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Commentary

ER-mitochondria tethering and Ca^{2+} crosstalk: The IP_3R team takes the field

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ABSTRACT

Inter-organelle communication represents a booming topic in cell biology research, with endoplasmic reticulum (ER)-mitochondria coupling playing the lion's share. In a recent work, Bartok and colleagues found that inositol trisphosphates receptors (IP_3Rs), in addition to their well-known involvement in ER-mitochondria Ca^{2+} transfer, are endowed with structural properties at organelles' interface.

The intimate interplay between ER and mitochondria is fundamental, among other processes, for guaranteeing cell Ca^{2+} homeostasis and maintaining mitochondrial function [1]. ER-mitochondria contacts, investigated by an increasing number of methodologies, have been revealed to be dynamic in nature and able to adapt during different metabolic cell states, as well as to be maintained by different specialized molecular structures [1].

In zones in which ER and mitochondria are in close contact, microdomains of high Ca^{2+} concentration can be formed at the outer mitochondrial membrane (OMM) upon stimulation of ER Ca^{2+} release [2,3]. These allow the activation of the mitochondrial Ca^{2+} uniporter (MCU) complex and the following mitochondrial Ca^{2+} uptake, which is a fundamental signal for cell survival/death events. In particular, it has been demonstrated that ER resident IP_3Rs, especially the IP_3R3, physically interact with the cytosolic fraction of the mitochondrial chaperone Grp75 and the voltage-dependent-anion channel 1 (VDAC1) of the OMM [4], forming a multi-protein complex which is critical for an efficient IP_3-dependent ER-mitochondria Ca^{2+} coupling. Initially, the intriguing possibility that this functional complex may additionally tether the two organelles was ruled out by the observation that the number and extension of ER-mitochondria contacts, evaluated by electron microscopy (EM), were similar between WT and IP_3Rs triple knock-out (TKO) DT40 cells [5]. Recently, however, a more in-depth ultrastructural analysis of the same cells, as well as of DT40-TKO clones rescued by the re-expression of individual mammalian IP_3R isoforms, revealed that each receptor isoform strengthens ER-mitochondria contacts [6]. Importantly, the IP_3Rs tethering function does not depend on their Ca^{2+}-transfer activity, since it is conserved by non-conductive IP_3R1 and IP_3R2 mutants.

By single-molecule localization super-resolution microscopy and TEM, Bartok et al. showed that all the IP_3R isoforms form clusters in proximity to mitochondria and they are critical for maintaining very close contacts between ER and mitochondria, whose frequency is decreased in DT40-TKO cells (Fig. 1). EM analysis revealed that the re-expression of mammalian IP_3R1 and IP_3R2 in DT40-TKO cells enhances ER-mitochondria close juxtaposition, while the effect of IP_3R3 was minimal [6]. Surprisingly, IP_3Rs expression mostly affects the closest contacts (*i.e.*, within 0–10 nm ER-mitochondria distance). Because functional IP_3Rs are large tetrameric channels, with a diameter of ~25 nm protruding in the cytosol ~13 nm [7], the question raises of whether these regions directly accommodate IP3Rs or are surrounded and stabilized by adjacent IP_3R-containing complexes. Moreover, considering that, in WT cells, 1) different isoforms can assembly into hetero-tetramers of variable composition, and 2) the extent of close ER-mitochondria contacts is apparently still larger than in single-isoform rescued clones, future investigations may focus on the relative efficacy of the different hetero-complexes.

By enhanced-resolution confocal microscopy, the authors showed also that, in plated DT40-TKO cells, the relative Z-distribution of ER and mitochondria is different compared to controls, possibly due to the lack of IP_3R-dependent linkers between the two organelles [6]. These alterations were rescued by the re-expression of each single IP_3R isoform, supporting a specific effect on this feature. It would be interesting to test whether the destruction of other known ER-mitochondria tethers triggers a similar modification, as well as to evaluate possible changes in ER and/or mitochondria morphology induced by IP_3R ablation, which could rearrange organelle networks' distribution and interaction with the cytoskeleton.

The other relevant finding presented by Bartok and collaborators [6] is the selective involvement of the three IP_3R isoforms in different Ca^{2+} pathways (Fig. 1). IP_3R1 is less effective, compared to type 2 and 3, in promoting ER to mitochondria Ca^{2+} transfer but more efficient in sustaining Ca^{2+} delivery to mitochondria from store operated Ca^{2+} entry (SOCE), due to its particularly enriched location in cortical ER

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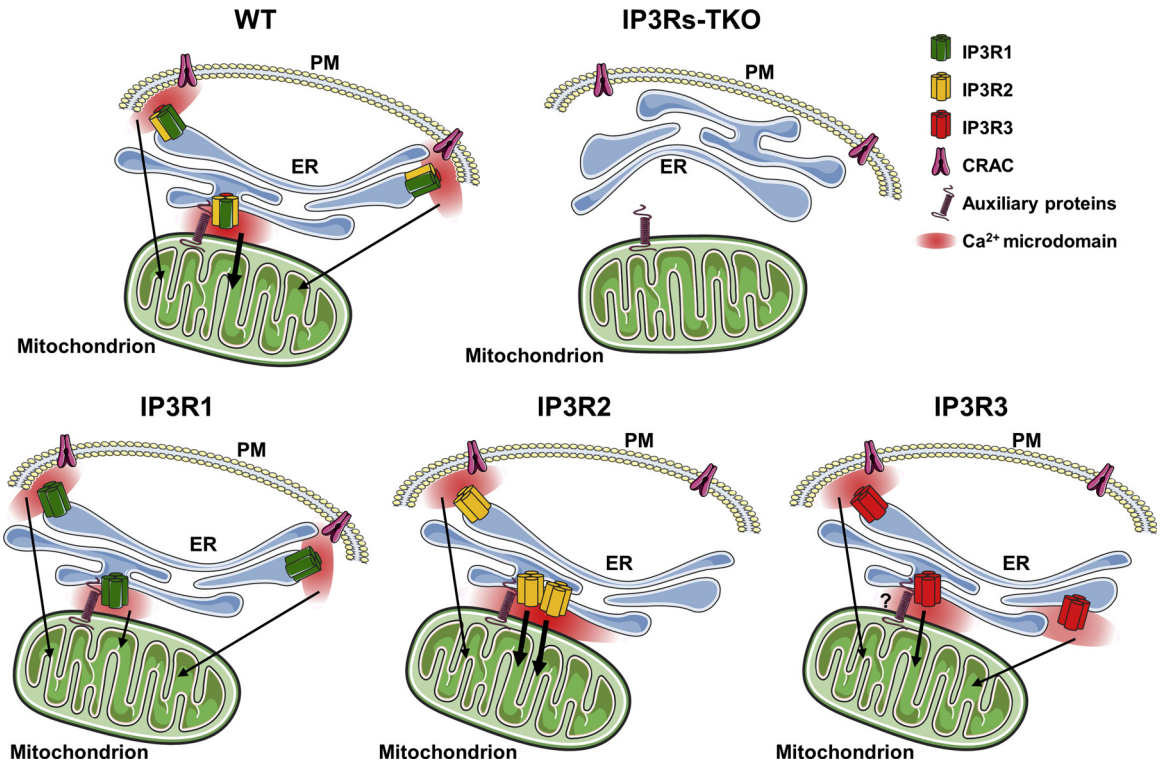

Fig. 1. Physical and functional properties of each IP_3R isoform at ER-mitochondria and ER-PM interface.

close to the plasma membrane (PM). Moreover, they shed light on the role of IP_3R2 in ER-mitochondria Ca^{2+} cross-talk: this so-far neglected isoform is enriched in the close vicinity of mitochondria (< 25 nm) and is the most efficient receptor type in mediating pure ER to mitochondria Ca^{2+} delivery. DT40-TKO cells re-expressing only IP_3R2 showed indeed the shorter coupling time between similar IP_3-mediated cytosolic and mitochondrial Ca^{2+} rises, compared to cells re-expressing respectively IP_3R1 or 3 [6]. This last result is surprising, considered previous findings suggesting that IP_3R3 and 1 are predominantly involved in Ca^{2+} shuttling from the ER to mitochondria [5,8,9], being specifically enriched at mitochondria-associated membranes (MAM) [1].

Overall, the interesting observations by Bartok and colleagues substantially increase our knowledge of the multifaceted roles of IP_3Rs at the ER-mitochondria interface, fostering likewise novel questions:

Does the IP_3R tethering function involve the known IP_3Rs-VDAC1 complex (or other proteins on the OMM)?

Do IP_3Rs work as direct ER-mitochondria tethers or represent scaffold molecules for other linker/spacer proteins?

Do other known IP_3R-interacting proteins, such as IRBIT, modulate its tethering function, as they do for its Ca^{2+} channel activity [10]?

What is the distribution, and the functional contribution, of the different IP_3R isoforms in other cell types characterized by a more complex morphology/function?

Future investigations on these aspects will be of great interest for the field of intracellular contact sites, introducing new characters in the blowing family of organelle tether proteins.

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