CELL BIOLOGY

Mediating ER-mitochondrial cross-talk

An ER-mitochondrial tethering protein regulates mitochondrial calcium uptake in neurons

By **Alyssa A. Lombardi** *and* **John W. Elrod**

Cellular function and survival require the highly ordered subcellular localization of organelles. In particular, there is considerable interest in understanding the interconnectedness of the endoplasmic reticulum (ER) and mitochondria. The direct interaction of ER and mitochondrial membranes is referred to as tethering, typically defined as an interorganelle distance of less than 30 nm. Tethering has been implicated in numerous physiological processes, including lipid synthesis and transfer, coupling of Ca^{2+} transfer, autophagosome formation, inflammatory signaling, mitochondrial morphology, and mitochondrial DNA (mtDNA) synthesis and distribution (*1*–*5*). Although the functional relevance of ER-mitochondrial tethering is widely accepted, identification of the proteins that constitute these interconnected structures has remained elusive. On page 623 of this issue, Hirabayashi *et al.* (*6*) show that PDZ domain containing protein 8 (PDZD8) is required for the tethering of ER and mitochondrial membranes and is critical for Ca^{2+} transfer from ER to mitochondria.

> ***"We now have the first description of a protein that appears to primarily function as a member of an ER-mitochondrial tethering complex..."***

The most well-defined ER-mitochondrial tethering complex is the yeast ER-mitochondria encounter structure (ERMES). The ERMES complex is composed of the ER-membrane–bound protein maintenance of mitochondrial morphology protein 1 (Mmm1), the cytosolic linker mitochondrial distribution and morphology protein 12 (Mdm12), and the outer mitochondrial membrane proteins Mdm34 and Mdm10. To date, no ERMES functional ortholog has been identified in metazoans (multicellular organisms). Using bioinformatics and structural modeling approaches, Hirabayashi *et al.* identified PDZD8 as a mammalian protein likely to contain an SMP (synaptotagmin-like mitochondrial lipid binding protein) domain, a functional feature synonymous with yeast ERMES proteins. Through a series of elegant experiments, they demonstrated that PDZD8 is an ortholog of the yeast ERMES complex protein Mmm1. *MMM1*-deficient yeast cells exhibit a dramatic phenotype attributed to loss of ER-mitochondrial contacts, including collapse of the mitochondrial network, inheritance defects, and complete loss of mtDNA (*7*). Expression of a yeast-*MMM1*–mouse-PDZD8 chimeric protein in *MMM1*-null yeast rescued the phenotype. These experiments prove that the SMP domain from mammalian PDZD8 is functionally equivalent to members of the yeast ERMES complex.

Next, they showed that PDZD8 in mammalian cell lines localized to ER membranes at sites in close proximity to mitochondria (see the figure). Further, the authors showed that mammalian cells lacking PDZD8 had significantly fewer ER-mitochondrial contact sites. Using three-dimensional serial electron microscopy, they showed that loss of PDZD8 markedly altered mitochondrial surface area in contact with the ER and that the reciprocal ER-mitochondrial interface was reduced. This dramatic difference (~80% reduction) in ER-mitochondrial contacts has never been shown for any of the candidate genes proposed to tether ER and mitochondria in mammalian systems. Furthermore, the difference in contact sites was not due to changes in either mitochondrial or ER morphology. This is of particular importance as alterations in mitochondrial structure have hampered the interpretation of other genes implicated in tethering (*5*). The alterations in tethering were causally linked to a decrease in ER-mitochondrial Ca^{2+} transfer. Importantly, loss of PDZD8 had no effect on Ca^{2+} release from the ER or ER-independent mitochondrial Ca^{2+} uptake, indicating that PDZD8 is specifically necessary for ER-dependent mitochondrial Ca^{2+} uptake.

Ca^{2+} cross-talk between ER and mitochondria is arguably the most important function of ER-mitochondrial tethering. Ca^{2+} is an essential cellular signaling molecule, the effects of which are dependent on local changes in its concentration. Receptor-activated Ca^{2+} release from ER creates high-Ca^{2+} microdomains at discrete subcellular locations (*8*, *9*). Indeed, it is estimated that ER Ca^{2+} release increases the Ca^{2+} concentration at ER-mitochondrial junctions more than 20-fold compared to the rest of the cytosol (*8*). Local elevations in Ca^{2+} regulate a vast array of effector molecules either directly through Ca^{2+}-binding sites or indirectly by modulating Ca^{2+}-dependent enzymatic processes (*10*). Thus, the spatiotemporal pattern of Ca^{2+} has profound effects on cellular function and survival, and ER-mitochondrial tethering is an important regulatory mechanism of Ca^{2+} dynamics and cellular physiology. The functional importance of subcellular Ca^{2+} domains is especially evident in the regulation of neurotransmitter release at neuronal synapses (for communication between neurons) (*11*, *12*). When an action potential (electrical signal) reaches the presynaptic terminal, voltage-gated Ca^{2+} channels open, allowing for Ca^{2+} entry. The local rise in intracellular Ca^{2+} triggers fusion of neurotransmitter-filled vesicles with the presynaptic membrane (*10*). In addition, the duration and amplitude of Ca^{2+} spikes in dendrites (sites of postsynaptic neurotransmitter binding in neurons) is necessary for synaptic integrity and plasticity mechanisms that underlie memory allocation and storage (*13*, *14*). Hirabayashi *et al.* show that PDZD8-dependent ER-mitochondrial tethering is also important for shaping Ca^{2+} microdomains in neuronal dendrites. Upon untethering mitochondria from ER, by decreasing PDZD8 expression, Ca^{2+} transfer was significantly reduced and cytosolic Ca^{2+} concentration was elevated, suggesting that PDZD8 is important for synaptic signaling. Future studies are likely already underway to determine the impact of the PDZD8-containing tethering complex on numerous neuronal functions.

Moving beyond cellular physiology, a better overall understanding of ER-mitochondrial associations may also shed light on mechanisms of disease. The ER-mitochondrial interface is the site of many

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biochemical processes that have been implicated in neurodegenerative diseases such as Ca^{2+} homeostasis, autophagy (the process of cellular organelle recycling), and mitochondrial dynamics (*15*). More importantly, it is known that ER-mitochondrial tethering is disturbed in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis with associated frontotemporal dementia (*15*). However, the molecular mechanisms underlying ER-mitochondrial disruption are not fully understood. Although ER-mitochondrial contact sites represent a nexus for many signaling cascades and biochemical reactions, it is yet to be determined whether a disruption in tethering is causative in neurodegenerative disease initiation or represents a secondary alteration that occurs during disease progression. Clearly, this discovery will provide new tools to better understand the ER-mitochondrial axis with respect to physiology and disease across cell types.

Although several mammalian ER-mitochondrial tethering proteins have been proposed, most lack clear indisputable evidence, and the identification of bona fide ER-mitochondrial tethers has remained elusive. We now have the first description of a protein that appears to primarily function as a member of an ER-mitochondrial tethering complex that can be genetically manipulated without confounding alterations in ER or mitochondrial integrity. This exciting discovery will provide not only new molecular tools to begin to define the physiological functions of ER-mitochondrial connections but also stimulate the search for the mitochondrial interaction partner of PDZD8 and other potential yeast ERMES homologs in mammals. ■

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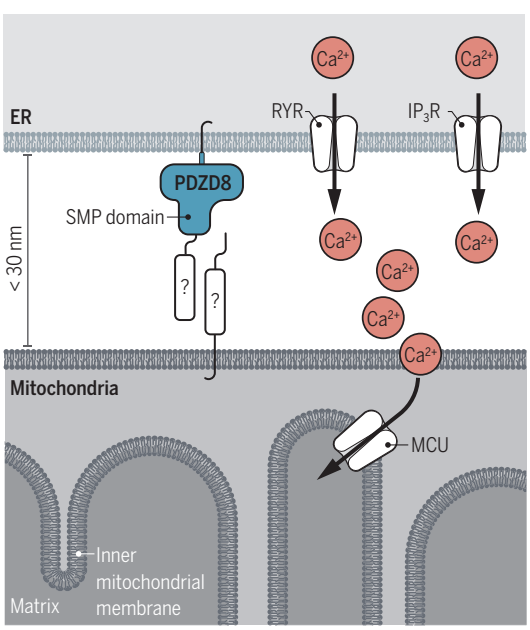
Coupling ER and mitochondrial membranes

The proteins that mediate the close coupling of ER and mitochondrial membranes (tethering) in mammalian cells have remained elusive. PDZD8 is an ER-bound protein that is critical for the tight association of ER and mitochondrial membranes. This will now allow the search for other possible binding partners and regulators that make up this newly identified tethering complex in mammalian cells. The close proximity of the ER and mitochondria is essential for several cellular processes. IP_3R, inositol-1,4,5-trisphosphate receptor; MCU, mitochondrial calcium uniporter; RYR, ryanodine receptor.



ER-mitochondrial tethering

- Calcium transport
- Subcellular signaling domains
- Lipid/membrane homeostasis
- Neurotransmitter release
- Cell death
- Autophagy
- Mitochondrial dynamics
- Inflammation

ARCHAEOLOGY

Finding the first Americans

The first humans to reach the Americas are likely to have come via a coastal route

By **Todd J. Braje,[1] Tom D. Dillehay,[2] Jon M. Erlandson,[3] Richard G. Klein,[4] Torben C. Rick[5]**

For much of the 20th century, most archaeologists believed humans first colonized the Americas ~13,500 years ago via an overland route that crossed Beringia and followed a long and narrow, mostly ice-free corridor to the vast plains of central North America. There, Clovis people and their descendants hunted large game and spread rapidly through the New World. Twentieth-century discoveries of distinctive Clovis artifacts throughout North America, some associated with mammoth or mastodon kill sites, supported this "Clovis-first" model. North America's coastlines and their rich marine, estuarine, riverine, and terrestrial ecosystems were peripheral to the story of how and when the Americas were first settled by humans. Recent work along the Pacific coastlines of North and South America has revealed that these environments were settled early and continuously provided a rich diversity of subsistence options and technological resources for New World hunter-gatherers.

Confidence in the Clovis-first theory started to crumble in the late 1980s and 1990s, when archaeological evidence for late Pleistocene seafaring and maritime colonization of multiple islands off eastern Asia (such as the Ryukyu Islands and the Bismarck Archipelago) accumulated. By the early 2000s, the Clovis-first theory collapsed after widespread scholarly accep-

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