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SPOTLIGHT

Mitochondrial membrane biogenesis: A new pathway for lipid transport mediated by PERK/E-Syt1 complex

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Despite decades of extensive research, mitochondrial lipid transport is a process far from fully understood. In this issue, Sassano et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202206008) identified a new complex, composed of E-Syt1 and PERK, which mediates lipid transport at ER–mitochondria contact sites and regulates mitochondrial functions in human cells.

Mitochondrial biogenesis requires the import of the vast majority of their constituents, including proteins and lipids from other cell compartments. Whereas the mechanisms involved in mitochondrial protein import are well described, much less is known about lipid transport. How lipid transport is regulated is an important question as lipids modulate many aspects of mitochondria biogenesis and functions (1). Lipids are mainly synthesized in the ER and are then transported to mitochondria by non-vesicular routes at ER–mitochondria contact sites (EMCSs). Thus, the formation of EMCSs is a prerequisite to allow the efficient exchange of lipids by lipid transfer proteins (LTPs). Up to date, only a few LTPs have been identified as key players in the regulation of mitochondrial lipid homeostasis (2). In their work, Sassano et al. (3) identified in human cells the ER-kinase PERK and one of its partners, the LTP extended synaptotagmin 1 (E-Syt1), as new actors involved in EMCSs lipid transfer, opening important new perspectives in this field.

E-Syt1 is an ER-anchored protein containing a synaptotagmin-like mitochondrial lipid-binding protein (SMP) lipid transport domain and five C2 domains (C2A to E). The C-terminal C2E domain is involved in phosphatidylinositol-(4,5)-bisphosphates ($PI(4,5)P_2$) binding. The function of E-Syt1 at ER–PM contact sites (EPCSs) is well described: E-Syt1 promotes (1) the tethering of ER and PM and (2) the transfer of lipids mediated by its SMP domain. Both processes are regulated by Ca^{2+} binding to C2A and C2C domains (4). PERK plays a primary role in the activation of the unfolded protein response (UPR) induced by ER stress and was previously shown to be located at EMCSs and to maintain mitochondrial homeostasis during stress (5, 6). Previous PERK interactome analyses suggested that E-Syt1 could be a partner of PERK (7). However, a link between E-Syt1 and PERK in the regulation of mitochondrial lipid homeostasis had never been established.

By a combination of cell fractionation and imaging techniques, Sassano et al. (3) showed that PERK and E-Syt1 were both located at EMCSs. Using immunogold labeling, they demonstrated that indeed, in HeLa cells, around 10% of the E-Syt1 pool is present at EMCSs, while a similar proportion is located at EPCSs. However, the absence of PERK, but not of E-Syt1, decreased the proximity between ER and mitochondria, showing that E-Syt1 did not mediate tethering at EMCSs, in contrast to its role at EPCSs. Pull-down assays confirmed interaction between E-Syt1 and PERK. Most importantly, the authors demonstrated that this interaction was required to target E-Syt1 to EMCSs. They further showed that the C2D and C2E domains of E-Syt1, but not its SMP domain, were important for PERK binding and EMCSs localization, revealing that E-Syt1 EMCSs localization mainly relied on protein–protein interactions. This targeting mechanism therefore differs from that of E-Syt1 to EPCSs, which mainly relies on C2 domains binding to $PI(4,5)P_2$ and to negatively charged lipids enriched in the PM (4). Finally, the authors showed that the kinase activity of PERK was not necessary to regulate ER–mitochondria proximity nor the interaction with E-Syt1 and its recruitment to EMCSs, indicating that those processes are regulated independently of PERK function in UPR.

Most interestingly, using several cell lines depleted (shRNA or KO) for E-Syt1 or PERK, the authors investigated the involvement of these two proteins in mitochondrial calcium transfer and lipid homeostasis, which are key roles of EMCSs. As previously shown, the absence of PERK led to a perturbation of mitochondrial calcium homeostasis (6), likely due to the decrease of ER–mitochondria proximity. However, no defect was observed in absence of E-Syt1, suggesting a minor role of E-Syt1 in this process. Lipidomic analyses on isolated mitochondria revealed that the absence of PERK or E-Syt1 triggered a similar perturbation of the mitochondrial lipidome. Indeed, the level of the major mitochondrial phospholipids was decreased in all lines. Using an elegant in vivo assay based on fluorescently labeled phosphatidylserine

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(NBD-PS), the authors demonstrated that this phenotype was likely linked to a defect in lipid transport. In mammalian cells, NBD-PS uptake from the culture media is first incorporated into the ER before being in part transported to mitochondria, where the inner membrane-localized PS decarboxylase converts NBD-PS to NBD-phosphatidylethanolamine (NBD-PE). By performing in vivo imaging, the authors showed that the proportion of NBD lipids co-localizing with mitochondria was reduced in the absence of PERK or E-Syt1. In addition, a decrease of the NBD-PS to NBD-PE conversion was observed, highlighting a defect in the mitochondrial transport of NBD-PS to the mitochondrial inner membrane. Interestingly, the phenotypes observed were rescued by the expression of full-length E-Syt1 protein but not by deletion mutants lacking the SMP domain or the C2E/D domains, showing that both the lipid transfer activity of E-Syt1 and its association to PERK via the C2E/D domains are required for ER–mitochondria lipid transport.

Finally, because of the important role of lipids in the regulation of mitochondrial functions, the authors analyzed mitochondrial respiration in the different cell lines and showed that the absence of PERK or E-Syt1 decreased respiration as well as ATP production. Complementation experiments showed that the kinase activity of PERK was not required. However, as for the lipid transport function, both the SMP and C2D/E domains of E-Syt1 were necessary for efficient mitochondrial respiration, suggesting that the mitochondrial respiratory defect was linked to the modification of the mitochondrial lipidome.

Overall, this paper highlights the importance of PERK/E-Syt1 complex in the regulation of mitochondrial lipid homeostasis and function, revealing (1) new roles of PERK in the regulation of mitochondria biogenesis in non-stress conditions and (2) the unexpected role of E-Syt1 at EMCSs in addition to EPCSs. It is likely that other partner(s), at least one at the mitochondrial surface, are required to localize the PERK/E-Syt1 complex at EMCSs. In that direction, a recent preprint suggests that E-Syt1 can interact with the mitochondrial outer membrane protein SYNJ2BP to regulate mitochondrial lipid homeostasis (8). Interestingly, three other LTPs (ORP5, ORP8, and VPS13A) also localize at both EMCSs and EPCSs (2, 9). In addition, VPS13 family proteins are also localized at a wide diversity of CSs (10). This shows an intimate communication between EPCSs and EMCSs, and more generally between all CSs, to regulate CS effectors localization. As illustrated here for E-Syt1, the localization of LTPs at multiple CSs relies on different mechanisms: particular membrane lipid compositions and/or by binding to protein partners, and can be regulated by different signaling molecules such as Ca^{2+} (see Fig. 1). In the future, it will be important to understand (1) which conditions promote the recruitment of an LTP at particular CSs rather than others and (2) whether and how all the identified LTPs act in concert to coordinate mitochondrial lipid transport (i.e., what is their specificity of transport, are they interacting together, can they complement each other…?). To conclude, many questions remain open in the exciting field of mitochondrial lipid trafficking, and more generally, in the field of non-vesicular lipid transport.

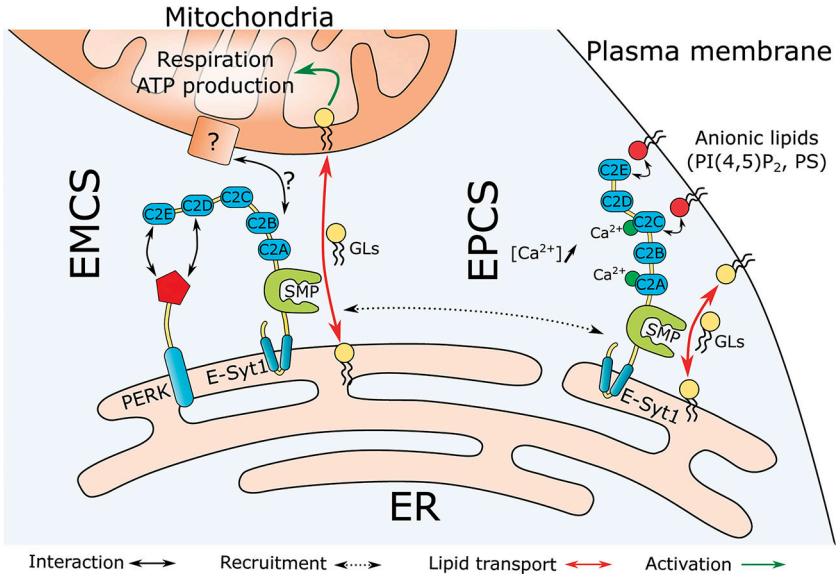

Figure 1. **The PERK/E-Syt1 complex promotes lipid transport to mitochondria and the maintenance of mitochondrial functions in human cells.** E-Syt1 is an ER anchored protein known to promote EPCSs expansion and to transport lipids at this site upon increase of intracellular Ca^{2+} concentration. The association of E-Syt1 to PM is mediated by the binding of C2E and C2C domains to $PI(4,5)P_2$ and negatively charged lipids enriched in the PM (4). In their work, Sassano et al. (3) showed that E-Syt1 is also important to mediate lipid transport at EMCSs. E-Syt1 is recruited at EMCSs by PERK, an ER kinase known to regulate mitochondria homeostasis during stress. E-Syt1 recruitment to EMCSs by PERK and its lipid transport activity are both required for efficient mitochondrial respiration and ATP production. GLs, glycerolipids; $PI(4,5)P_2$, phosphatidylinositol-(4,5)-bisphosphate; PS, phosphatidylserine.

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