

The PTPIP51 TPR-Domain: A Novel Lipid Transfer Domain?

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During the last decade, mitochondria-associated ER membranes (MAMs) have emerged as critical signaling, metabolic and trafficking hubs involved in the regulation of multiple cellular processes including autophagy, inflammation, signaling and apoptosis (Csordas et al., 2018; Phillips & Voeltz, 2016; Rowland & Voeltz, 2012). MAMs are zones of close membrane proximity where the ER and mitochondria membranes are tethered by multiple linker proteins, allowing direct exchange of key metabolites and ions, such as lipids and Ca^{2+} , between these two organelles that are not connected by the classical vesicle-transport routes. While the mechanism and proteins involved in Ca^{2+} transport at the MAMs are well characterized, our knowledge on how lipids are exchanged between these two organelles is still rudimentary, especially in metazoa, as the lipid transfer proteins (LTPs) have just started to be identified. Writing in EMBO Reports, Yeo and colleagues (Yeo et al., 2021) reveal a new role of the mitochondrial protein PTPIP51 (also known as Regulator of Microtubule Dynamics, RMD3) in lipid transfer at MAMs. PTPIP51 has been previously shown to be a tether that bridges ER and mitochondria membranes via interaction with the ER protein VAPB, thus facilitating Ca^{2+} transport to mitochondria (Stoica et al., 2014). However, the exact biochemical function of PTPIP51 beyond ER-mitochondria tethering had so far been unclear.

PTPIP51 possesses tandem FFAT motifs involved in the binding to VAP (Di Mattia et al., 2020; Mikitova & Levine, 2012), a coiled coil (CC) domain and a large C-terminal globular domain, the tetratricopeptide repeat (TPR) whose role in PTPIP51 function remains enigmatic. With over 100 TPR structures deposited in the protein data bank, the TPR domain has established itself as a major protein-protein interaction module (Blatch & Lassle, 1999). A diverse array of protein ligands has been seen to bind within the TPR cleft. These do not share any common sequence or secondary structure. Moreover, the diversity of the ligand and the amino-acid residues that line the binding cleft of the TPR domain produce highly specific TPR binding domains. Yeo and colleagues reveal a novel role for the TPR of PTPIP51 in binding and transferring phospholipids that is unusual if compared to the established protein-protein interaction mode of other TPR domains. On this line,

Yeo and colleagues show that interaction of PTPIP51 with VAPB is not mediated by the TPR domain but by the tandem FFAT-like motif. Then, they provide evidence that suggests that the PTPIP51 TPR-domain is involved in phospholipid binding and transfer at MAMs. The authors present the X-ray structure of the TPR domain of PTPIP51 and biochemical evidence *in vitro* and *in situ* for a lipid binding and transfer function. They propose that the PTPIP51 – VAPB complex might be the counterpart of the yeast ER–mitochondria encounter structure (ERMES) complex, responsible for phospholipid transportation at MAMs (AhYoung et al., 2015; Kornmann et al., 2009; Tatsuta et al., 2014). Although other LTPs are also present at contact sites between ER and mitochondria (i.e. ORP5/8, PDZD8, VPS13A), the unexpected finding that the PTPIP51 TPR domain could be involved in lipid trafficking at the same interface is very intriguing and naturally deserves closer scrutiny.

However, before we discuss this, it would be useful to draw some information from structures that are known to be lipid trafficking proteins (LTPs) at regions of close membrane apposition, including MAMs. There are several major families of LTPs that localize at membrane contact sites, each containing a core lipid-binding/transfer domain (Giordano, 2018), that include oxysterol-binding protein (OSBP)-related proteins (ORD), START, START/VASt, PITPs, PRELI-like, and SMP domains, that collectively act as tethers, lipid sensors, or transporters at multiple contact sites (Giordano, 2018). Looking at examples of such

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structures (Osh6, the yeast orthologous of ORP5/8, PDB 4PH7; human phosphatidylcholine transfer protein, PDB 1LN1; Ysp2p, PDB 6CAY and the SMP domain of Mmm1, PDB 5YK6) we see that these structures suggest a common theme by which MAM associated phospholipid transfer proteins bind their ligands within solvent inaccessible sites. Furthermore, LTPs such as PITP-alpha and Beta (PDB, 1KCM and 2AIL, respectively) also bury their bound ligand. However, an exception to this rule is seen for mitochondrial PRELID1 (PDB, 6I3Y), where the phosphatidylserine (PS) is bound in a deep cleft, but is solvent exposed. This latter example indicates that a TPR domain, with a solvent exposed channel, might be implicated in binding phospholipid. Furthermore, it is not inconceivable that such a binding event could occur as this has been demonstrated for MHC and CD1 proteins (Grant et al., 1999; Rossjohn et al., 2015; Salomonsen et al., 2005)

The structural data shown by Yeo and colleagues reveal that the TPR in PTPIP51 consists in 12 alpha-helices forming several antiparallel TPR units, two of them (TPR2 and TPR3) generating a deep channel-shaped cavity covered on one side by the alpha-helix 8 ($\alpha 8$) as a plug. Another unusual feature of this TPR domain is helix 12, which lies across the top of the TPR cleft and restricts it somewhat. The significance of helix 8 and 12 remains unknown. Structural evidence for a lipid-like molecule being bound to the TPR cleft of PTPIP51 was a weak, tube-shaped, serpentine electron density observed in the electron density map of the structure. The crystals were grown in the presence of Sokalan 42, a modified polycarboxylate, which might account for this electron density, but in doing so was mimicking the binding of a phospholipid. Alternatively, a lipid from the expression host could have been weakly bound. However, attempts to refine this or determine the molecule responsible for this electron density failed. Electron density for a bound head group, assuming such a lipid was bound, was unfortunately lacking, indicating that a head group was either absent or disordered. As with the Mmm1 structure, the head groups of its bound phospholipid might be exposed to solvent, which may indicate a low specificity for phospholipid, where the head group could be quite variable.

In support for the PTPIP51 TPR domain being involved in phospholipid binding, a series of biochemical assays were conducted. First, the authors performed PIP-strip lipid-binding assays, using purified recombinant proteins, PTPIP51_ΔTM, PTPIP51_TPR, and PTPIP51_ΔTPR (residues 36–235) proteins. They found that the PTPIP51 TPR domain has a binding preference for PtdIns(4)P, PtdIns(5)P, and phosphatidic acid (PA), over cardiolipin (CL), di-PtdIns, phosphatidylethanolamine (PE) and phosphatidylcholine (PC). The construct lacking the TM domain showed a wider spectrum of affinity, binding a variety of PtdIns, PA, PS and CL. Only PtdIns(5)P interacted with all three constructs of PTPIP51. Although PIP-strip lipid binding assays allow us to address the ability of a

protein surface to interact with lipids they do not allow us to determine the ability of the TPR domain to extract and bind lipids within its cleft. Thus, the authors also conducted in vitro liposomes-based lipid precipitation assays using PTPIP51_ΔTM and PTPIP51_TPR, confirming the ability of PTPIP51_ΔTM to bind PA, CL and PtdIns(4)P, but not PS, PC and PE. The interaction between PTPIP51_ΔTM and PA was further investigated, and confirmed, by a sucrose gradient liposome flotation assay in vitro. Collectively, these experiments showed that PTPIP51 has a broad lipid binding affinity suggesting that some variability in the head group interaction with the TPR domain is possible.

Finally, the authors evaluate the PA transfer activity of PTPIP51 using a FRET-based in vitro PA transfer assay. The assay showed a clear dose-dependent PA transfer by PTPIP51, which was higher with the PTPIP51_ΔTM construct than with PTPIP51_TPR. However, a negative control, such as a TPR mutant, was not used in these experiments. It was also noted that the kinetics of PA transfer was rather slow to be physiological, but that this was probably due to fundamental limitations of the in vitro assay. Finally, an alternative bead pulldown-based fluorescence lipid transfer assay was performed, in vitro, which showed a PA and monolysocardiolipin transfer activity of PTPIP51. The authors finally conclude that PTPIP51 can bind and transfer lipids such as PA in vitro.

PA, that is synthesized in the ER, needs to be shuttled from the ER to the mitochondria at contact sites as it is the precursor of CL, a key lipid of mitochondria required to maintain their structural and functional integrity. By using a spectrophotometric assay the authors found that PTPIP51 knockdown in HeLa cells decreases levels of CL. The decrease was weak but statistically significant. Although further lipidomic analysis did not measure a significant decrease in CL upon PTPIP51 downregulation, an increase in CL levels was found upon re-expression of PTPIP51, possibly as a result of both its lipid transfer and tethering activity (overexpression of PTPIP51 strongly increases ER-mitochondria contact sites). Yeo and colleagues conclude that PTPIP51 mediates PA transfer at ER-mitochondria junctions in situ. Unexpectedly, a mutant deleted in the FFAT motifs was also able to rescue CL decrease, indicating that the function of PTPIP51 on mitochondrial CL does not require its interaction with VAPB. This finding highlights a peculiarity of PTPIP51 versus other FFAT-proteins, that instead seem to require FFAT-mediated interaction with VAP for their lipid transfer function (for example, STARD3 requires FFAT-mediated interaction with VAP for its sterol transfer function both in vitro and in vivo (Di Mattia et al., 2020)). PTPIP51 has been shown to also interact with ORP5/8 (Galmes et al., 2016), which could bind PTPIP51 TPR domain and compensate for the loss of interaction with VAPB. Further studies would be needed to address this possibility and to analyse

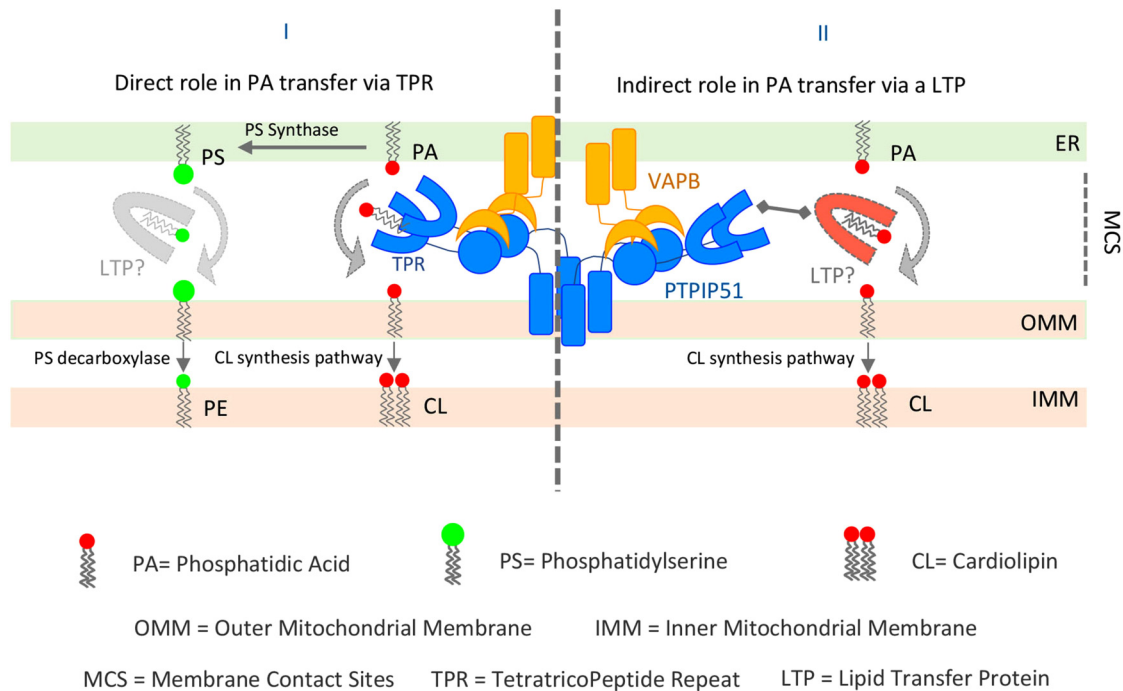


Figure 1. Cartoon showing the possible role of PTPIP51 in PA transfer, involving a direct role of its TPR domain (II) or an indirect role utilising another lipid transfer protein (LTP) intermediate (I).

the effects of the TPR and FFAT mutants on ORP5/8 binding and on the morphology of ER-mitochondria contact sites.

Overall, the experiments performed by Yeo and colleagues show that the exact lipid transfer activity of PTPIP51 appears to be construct dependant, which can be difficult to rationalise. This raises specific questions and further research, in our opinion, is required before this TPR domain is categorised as lipid binding. A next step would be to use a simple mutation in the cavity of the TPR domain, close to the serpentine electron density, that would sterically prevent lipid binding and confirm binding within the TPR cleft. Crystallization of the TPR domain with phospholipid would ultimately identify the exact interaction site on the TPR domain. The TPR mutant could be also used for rescue experiments in situ to confirm a direct role of PTPIP51 in lipid transfer (Figure, left (I)) and to exclude possible indirect effects due to its tethering function or its interaction with other LTPs, such as ORP5/8 (Figure 1, right (II)).

To conclude, while the results are exciting in that a TPR domain can interact with phospholipid, direct structural evidence for this is still tantalisingly out of reach. Hence, the authors lay a platform from which additional experimentation is evident to strengthen the argument for the role of PTPIP51 TPR in non-vesicular lipid transfer in vitro and at ER-mitochondria contact sites.

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