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Review

MAM (mitochondria-associated membranes) in mammalian cells: Lipids and beyond

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ABSTRACT

One mechanism by which communication between the endoplasmic reticulum (ER) and mitochondria is achieved is by close juxtaposition between these organelles via mitochondria-associated membranes (MAM). The MAM consist of a region of the ER that is enriched in several lipid biosynthetic enzyme activities and becomes reversibly tethered to mitochondria. Specific proteins are localized, sometimes transiently, in the MAM. Several of these proteins have been implicated in tethering the MAM to mitochondria. In mammalian cells, formation of these contact sites between MAM and mitochondria appears to be required for key cellular events including the transport of calcium from the ER to mitochondria, the import of phosphatidylserine into mitochondria from the ER for decarboxylation to phosphatidylethanolamine, the formation of autophagosomes, regulation of the morphology, dynamics and functions of mitochondria, and cell survival. This review focuses on the functions proposed for MAM in mediating these events in mammalian cells. In light of the apparent involvement of MAM in multiple fundamental cellular processes, recent studies indicate that impaired contact between MAM and mitochondria might underlie the pathology of several human neurodegenerative diseases, including Alzheimer's disease. Moreover, MAM has been implicated in modulating glucose homeostasis and insulin resistance, as well as in some viral infections.

1. Introduction

The normal functioning and survival of eukaryotic cells depend on compartmentalization of metabolic processes within specific subcellular organelles. This compartmentalization is achieved by restriction of proteins involved in these processes to distinct intracellular organelles. In addition to the spatial separation of metabolic processes in defined organelles, further metabolic compartmentalization occurs *within* organelles. For example, many proteins are non-uniformly distributed throughout the endoplasmic reticulum (ER). Consequently, the rough ER, which is bound to ribosomes, is morphologically and functionally distinct from the smooth ER which lacks ribosomes. In addition, the "transitional" ER packages proteins for export to the Golgi and subsequently for secretion, whereas another domain of the ER is involved in ER-associated protein degradation, and a different region of the ER is contiguous with the nuclear envelope. Thus, the ER consists of multiple domains that execute specific functions. In mitochondria, metabolic processes are also segregated to distinct sub-compartments so that the outer and inner membranes of mitochondria have distinct lipid and protein compositions and perform different functions. For example, proteins utilized for ATP production via the electron transport chain and oxidative phosphorylation are restricted to mitochondrial inner membranes. Despite this extensive segregation of metabolic processes to and within specific intracellular organelles, inter-organelle communication between the ER and mitochondria is crucial for processes such as lipid synthesis and transport, mitochondrial functions, the regulation of calcium homeostasis and apoptosis. Experimental evidence indicates that one mechanism by which inter-organelle communication is achieved is by close apposition, or transient contact, between membranes of different types of organelle. This review will focus on the membrane contacts that are formed between the ER and mitochondria in mammalian cells. The mechanisms by which these zones of contact participate in key cellular processes such as lipid synthesis, calcium homeostasis, mitochondrial function and survival will be discussed.

2. Discovery of ER–mitochondria contact sites and mitochondria-associated membranes (MAM)

Over the past 4 decades, numerous ultrastructural/electron microscopic studies have detected regions of close apposition between the ER and mitochondria [1,2]. However, since these studies provided only

Abbreviations: APP, amyloid precursor protein; CHO, Chinese hamster ovary; DRP, dynamin-related protein; ER, endoplasmic reticulum; MAM, mitochondria-associated membranes; MFN, mitofusin; mTORC, mammalian target of rapamycin complex; PACS, phosphofurin acidic cluster protein; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PSD, phosphatidylserine decarboxylase

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morphological data, the reports were frequently dismissed as being artefactual. Nevertheless, subsequent subcellular fractionation studies on rat liver using differential centrifugation have revealed that, indeed, mitochondria can be isolated that are tightly associated with elements of the ER [3–8]. In the course of studies designed to evaluate a potential role for ER–mitochondria contacts in the import of phospholipids from the ER into mitochondria (Section 3.2) a protocol was developed for isolation of a novel population of ER-like membranes that co-isolated with mitochondria from rat liver [9–11] and Chinese hamster ovary (CHO) cells [12]. These membranes are now frequently called mitochondria-associated membranes or "MAM". During this procedure, mitochondria were pelleted by the traditional method of centrifugation of a cellular homogenate at 10,300 ×*g* for 10 min, after which the crude mitochondria were centrifuged on a Percoll gradient for 30 min at 95,000 ×*g*, thereby separating the MAM from highly purified mitochondria. The MAM were collected from the Percoll gradient and pelleted by centrifugation for 1 h at 100,000 ×*g*. Slight modifications of this procedure for isolation of MAM have been reported [13].

The MAM exhibit many, but not all, characteristics of the ER, including glucose-6-phosphate phosphatase activity and high activities of several lipid-synthesizing enzymes. However, the specific activity of a typical ER marker enzyme, NADPH:cytochrome c reductase, in the MAM was only ~30% of that in the bulk of the ER [9,11]. Marker proteins for other organelles such as mitochondria, lysosomes, plasma membrane and Golgi, were present at very low abundance in the MAM [9,12]. Compared to the bulk of the ER, mammalian MAM are several-fold enriched in certain lipid biosynthetic enzyme activities such as acyl-CoA:cholesterol acyltransferase and diacylglycerol acyltransferase [11,14] as well as phosphatidylserine (PS) synthase [15]. The MAM also contain secretory proteins such as apolipoprotein B [11]. Moreover, electron microscopy of hepatoma cells has revealed that lipid droplets lie in close proximity to both mitochondria and elements of the ER [16]. These observations suggest that MAM might participate in the assembly/secretion of hepatic lipoproteins and/or the formation of lipid storage droplets.

Identification of marker proteins that reliably distinguish MAM from the bulk of the ER and other membranes has been a challenge (Table 1). Serendipitously, however, phosphatidylethanolamine-*N*-methyltransferase-2, was identified in rodent liver and primary hepatocytes as a specific marker protein for MAM. Immunoblotting revealed that this protein is highly enriched in MAM but is essentially undetectable in the bulk of ER or mitochondria [17]. However, this methyltransferase appears to be expressed at significant levels only in liver and primary hepatocytes [18,19]. Thus, use of this protein as a marker of MAM in other cell types, including hepatoma cell lines [20], is unreliable. Table 1 indicates several mammalian proteins that are not highly specific for, but are enriched in, MAM compared to the bulk of the ER and other organelles and have been used as markers of MAM including: the inositol-1,4,5-*tris*-phosphate receptor [21], calnexin [22], phosphofurin acidic cluster protein (PACS2) [23], sigma receptor-1 [24], the signaling kinase Akt [25], long-chain fatty acyl-CoA synthetase-4 [26], p66shc [13], acyl-CoA:cholesterol acyltransferase-1 [11], diacylglycerol acyltransferase-2 [14] and phosphatidylserine synthase-1 and -2 [15]. A detailed proteomic analysis of MAM isolated from mouse brain has recently been reported [27]. However, most of the proteins that are present in MAM are not restricted to MAM but are also detected in various amounts in the bulk of the ER and/or other organelles.

MAM have now been identified in species ranging from yeast [28,29] to mammals [23,30–32]. In addition, contact sites between the ER and chloroplasts have been implicated in lipid trafficking in plants (reviewed in [33]). Three-dimensional reconstructions of rat liver cells analyzed by electron microscopy tomography showed that mitochondria are frequently bordered by ER membranes [34,35]. Indeed, as much as 20% of the total mitochondrial network in HeLa cells was estimated to be in close apposition to the ER [31]. Debate has continued about whether or not the ER that is associated with mitochondria contains ribosomes [2,4,6,25,27]. High resolution electron microscopy studies have shown that the distance between the smooth ER and mitochondria is 9–16 nm at the contact points, whereas between the rough ER and mitochondria the separation is 19–30 nm [36,37]. These dimensions are consistent with the idea that a protein bridge tethers the two apposing membranes [34]. Although the association between the ER and mitochondria is quite stable, the two organelles can be separated by incubation of crude mitochondria with detergent, a high salt concentration or by limited proteolysis [36,38]. Thus, the close linkage between the ER/MAM and mitochondria is reversible and does not involve membrane fusion. Live cell imaging has revealed that the ER–mitochondria contact sites are constantly in flux and that the ER and mitochondria remain tethered during the movement of mitochondria along the cytoskeleton [39]. Even when the integrity of microtubules is compromised, the close association between the ER and mitochondria persists [40]. An interesting question arising is whether or not the mitochondria that associate with the MAM have properties that are distinct from those of mitochondria that are not associated with the MAM.

Table 1
Proteins enriched in MAM of mammalian cells.
(abbreviations are defined in the text).

ABCC6	[188]
Acyl-CoA:cholesterol acyltransferase	[11,76]
Akt protein kinase	[25]
Autophagy protein, ATG14	[133]
Calnexin	[22,88]
Cytomegalovirus protein	[87,91,184]
Diacylglycerol acyltransferase	[11,14]
Dynamin-related protein, DRP1	[93,107]
HIV protein R	[185]
Inositol *tris*-phosphate receptor, IP3R	[21,31]
Long-chain acyl-CoA synthetase-4	[26]
Mammalian target of rapamycin mTORC	[25]
Mitofusin MNF2	[105]
Oxidoreductase Ero1	[94]
Phosphofurin acidic cluster protein, PACS-2	[23]
Phosphatidylethanolamine-*N*-methyltransferase	[17]
Phosphatidylserine synthase-1 and -2	[15]
Sigma-1 receptor	[24]
Stimulator of interferon genes STING	[186]
Thioredoxin-related oxidoreductase, TMX	[22,88]
Voltage-dependent anion channel, VDAC1	[95]

3. Involvement of MAM in lipid transport

3.1. Mechanisms of inter-organelle lipid trafficking

Major alterations in the membrane content of most lipids cannot be tolerated by cells or organelles. Consequently, the lipid composition of organelle membranes is tightly regulated. The synthesis of the majority of membrane phospholipids in eukaryotic cells occurs on ER membranes and these lipids are subsequently distributed to other organelles by mechanisms that, for the most part, have remained elusive (reviewed in [41,42]). Indeed, far more is understood about the molecular mechanisms that govern inter-organelle protein transport than about lipid trafficking. Thus, a fundamental question in cell biology is: how do hydrophobic lipid molecules move between organelles through an aqueous milieu (i.e. the cytosol)? The spontaneous diffusion of phospholipids between organelles through the cytosol is an energetically unfavorable process and therefore occurs very slowly, if at all. Lipids can, presumably, be transported between organelles by the well-characterized vesicle-mediated processes that are used for protein trafficking, but mitochondria are not known to be connected to the ER by vesicular trafficking pathways. It is now becoming apparent, however, that non-vesicular mechanisms are quantitatively important for the trafficking of lipids between organelles. Although the cytosol contains several so-called