. ER tubules mark sites of mitochondrial division. *Science*. 2011; 334:358–362. [PubMed: 21885730]
- Gandre-Babbe S, van der Blik AM. The novel tail-anchored membrane protein Mff controls mitochondrial and peroxisomal fission in mammalian cells. *Molecular Biology of the Cell*. 2008; 19:2402–2412. [PubMed: 18353969]
- Hoppins S, Nunnari J. Mitochondrial dynamics and apoptosis—the ER connection. *Science*. 2012; 337:1052–1054. [PubMed: 22936767]
- Ingerman E, Perkins EM, Marino M, Mears JA, McCaffery M, Hinshaw JE, Nunnari J. Dnm1 forms spirals that are structurally tailored to fit mitochondria. *Journal of Cell Biology*. 2005; 170:1021–1027. [PubMed: 16186251]
- Jena BP, Gumkowski FD, Konieczko EM, Von Mollard GF, Jahn R, Jamieson JD. Redistribution of a Rab3-like GTP binding protein from secretory granules to the Golgi complex in pancreatic acinar cells during regulated exocytosis. *Journal of Cell Biology*. 1994; 124:43–53. [PubMed: 8294505]
- Jena BP, Cho SJ, Jeremic A, Stromer MH, Abu-Hamdah R. Structure and composition of the fusion pore. *Biophysical Journal*. 2003; 84:1337–1343. [PubMed: 12547814]
- Jeremic A, Kelly M, Cho SJ, Stromer MH, Jena BP. Reconstituted fusion pore. *Biophysical Journal*. 2003; 85:2035–2043. [PubMed: 12944316]
- Kaasik A, Safiulina D, Zharkovsky A, Vekslar V. Regulation of mitochondrial matrix volume. *American Journal of Physiology Cell Physiology*. 2006; 292:C157–C163. [PubMed: 16870828]
- Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, Weissma JS, Walter P. An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science*. 2009; 325:477–481. [PubMed: 19556461]
- Lackner LL, Nunnari JM. The molecular mechanism and cellular functions of mitochondrial division. *Biochimica et Biophysica Acta*. 2009; 1792:1138–1144. [PubMed: 19100831]
- Mannella CA, Buttle K, Rath BK, Marko M. Electron microscopic tomography of rat-liver mitochondria and their interaction with the endoplasmic reticulum. *Biofactors*. 1998; 8:225–228. [PubMed: 9914823]
- Martins de Brito O, Scorrano L. An intimate liaison: spatial organization of the endoplasmic reticulum-mitochondria relationship. *EMBO Journal*. 2010; 29:2715–2723. [PubMed: 20717141]
- Meier PJ, Spycher MA, Meyer UA. Isolation and characterization of rough endoplasmic reticulum associated with mitochondria from normal rat liver. *Biochimica et Biophysica Acta*. 1981; 646:283–297. [PubMed: 6170330]
- Merz S, Hammermeister M, Altmann K, Durr M, Westermann B. Molecular machinery of mitochondrial dynamics in yeast. *Biological Chemistry*. 2007; 388:917–926. [PubMed: 17696775]

- Okada S, Misaka T, Tanaka Y, Matsumoto I, Ishibashi S, Sasaki S, Abe K. Structural and functional features and significance of the physical linkage between ER and mitochondria. *FASEB Journal*. 2008; 22:3672–3684. [PubMed: 18606867]
- Park SH, Blackstone C. Further assembly required: construction and dynamics of the endoplasmic reticulum network. *EMBO Reports*. 2010; 11:515–521. [PubMed: 20559323]
- Schneider SW, Sritharan KC, Geibel JP, Oberleithner H, Jena BP. Surface dynamics in living acinar cells imaged by atomic force microscopy: identification of plasma membrane structures involved in exocytosis. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94:316–321. [PubMed: 8990206]
- Shore GC, Tata JR. Two fractions of rough endoplasmic reticulum from rat liver. I. Recovery of rapidly sedimenting endoplasmic reticulum in association with mitochondria. *Journal of Cell Biology*. 1977; 72:714–725. [PubMed: 838772]
- Skach WR, Shi L, Kalayag MC, Frigeri A, Lingappa VR, Verkman AS. Biogenesis and transmembrane topology of the CHIP28 water channel at the endoplasmic reticulum. *Journal of Cell Biology*. 1994; 125:803–815. [PubMed: 7514605]
- Tang HL, Lung HL, Wu KC, Le AH, Tang HM, Fung MC. Vimentin supports mitochondrial morphology and organization. *Biochemical Journal*. 2008; 410:141–146. [PubMed: 17983357]
- Wang S, Lee JS, Bishop N, Jeremic A, Cho WJ, Chen X, Mao GJ, Taatjes DJ, Jena BP. 3D organization and function of the cell: porosome and vesicle biogenesis to Golgi budding and mitochondrial division. *Histochemistry and Cell Biology*. 2012; 137:703–718. [PubMed: 22527693]
- Westermann B. Mitochondrial fusion and fission in cell life and death. *Nature Reviews Molecular Cell Biology*. 2010; 11:872–884.
- Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. *Science*. 2012; 337:1062–1065. [PubMed: 22936770]

Fig. 1. Representative electron micrographs of mouse (a) and rat (b) pancreatic acinar cells demonstrating polarized morphology. Note the presence of microvilli (MV) and electron dense secretory granules called zymogen granules (ZG), at the apical (AP) part of the cell. At the basolateral (BL) end is a prominent cell nucleus (N), and present throughout the cell are mitochondria (M) and a network of predominantly rough endoplasmic reticulum (ER). Also note the presence of nucleoli within the cell nucleus. Scale bar = 1 μ m.

Fig. 2. Representative electron micrographs of resting (a), and cholecystokinin-stimulated (b) rat pancreatic acinar cells, demonstrating partial loss of zymogen granule (ZG) contents following secretion. The apical lumen (AL) of acini expressing microvilli is also observed in the micrographs. Note, little or no change in the endoplasmic reticulum (ER) is demonstrated in the stimulated acinar cells. Scale bar = 1 μ m.

Fig. 3.

AFM micrograph demonstrating ER-induced mitochondrial fission in mouse exocrine pancreas. AFM amplitude image of the basolateral region of a mouse pancreatic acinar cell with the plasma membrane removed, exposing the underlying organelles such as the endoplasmic reticulum (ER) network (black arrows, some ER in green pseudo color), and of ER tubules wrapping around (red arrow, ER in green pseudo color) mitochondria (M) to induce mitochondrial division. Scale bar = 1 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. ER-induced mitochondrial fission in mouse (a–d) and rat (e–h) exocrine pancreas. Electron micrographs, depicting several mitochondria undergoing division at sites depicted by red arrowheads. Note that while most mitochondria divide into two, some (seen in a and d) are undergoing fission simultaneously at more than one site. The mitochondrion in (b) appears to be undergoing fission of a projection from the parent mitochondria body. Scale bar = 1 μ m. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 5.

Electron micrographs of mitochondria undergoing fission and depicted in Fig. 3 are shown here at greater magnification. A number of mitochondria undergoing ER-induced fission have been magnified to demonstrate fission sites (blue arrowheads). The ER lumen is filled (green) and outlined (yellow lines) for clarity, and the ER membrane and tethers (colored yellow) connecting ER and mitochondria are shown. Close examination of the micrographs at mitochondrial fission sites reveals ring-like structures (red), each measuring approximately 100 nm in diameter, and may represent the GTPase Drp1 oligomeric helices. Note the presence of swollen ER at mitochondrial fission sites. Scale bars = 200 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 6.

Electron micrographs demonstrating ER-induced mitochondrial fission in exocrine pancreas following caerulein-induced pancreatitis. Control pancreatic tissue (a and b), and at 2 h (c and d) following exposure to supramaximal dose of caerulein exposure known to induce ER stress, leading to pancreatitis. Note that 2 h following the supramaximal stimulation of the exocrine pancreas, the ER appear beaded and detach from each other, establishing tighter interactions with the mitochondria. Measurements of mitochondria lengths (mean $\pm$ SEM; $n = 40$, each for control and 2 h), demonstrates that 2 h following caerulein exposure, mitochondrial size is significantly ($*p < 0.001$) reduced and they appear more spherical (d and e). Interestingly at the 2 h post-caerulein exposure, the ER appears vesiculated, suggesting ER stress. Scale bar = 1 μ m.

Fig. 7.

Fluorescent light micrographs of Mito Red transfected HEK293 cells, demonstrating ER stress-induced mitochondrial fission and abrogation in the presence of mercuric chloride. Only adequately transfected cells expressing detectable red fluorescence of the tubular mitochondrial network, were used in the study. Note in the top panel in control untreated cells, the mitochondrial network within the cell exhibit no visible time-dependent changes in shape or distribution. In contrast, exposure of cells to the ER stress-inducing drug tunicamycin (Tm) (central panel), results in a time-dependent increase in mitochondrial fission, as observed in the fluorescent micrographs. In the bottom panel, cells pre-exposed to the water channel inhibitor mercuric chloride (HgCl_2), demonstrate no Tm-induced mitochondrial fission, suggesting the role of water channels or aquaporins (AQP) in mitochondrial fission. Scale bar = 20 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 8.

Schematic diagram and EM inset of ER-induced mitochondrial fission. The entry of water into ER via water channels or aquaporins, and a concomitant loss of water from the mitochondria also via aquaporins at the ER-mitochondria contact sites, would lead to constriction of the mitochondrial diameter at that location, enabling the formation of Drp1 oligomeric helices, leading to GTP-driven mitochondrial fission. Note in the inset, an EM micrograph of a mitochondrion undergoing fission, and depicting two ring-like structures outlined in red, pointed by four red arrowheads. Each ring measures approximately 100 nm in diameter, and may represent the GTPase Drp1 oligomeric helices. Also note the ER-mitochondria tethers shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)