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Review

Mitochondrial reticulum network dynamics in relation to oxidative stress, redox regulation, and hypoxia

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ABSTRACT

A single mitochondrial network in the cell undergoes constant fission and fusion primarily depending on the local GTP gradients and the mitochondrial energetics. Here we overview the main properties and regulation of pro-fusion and pro-fission mitodynamins, i.e. dynamins-related GTPases responsible for mitochondrial shape-forming, such as pro-fusion mitofusins MFN1, MFN2, and the inner membrane-residing long OPA1 isoforms, and pro-fission mitodynamins FIS1, MFF, and DRP1 multimers required for scission. Notably, the OPA1 cleavage into non-functional short isoforms at a diminished ATP level (collapsed membrane potential) and the DRP1 recruitment upon phosphorylation by various kinases are overviewed. Possible responses of mitodynamins to the oxidative stress, hypoxia, and concomitant mtDNA mutations are also discussed. We hypothesize that the increased GTP formation within the Krebs cycle followed by the GTP export via the ADP/ATP carrier shift the balance between fission and fusion towards fusion by activating the GTPase domain of OPA1 located in the peripheral intermembrane space (PIMS). Since the protein milieu of PIMS is kept at the prevailing oxidized redox potential by the TOM, MIA40 and ALR/Erv1 import-redox trapping system, redox regulations shift the protein environment of PIMS to a more reduced state due to the higher substrate load and increased respiration. A higher cytochrome *c* turnover rate may prevent electron transfer from ALR/Erv1 to cytochrome *c*. Nevertheless, the putative links between the mitodynamin responses, mitochondrial morphology and the changes in the mitochondrial bioenergetics, superoxide production, and hypoxia are yet to be elucidated, including the precise basis for signaling by the mitochondrion-derived vesicles.

Contents

Abbreviations: AMPK, AMP-activated protein kinase; ALR, "augmenter of liver regeneration", a sulfhydryl oxidase; BCL, B cell lymphoma (family); Bi, BCL-inhibitor; CDK, cyclin-dependent kinase; DRP1, dynamin-related protein 1; DNM1, yeast (*S.c.*) DRP1 ortholog; FIS1, fission protein-1; GDAP1, ganglioside-induced differentiation-associated protein 1; GED, GTPase effector domain; GRX, glutaredoxin; GPX, glutathione peroxidase; GST, selenium-independent glutathione-S-transferases; HIF, hypoxia inducible factor; IBM, inner boundary membrane; ICM, intracristae part of inner membrane; ICS, intermembrane space intracristae part (cristae sacks interiors); IM, inner membrane; MAP, matrix targeting sequence motifs; MAPL, an outer membrane-anchored protein ligase; MAVS, an outer membrane antiviral signaling protein; MDVs, mitochondrial derived vesicles; MIB, mitofusin-binding protein; MFF, mitochondrial fission factor; MFN, mitofusin; MICS1, mitochondrial morphology cristae structure (protein) 1; MnSOD, Mn superoxide dismutase; mtDNA, mitochondrial DNA; PIMS, peripheral (part) of intermembrane space; OM, outer membrane; OPA1, optic atrophy (protein) 1; OXPHOS, oxidative phosphorylation; PARL protease, presenilins-associated rhomboid-like protein protease; PHB2, prohibitin 2; PIN1, peptidyl-prolyl-isomerase 1; PKA, protein kinase A; PLD, phospholipase D; PRX, peroxiredoxin; pVHL, von Hippel–Lindau tumor suppressor protein; RIG-I, intracellular receptor for viral RNA; ROS, reactive oxygen species; STOML2, stomatin-like protein 2; SUMO, small ubiquitin-like modifier; TRX, thioredoxin; VDAC, voltage-dependent anion channel.

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1. Introduction—21st century view of mitochondrion

A 21st century integrated view of a mitochondrion is required for understanding relationships between mitochondrial bioenergetics, biogenesis plus morphology, information signaling, and pathogenesis. A traditional, derived from electron microscopy view of mitochondria as isolated kidney-shaped organelles with inner structures of folded sheet-like cristae no longer represents the reality. These solitary shapes are now considered as sections of the mitochondrial tubules (Bereiter-Hahn et al., 2008) and in a typical cell, a mitochondrion comprises a single mitochondrial reticulum network. Thus isolated mitochondria may be considered as a fractionation artefact due to the inevitable presence of vesicles that have been cut and fused from the original mitochondrial network present within the cell.

1.1. A single mitochondrion within the cell?

Based on our observations in cell cultures of the highly oxidatively phosphorylating insulinoma INS-1E cells using three-dimensional (3D) high resolution 4Pi microscopy (Fig. 1A; Plecitá-Hlavatá et al., 2008), we proposed that a single mitochondrion consisting of a continuous mitochondrial reticulum exists in a healthy intact cell in the absence of stress signaling. Further experiments are required to confirm this hypothesis by investigation of the mitochondrial network in intact organs or tissues. The stress responses, energetic variations and retrograde migration in neuronal axons initiate fission, i.e. disintegration of the single mitoreticulum by activating pro-fission protein machinery. Fission never exists without the parallel ongoing fusion, which establishes the dynamic equilibrium determining the instant mitochondrial morphology. Activation of the pro-fusion proteins leads either to reintegration of short tubules back into the single mitochondrion, or under stress signaling and pathological conditions, the formation of rings, red-blood-shape like vessels, or much bulkier flat or spherical cisternae that form after fission (Fig. 1B; Plecitá-Hlavatá et al., 2008). Since both fission and fusion depend on the local GTP gradients and superimposed information signaling, which are both altered under numerous pathogenic conditions, morphology of the mitochondrial network may reflect certain pathologies (Benard et al., 2007; Benard and Rossignol, 2008). Consequently, it may be used as a diagnostic tool, provided that the necessary imaging techniques are developed and distinct causal patterns can be recognized (Koopman et al., 2005; Guillery et al., 2008).

1.2. Unique mitochondrial morphology allows integrated molecular physiology responses

In most of the cell types mitochondria form a reticular network or several such networks with additional solitary small tubular remnants, mostly resulting from fission of the central mitochondrial reticulum (Benard and Rossignol, 2008; Bereiter-Hahn et al., 2008; Herzig and Martinou, 2008; Knott et al., 2008; Plecitá-Hlavatá et al., 2008; Soubannier and McBride, 2009). Electron tomography revealed details of the internal structure (Mannella, 2006; Zick et al., 2009) and established that cristae, visualized previously by 2D electron microscopy, represent sacks protruding deeply into the central matrix space of the mitochondrial tubules. Consequently, the major morphology features of a mitochondrion can be distinguished, such as the outer membrane (OM), topologically contouring the tubular surface; the inner membrane (IM), with its peripheral IM part termed inner boundary membrane (IBM) and intracristae parts (ICM); the intermembrane space, with its peripheral part (PIMS), located between OM and IBM, and the intracristae part (cristae sacks interiors, ICS); finally, the matrix filling the IM-engulfed space (Fig. 1C).

The IM with its enormous surface capacity is the site of oxidative phosphorylation (OXPHOS). The OXPHOS machinery or "chip" is localized predominantly in the ICM (Benard and Rossignol, 2008), and includes mainly the respiratory chain, ATP synthase, and membrane carrier proteins. The respiratory chain Complexes I to IV ensure electron transport from NADH ($FADH_2$) to O_2 by the cytochrome *c* oxidase (Complex IV). As a result of H^+ pumping activity of Complexes I, III, and IV, the proton-motive force Δp ($\Delta p = \Delta\Psi - 60\Delta$pH, in mV at 30 °C, where $\Delta\Psi$ and ΔpH correspond to membrane potential and pH gradient, respectively) is established across the IM, which becomes negatively charged and alkaline surface inside. The Δp is used by the ATP synthase membrane complex (F_O) to drive rotation of the F_1 subunits protruding to the matrix, which induces sequential conformation changes essential for the ATP synthesis. In addition, Δp drives substrate and protein import into the matrix and affects numerous additional reactions including those leading to the inevitable formation of superoxide within the respiratory chain Complexes I and III (Ježek

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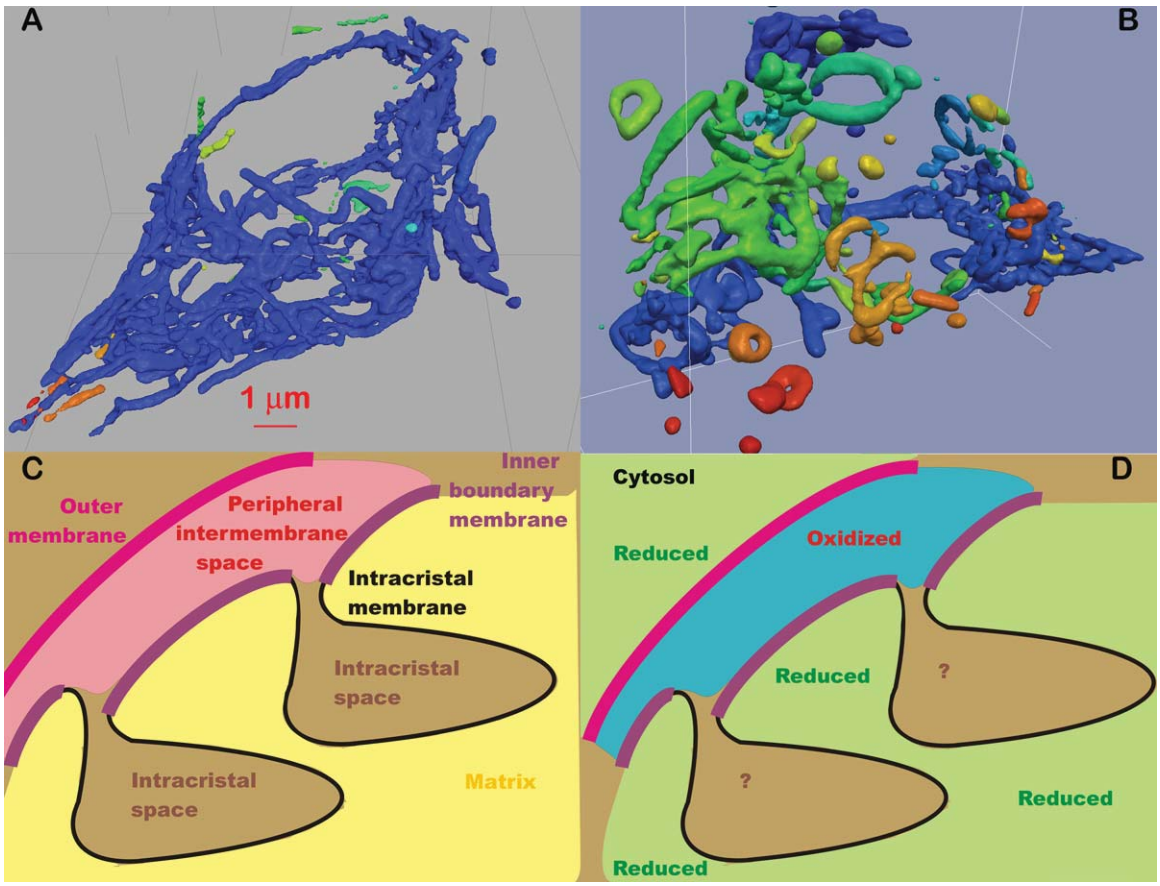

Fig. 1. 4Pi microscopic images of mitochondrial network and schemes of internal compartments. (A) Mitochondrial network in intact INS-1E cells; (B) after uncoupler-induced disintegration, 3D images have been taken as described in Plecitá-Hlavatá et al. (2008) using 4Pi microscopy with fixed INS-1E cells transfected by mitochondria targeted redox-sensitive GFP. The surface rendering was performed using a Paraview software (Sandia Corporation, Kitware Inc., NM, USA) illustrating the connectivity of tubules. Each color represents a different object. For control cells (A) average tubule diameter was 270 nm (Plecitá-Hlavatá et al., 2008). For (B) 1 μM carbonylcyanide-*p*-(trifluoromethoxy)phenylhydrazone was applied. Formation of rings appears to be a hallmark of the resulting uncoupler-induced network disintegration. A fusion proceeds simultaneously or shortly after fission to sew short segments into the rings (probably in concert with cytoskeleton). (C) Distict compartments of a mitochondrion are illustrated using the same color for each object and its description on a schematic drawing of a sectioned tubule of mitochondrion: outer membrane (OM), peripheral intermembrane space (PIMS) and its part within the cristae sacks, the intracristal space (ICS) are recognized, as well as two parts of the inner membrane, the peripheral, i.e. inner boundary membrane (IBM), and the intracristal membrane (ICM), and finally the matrix. Sectioning the tubule, one can view either a unique peripheral sandwich OM–PIMS–IBM-matrix; or below OM one may point to cristae (sack) outlets and ICS, below which ICM and finally matrix layers are recognized. (D) Typical redox sandwich: formed by reduced cytosol, "oxidized" PIMS, and reduced matrix of a mitochondrion. The shift to more oxidized redox potential of the protein milieu in PIMS is due to MIA40–ALR redox trapping system.

and Hlavatá, 2005). The relative isolations of cristae sacks interiors may substantiate a basis for a localized chemiosmosis, whereas IM continuity maintaining equal $\Delta\Psi$ to the whole connected network is acting against it.

Apart from its role in filtering incoming molecules by porin/VDAC channels, the OM represents an external "information keyboard" or a "switchboard", where the integrative response of the BCL-family and other information proteins triggers induction or inhibition of various cellular processes, such as apoptosis, autophagy, and mitoptosis. At the OM, the information responses are also integrated with the activities of the major GTPase proteins, thereafter called mitodynamins, which dynamically affect shape-forming of the mitochondrial reticular network. In this way, mitodynamins specifically participate in cell death processes, whereas the BCL-family proteins BAX and BAK play a role in shaping of the mitochondrial network (Karbowski et al., 2006; Oka et al., 2008; Youle and Karbowski, 2005). The very first study of the heterotrimeric G proteins focused on information signaling involving mitochondria (Andreeva et al., 2008). Thus, upon a single point mutation (Q229L), the Gα12 proteins localized at the OM induce mitochondrial fission, as the mutated proteins are unable to signal to RhoGEFs during the lysophosphatidic acid-induced signaling cascade (Andreeva et al., 2008).

The surrounding cylinders formed by the OM, PIMS, and IBM sandwich around the cristae outlets are termed contact sites. Here the TOM–TIM protein-import complexes span the whole OM–PIMS–IBM sandwich (Bolender et al., 2008). Various import proteins recognize the imported proteins according to the import motifs present within the pre-proteins and mature processed polypeptides. Upon participation of different TOM/TIM subunits, especially those acting as the molecular import engines and chaperones, the imported proteins are released to the OM, PIMS, IM, or matrix. The tail-anchored OM proteins do not require this machinery. Certain tRNAs are imported to the matrix, probably via a specific mechanism for nucleic acid import (Rubio et al., 2008). Upon oxidative stress stimuli, the (human) telomerase reverse transcriptase, hTERT, has been found to be imported into the matrix where its function is still enigmatic (Ahmed et al., 2008). The matrix harbors nucleoids formed by an average of six ds circular mitochondrial DNA copies, accessory proteins, and proteins of mtDNA replication and transcription machinery (Bogenhagen et al., 2008; He et al., 2007; Iborra et al., 2004). The mtDNA encodes 13 essential subunits of the respiratory chain and ATP synthase (Wong, 2007), and represents the most vulnerable entity susceptible to the oxidative stress.

Like the cell cytosol, the matrix contains two major redox buffer systems of glutathione and thioredoxin (Go and Jones, 2008) which

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establish the reduced environment and protect the matrix constituents, such as mtDNA, from the oxidative damage. These two buffering systems counteract the constant release of superoxide into the matrix and its dismutation to H_2O_2 by the matrix Mn superoxide dismutase (MnSOD). As a consequence, an intricate albeit easily destructible redox balance is established in PIMS or ICS which acts as the "inner redox regulatory element". CuZnSOD residing in PIMS/ICS is activated by the formation of the intramolecular S–S bridge (Iñarrea et al., 2007). This enzyme, together with a mutually amplifying action of an import receptor MIA40 and sulfhydryl oxidase ALR ("augmenter of liver regeneration", yeast Erv1), keeps the local surrounding protein milieu oxidized (in spite of reduced glutathione penetration; Hu et al., 2008). MIA40 establishes the S–S bridges within numerous proteins imported to PIMS. This protein is regenerated by ALR/Erv1, and can be either directly oxidized by O_2, releasing H_2O_2, or transfers electrons to cytochrome *c* (Bihlmaier et al., 2007; Hell, 2008; Müller et al., 2008; Lange et al., 2001). As a result, a "redox sandwich" is established, comprising the reduced peri-OM cytosol, oxidized PIMS, and the reduced matrix (Fig. 1D), which allows tight redox regulations dependent on variations of the respiratory electron flow and the superoxide formation within the respiratory chain. Exclusive electron transfer to cytochrome *c* from ALR/Erv1 without generation of H_2O_2 is very important, especially in hypoxia (Bihlmaier et al., 2007; Herrmann et al., 2009). In turn, $p66^{Shc}$ is a typical example of an oxidoreductase dependent on the S–S bridges status of its tetrameric H_2O_2-forming isoform which accepts electrons from cytochrome *c* and promotes apoptosis upon its recruitment to PIMS during the oxidative stress (Gertz et al., 2008). The physically compartmented cytochrome *c* itself might serve for redox regulations considering the heterogeneity of respiratory chain supercomplexes and different functional pools of cytochrome *c* at state 3 (Benard and Rossignol, 2008).

1.3. Mitodynamins

The reader may refer to the other pertinent reviews in this series or published in the past, describing expanding knowledge on mitochondrial dynamins (mitodynamins) including their regulator/interacting proteins, phosphorylation, proteolytic cleavage, and other signaling processes. Here, we review the most important properties of mitodynamins (Table 1). The majority of mitodynamins are large proteins containing the N-terminal GTPase domain and the GTPase effector domain (GED), usually with coiled coil structures proximal to the C-terminus. The GTPase domain provides the mechanical force and probably acts as a molecular switch. Pro-fusion mitodynamins include the mitofusins MFN1 and MFN2 (De Brito and Scorrano, 2008; Hoppins et al., 2007; Zhang and Chan, 2007) and IM-residing long OPA1 isoforms (with GTPase and coiled coil domains exposed to PIMS-ICS; Cipolat et al., 2004, 2006; Frezza et al., 2006; Olichon et al., 2007a,b; Pellegrini and Scorrano, 2007), supported by ring-like prohibitin PHB2 complexes (Merkwirth et al., 2008; Merkwirth and Langer, 2008, 2009). The IM-residing protein MICS1 lacks the GTPase domain and is required for mitochondrial tubular network and cristae organization (Oka et al., 2008). The OM is uniformly coated by yet another non-GTPase mitodynamin, FIS1 (Yu et al., 2005; Lee et al., 2007) which promotes fission. However, the key fission player promoting the tubular scission is the dynamin-related protein, DRP1 (in mammals acting independently of FIS1; Hoppins et al., 2007; Santel and Frank, 2008) which upon its recruitment from the cytosol to the BAX-enriched OM foci is inhibited by MFN2, the latter also sequestered by BAX into these foci (Karbowski et al., 2006). In this way, fission may occur without inducing apoptosis, whereas apoptosis seems to be generally accompanied by fission of the mitochondrial network. The recently discovered mitochondrial fission factor (MFF) possessing conserved motifs for protein binding, the coiled coil domain and transmembrane spanning domain (probably no GTPase domain) acts independently of DRP1 (Gandre-Babbe and van der Bliek, 2008).

Mitofusins MFN1 and MFN2 contain two transmembrane segments anchoring them to OM and exposing larger portion of their U shape to the cytosol (Bach et al., 2003; Cipolat et al., 2004; Chen et al., 2003, 2005; Choi et al., 2006; Detmer and Chan, 2007; Karbowski et al., 2002, 2006; Koshiba et al., 2004). Thus the GTPase and the first coiled-coil domain form one arm, while the second coiled-coil domain provides the other arm. Mitofusins exist as monomers or form homo- or heterodimers. They promote fusion by docking two juxtaposed mitochondrial tubules ~10 nm apart via the second coiled coil domain (Koshiba et al., 2004). An alternative mechanism has been suggested which is based on a hydrophobic paddle formed by the two transmembrane domains (Knott et al., 2008). BAX is essential for the correct assembly of the MFN2 oligomers while MFN2 also interacts with $Bclx_L$ (Delivani et al., 2006). The MFN1 protein has a higher GTPase activity and exhibits higher tethering, i.e. mechano-chemical action, than the 81% homologous MFN2 (Ishihara et al., 2004). In turn, MFN2 has a greater affinity for GTP. GTP binding, but not hydrolysis, is required for the tethering function of MFN2 (Neuspiel et al., 2005). We hypothesized that the higher activity is required for sewing greater size non-tubular objects, whereas lower tethering is sufficient to sew together regular objects (Plecitá-Hlavatá et al., 2008). MFN2 contains the RAS-binding domain (Chen et al., 2004), allowing MFN2 to act as a proliferation suppressor in vascular cells. MFN2 also keeps interaction between mitochondria and their axonal transport machinery and controls mitochondrion-endoplasmic reticulum interactions (De Brito and Scorrano, 2008). The loss of either mitofusin is lethal (Chen et al., 2003). MFN1 seems to act in concert with OPA1 (Cipolat et al., 2004) and is inhibited by the MFN-binding protein (MIB, Eura et al., 2006), whereas MFN2 acts alone (being probably also inhibited by MIB). MIB prevents the MFN1 fusion activity, probably by interacting with GTP bound within the GTPase domain (Eura et al., 2006). MIB is a member of the medium-chain dehydrogenase/reductase superfamily contaning a NADPH binding domain, predicted to bind GTP. MIB in a certain way stabilizes the OM position vs. IM, since its knockdown results in the extension of mitochondrial reticulum tubules and separation of OM to their leading edge during the cell growth arrest (Eura et al., 2006). Stomatin-like protein 2, STOML2, which also associates with mitofusins (Hajek et al., 2007), undergoes processing upon its import via OM and orients towards the PIMS.

OPA1 coordinates IBM fusion in concert with MFN1 which promotes the OM fusion (Cipolat et al., 2004; De Brito and Scorrano, 2008). If OPA1 also mediates the ICM fusion, such process could only happen independently of the distant OM-residing MFN1. Yeast mitochondria deficient of homologous Mgm1 can fuse their OM but not IM (Meeusen et al., 2006). The active long isoform OPA1 resides in IM (IBM) being anchored by its single transmembrane domain. Its GTPase domain, the middle domain, and the GED (tethering and complex forming) domain containing coiled coil at the C-terminus are both exposed to ICS/PIMS. An interaction in trans between the middle and GTPase domains of two OPA1 molecules attracts two IM portions and mediates their fusion (Meeusen et al., 2006).

The OPA1 expression involves an alternative splicing that produces eight human (Delettre et al., 2001) and four mouse mRNA isoforms (Akepati et al., 2008). The translated proteins contain matrix targeting motifs (MAP) preceding transmembrane domains, hence the MAP-containing parts are imported into the matrix. The MAP sequences are cleaved off in the matrix by the mitochondrial processing peptidase and the resulting long isoforms are anchored in the IM. Two such long isoforms 1 and 7 can be found in various human and mouse tissues. Proteolytic cleavage in vivo releases short OPA1 isoforms from the membrane. These isoforms possess

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Table 1
Links between signaling and function of mitochondria-shaping proteins, mitodynamins.

Mitodynamin	Possible link	Consequences	
MFN1	Binding of MIB	Promotes *fission*	
MFN2	RAS binding domain	Decreased Akt phosphorylation	Anti-LDL oxidation
	ERK/JNK pathway	Formation of megamitochondria	i.e. pro-fusion
MIB	NADPH binding domain, if $NADP^+$ does not bind	Binding to GTP-MFN1 NADPH release from MIB: => inhibition of MFN1, i.e. *fission*	NADPH binding to MIB: => protects inhibition of MFN1, i.e. *pro-fusion*
OPA1 L1	Paraplegin (mAAA)-mediated cleavage	Three short isoforms lacking the fusion ability	At low ATP ($\Delta\Psi_m$) => *fission*
	YME1L(iAAA)-mediated cleavage	Short isoforms	At low $\Delta\Psi_m$ => *fission*
	PARL-mediated cleavage	Guarding of cristae outlets	Regulation of cytochrome *c* ICS/PIMS distribution and/or apoptosis
PHB2 prohibitin	Ring like support tor OPA1		i.e. pro-fusion
MICS1	Cristae organization		
mtPLD	Cardiolipin hydrolysis	Promotes trans-membrane adherence	i.e. pro-fusion
DRP1	CDK1/cyclin B-mediated phosphorylation	Self-assembly of DRP1 to circles or spirals	G2, mitosis => i.e. *fission* dephosphorylation = other phases of cell cycle
	Ca^{2+}/calmodulin-dep. PKIα	Link to Ca^{2+} homeostasis	
	PKA-mediated phosphorylation; vs. Ca^{2+} dep. calcineurin-mediated dephosphorylation	Link to Ca^{2+} homeostasis	
	SUMOylation, ubiquitylation	Proteolytically balanced levels	
MTP18	PI3 kinase target	Promotes *fission*	
Endofilin B1		Promotes *fission*	
GDPAP1		Promotes *fission*	
FIS1		Promotes *fission*	
MFF		Promotes *fission*	

a distinct function. So far, PARL (Cipolat et al., 2006; Frezza et al., 2006), i-AAA protease YME1L (Griparic et al., 2007; Song et al., 2007) and m-AAA metalloprotease paraplegin were found to cleave OPA1. Paraplegin cleaves OPA1 to three short isoforms lacking the fusion ability (Duvezin-Caubet et al., 2006; Ishihara et al., 2006). This cleavage is initiated by the ATP level decrease (Baricault et al., 2007), therefore proceeds at a decreased $\Delta\Psi$ (Δp) or upon apoptotic initiation. Nevertheless, the essential structural support for the essential long OPA1 isoform 1 (L1-OPA1) determining its presence in IM is provided by the prohibitin ring-like complexes. L1-OPA1 interaction with prohibitin PHB2 is crucial for the OPA1 pro-fusion activity (Merkwirth et al., 2008; Merkwirth and Langer, 2008, 2009), correct cristae folding and the antiapoptotic role. Probably, prohibitins organize lipid structure of IM and atract the m-AAA metalloprotease through these mutual interactions. Notably, the heterocomplex of two long and one short (PARL-cleaved) OPA1 isoforms (probably supported by prohibitins) guards the cristae outlets as well as upon its disruption induces the release of cytochrome *c*, thus enabling pro-apoptotic signaling (Cipolat et al., 2004, 2006; Frezza et al., 2006; Pellegrini and Scorrano, 2007).

The OM protein MISC, a member of the BCL-inhibitor (Bi) family, also participates in apoptosis initiation by promoting SMAC/DIABLO and cytochrome *c* release (Oka et al., 2008). Mitochondrial phospholipase D is also involved in the OM (IBM) fusion (Choi et al., 2006). Mitochondrial PLD targets to the external face of mitochondria and promotes trans-mitochondrial membrane adherence in a MFN-dependent manner by hydrolyzing cardiolipin of IBM to generate phosphatidic acid. The latter creates locally negatively charged OM, which might facilitate attraction of the positively charged outer leaflet of the energized IBM (negatively charged inner leaflet).

Fission produces solitary small objects or at least several reticula derived from the main mitochondrial reticulum. This process is usually accompanied by cristae remodeling, such as dilatation, vesiculation or even ICM disappearance (Knott et al., 2008; Zick et al., 2009). The tubular scission is executed by a large cytosolic GTPase DRP1, recruited to associate with large localized foci of IM independently of another pro-fission protein FIS1 (Chang and Blackstone, 2007; De Vos et al., 2005; Karbowski et al., 2007; Knott et al., 2008; Wasiak et al., 2007). DRP1 may also indirectly interact with FIS1. After translocation towards mitochondrial tubules, the DRP1 multimers associated into the rings (belts) or spirals wrap around the tubules, constrict them, wrap more tightly around the constricted part and ultimately induce fission (Hoppins et al., 2007). The middle and GED domains mediate assembly of the DRP1 proteins into the multimeric complexes (De Brito and Scorrano, 2008; Hoppins et al., 2007). Mutations preventing GTP binding to the yeast homolog Dnm1 prevent spiral formation and hence fission (Smirnova et al., 2001; Naylor et al., 2006). FIS1 in mammals is also essential for fission, probably acting downstream of DRP1 recruitment (Wasiak et al., 2007). The FIS1 protein is spread evenly on the OM surface and contains a tetratricopeptide (TPR) domain involved in protein-protein interactions (Jofuku et al., 2005; Karren et al., 2005; Lee et al., 2007; Suzuki et al., 2003; Yu et al., 2005).

To date, three types of post-translational modifications were found to regulate the DRP1 activity: phosphorylation, SUMOylation, and ubiquitylation. Recruitment of DRP1 was found to be initiated by the mitosis promoting factor, by the cyclin-dependent kinase 1 (CDK1)/cyclin B-dependent phosphorylation of Ser^{585} during mitosis in rat (Taguchi et al., 2007); by Ca^{2+}/calmodulin-dependent protein kinase Iα phosphorylating Ser^{630} in cultured rat neurons (Han et al., 2008); and by CDK5 (Meuer et al., 2007). In turn, DRP1 recruitment was inactivated by the protein kinase A (PKA) in quiescent cells phosphorylating Ser^{637} of the human variable domain (Chang and Blackstone, 2007) or Ser^{656} of the rat GED domain (Cribbs and Strack, 2007). When these PKA-sites were dephosphorylated by the Ca^{2+}-dependent phosphatase calcineurin, DRP1 recruitment was reestablished followed by fission (Cribbs and Strack, 2007). Note that these latter findings together with those of Han et al. (2008) have demonstrated for the first time a connection between Ca^{2+} signaling and mitochondrial network morphology. The FIS1 and DRP1 levels are also proteolytically balanced by E3 ubiquitin ligase MARCH-V (RING-CH-V) (Karbowski et al., 2007) followed by the subsequent action of proteasome system and by SUMOylation (Wasiak et al., 2007). SUMO (small ubiquitin-like modifier) is a small protein, which, after conjuga-

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tion to target proteins, usually changes their conformation and prolongs their half-life. A PIMS/ICS protein MTP18 anchored to IM which is a target of PI3 kinase (Tondera et al., 2004), an OM fatty acyl transferase termed endofilin B1 (Karbowski et al., 2004), and ganglioside-induced GDAP1 (Niemann et al., 2005) participates downstream of DRP1 and promote mitochondrial fission.

1.4. Mitochondrial superoxide sources and the inevitable vicious cycle

Senescence is given by a "tax for life", by which the mitochondrial superoxide ($O_2^{-\bullet}$) production, the inevitable byproduct of cell respiration, is constitutively produced at low level in this organelle. Superoxide is not reactive per se, but its conjugated acid, arising in ~1% from $O_2^{-\bullet}$, the hydroperoxyl radical ($HO_2^{\bullet}$) attacks constantly mitochondrial DNA, proteins, and initiates lipid peroxidation (De Grey, 2002; Ježek and Hlavatá, 2005). Under oxidative or xenobiotic stress conditions, the $O_2^{-\bullet}$ dismutation product H_2O_2 can subsequently react with free iron and other transient metals and form the most reactive hydroxyl radical $^{\bullet}OH$, which evokes additional mtDNA damage, protein modifications and lipid peroxidation in the very proximal sites. These damages are accumulated over the years. Oxidized mtDNA bases, such as 8-oxo-G and FapyG (the two most frequent ones), are formed with the frequency of 100–500 per day and in spite of the mtDNA repair mechanisms, a pool ends up as mtDNA mutations (Hu et al., 2005). This is due to the activity of the mitochondrial DNA polymerase (polγ) which inserts A instead of G with the frequency of 27%. In addition, since 8-oxo-G mispairs with A in the syn conformation, the oxidative modifications of mtDNA lead to G:C → A:T transversal mutations. The resulting mtDNA mutations accumulate until overcoming a threshold (% heteroplasmy). Mutations in the tRNA and rRNA mt genes (mtDNA encodes for 2 rRNAs, 22 tRNAs) cause the overall impairment of mtDNA expression, while mutations in the mitochondria-coded protein subunits (Wong, 2007) are even more deleterious, leading to diseases and cell death. These13 proteins of the OXPHOS machinery subunits include 7 subunits of respiratory chain Complex I, ND1 to ND6 and ND4L; cyt b, i.e. subunit of Complex III, 3 subunits of Complex IV, i.e. cytochrome *c* oxidase, COX1, 2, 3; as well as the ATP synthase subunits 6 and 8 (Wong, 2007). Accumulation of either inherited mutations (carried over through the purifying bottleneck effect, see Section 4.3) or those superimposed due to the mtDNA oxidative modifications over a certain threshold is indeed lethal, as proven by numerous mitopathies (Wong, 2007; Zhadanov et al., 2007).

Generally, a slowdown of electron transport (with simultaneous essential suppression of H^+ pumping activity by Complex I due to either high Δp or mtDNA mutations) leads to the elevated $O_2^{-\bullet}$ production at Complexes I and III and the oxidative stress (Dlasková et al., 2008; Ježek and Hlavatá, 2005). Consequently, dysfunctional mutations, particularly those of the H^+-pumping subunits (ND2, ND4, ND5, and probably nearly all NDs) and cyt b, further increase $O_2^{-\bullet}$ formation. This effect is also enhanced in the non-phosphorylating state 4, when the dysfunctional mutations of ATPase subunit 6 and 8 additionally lead to the elevated $O_2^{-\bullet}$ formation. When $O_2^{-\bullet}$ production exceeds its dismutation of this radical and redox buffer capacities, the oxidative stress is established. Excessive formation of reactive oxygen species (ROS) leads to the so-called vicious cycle (Dlasková et al., 2008), a self-accelerating oxidative stress leading to even more intensive oxidative damage, as the additional mtDNA mutations accumulate within the respective subunit-coding regions of mtDNA (or within tRNA and rRNA regions affecting expression of all mt genes). Hence, the cycle evolves continuously and provides the basis for etiology of diseases resulting from the oxidative stress or from inherited mtDNA mutations. The mitochondrial network dynamics are likely to be affected by this vicious cycle due to the disruption of redox homeostasis. Moreover, the impairment of the redox homeostasis specifically affects mtDNA–protein clusters, the so-called mtDNA nucleoids, and their distribution within the mitochondrial network, which, among other factors, affects mtDNA heteroplasmy in the daughter cells upon cell division. When enriching for mutant mtDNA, pathogenetic development is self-accelerated once again.

1.5. Mitochondrial redox buffer systems and hypothetical basic redox regulations

The major redox buffer system maintaining reduced matrix is provided by glutathione (GSH) accounting for 10–15% of the total glutathione in liver (García-Ruiz et al., 1994), but processing only ~15% of the total H_2O_2 production rate (Cadenas, 2004), thus allowing H_2O_2 to diffuse out of the mitochondrial matrix. GSH must be imported into mitochondria by the oxoglutarate (Coll et al., 2003) and dicarboxylate carriers (Lash et al., 2002). Matrix NADPH reduces impermeant GSSG to GSH via glutathione reductase. NADPH is regenerated either by Δp-driven transhydrogenase (Jackson, 2003), or by the $NADP^+$-dependent isocitrate dehydrogenase and the malic enzyme in the matrix, which also indirectly dissipates Δp. It is known that GSH interacts with the BH3 groove of BCL2, hence it combines an antioxidant and anti-apoptotic function (Zimmermann et al., 2007). The mitochondrial GSH peroxidases GPX1 and GPX4, the matrix glutaredoxin 2 (GRX2) together with PIMS-located GRX1 (Pai et al., 2007), and selenium-independent glutathione-S-transferases (GST, Yang et al., 2003) represent other branches of the system protecting mitochondria against oxidative stress (Go and Jones, 2008; Koehler et al., 2006). GRX2 catalyzes reversible oxidation and glutathionylation of membrane proteins. The GSTs catalyze GSH-dependent reduction of phospholipid hydroperoxides, fatty acid hydroperoxides, and their respective aldehydes.

The second redox buffer system is sustained by thioredoxin TRX2 (Tanaka et al., 2001, 2002) and it represents a thiol-specific antioxidant that reduces disulfide bridges of proteins subjected to the oxidative stress. While glutathione forms inter-molecular disulphides, thioredoxin produces mostly intramolecular S–S bridges and has different substrate preferences. Thioredoxin's active site of Trp-Cys-Gly-Pro-Cys-Lys is maintained in the reduced form by electron transfer from NADPH catalyzed by thioredoxin reductase-2 (TRXR2). Other, selenium- and heme-free thioredoxin-dependent peroxide reductases, peroxiredoxins PRX3 and PRX5 (Cao et al., 2007; Wood et al., 2003), reduce H_2O_2, fatty acid hydroperoxides, and peroxynitrite.

In contrast, the protein milieu of PIMS (ICS?), is kept at the oxidized redox potential by the import receptor MIA40 regenerated by the sulfhydryl oxidase ALR (yeast Erv1; Bihlmaier et al., 2007; Hell, 2008; Herrmann et al., 2009; Müller et al., 2008). After TOM-mediated import, MIA40 forms S–S bridges on proteins with twin Cys-X_3-Cys segments juxtaposed in antiparallel α helices. In this way a mature form of a hairpin-like structure is formed which aggregates into hetero-hexamers, such as those of yeast *S. cerevisiae* (*S.c.*) Tim8, Tim 9, Tim10, Tim12, and Tim13 proteins. Moreover, the proteins harboring twin Cys-X_9-Cys motif are imported, including copper chaperone Cox17, Complex IV assembly proteins Cox19 and Cox23, Cox 12 (Cys-X_9-Cys-Cys-X_{10}-Cys), a copper donor Cox11 and Cu binding protein Sco1 (Cys-X_3-Cys). Some proteins lacking the above twin motif may also be imported, such as the Rieske FeS protein and the subunit 8 (hinge protein) of Complex III (Hell, 2008). All these proteins establish mature S–S bridges. Certain proteins recruited into PIMS (such as $p66^{Shc}$, see Section 3.3.3) and out of PIMS (hypothetically CIDE proteins, Valoušková et al., 2008) might be imported by the TOM–MIA40–ALR system and sequestered in

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the PIMS with S–S intramolecular bridges or as the S–S stabilized oligomers. Finally, PIMS/ICS CuZnSOD may be a subject of MIA40 and ALR, forming assembled S–S connected homodimer, requiring a copper chaperone Ccs1 (*S.c.*) with two disulfide bonds, one being in the Cys-X_2-Cys motif. In liver, thioredoxin-1-mediated formation of these S–S bridges was found to activate the enzyme (Iñarrea et al., 2007). In both cases, it strengthens the "oxidized state" of PIMS providing H_2O_2 locally.

The "oxidized" PIMS which establishes a "redox sandwich" (Fig. 1D) is not only beneficial for the S–S bridges formation in a specific subset of proteins. We hypothesize that due to the various extent of electron transfer from ALR to cytochrome *c* this system also allows for the putative: (i) sensing of the respiration rate via electron transfer to cytochrome *c* (Fig. 2), (ii) sensing ROS released into PIMS/ICS, (iii) retrograde signaling upon reduction of the S–S bridges in multimeric signaling proteins such as CIDE multimers (Ježek et al., unpublished) that are subsequently exported for signaling to cytosol or nucleus in the reduced monomeric forms. The export from PIMS would be expected under conditions of the reduced redox potential, such as during decreasing respiration or decreased $O_2^{-\bullet}$ production (see below), hence it could be initiated during starvation or hypoxia, provided that the relatively high (albeit decreased) $O_2^{-\bullet}$ production does not reestablish the S–S bridges. These aspects extend our original hypothesis (Ježek and Hlavatá, 2005) which predicted influence of cytosol and extracellular space due to diffusion of H_2O_2 and residual $O_2^{-\bullet}$ at elevated matrix- and PIMS-(ICS)-released $O_2^{-\bullet}$.

The increased turnover of cytochrome *c* between Complexes III and IV due to an unobstructed high substrate load, i.e. under high respiration conditions, may prevent ALR/Erv1 from donating the electrons to the oxidized cytochrome *c* (Fig. 2B). This would partially prevent MIA40-mediated S–S bridges formation, however it does not stop the direct reaction of ALR/Erv1 with O_2. Hence, the phenomenon would dominantly occur at hypoxia. In contrast, at a low or intermediate substrate flux and/or respiration, a larger cytochrome *c* fraction can accept electrons from ALR/Erv1, leading to re-establishment of the S–S bridges. Since until saturation, $O_2^{-\bullet}$ production at "equal retardation at given redox centers" is directly proportional to the respiration rate (Dlasková et al., 2008), ROS seem to indirectly indicate mitochondrial respiration or substrate load under these conditions (Jones, 2006), by oxidizing redox buffers and promoting S–S bridge formation in PIMS. Under the conditions of the retarded electron flow or inhibition of Complex I proton pumping activity, i.e. when high $O_2^{-\bullet}$ formation is induced at inhibited respiration (Dlasková et al., 2008), low cytochrome *c* turnover promotes the MIA40-mediated S–S bridges formation. During saturated respiration with a high substrate load, relatively high Complex I $O_2^{-\bullet}$ formation exists (Dlasková et al., 2008), which would induce the formation of S–S bridges. However, a still high cytochrome *c* turnover would act against it.

2. Mitodynamin responses to mitochondrial energetics

Since the vast majority of mitodynamins are GTPases (except of FIS1 and MISC) morphology of the mitochondrial network may change depending on the local GTP gradients. Generally, the GTP levels are proportional to the ATP levels or the ATP/ADP level ratios, since GTP is formed from ATP by matrix and cytosolic nucleoside diphosphate kinases. Independently, the succinyl-CoA-synthetase of the Krebs cycle produces GTP in the matrix (Bridger et al., 1987). Locally, the GTP levels may be decreased by the GTP-binding proteins, which is cell-wide negligible when compared to the robust mitochondrial energetics. Consequently, mitochondrial morphology is determined by the mitochondrial energetics (for the opposite relationships see Section 4).

During fission, DRP1 is recruited towards the OM proximity depending on the local cellular energetics and information signaling. Subsequently, the local cytosolic GTP concentrations in the close OM perimeter govern fission. With already multimerized tubule-encircling DRP1 a dysbalance between fusion/fission cannot be governed by these GTP levels, since the pro-fusion mitodynamins are equally affected (apart from influence of the BCL protein family). However, as the long OPA1 isoforms sense elevation of the local PIMS/ICS GTP levels, the OPA1-mediated fusion may dominate (Fig. 3), whereas the GTP decrease in PIMS/ICS may suppress this fusion and the overall fission would prevail. Further insight may be provided by studying fission inhibitors, such as Mdivi-1 which bind to the allosteric site of DRP1 and its yeast (*S.c.*) ortholog DNM1, a site which blocks or retards conformational changes required for self-assembly and GTP hydrolysis (Cassidy-Stone et al., 2008).

2.1. OPA1 functions as a GTPase within the inner membrane

OPA1-mediated sensing of GTP in PIMS may play a central role in a fusion/fission dysbalance. Hypothetically, the PIMS GTP may be elevated at a higher turnover rate of the Krebs cycle vs. ATP synthesis rate. During higher respiration the IBM-located ADP/ATP carrier would export GTP^{4-}, exchanging it for ADP^{3-} or GDP^{3-} (Fig. 3A). This would be possible under a higher substrate load (e.g. in high glucose conditions) but not during uncoupling, i.e. when GTP^{4-} export is no longer driven by $\Delta\Psi_m$. Another type of variation of the PIMS GTP levels lies in an elevated or suppressed turnover of the IBM-located ADP/ATP carrier. The driving force for the carrier is the $\Delta\Psi$ (Klingenberg, 2008), hence diminishing of $\Delta\Psi$ by lowering substrate supply (including inhibition of respiration), its collapse by opening of numerous IM-residing K^+ channels, its decrease/collapse by uncoupling, etc., all lead to the GTP decrease in PIMS and establishment of fusion/fission dysbalance in favor of fission (Fig. 3B) as observed (see De Vos et al., 2005). At the same time, overall decrease in peri-mitochondrial ATP and hence in GTP does not disturb the fusion/fission dysbalance, but ATP (transferred into GTP) produced in glycolysis may still drive the DRP1 recruitment.

2.2. Responses of the outer membrane GTPases

The OM GTPases of the two pro-fusion (MFN1, MFN2) and one pro-fission (DRP1) mitodynamins sense the equal local GTP concentrations, hence the dysbalance of mitochondrial dynamics should not occur upon GTP level variations. At higher OXPHOS, elevated GTP would be also produced by the activated cytosolic nucleoside diphosphate kinase, which could lead to preferred DRP1 recruitment, its multimerization, and ultimately fission. Information signaling provides yet another level of regulation, such as DRP1 phosphorylation/dephosphorylation control over the DRP1 recruitment (see above). MFN2- (but not MFN1-) related signaling was reported to modulate metabolism through function of respiratory chain Complexes I, and IV and ATP synthase (Bach et al., 2003; Pich et al., 2005). However, to gain a complete insight into this signaling further studies are required.

3. Mitodynamin responses to oxidative stress and redox regulations

To date, it is still debatable whether the mitochondrial network (cristae) morphology changes upon oxidative stress and whether, in turn, morphology changes may influence mitochondrial production of superoxide (or other ROS) or impair redox buffer systems. Some evidence suggests that the oxidative stress initiates fission in cerebellar granule neurons, while such fission is prevented by the MFN2 overexpression (Jahani-Asl et al., 2007). Other reports demonstrate

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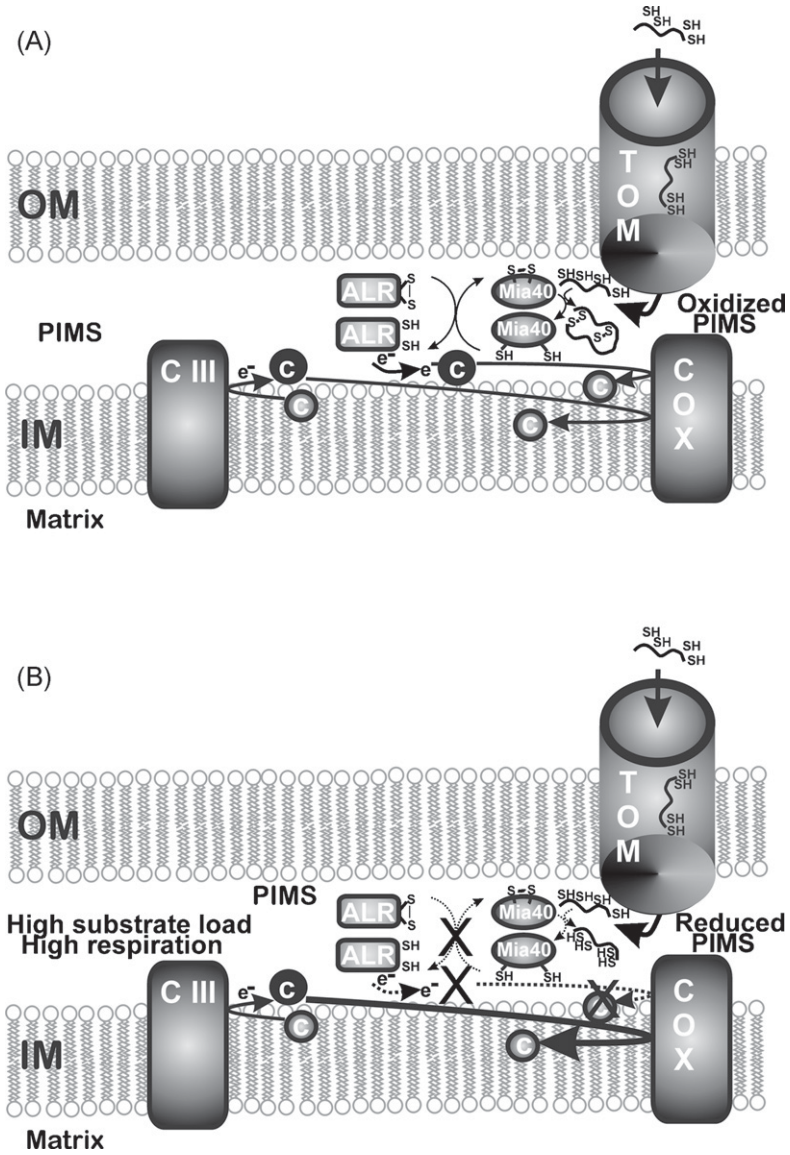

Fig. 2. Redox regulations by shifting protein milieu of PIMS to more reduced redox potential: (A) at intermediate respiration and/or substrate load, an unobstructed function of the TOM–MIA40–ALR/Erv import-redox trapping system leads to the prevailing oxidized redox potential of PIMS (which may be amplified by CuZnSOD activation by intermolecular S–S bridge formation). MIA40 is regenerated as depicted by ARL donating electron to the cytochrome *c* ("c") which becomes reduced (black color) and is oxidized by cytochrome oxidase ("COX"). Following intermediate respiration there is still a sufficient cytochrome *c* pool that can be used to reduce PIMS. (B) At high respiration and high substrate load, an increased cytochrome *c* turnover between Complex III ("CIII") and COX (depicted by a thick arrow) does not allow the ARL to merge, slowing down import and the subsequent S–S bridge formation. Consequently, PIMS shifts to a more reduced environment. This may provide an initiating impulse for putative retrograde signaling by the reduced protein monomers, post-translational modifications, etc.

that the oxidative stress leads to fission upon induction with NO (Barsoum et al., 2006) and 6-hydroxydopamine (Gomez-Lazaro et al., 2008). In addition, the DRP1-mediated fission accompanies respiration increase and ROS production induced by a high level of glucose (Yu et al., 2006, 2008a). The cells exposed to extracellular or intracellular ROS were shown to have impaired morphology of mitochondrial reticulum, depending on the load of ROS and duration of the treatment (Jendrach et al., 2008). Using a non-apoptotic concentration of H_2O_2 in a transient treatment, original long interconnected mitochondrial tubules were transiently shortened and weakly fragmented (Jendrach et al., 2008), implying a signaling role for such a transient ROS burst. The re-establishment of the tubular phenotype involved upregulation of MFN1 and DRP1, hence an increase in mitochondrial dynamics as well as formation of new mitochondria (assessed from an increase in PGC-1α mRNA levels). However, the precise mechanism of the described morphology changes is largely unknown.

3.1. Hypothetical redox regulations of mitodynamins sensing the intermembrane space

Short sequence segments of either MFN1 or MFN2 protruding to PIMS are unlikely to contain redox-response elements. Hence, speculations on redox regulations of OPA1 within the PIMS or ICS are more plausible. Due to the prevailing oxidized redox potential one would expect the initiating impulse to be given by a shift to the more reduced environment (Fig. 2). This may slowdown import and the subsequent S–S bridge formation of proteins directed to PIMS (ICS), possibly including protein kinases and phosphatases. Such a shift could be produced by NADH/NADPH accumulation in the PIMS (ICS), reduction of the intramolecular S–S bridge within the residing CuZnSOD, due to the slowndown of sulfhydryl reductase activity of ALR, and high cytochrome *c* turnover, as described above. Downregulation or inhibition of MIA40–ALR may also halt the recruitment of alternative electron acceptors, such as $p66^{Shc}$,

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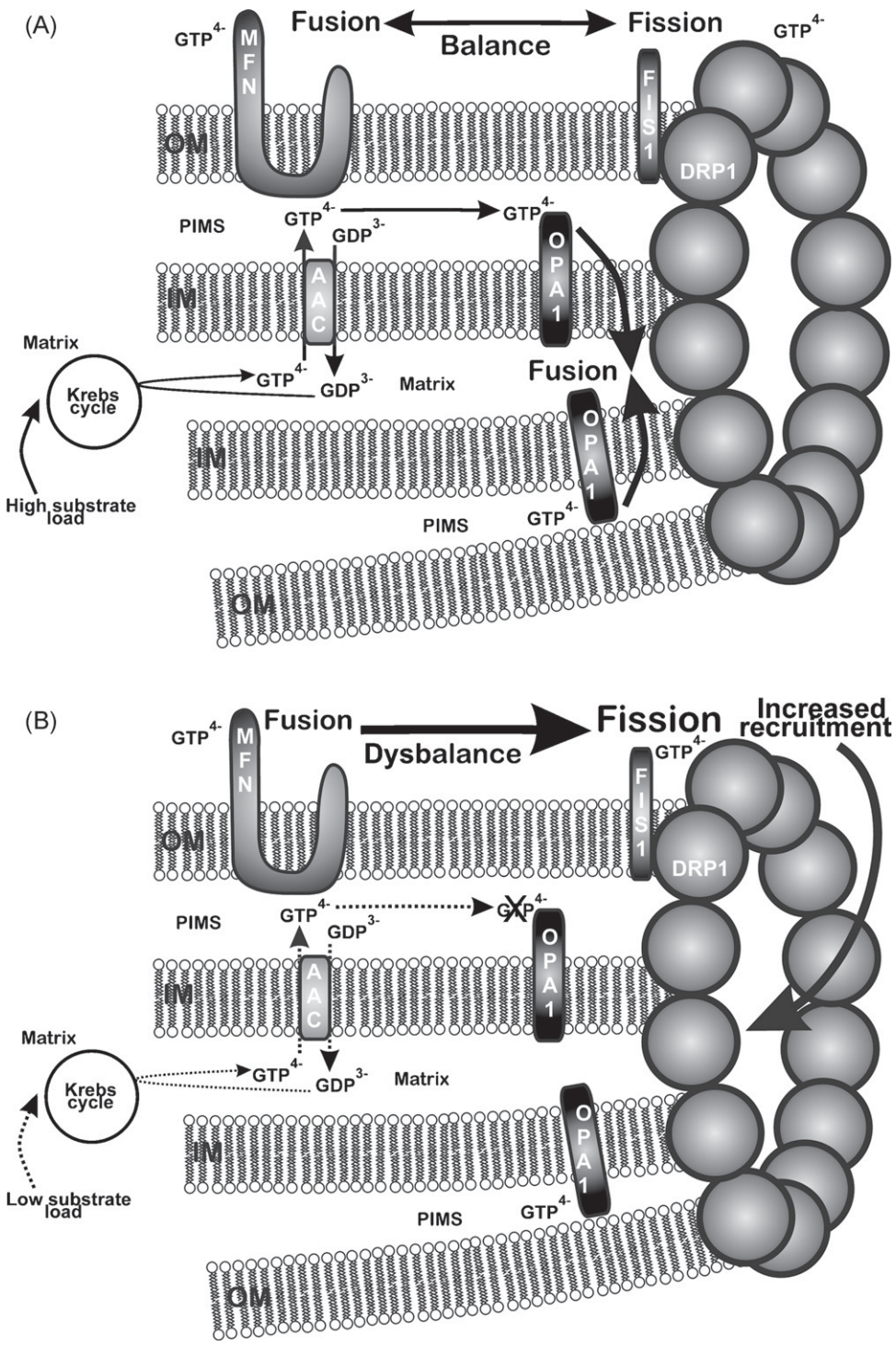

Fig. 3. Fusion/fission dysbalance shifts by variations of a GTP level in PIMS. (A) Hypothetical induction of OPA1-mediated fusion after increased GTP formation in Krebs cycle and/or increased export by the ADP/ATP carrier – with already multimerized tubule-encircling DRP1 a dysbalance between fusion/fission cannot be governed by the GTP levels at the OM perimeter, since pro-fusion OM mitodynamins MFN1 and MFN2 are equally affected. However, the long OPA1 isoforms sense elevation of the local PIMS/ICS GTP levels, hence OPA1-mediated fusion may dominate, when GTP export is allowed by the ADP/ATP carrier (AAC). The increased GTP export may result from the increased GTP formation within the Krebs cycle at its high turnover due to a higher respiration and substrate load. In turn, GTP decrease in PIMS/ICS may suppress this fusion and the overall fission would prevail. (B) Hypothetical pro-fission mechanism upon decreased GTP formation in the Krebs cycle and/or GTP export by the ADP/ATP carrier – decrease in the Krebs cycle turnover and respiration, e.g. by lowering substrate supply, at hypoxia, etc., or independent $\Delta\Psi$ decrease (diminishing GTP export to PIMS) may establish fusion/fission dysbalance in favor of fission. Concomitant decrease in peri-mitochondrial ATP and hence GTP does not disturb this fusion/fission dysbalance, but ATP (transfered into GTP) from glycolysis may still drive the DRP1 recruitment.

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stop the concomitant H_2O_2 formation and shift the oxidized redox potential of PIMS toward more reduced.

3.2. Mitodynamins sensing oxidative stress and redox regulations at the perimeter of the outer membrane

To date, there are no reports demonstrating the direct redox regulation of the OM-residing mitodynamins. It is tempting to speculate that MIB, being a member of the medium-chain dehydrogenase/reductase superfamily contaning a NADPH binding domain (Eura et al., 2006), might be involved in certain redox regulations, providing that this domain would specifically respond to reduced nucleotides. Speculatively, NADPH release from the MIB may trigger its interaction and inhibit MFN1 (and MFN2), thus promoting fission. Interference with information signaling was manifested by the ability of MFN2 to bind RAS and hence, to decrease Akt phosphorylation in vascular smooth muscle cells (Chen et al., 2004). The ROS-dependent formation of megamitochondria might involve the ERK–JNK pathway (De Brito and Scorrano, 2008). Mitochondrial DRP1-mediated fission was involved in high glucose-induced respiration and elevation of the superoxide production (Yu et al., 2006, 2008a). Thus upregulation of pro-fusion proteins may be one of the counterbalancing responses against the oxidative stress. It remains to be elucidated, whether ROS-responding pathways, such as ERK-JNK, are directly involved. Overfused megamitochondria, such as observed after the MFN2 overexpression, were reported to originate from ROS overload (De Brito and Scorrano, 2008). Alternatively, they may form following the suppressed autophagy and do not fuse with the main mitorecticulum due to the lower OPA1 content (Navratil et al., 2008).

3.3. Integrating redox regulations

3.3.1. Does the fragmented mitochondrial reticulum produce more ROS?

Yu et al. (2006) did not correlate the observed fission during a high glucose-induced transient increase in the presence of ROS with the ROS production itself, but suggested that the disintegrated mitoreticulum is unable to increase ROS proportionally to the high pyruvate load from glucose metabolism. Our experiments have demonstrated similar pyruvate-induced fission within the INS-1E cells (Plecitá-Hlavatá, unpublished data). Indeed, the DRP1 inactivation inhibited pyruvate uptake into the matrix with a concomitant respiratory burst accompanied by the elevated ROS production (Yu et al., 2006). This might originate from much higher density of the pyruvate dehydrogenase complex, ATP synthase, and respiratory chain complexes within ICM (in cristae sacks) compared to IBM, provided that the newly formed small fragments of mitoreticulum would contain only the IBM but no cristae sacks. Alternatively, information signaling derived from glucose metabolism might be involved in this process via phosphorylation control of DRP1.

3.3.2. Mitochondrial ROS burst as oxidative signaling to block the cell cycle

Besides the AMP-dependent protein kinase activation, an elevated mitochondrial ROS level may serve as a signal to block the cell cycle progression, as recently demonstrated in Drosophila (Owusu-Ansah et al., 2008). On the other hand, the transition of mitochondrial network morphology to the fragmented type is inherent to the G2 phase of the cell cycle and mitosis (Taguchi et al., 2007). CDK/cyclin B phosphorylation at Ser-585 of DRP1 is involved in this process, leading to the enhanced interaction with the other pro-fission proteins or DRP1 itself, ultimately promoting self-assembly of DRP1 to circles or spirals. Another type of ROS-mediated signaling occurs during the defense against viral infection. The OM antiviral signaling protein, MAVS, acts in a pathway of the intracellular receptor for viral RNA, RIG-I, by activating the NF-κB and IRF3 signaling pathways in response to the viral infection (Xu et al., 2005). Another OM protein, NLRX1, interacts with MAVS inhibits the antiviral response (Moore et al., 2008), possibly via stimulation of the mitochondrial ROS production, in a similar manner as during NLRX1 overexpression (Tattoli et al., 2008). One may speculate that a distinct outcome of either overcoming or surrendering to viral infection of the cell will be reflected by its mitochondrial network or cristae morphology.

3.3.3. $p66^{Shc}$ function

Usually ~20% of the $p66^{Shc}$ protein is found in PIMS/ICS of mammalian fibroblasts (Orsini et al., 2006). Its further recruitment sensitizes cells to apoptotic stimuli (Pinton and Rizutto, 2008). Oxidative stress activates PKCβ which then directly phosphorylates cytosolic $p66^{Shc}$ leading to its conformational changes catalyzed by peptidyl-prolyl-isomerase-1 (PIN1, Pinton et al., 2007). The isomerized $p66^{Shc}$ protein is subsequently imported into mitochondrial PIMS or ICS, probably using MIA40–ALR, where it serves as an oxidoreductase accepting electrons directly from cytochrome *c* and relaying them to O_2 whilst generating H_2O_2 (Giorgio et al., 2005). The oxidoreductase function of $p66^{Shc}$ is activated by tetramerization, involving a disulfide bond formation (Gertz et al., 2008). In turn, GSH and thioredoxins reduce these S–S bridges and inactivate $p66^{Shc}$. Thus, a self-accelerating vicious cycle is induced in this pathway upon a high oxidative stress overcoming redox state of GSH and thioredoxins in PIMS. The role of $p66^{Shc}$ is clearly pointed out by its ablation in KO mice. These mice exhibit an extended lifespan probably due to the lack of the $p66^{Shc}$-induced oxidative stress and/or apoptosis (Migliaccio et al., 1999). Apoptosis induction due to $p66^{Shc}$ redistribution to PIMS/ICS eventually leads to mitochondrial fragmentation (Giorgio et al., 2005; Pinton et al., 2007; Pinton and Rizutto, 2008). Moreover, protein redistribution between ICS and PIMS may play a significant role physiologically, not only under apoptotic conditions. Thus heterotrimers of OPA1, when opening cristae outlets, may allow for relocation of cytochrome *c* from ICS to PIMS. The DRP1-induced fission and cristae remodeling may also redistribute cytochrome *c* from ICS to PIMS, resulting in a higher content of $p66^{Shc}$ and increased H_2O_2 production (De Brito and Scorrano, 2008).

4. Mitochondrial dynamics and morphology in the context of mtDNA damage and related dysfunctions

4.1. Mitochondrial network dynamics as a prerequisite for intact mtDNA expression and maintenance

The processes of fusion and fission maintain the integrity of a mitochondrion, IM electrical connectivity and metabolic and biochemical connectivity of all the mitochondrial compartments including matrix. However, the most important are two counteracting aspects. On the one hand, there is a possibility of segregation of mutated mtDNA upon fission (Barsoum et al., 2006; Knott et al., 2008; Twig et al., 2008a,b). On the other hand, full accessibility is beneficial, i.e. when the entire matrix compartment of a single mitochondrial reticulum is accessible to products of mtDNA replication and transcription, mitochondrial ribosomes, enzymes of mtDNA maintenance, transcription and translation machinery, etc. Pro-fusion mitodynamins ensure spreading of the new mt DNA nucleoids (hypothetically arising from mtDNA replication) to the entire network and allow common access of mitochondrial ribosomes to the mtDNA transcripts. Fusion also allows access to all the ICM locations for 13 translated subunits of OXPHOS machinery. These subunits require a coordinated assembly with

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OM attached nuclear ribosomes at contact sites which provide the nuclear encoded subunits. When such accessibility is missing, pathological states are established. Indeed, the loss of mtDNA and lack of the general ability to respire together with largely eliminated mitochondrial fusion were found in the diseases, such as Parkinson's, Charcot–Marie Tooth Type 2A (mutations in MFN2), and autosomal dominant optic atrophy (mutations of OPA1; Chen et al., 2003, 2005; Davies et al., 2007). Also, MFN2-deficient Purkinje cells exhibited greater number of mitochondria lacking mtDNA (Chen et al., 2007). Mutations in the OPA1 GTPase domain were found to increase breaks and truncations of mtDNA (Amati-Bonneau et al., 2008; Hudson et al., 2008). In contrast, unlike in neurons, mtDNA molecules containing deletions accumulate during aging in the dividing rat cells. They replicate more rapidly and spread due to the regular mitochondrial fusion (Diaz et al., 2002; Kowald et al., 2005).

4.2. Mitochondrial energetic dependence on mitochondrial network morphology

The OPA1 cleavage and subsequent fission together with mitochondrial de-energization was found in the cells which contained the mutated genes of the mitochondrial transcription factor A, TFAM, and mitochondrial DNA polymerase, i.e. polγ (Duvezin-Caubet et al., 2006). Fission frequently results in impaired respiration, especially in the mitodynamin-linked diseases (Benard et al., 2007; Cartoni et al., 2005; Chen et al., 2003, 2005; Davies et al., 2007; Pich et al., 2005). The mitochondrial bioenergetics in the DRP1-depleted cells is largely impaired (Benard et al., 2007), however, the detailed mechanism remains to be elucidated. Hypothetically, the respiratory deficiencies due to the insufficient mixing of mtDNA and its expression products (the 13 "VIP" subunits), may be a primary cause. The classic observations of the "orthodox" vs. "condensed" state of mitochondrial cristae have been recently elucidated, uncovering the machinery of proteins involved in the ICM shaping (Mannella, 2006; Zick et al., 2009).

4.3. Benefits of mitochondrial DNA segregation

The segregation of mtDNA at heteroplasmy to decrease the mutant mtDNA seems to be an attractive hypothesis (Barsoum et al., 2006; Knott et al., 2008; Twig et al., 2008a,b). Such a segregation occurs during the so-called bottleneck effect (Cree et al., 2008; Stewart et al., 2008a,b), in which the mtDNA replication/transcription/repair machinery is likely to be involved. The mitochondrial genetic bottleneck refers to the process in which the number of 200 000 mtDNA copies existing in the human egg is reduced to ~200 mtDNA copies in primordial cells from which the future egg develops during the early female embryogenesis. These 200 mtDNA copies are, however, highly "purified", most likely by elimination of non-synonymous changes in the protein-coding genes (Stewart et al., 2008a,b). Due to the essentially maternal mtDNA inheritance and the bottleneck effect during early oogenesis, the newly arising mitochondrial DNA (mtDNA) mutations segregate rapidly in metazoan female germlines.

Hypothetically, mtDNA could be divided with the help of a yet unknown mechanism of mutation recognition into two pools, one with the intact DNA for preservation and for transfer to daughter cells upon cell division, and the second one with the mutant mtDNA. This process might be mediated by capturing mtDNA nucleoids into small isolated objects disintegrated from the main mitochondrial reticulum. The isolated mitochondria bearing the mutated mtDNA would be marked for mtDNA degradation. In addition, mtDNA replication could be accelerated to ensure the intactness of the mtDNA pool, thus allowing enumeration of "healthy" nucleoids which would spread by fusion to the continuous reticulum.

5. Mitodynamin responses to transition between hyperoxia, normoxia, and hypoxia

5.1. Cell survival/adaptation responses to hypoxia and resulting bioenergetics

Atmospheric conditions (20.9% O_2; pO_2 pressure of 167 mmHg) under which cells are cultured are in fact hyperoxic for most of tissues (but lung), exposed to pO_2 of 1–90 mmHg (Brahimi-Horn and Pouysségur, 2007). Especially, tumor cells possess extensive regions of low O_2, with limited diffusion originating from the rapid cell proliferation and from the distorted/irregular vascular system (Vaupel, 2004). Under hypoxia a failure of either delivery or use of O_2 limits normal tissue function. "Physiological hypoxia" refers to pO_2 suppression (down to 10–30 mmHg), which after pO_2 restoration back to normoxic conditions maintains the cells in a non-lethal state and confers mainly a regulatory/signaling function (such as in fetal stage). In turn, for adaptation and survival, cells respond to hypoxia by metabolic adjustments and/or gene expression pattern changes mediated by the AMP-activated protein kinase (AMPK) pathway and the hypoxia inducible factor (HIF). AMPK, as a sensor of AMP: ATP ratio, responds to an immediate ATP shortage resulting from O_2 dropping and promotes catabolic processes while inhibiting anabolic metabolism. AMPK especially up-regulates glucose energy metabolism and transcriptional activity of HIF. The HIF pathway involves stabilization and activation of transcriptional factor HIF at the pO_2 below the level of 5% atmospheric O_2. HIF is a heterodimeric DNA-binding complex composed of hypoxia-inducible HIF1/2α and constitutively expressed HIF1β subunits (Semenza, 2003; Wenger et al., 2005). The HIFα with the O_2-dependent degradation domain forms a regulatory part required for the O_2-dependent stability. Under normoxic conditions HIF interacts with the von Hippel–Lindau tumor suppressor protein (pVHL) upon hydroxylation of two prolyl residues, and is then recognized by ubiquitin machinery and targeted to the proteasome. Hydroxylation is conducted by the prolyl hydroxylase domain enzymes (PHD1-3) that utilize O_2 and 2-oxoglutarate as co-substrates and iron (Fe^{II}) and ascorbate as co-factors. Hypoxia, however, impedes this hydroxylation and thus stabilizes the HIF protein while inducing expression of proteins involved in providing the continuous O_2 supply (iron transport, angiogenesis, erythropoiesis and blood flow), cellular metabolism (glycolysis, glucose uptake, pH regulation, and xenobiotic metabolism), transcription, cell survival (pro- as well as antiapoptotic proteins, cell cycle, DNA repair), cell adhesion and motility and O_2 sensing (Kaluz et al., 2008). The Krebs cycle intermediates (pyruvate and oxalacetate) act as competitive inhibitors of PHDs and HIF1 whilst inducing HIF system (Dalgard et al., 2004). HIF is also known to suppress the mitochondrial metabolism in hypoxic cells, thus modulating the reciprocal relationship between glycolysis and OXPHOS (Kim et al., 2006; Papandreou et al., 2006; Seagroves et al., 2001; Yu et al., 2008b).

5.2. Consequences of hypoxia for mitochondrial morphology and dynamics

To date, mitochondrial dynamics under hypoxic conditions has not been studied. We may only predict that changes in bioenergetics and redox regulations upon hypoxia should be accompanied by morphological adjustments reflecting the physiological state even under physiological hypoxia or pathogenesis progression at hypoxia. The factors having impact on mitochondrial network integrity might be primarily GTP formation in the Krebs cycle and its export to PIMS (Fig. 3), Δp ($\Delta\Psi_m$), energy supply, respiration rate, accompanied ROS production, etc. All these factors are impaired under hypoxia. In addition, an energy shortage might

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cause fission either due to the OPA1 cleavage induced by dropping $\Delta\Psi_m$ (Baricault et al., 2007; Duvezin-Caubet et al., 2006; Griparic et al., 2007; Ishihara et al., 2006; Song et al., 2007) or the GTP level decrease in PIMS (Fig. 3B). $\Delta\Psi_m$ was found to be depolarized during the long term (several hours) hypoxia (Chandel et al., 1997), hence we may expect fission to occur, which was indeed observed (Plecitá-Hlavatá, unpublished results). This fission could be accompanied by fusion, as shown for chemical depolarization of mitochondria by an uncoupler (Plecitá-Hlavatá et al., 2008). However, the direct regulation of mitodynamins in the HIF pathway or hypoxic conditions has not been fully revealed as yet.

6. Possible signaling via mitochondrial dynamics

6.1. Small fission-cleaved mitochondria as hypothetical signaling devices

Recently, raft-like microdomains were reported to exist in the mitochondrial membranes following the CD95/FAS triggering (Garofalo et al., 2007). The mitochondrion appears as a dynamic and sub-compartmentalized organelle in which microdomains might act as controllers of apoptosis-associated fission that results in the release of apoptogenic factors (Skulachev et al., 2004). Rafts may naturally occur in IM due to its high content of proteins that divide the continual lipid membrane into patches. It has been hypothesized that some "small" mitochondria, or the mitochondrial-derived vesicles (MDVs) may form a new signaling platform (Soubannier and McBride, 2009). Hypothetically, MDVs derived from a fission process of the original highly connected network might reach the nuclear envelope and tightly interact with this membrane. MDVs could act as a signaling "device" which contributes to the molecular trafficking of molecules, namely pro-apoptotic proteins, and raft-like components, during apoptosis. Exemplar evidence for MDVs of 70–100 nm size was reported by Neuspiel et al. (2008), who described a DRP1-independent fission (budding) into vesicles containing the OM-anchored protein ligase (MAPL) free of TOM20. These MDVs were composed of either one or both IM and OM, and were free of cristae and underwent fusion with peroxisomes, demonstrating a cross-talk between the organelles. Speculatively, MDVs could act as capturing mitochondrial DNA nucleoids marked for mtDNA degradation.

6.2. Mitoptosis

When MDV or, in general, a part of mitochondrial reticulum is degraded in the absence of cell dead, one can hypothesize a concept of mitochondrial death, termed *mitoptosis* (Lyamzaev et al., 2008). Also autophagic delivery to lysosomes, termed *mitophagy*, seems to be the major degradative pathway in mitochondrial turnover (Kim et al., 2007; Van der Vaart et al., 2008). Although long assumed to be a random process, increasing evidence indicates that mitophagy is a selective process (Kim et al., 2007). Recently, mitoptotic elimination of malfunctioning mitochondria has been observed, e.g. in highly glycolyzing HeLa cells surviving after inhibition of respiration and uncoupling for 2–4 days (Lyamzaev et al., 2008). The survival was accompanied by selective elimination of mitochondria in the following steps, including (i) fission of mitochondrial filaments, (ii) clustering of the resulting roundish mitochondria in the perinuclear area, (iii) occlusion of mitochondrial clusters by a membrane, (iv) decomposition of mitochondria inside this body to small membrane vesicles, (v) protrusion of the body from the cell, and (vi) disruption of the body boundary membrane. Tinari et al. (2007) hypothesized that in T cells two main pathways of mitoptosis could occur: an inner membrane mitoptosis, in which only the internal matrix and cristae are lost while the external mitochondrial envelope remains unaltered, and an outer membrane mitoptosis, where only swollen internal cristae are detected as remnants. It remains to be established, whether mitoptosis indeed exists in vivo and contributes to physiological phenomena or pathophysiology, namely whether it can degrade mitochondrial DNA.

7. Future perspectives

Further detailed knowledge on the mitochondria shaping proteins is to be gathered as well as concerning their regulation. Microscopic studies will be required that reach the highest possible 3D space resolution on the one hand, and the time resolution on the other hand. Electron or light microscopic tomography should separately define the 3D topology of OM, cristae (IBM plus ICM), and matrix in relation to the instant proteomics within these compartments. The almost unknown universe of mtDNA must be studied as well as in connection to the mitochondrial dynamics. The acquired knowledge should be projected to the studies of pathological situations in which impaired mitochondrial function takes place.

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