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Review

The endoplasmic reticulum–mitochondria connection: One touch, multiple functions ☆

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ABSTRACT

The endoplasmic reticulum (ER) and mitochondria are tubular organelles with a characteristic "network structure" that facilitates the formation of interorganellar connections. The ER and mitochondria join together at multiple contact sites to form specific domains, termed mitochondria-ER associated membranes (MAMs), with distinct biochemical properties and a characteristic set of proteins. The functions of these two organelles are coordinated and executed at the ER–mitochondria interface, which provides a platform for the regulation of different processes. The roles played by the ER–mitochondria interface range from the coordination of calcium transfer to the regulation of mitochondrial fission and inflammasome formation as well as the provision of membranes for autophagy. The novel and unconventional processes that occur at the ER–mitochondria interface demonstrate its multifunctional and intrinsically dynamic nature. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction

The definition of the word *reticulum*, "a net-like formation or structure; a network", clearly describes the endoplasmic reticulum (ER), the largest of the membrane-bound organelles within the cell [1]. The ER can connect to and consequently act synergistically with other membranous structures. Among the various intracellular organelles that interact with the ER, which include the Golgi apparatus, mitochondria, peroxisomes, endosomes and lysosomes, the mitochondria has one of the most extensively studied and well-characterized connections with the ER. The first observation of their physical interaction more than 50 years ago [2] suggested that these two organelles might share regulatory factors and, notably, that their functions might be performed or regulated mutually. At the molecular level, proteins residing in different organelles can interact and facilitate the formation of multi-organellar domains with new properties and functions. The distance between the ER and mitochondria was originally estimated to be approximately 100 nm [3], but later, high speed digital imaging microscopy [4] and electron tomography [5] studies suggested that it was even smaller, approximately 10 to 25 nm. The close proximity of the ER and the outer mitochondrial membrane (OMM) further explains how proteins situated on the opposing membrane faces could interact and thus "tether" the two organelles [6]. However, although the ER and mitochondrial membranes form specific contact sites, they do not fuse, maintaining the organelles' distinct structures.

Today, biochemical techniques allow us to isolate the ER–mitochondria contact sites, also known as mitochondria-associated ER membranes (MAMs) [7–10] (for a detailed isolation protocol, see [11]). The optimized method is based on seminal work by J.E. Vance, who described the isolation of a particular fraction ("fraction X") with many similarities to microsomes but which sediments with the mitochondria upon centrifugation [12]. Vance speculated that a subfraction of the ER could be associated with the mitochondria. More recently, this hypothesis has been corroborated by several studies demonstrating the importance of MAMs in lipid synthesis and trafficking [13,14]. Mitochondria require a continuous and coordinated supply of membrane lipids to carry out their physiological processes and maintain their membrane integrity. The transport of phospholipids between membranes of the ER and mitochondria involves MAMs, although the mammalian macromolecular complexes responsible for directing lipid exchange have not yet been elucidated (for reviews, see [10,15]). Identification of the various proteins that reside within the MAMs might help to advance this field. Recently, two different proteomic studies of MAMs isolated from cells [16] and the mouse brain [17] each identified approximately 1000 "MAM proteins" (991 and 1212 proteins, respectively), but only 44% of these overlapped (i.e., were found in both studies), likely due to the different cellular

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sources used and to the difficulty of obtaining pure MAM preparations. However, all of the proteins identified are involved in relevant biological pathways, such as calcium (Ca^{2+}) handling and inflammasome formation. In this review, we discuss the role played by ER–mitochondria contacts in the regulation of four different processes: mitochondrial fission, Ca^{2+} transfer, autophagy and inflammation. These important, and in some respects novel and unconventional, processes that have been characterized at the ER–mitochondria interface indirectly reveal its multifunctional and intrinsically dynamic nature.

2. Regulation of mitochondrial fission

The first direct evidence that the ER and mitochondria are sufficiently close to mediate a synapse-like interaction in living cells was obtained using a GFP-based approach [4]. This idea was greatly strengthened in a seminal paper from the Voeltz research group [18]. This study analyzed the three-dimensional structure of ER–mitochondria contacts in *Saccharomyces cerevisiae* using electron microscopy and tomography, revealing that ER tubules were associated with mitochondria and might mediate the formation of mitochondrial constriction sites [18]. A reduction in mitochondrial diameter was observed at sites in which the ER was almost completely wrapped around the mitochondrial membrane (from ~210 nm for uncircumscribed mitochondria to ~140 nm for circumscribed mitochondria) [18]. Thus, the mitochondria appeared to be constricted at the point of contact with the ER, pointing to a crucial role for the ER–mitochondria association in the initiation of mitochondrial fission. The presence of ER tubules at sites of mitochondrial contraction and fission has been confirmed using a two-color STORM super-resolution approach [19]. This technique also allows the visualization of the actively extending ER network; the newly formed tubules appear to be thinner than pre-existing tubules [19].

Mitochondria are dynamic organelles, continuously undergoing fusion and fission. These opposing processes maintain the shape, size and number of mitochondria as well as their physiological functionality [20,21]. Dynamin-related protein (Drp1; also termed Dlp1) localizes primarily to the cytoplasm and is recruited to the mitochondria to regulate mitochondrial fission [22]. Drp1 associates with the OMM, where it forms multimeric ring-like structures or oligomers that wrap around the constricted portions of the mitochondria [23]. Drp1 possesses GTPase activity, and its hydrolysis of GTP causes a conformational change in the oligomer that bisects the membrane and leads to a fission event. The ability to form multimeric spirals is a general property of dynamin family members [24], and the self-assembly of Dnm1 (the Drp1 yeast ortholog) oligomers also drives the constriction of mitochondria during mitochondrial division [25]. Although the molecular machinery responsible for the recruitment of Dnm1 to the mitochondria has been thoroughly described in yeast, the mechanistic roles of many fission-related proteins in mammals remain obscure. In yeast, both fission protein 1 (Fis1) and mitochondrial division protein 1 (Mdv1) target Dnm1 to the mitochondrial membrane, promoting its oligomerization and the subsequent fission event [26,27]. There is no mammalian ortholog of Mdv1, and the role of human Fis1 (hFis1) has not been fully elucidated (reviewed in [22]); for example, the introduction of hFis1 into fis1Δ yeast cells is unable to rescue the mutant phenotype [28], suggesting that the two proteins are structurally divergent or act through different mechanisms. Drp1 is anchored to the mitochondria through its interaction with mitochondrial fusion factor (Mff) [29,30] or mitochondrial dynamics 51 (MiD51) [31], but the MiD51 present in the Drp1–MiD51 complex has also been proposed to play an inhibitory role by blocking the GTPase-dependent fission activity of Drp1 and promoting fusion [32]. However, in both yeast and mammalian cells, Dnm1/Drp1 localizes to the site at which ER tubules circumscribe the mitochondrial membrane, and the ER–mitochondria interaction is fully independent of the mitochondrial fission machinery [18]. In fact, the ER remains able to wrap around the mitochondrial tubules even when Drp1 or Mff is down-regulated [18], indicating that ER–mitochondria contact represents a conserved platform for the regulation of mitochondrial division. A recent paper published in *Science* proposed a potential mechanism for ER association-induced mitochondrial fission, involving actin polymerization and the ER-localized protein inverted formin 2 (INF2) [33]. This model is based on two major observations: i) INF2 siRNA significantly elongates the mitochondria, and ii) actin filaments seem to aggregate between the mitochondria and INF2-enriched ER membranes at fission loci [33]. Thus, at the mitochondria–ER contact site, INF2 is activated to polymerize actin, which in turn might generate the driving force for initial mitochondrial constriction (Fig. 1).

A growing body of evidence now supports a role for ER–mitochondria interactions in mitochondrial fission, and mitochondrial fusion is emerging as a process that could also be influenced by ER contact. Mitochondrial fusion is mainly orchestrated by mitofusins 1 (MFN1) and 2 (MFN2). Both proteins localize predominantly to the OMM, but while MFN1 plays a critical role in mitochondrial docking and fusion, MFN2 coordinates the interactions between mitochondria, leading to the stabilization of the whole mitochondrial network [34]. Notably, MFN2 expression is crucial for tethering the ER to the mitochondria and stabilizing MAM formation. MFN2 localizes not only to the mitochondria but also to the ER and MAMs, forming both homo- and heterotypic interactions with mitochondrial MFN2 and MFN1 [35]. MFN2 activity at the ER–mitochondria interface is regulated by a mitochondrial ubiquitin ligase called MITOL [36]. MITOL interacts with mitochondrial MFN2 but not with ER-localized MFN2, mediating the addition of lysine 63-linked polyubiquitin chains to MFN2 but not its proteasomal degradation. This polyubiquitination event induces MFN2 oligomerization, a fundamental step in MFN2-induced ER–mitochondria tethering [36]. Thus, MITOL regulates MAM formation by enhancing MFN2 activity, as also illustrated by the reduction in ER Ca^{2+} transfer that occurs in MITOL-deficient cells. The observation of dual roles for MFN2 in both mitochondrial fusion and ER–mitochondria tethering suggests that the establishment of ER–mitochondria contact might be a critical event in MFN2-dependent mitochondrial fusion.

Overall, these findings indicate that the ER–mitochondria connection plays a fundamental role in the regulation of mitochondrial dynamics. In particular, the wrapping of ER tubules around constricted mitochondria is now accepted to be one of the key early events in the mitochondrial fission process (Fig. 1).

3. Ca^{2+}-transfer

The rapid and large accumulation of Ca^{2+} in the mitochondrial matrix is one of the main features of the relationship between the mitochondria and this cation. Mitochondria in living cells undergo rapid and dramatic increases in their matrix Ca^{2+} levels, reaching peak levels that are one or two orders of magnitude higher than those observed in the cytoplasm [37,38]. The presence of a gated Ca^{2+}-selective ion channel in the inner mitochondrial membrane [39,40] allows Ca^{2+} to enter the mitochondrial matrix. The molecular identity of this channel has now been clarified [41,42], and the protein, previously known as CCDC109A, has been re-named MCU (mitochondrial calcium uniporter). The principal properties of the Ca^{2+} transporter are its sensitivity to Ruthenium Red and low affinity for Ca^{2+} (K_D of 20–50 μM) [43–45]. The latter characteristic inevitably raises the question of how a low-affinity Ca^{2+} system can ensure a high mitochondrial Ca^{2+} concentration ($[Ca^{2+}]$). The answer to this question has been found in ER–mitochondria contact sites. Microdomains of high $[Ca^{2+}]$ (>10 μM) can form transiently in regions of close apposition between the mitochondria and the Ca^{2+} channels of the ER/SR (sarcoplasmic reticulum) or of the plasma membrane [37]. The ER has been identified as the major Ca^{2+} storage unit inside the cell [46], with a steady-state $[Ca^{2+}]$ of approximately 1 mM, which is close to concentrations in the extracellular milieu, and significant heterogeneity in Ca^{2+} levels among its different regions [47]. At resting state, cytosolic $[Ca^{2+}]$ is

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