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Mitochondrial morphology and dynamics in hepatocytes from normal and ethanol-fed rats

Sudipto Das,

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA

Nora Hajnóczky,

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA

Anil Noronha Antony,

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA

György Csordás,

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA

Lawrence D. Gaspers,

Department of Pharmacology and Physiology, New Jersey Medical School, Newark, NJ, USA

Dahn L. Clemens,

Research Service, Veterans Administration Medical Center, 4101 Woolworth Avenue, Omaha, NE 68105, USA

Jan B. Hoek, and

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA

György Hajnóczky

Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, USA

György Hajnóczky: gyorgy.hajnoczky@jefferson.edu

Abstract

Mitochondrial structure and function are central to cell physiology and are mutually interdependent. Mitochondria represent a primary target of the alcohol-induced tissue injury, particularly in the liver, where the metabolic effects of ethanol are predominant. However, the effect of ethanol on hepatic mitochondrial morphology and dynamics remain to be established. In the present work, we employed the organelle-targeted photoactivatable fluorescent protein technology and electron microscopy to study hepatic mitochondrial structure and dynamics. Hepatocytes in perfused liver as well as in primary cultures showed mostly discrete globular or short tubular mitochondria. The mitochondria showed few fusion events and little movement activity. By contrast, human hepatoma (HepG2)-derived VL-17A cells, expressing the major

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Correspondence to: György Hajnóczky, gyorgy.hajnoczky@jefferson.edu.

Sudipto Das, Nora Hajnóczky, and Anil Noronha Antony contributed equally to this paper.

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hepatic ethanol metabolizing enzymes, alcohol dehydrogenase and cytochrome P450 2E1, have elongated and interconnected mitochondria showing matrix continuity and many fusion events. Hepatocytes isolated from chronically ethanol-fed rats showed some increase in mitochondrial volume and exhibited a substantial suppression of mitochondrial dynamics. In VL-17A cells, prolonged ethanol exposure also caused decreased mitochondrial continuity and dynamics. Collectively, these results indicate that mitochondria in normal hepatocytes show relatively slow dynamics, which is very sensitive to suppression by ethanol exposure.

Keywords

Mitochondria; Fusion; Motility; Alcohol; Oxidative stress; Alcoholic liver disease

Introduction

Mitochondrial morphology and dynamics in living cells rely on processes of membrane fusion and fission that result in structures ranging from an integrated mitochondrial reticular network to a largely dispersed mitochondrial morphology and allows for the dynamic interchange of inner and outer membrane constituents and matrix content between individual mitochondria [2, 22, 23, 26, 35]. Mitochondrial fusion proteins and their activity are essential for normal cell function [5, 7]. Mitofusins 1 and 2 (Mfn1 and Mfn2) are integral proteins of the outer mitochondrial membrane [42] shown to act in trans in either homo- or heterotypic interactions to tether apposing mitochondria and initiate fusion [30]. Mutations in Mfn2 cause Charcot–Marie–Tooth neuropathy type 2A [54]. Optic atrophy 1 (Opa1), is an inner mitochondrial membrane (IMM) protein, that is required to tether and fuse the IMMs [36]. Mutations of Opa1 cause dominant optic atrophy [11]. Although the most severe consequences of Mfn and Opa1 mutations appear in the nervous system, the function of many other tissues also seems to be impaired by the shortage of fusion proteins [6, 7, 13, 53].

Mitochondrial fusion has been visualized and quantitated in several cell lines [25, 33, 43, 46] but only very few primary cells [34, 47]. Strikingly, it has not been visualized or quantified in the liver or muscle, where tissue function is greatly dependent on mitochondrial bioenergetics.

Recent studies [27, 28, 31, 48, 52] have highlighted the essential function of mitochondrial dynamics in the management of cellular integrity and in restricting the dispersal and disposal of defective constituents in the mitochondrial network. Alcoholic liver disease (ALD), which results from the chronic consumption of excess ethanol, is associated with defects in mitochondrial morphology and function in liver cells, including the appearance of misshapen and enlarged mitochondria (megamitochondria), often with par-acrystalline inclusions [14]. Chronic ethanol consumption is also associated with defects in liver mitochondrial energy metabolism [1, 19, 20]. However, a possible contribution of defects in mitochondrial fusion and fission to the development of ALD has not been investigated. In this study, we report observations that support a significant impairment in mitochondrial dynamics in hepatocytes isolated from chronically ethanol-fed rats and in liver cell lines that are exposed to ethanol.

Methods

Animal treatment

Adult male Sprague Dawley rats were fed with an ethanol-containing, nutritionally adequate liquid diet that contained ethanol as 36 % of total calories for 6–9 months. Pair-fed control

littermates received a liquid diet in which ethanol calories were isocalorically replaced with maltose-dextran [10]. No other liquid was provided during the treatment.

Cell culture and transfections

Hepatocytes were isolated from the rats by collagenase perfusion, essentially as described by Meyer et al. [3, 38]. Briefly, after the rat was anesthetized (5 % Isoflourane), the portal vein was cannulated, and the liver was perfused for 8 min with a Ca^{2+} -free Krebs-Ringer buffer (100 mM NaCl, 23.8 mM NaHCO_3 , 5.5 mM glucose, 10 mM HEPES, 4.8 mM KCl, 1.2 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.2 mM KH_2PO_4 , pH 7.4) containing 1 mM EDTA and 2 mM EGTA, equilibrated with 95 % O_2 /5% CO_2 at a rate of 30 ml/min, followed by approximately 10 min perfusion with collagenase buffer (Krebs-Ringer buffer containing 0.1 mM CaCl_2 and 0.17 U/ml Collagenase; Liberase Roche Cat. no - 05 466 202 001). The liver was then removed from the rat and immersed in ice-cold wash buffer (Krebs-Ringer buffer containing 0.1 mM CaCl_2). The capsule surrounding the liver was torn gently to release the hepatocytes. The cells were filtered through a 70 μm mesh filter and then centrifuged ($50 \times g$) for 2 min at 4 °C. The pellet was washed and resuspended in Krebs-Ringer buffer containing 1 mM CaCl_2 and kept on ice. Hepatocytes were counted in a hemocytometer, and viability was determined using the trypan blue exclusion method. Viability was always >90 %. Hepatocytes were cultured as described earlier [18].

The details of the liver isolation procedure, perfusion apparatus and imaging chamber [16], and the culture procedures for VL-17A HepG2 cells have been described earlier [12]. mtDsRed and mtPAGFP were introduced by adenovirus and plasmid DNA into primary rat hepatocytes and VL-17A cells, respectively [15].

Electron microscopy

Rat livers were perfusion-fixed as follows. The animals were anesthetized (5 % Isoflourane), and upon portal vein cannulation, the liver was perfused with 0.067 M cacodylate buffer, pH 7.4 for 5–10 min at a rate of 30 ml/min. The perfusate was then switched to the fixative composed of 2.5% glutaraldehyde in 0.067 M Na-cacodylate buffer pH 7.4 for 5 min. Around 1 mm^3 pieces were cut from the liver and incubated in the fixative for another 10 min. After that, samples were washed thrice with 0.067 M Na-cacodylate buffer pH 7.4 and stained with 1% osmium tetroxide in phosphate buffer 0.2M for 1 h at 4 °C. Stained samples were dehydrated on an acetone dilution series and embedded to Epon/Araldite resin (Epon 812-Polybed and Araldite 6005, dodecenyl succinic anhydride DDSA 1: 1: 2.66 v/v ratio) according to manufacturer's (Electron Microscopy Sciences) suggestions. Thin sections (60–100 nm) were cut from the embedded specimens, mounted on electron microscopy grids, and examined using a FEI Tecnai 12 TEM equipped with a phosphor plate (Advanced Microscopy Techniques) and Hamamatsu Orca 8 Mpx digital camera. Image analysis and morphometric evaluation of the mitochondria and ER-mitochondrial associations in randomly selected hepatocytes of the liver specimen derived from the alcoholic and control rats was done as described earlier [8].

Cellular pyridine nucleotide fluorescence measurements

Cellular NAD(P)H fluorescence was imaged on a Bio-Rad radiance 2002-MP confocal system equipped with a 10-W Mira/Verdi Ti:sapphire laser from Coherent. Pyridine nucleotide images were acquired with 710 nm multi-photon excitation and 450 ± 80 nm emission as described earlier [17].

Live cell microscopic imaging

Confocal imaging experiments were performed using a Bio-Rad radiance system fitted to an Olympus IX70 microscope using a 40× objective or a Zeiss LSM780 system using a 63× objective. The laser source was used for imaging of mtPAGFP at ex:488 nm and mtDsRed at ex:568 nm or 561 nm. At the Radiance system, a HeCd laser (442 nm) was used for photoactivation of photoactivatable GFP (PAGFP) [33]. At the LSM780 system, a Chameleon pulsed laser system (Coherent, 760 nm) was used for photoactivation. To create 3D-like reconstructions, a sequence of images was collected from below the adherent surface up in 0.367 μm steps (30 steps, Z series). Image analysis was done in Spectralyzer imaging software.

Statistics

Experiments were carried out with at least three different cell preparations. Traces represent single cell responses unless indicated otherwise. Data are presented as means $\pm$ S.E.M. Significance of differences from the relevant controls was calculated by Student's *t* test.

Results

Ultrastructure of hepatocytes isolated from normal- and ethanol-fed rats

Transmission electron microscopy of rat hepatocytes in the perfusion-fixed liver showed a large number of mostly round mitochondrial cross sections, which occupied ~20 % of the cytoplasmic area (Fig. 1a, b). The ER stacks appeared as islands, which were most visible around the nucleus (Fig. 1a). In the liver of ethanol-fed (9 months) rats, several large, clear structures appeared, which represent lipid vesicles (Fig. 1b, c). Furthermore, the fraction of cellular area occupied by mitochondria and ER and the cross-sectional area of the individual mitochondria was slightly increased (Fig. 1b, c). Alcohol-induced lipid storage and ER proliferation likely account for some of these changes, whereas matrix swelling in a fraction of the mitochondria might be the cause of the mitochondrial alterations.

Mitochondrial morphology of rat hepatocytes in situ

To evaluate if isolated hepatocytes were a faithful model for studying the effects of alcohol on the mitochondrial morphology in the liver, we also visualized mitochondria in both perfused liver and isolated hepatocytes by two-photon excitation of NAD(P)H fluorescence. The images showed cells packed with short tubular and globular mitochondria in the perfused liver as well as isolated hepatocytes (Fig. 2). Thus, the isolated hepatocytes maintained the typical mitochondrial morphology of the hepatocytes present in the liver. Also, the mitochondrial structure observed in the EM images is similar to the one untreated with a fixative.

Mitochondrial morphology and dynamics in normal hepatocytes

To monitor mitochondrial dynamics, hepatocytes isolated from control rats were infected with mitochondrial matrix targeted DsRed and PAGFP. Based on the red fluorescence, the cells were densely packed with short, tube-like or globular mitochondria (Fig. 3a mtDsRed images i–iv). Therefore, identifying individual mitochondria was a difficult task. To better visualize single organelles, mtPAGFP was turned on in small square-shaped regions (5 $\times$ 5 μm) by two-photon photoactivation (at 30 s). For several seconds, mtPAGFP fluorescence remained within the regions of photoactivation (Fig. 3a image vi). Matrix continuity among neighboring mitochondria would allow the fluorescent signal to travel from one mitochondrion to the next. The lack of rapid fluorescence spreading indicates that hepatocyte mitochondria have isolated matrix content. This pattern is in sharp contrast to the mitochondrial reticular structures commonly observed in cell lines in culture, including

HepG2 hepatocarcinoma cells (Fig. 5). Mitochondrial fusion events among adjacent mitochondria can also provide a mechanism for spreading of the soluble matrix content as seen at 48 s (Fig. 3a image vii). To discriminate between mitochondrial fusion and other fluorescence transfer events, evaluation of both the mtPAGFP and mtDsRed fluorescence was used. Simultaneously with PAGFP photoactivation, the DsRed was photobleached in the same mitochondria and, therefore, matrix content exchange among these mitochondria and mitochondria outside the region results in opposing changes in green and red fluorescence. In the example shown in Fig. 3b, the mitochondrion on the right side donated photoactivated PAGFP and received fluorescent DsRed from the mitochondrion on the left that at the same time gained PAGFP fluorescence and lost DsRed fluorescence. We could confirm less than one clear fusion event per cell during the 7 min of the recording. We estimate that there were some additional events (1–2 fusions per cell); however, we were unable to confirm this due to the high density of the mitochondria. In any case, this number is much lower than what we counted in previous studies on primary cells or H9c2 and INS1 cell lines [33, 47]. Taken together, these results indicate that fusion events are relatively rare in hepatocytes. MtGFP spreading may also occur with mitochondrial movement. Comparison of the PAGFP images collected at varying times (33 s vs 258 s) gave evidence that movement did in fact occur (Fig. 3a vi vs viii). To show only the sites of movement, the difference between the two images was calculated ($F_{258s} - F_{33s}$; negative values shown in red, positive values in blue, respectively, Fig. 3c). The amount and distribution of red and blue pixels in the difference image corresponds with the movement of the organelles. Specifically, blue color highlights the new location of several mitochondria that showed significant displacement (Fig. 3c). Notably, the movement activity in primary hepatocytes was much less than that in previously studied cell lines (e.g. H9c2 cells, [51]).

Mitochondrial morphology and dynamics in hepatocytes of ethanol-fed rats

The morphology of mitochondria in hepatocytes derived from ethanol-fed rats was similar to that seen in control rats. However, a varying fraction of the mitochondria tended to be swollen (Fig. 4a, mtDsRed images). These mitochondria were intermingled with many more voids than in the control hepatocytes, which likely correspond to lipid vesicles. The PAGFP time-course images showed that little spreading occurred; there was no indication of matrix continuity, no fusion, and little movement (Fig. 4a, v–viii). To further evaluate the spreading of the fluorescence, a Z series of images was collected on a cell 7 min after being exposed to PAGFP photoactivation (Fig. 4b). Reconstruction in three dimensions confirmed that minimal spreading is detectable in hepatocytes of ethanol-fed rats. Finally, to quantitatively compare mitochondrial dynamics in both control and ethanol-fed conditions, the time course of PAGFP fluorescence was calculated for each region of photoactivation (Fig. 4c). The fluorescence in each region was normalized to the post photoactivation point before the mean decay kinetics was calculated. The graphs produced show the average for 10–15 cells (Fig. 4c). The results showed a continuous decrease in the control leading to 25 % decrease during the recording time period. By contrast, the hepatocytes of the ethanol-fed rats showed negligible decay in fluorescence (Fig. 4c). This difference between the two conditions is further supported by the final responses calculated for two separate cultures for each (20–30 cells, Fig. 4c bar charts). Thus, mitochondrial dynamics is greatly suppressed in hepatocytes of ethanol-fed rats.

Mitochondrial morphology and dynamics in control and ethanol-treated VL-17A cells

To test whether hepatic cell lines show mitochondrial dynamics and ethanol sensitivity similar to normal hepatocytes, we used human hepatoma (HepG2)-derived VL-17A cells, which express the major ethanol metabolizing enzymes of the liver, alcohol dehydrogenase and cytochrome P450 2E1 [12]. VL-17A cells displayed elongated and interconnected mitochondria with matrix continuity and many fusion events (Fig. 5a upper row of images).

Prolonged exposure to ethanol (50 mM for 72 h) caused decreased mitochondrial continuity and fusion activity (Fig. 5a lower row of images). Quantitative assessment confirmed faster mitochondrial dynamics in VL-17A than in primary hepatocytes (Fig. 5b). Furthermore, the mitochondrial dynamics was greatly suppressed upon prolonged ethanol exposure in VL-17A cells (Fig. 5b) like in primary hepatocytes of ethanol-fed rats.

Discussion

A major finding of this study is that mitochondrial fusion and movement activity is low in primary rat hepatocytes. Fusion activity is less than it is in hepatocarcinoma cells expressing the key enzymes of ethanol metabolism or in other cell lines or primary cells, such as cells [47]. This probably does not result from movements producing few intermitochondrial contacts, since the high density of mitochondria allows for many contact events in hepatocytes. Furthermore, this work provides evidence that chronic ethanol exposure effectively eliminates mitochondrial fusion and motility dynamics in primary hepatocytes and also greatly suppresses mitochondrial dynamics in hepatocarcinoma cells. Overall, these studies indicate that defective mitochondrial fusion might impair mitochondrial quality control in hepatocytes from chronically ethanol-fed rats and may serve as an important mediator causing ethanol-induced liver injury.

Multiple lines of evidence support the concept that mitochondrial fusion is required for the maintenance of healthy mitochondria [7, 44–46, 49]. Fusion depends on fusion proteins and on a positioning system that allows mitochondria to engage in contacts [33]. Our results show that fusion events are rare in hepatocytes, especially when it is normalized to the amount of mitochondria. No information is available to indicate that the fusion proteins would be scarce in liver mitochondria. Although directional mitochondrial movements are also infrequent in hepatocytes, many mitochondrial contact events take place among adjacent mitochondria due to the high mitochondrial density. Thus, low fusion activity in hepatocytes likely results from another, presently unknown factor.

Ethanol is primarily metabolized in the liver through enzymes that generate acetaldehyde and enhance oxidative stress conditions. Our studies indicate that some targets of ethanol or its metabolites are relevant for mitochondrial fusion and motility. Though the exact mechanisms remain to be determined, they seem to be preserved in both primary hepatocytes where the mitochondrial dynamics is low and in hepatocarcinoma cells where the dynamics is very active. As described above, it is unlikely that a single factor of mitochondrial dynamics would mediate the effects on both fusion and motility. Chronic ethanol treatment can cause a decrease in mitochondrial membrane fluidity [41] which may impair the fusion events required for their merging. Accumulation of oxidized lipid aldehydes resulting from ROS-induced membrane damage and changes in membrane cholesterol content may also have more direct effects on the processes required for fusion. Also, the accumulation of acetaldehyde and ROS resulting from ethanol metabolism [4, 9, 21, 50] may cause covalent modification of proteins required for mitochondrial dynamics. Furthermore, along the line of previous studies describing sensitization of the permeability transition pore during chronic ethanol exposure [29, 32, 39], signs of mitochondrial swelling were observed both in the present ultrastructural studies and in live cell imaging. Permeability transition pore opening results in depolarization of the mitochondria and ensuing decrease in ATP supply to interfere with fusion and motility, respectively [24, 33, 37]. Further studies are required to assess the contribution of these mechanisms to the ethanol-induced defects in mitochondrial dynamics in the liver and perhaps in other important targets of ethanol (e.g. exocrine pancreas, [40]).

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References

1. Bailey SM, Cunningham CC. Contribution of mitochondria to oxidative stress associated with alcoholic liver disease. *Free Radic Biol Med*. 2002; 32:11–16. [PubMed: 11755312]
2. Bereiter-Hahn J, Voth M. Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc Res Tech*. 1994; 27:198–219. [PubMed: 8204911]
3. Berry MN, Friend DS. High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. *J Cell Biol*. 1969; 43:506–520. [PubMed: 4900611]
4. Carmichael FJ, Orrego H, Israel Y. Acetate-induced adenosine mediated effects of ethanol. *Alcohol Alcohol Suppl*. 1993; 2:411–418. [PubMed: 7748331]
5. Chen H, Chan DC. Physiological functions of mitochondrial fusion. *Ann N Y Acad Sci*. 2010; 1201:21–25. [PubMed: 20649534]
6. Chen Y, Liu Y, Dorn GW 2nd. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circ Res*. 2011; 109:1327–1331. [PubMed: 22052916]
7. Chen H, Vermulst M, Wang YE, Chomyn A, Prolla TA, McCaffery JM, Chan DC. Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell*. 2010; 141:280–289. [PubMed: 20403324]
8. 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. *J Cell Biol*. 2006; 174:915–921. [PubMed: 16982799]
9. Cunningham CC, Bailey SM. Ethanol consumption and liver mitochondria function. *Biol Signals Recept*. 2001; 10:271–282. [PubMed: 11351133]
10. DeCarli LM, Lieber CS. Fatty liver in the rat after prolonged intake of ethanol with a nutritionally adequate new liquid diet. *J Nutr*. 1967; 91:331–336. [PubMed: 6021815]
11. Delettre C, Lenaers G, Griffoin JM, Gigarel N, Lorenzo C, Belenguer P, Pelloquin L, Grosgeorge J, Turc-Carel C, Perret E, Astarie-Dequeker C, Lasquelléc L, Arnaud B, Ducommun B, Kaplan J, Hamel CP. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet*. 2000; 26:207–210. [PubMed: 11017079]
12. Donohue TM, Osna NA, Clemens DL. Recombinant Hep G2 cells that express alcohol dehydrogenase and cytochrome P450 2E1 as a model of ethanol-elicited cytotoxicity. *Int J Biochem Cell Biol*. 2006; 38:92–101. [PubMed: 16181800]
13. Gall JM, Wang Z, Liesa M, Molina A, Havasi A, Schwartz JH, Shirihai O, Borkan SC, Bonegio RG. Role of mitofusin 2 in the renal stress response. *PLoS One*. 2012; 7:e31074. [PubMed: 22292091]
14. Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology*. 2011; 141:1572–1585. [PubMed: 21920463]
15. Garcia-Perez C, Roy SS, Naghdi S, Lin X, Davies E, Hajnoczky G. Bid-induced mitochondrial membrane permeabilization waves propagated by local reactive oxygen species (ROS) signaling. *Proc Natl Acad Sci U S A*. 2012; 109:4497–4502. [PubMed: 22393005]
16. Gaspers LD, Patel S, Morgan AJ, Anderson PA, Thomas AP. Monitoring generation and propagation of calcium signals in multicellular preparations. In: Tepikin A, editor. *Calcium signalling: a practical approach*. Oxford: Oxford University Press; 2001. p. 155–175.
17. Gaspers LD, Thomas AP. Calcium-dependent activation of mitochondrial metabolism in mammalian cells. *Methods*. 2008; 46:224–232. [PubMed: 18854213]
18. Hajnoczky G, Robb-Gaspers LD, Seitz MB, Thomas AP. Decoding of cytosolic calcium oscillations in the mitochondria. *Cell*. 1995; 82:415–424. [PubMed: 7634331]
19. Hoek JB, Cahill A, Pastorino JG. Alcohol and mitochondria: a dysfunctional relationship. *Gastroenterology*. 2002; 122:2049–2063. [PubMed: 12055609]

20. Hoek JB, Pastorino JG. Ethanol, oxidative stress, and cytokine-induced liver cell injury. *Alcohol*. 2002; 27:63–68. [PubMed: 12062639]
21. Holmuhamedov EL, Czerny C, Beeson CC, Lemasters JJ. Ethanol suppresses ureagenesis in rat hepatocytes: role of acetaldehyde. *J Biol Chem*. 2012; 287:7692–7700. [PubMed: 22228763]
22. Hoppins S, Lackner L, Nunnari J. The machines that divide and fuse mitochondria. *Annu Rev Biochem*. 2007; 76:751–780. [PubMed: 17362197]
23. Hyde BB, Twig G, Shirihai OS. Organellar vs cellular control of mitochondrial dynamics. *Semin Cell Dev Biol*. 2010; 21:575–581. [PubMed: 20079451]
24. Ishihara N, Fujita Y, Oka T, Mihara K. Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. *EMBO J*. 2006; 25:2966–2977. [PubMed: 16778770]
25. Karbowski M, Arnoult D, Chen H, Chan DC, Smith CL, Youle RJ. Quantitation of mitochondrial dynamics by photolabeling of individual organelles shows that mitochondrial fusion is blocked during the Bax activation phase of apoptosis. *J Cell Biol*. 2004; 164:493–499. [PubMed: 14769861]
26. Karbowski M, Youle RJ. Dynamics of mitochondrial morphology in healthy cells and during apoptosis. *Cell Death Differ*. 2003; 10:870–880. [PubMed: 12867994]
27. Kim I, Lemasters JJ. Mitochondrial degradation by autophagy (mitophagy) in GFP-LC3 transgenic hepatocytes during nutrient deprivation. *Am J Physiol Cell Physiol*. 2011; 300:C308–C317. [PubMed: 21106691]
28. Kim I, Rodriguez-Enriquez S, Lemasters JJ. Selective degradation of mitochondria by mitophagy. *Arch Biochem Biophys*. 2007; 462:245–253. [PubMed: 17475204]
29. King AL, Swain TM, Dickinson DA, Lesort MJ, Bailey SM. Chronic ethanol consumption enhances sensitivity to Ca(2+)-mediated opening of the mitochondrial permeability transition pore and increases cyclophilin D in liver. *Am J Physiol Gastrointest Liver Physiol*. 2010; 299:G954–G966. [PubMed: 20651005]
30. Koshiba T, Detmer SA, Kaiser JT, Chen H, McCaffery JM, Chan DC. Structural basis of mitochondrial tethering by mitofusin complexes. *Science*. 2004; 305:858–862. [PubMed: 15297672]
31. Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res*. 2005; 8:3–5. [PubMed: 15798367]
32. Lemasters JJ. Modulation of mitochondrial membrane permeability in pathogenesis, autophagy and control of metabolism. *J Gastroenterol Hepatol*. 2007; 22(Suppl 1):S31–S37. [PubMed: 17567461]
33. Liu X, Weaver D, Shirihai O, Hajnoczky G. Mitochondrial ‘kiss-and-run’: interplay between mitochondrial motility and fusion-fission dynamics. *EMBO J*. 2009; 28:3074–3089. [PubMed: 19745815]
34. Magrane J, Sahawneh MA, Przedborski S, Estevez AG, Manfredi G. Mitochondrial dynamics and bioenergetic dysfunction is associated with synaptic alterations in mutant SOD1 motor neurons. *J Neurosci*. 2012; 32:229–242. [PubMed: 22219285]
35. McBride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. *Curr Biol*. 2006; 16:R551–R560. [PubMed: 16860735]
36. Meeusen S, DeVay R, Block J, Cassidy-Stone A, Wayson S, McCaffery JM, Nunnari J. Mitochondrial inner-membrane fusion and crista maintenance requires the dynamin-related GTPase Mgm1. *Cell*. 2006; 127:383–395. [PubMed: 17055438]
37. Meeusen S, McCaffery JM, Nunnari J. Mitochondrial fusion intermediates revealed in vitro. *Science*. 2004; 305:1747–1752. [PubMed: 15297626]
38. Meijer AJ, Gimpel JA, Deleeuw GA, Tager JM, Williamson JR. Role of anion translocation across the mitochondrial membrane in the regulation of urea synthesis from ammonia by isolated rat hepatocytes. *J Biol Chem*. 1975; 250:7728–7738. [PubMed: 1182028]
39. Pastorino JG, Hoek JB. Ethanol potentiates tumor necrosis factor- α cytotoxicity in hepatoma cells and primary rat hepatocytes by promoting induction of the mitochondrial permeability transition. *Hepatology*. 2000; 31:1141–1152. [PubMed: 10796891]

40. Petersen OH, Tepikin AV, Gerasimenko JV, Gerasimenko OV, Sutton R, Criddle DN. Fatty acids, alcohol and fatty acid ethyl esters: toxic Ca^{2+} signal generation and pancreatitis. *Cell Calcium*. 2009; 45:634–642. [PubMed: 19327825]
41. Rubin E, Rottenberg H. Ethanol-induced injury and adaptation in biological membranes. *Fed Proc*. 1982; 41:2465–2471. [PubMed: 7044830]
42. Santel A, Fuller MT. Control of mitochondrial morphology by a human mitofusin. *J Cell Sci*. 2001; 114:867–874. [PubMed: 11181170]
43. Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC. Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. *Mol Biol Cell*. 2009; 20:3525–3532. [PubMed: 19477917]
44. Suen DF, Norris KL, Youle RJ. Mitochondrial dynamics and apoptosis. *Genes Dev*. 2008; 22:1577–1590. [PubMed: 18559474]
45. Tatsuta T, Langer T. Quality control of mitochondria: protection against neurodegeneration and ageing. *EMBO J*. 2008; 27:306–314. [PubMed: 18216873]
46. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J*. 2008; 27:433–446. [PubMed: 18200046]
47. Twig G, Liu X, Liesa M, Wikstrom JD, Molina AJ, Las G, Yaniv G, Hajnoczky G, Shirihai OS. Biophysical properties of mitochondrial fusion events in pancreatic beta-cells and cardiac cells unravel potential control mechanisms of its selectivity. *Am J Physiol Cell Physiol*. 2010; 299:C477–C487. [PubMed: 20445168]
48. Twig G, Shirihai OS. The interplay between mitochondrial dynamics and mitophagy. *Antioxid Redox Signal*. 2011; 14:1939–1951. [PubMed: 21128700]
49. Westermann B. Mitochondrial dynamics in model organisms: what yeasts, worms and flies have taught us about fusion and fission of mitochondria. *Semin Cell Dev Biol*. 2010; 21:542–549. [PubMed: 20006727]
50. Wu D, Cederbaum AI. Oxidative stress and alcoholic liver disease. *Semin Liver Dis*. 2009; 29:141–154. [PubMed: 19387914]
51. Yi M, Weaver D, Hajnoczky G. Control of mitochondrial motility and distribution by the calcium signal: a homeostatic circuit. *J Cell Biol*. 2004; 167:661–672. [PubMed: 15545319]
52. Youle RJ, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol*. 2011; 12:9–14. [PubMed: 21179058]
53. Zhang Z, Wakabayashi N, Wakabayashi J, Tamura Y, Song WJ, Sereda S, Clerc P, Polster BM, Aja SM, Pletnikov MV, Kensler TW, Shirihai OS, Iijima M, Hussain MA, Sesaki H. The dynamin-related GTPase Opa1 is required for glucose-stimulated ATP production in pancreatic beta cells. *Mol Biol Cell*. 2011; 22:2235–2245. [PubMed: 21551073]
54. Zuchner S, Mersiyanova IV, Muglia M, Bissar-Tadmouri N, Rochelle J, Dadali EL, Zappia M, Nelis E, Patitucci A, Senderek J, Parman Y, Evgrafov O, Jonghe PD, Takahashi Y, Tsuji S, Pericak-Vance MA, Quattrone A, Battaloglu E, Polyakov AV, Timmerman V, Schroder JM, Vance JM. Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat Genet*. 2004; 36:449–451. [PubMed: 15064763]

Fig. 1.

Ultrastructure of hepatocytes derived from normal and ethanol-fed rats. Representative transmission electron microscopy of livers from ethanol-fed (*EtOH*) (**b**) and paired-control rats (**a**). Labels: *LD* lipid droplets, *M* mitochondria. Bar charts show the total mitochondrial and lipid droplet area per cell normalized to the cytoplasmic area in both control and ethanol-fed samples (**c**). Histograms and bar charts show the distribution and mean of the single mitochondrial cross-sectional areas in both control and ethanol-fed samples (**d**, **e**)

Fig. 2. Mitochondrial morphology in hepatocytes in situ. Images show the subcellular distribution of NAD(P)H fluorescence in hepatocytes in the intact perfused rat liver (**a**) and in hepatocytes maintained in primary culture (**b**). Pyridine nucleotide fluorescence was imaged with two-photon excitation as described in [17]

Fig. 3.

Visualization of mitochondrial fusion and movement by photoactivated PAGFP in a normal rat hepatocyte. **a** Labeling of subsets of mitochondria by photoactivation of PAGFP in a primary rat hepatocyte expressing both mtDsRed and mtPAGFP (sites are marked by white boxes in image v). From the time series, red and green image pairs obtained before photoactivation (i, v), immediately after photoactivation (ii, vi), after a fusion event occurred (iii, vii) and 4 min after photoactivation (iv, viii) are shown. These images illustrate the spreading of mtPAGFP fluorescence by matrix continuity (vi), fusion (vii) and by movements (viii), respectively. **b** Time course of mitochondrial fusion event shown in vii. Graphs were created by masking the mtPAGFP donor (D, green) and acceptor (A, red)

organelles and calculating the time course of the green and red fluorescence for each. **c** Magnified image (258 s) shows both PAGFP fluorescence (*grayscale*) and the sites of mitochondrial movement calculated by subtraction of sequential images (*blue*, positive changes; *red*, negative changes)

Fig. 4.

Visualization of mitochondrial fusion and movement by photoactivated PAGFP in hepatocytes derived from ethanol-fed rats. **a** Labeling of subsets of mitochondria by photoactivation of PAGFP (sites are marked by *white boxes* in image *v*) in a pair of cells expressing both mtDsRed and mtPAGFP. From the time series, *red* and *green image pairs* obtained before photoactivation (*i*, *iv*), immediately (*ii*, *v*), and 4 min after photoactivation (*iii*, *vi*) are shown. These images illustrate the spreading of mtPAGFP fluorescence by matrix continuity (*v*), and by movements (*vi*), respectively. To better visualize the mitochondrial movements, a magnified image (*vii*) shows both PAGFP fluorescence

(*grayscale*) and the sites of mitochondrial movement calculated by subtraction of sequential images (*blue*, positive changes; *red*, negative changes). **b** 3D reconstruction of mtDsRed and mtPAGFP fluorescence in a chronic ethanol (*EtOH*) hepatocyte 7 min after photoactivation. **c** Time course of the mtPAGFP fluorescence in both normal and chronic ethanol conditions. Time course of the green fluorescence was calculated for every photoactivation area. After normalization to the maximal value reached after photoactivation, the mean was calculated for all cells recorded in a cell culture (10–15). The *bar charts* show the summary of 20–30 for each condition ($p<0.01$)

Fig. 5. Visualization of mitochondrial dynamics by photoactivated PAGFP in control and ethanol-treated (50 mM for 72 h) VL-17A HepG2 cells. **a** Labeling of subsets of mitochondria by photoactivation of PAGFP (sites are marked by *white boxes*) in cells expressing both mtDsRed and mtPAGFP. From the time series, overlaid red and green images obtained before and various times after photoactivation are shown for both control (*upper row*) and ethanol-treated (*EtOH*, *lower row*) cells. **b** Time course of the mtPAGFP fluorescence in both normal and prolonged ethanol conditions. Time course of the green fluorescence was calculated for every photoactivation area. After normalization to the maximal value reached

after photoactivation, the mean was calculated for all cells recorded in a cell culture (30–40 cells recorded in four different experiments, $p < 0.01$)