NAADP receptors: A one-two.

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NAADP is a potent Ca^{2+}-mobilizing messenger that activates two-pore channels (TPCs) on endo-lysosomes thereby regulating Ca^{2+} signaling in health and disease [1]. New work by Zhang *et al* [2] identifies LSM12 as an NAADP-binding protein that confers NAADP sensitivity to TPCs, joining JPT2 [3, 4] as long sought NAADP receptors.

It has been over a decade since TPCs were identified as the target channels for NAADP [5]. But soon after their discovery, it became clear that TPCs were not NAADP receptors i.e. they did not directly bind NAADP. This was based on photoaffinity NAADP probes, which labelled small 22-23 kDa proteins that associated with TPCs [6]. The hunt was on to identify these proteins at the molecular level. Up until this year they were yet to be defined. But now we have not one but two prime suspects [7]. The latest addition is LSM12 [2]. The LSM proteins are similar to the Sm proteins, hence their name ('Like Sm'), and are known to serve important roles in RNA processing [8].

Zhang *et al* transiently expressed human TPC1 or TPC2 in HEK293 cells and used mass spectrometry to identify proteins in immuno-precipitates and pull-downs with immobilized NAADP [2]. LSM12 was the only common hit in the four interactomes. Purified LSM12 bound NAADP with high affinity (*Kd* ~20-30 nM). NADP however was unable to compete with NAADP in binding recombinant LSM12 even at high (up to 100μM) concentrations. This selectivity is remarkable given commercial NADP preparations are contaminated by NAADP. In HEK cells where LSM12 was knocked out but transiently expressing wild type TPCs or mutants re-routed to the cell surface, microinjection of NAADP failed to produce any significant increases in cytosolic Ca^{2+} (from lysosomes) or Na^{+} currents (at the plasma membrane). But both NAADP responses were restored when LSM12 protein was co-injected or re-expressed. Through biochemical and functional assays, the N-terminal Lsm domain of LSM12 was sufficient for NAADP binding, included a small stretch of residues that appeared to be critical for interaction with TPCs (though not for NAADP binding), and was indispensable for NAADP-evoked Ca^{2+} release. Overall, the evidence for LSM12 as an NAADP receptor is robust.

LSM12 joins JPT2 as an NAADP receptor. JPT2 was independently identified as an NAADP-binding protein by two groups based on biochemical purification from blood-derived cells using a next generation NAADP photoaffinity probe [3, 4]. Like LSM12, it bound NAADP, co-immunoprecipitated with TPCs and was required for NAADP-mediated Ca^{2+} signaling [3]. JPT2 (but not the related, JPT1) was also required for SARS-Cov-2 entry [3] and appeared to associate with ryanodine receptors in T-cells during antigen stimulation to initiate early subcellular Ca^{2+} signals [4].

JPT2 and LSM12 are therefore both 'functional' NAADP receptors. But aside from their similar small size (consistent with photoaffinity labelling), they are unrelated. Figure 1 shows the predicted structure of LSM12 delineating its Lsm domain and its C-terminal anti-codon binding domain. JPT2 however is predicted as disordered. So how do both bind NAADP with high affinity and selectivity? And how do both interact with TPCs? LSM proteins are known to form ring-shaped oligomers consisting of six or seven protomers and this appears to be important for their function related to RNA processing [8]. The stoichiometry of LSM12 and TPCs remains to be defined. Interestingly, LSM12 associated equally well with TPC1 and TPC2 [2] but JPT2 appeared to interact preferentially with TPC1 [3]. This suggests that their interacting regions might be different.

Another issue relates to redundancy. In U2OS and HEK cells, knockdown of JPT2 reduced photoaffinity labelling by ~50% [3]. So it's possible that LSM12 could account for the residual binding. However, JPT2 knockdown in U2OS cells completely abrogated NAADP-mediated Ca^{2+} signals [3]. LSM12 is ubiquitously expressed so should we have not expected a residual Ca^{2+} response upon JPT2 depletion? Conversely, knockdown of LSM12 in SKBR3 cells and knockout in HEK cells expressing TPCs also fully abrogated NAADP action [2]. Similar results were observed in embryonic fibroblasts from mice with LSM12 deleted for the TPC-interacting region [2]. However, JPT2 is expressed in all of these cells and at relatively high levels in SKBR3 cells [3]. So, where is its contribution upon LSM12 depletion? These observations argue against parallel pathways for NAADP action by the two proteins. The LSM12 transgenic mice are neat as they will help to distinguish TPC dependent and -independent phenotypes.

Finally, eukaryotic genomes can contain up to 16 LSM isoforms [9] and the Lsm-domain is likely to be largely conserved structurally. It is therefore intriguing that only LSM12 was identified as an NAADP receptor. LSM12 together with LSM13-16 differ from other LSM isoforms by virtue of an extended C-terminus [9]. But the C-terminus is dispensable for NAADP functionality and TPC interactivity [2]. Knockdown of Lsm5 and 11 only marginally inhibited NAADP action but more work is required to determine whether other Lsm domain-containing proteins bind NAADP and/or associate with TPCs.

In sum, it's an exciting time for 'NAADPologists'. The identification of TPCs was a step-change in our understanding of NAADP signaling allowing application of sorely needed molecular approaches. This has paid off and we have many molecular tools at our disposal, including new TPC2 agonists [10]. The identification of LSM12 and JPT2 represents another step-change that will no doubt further advance the field.

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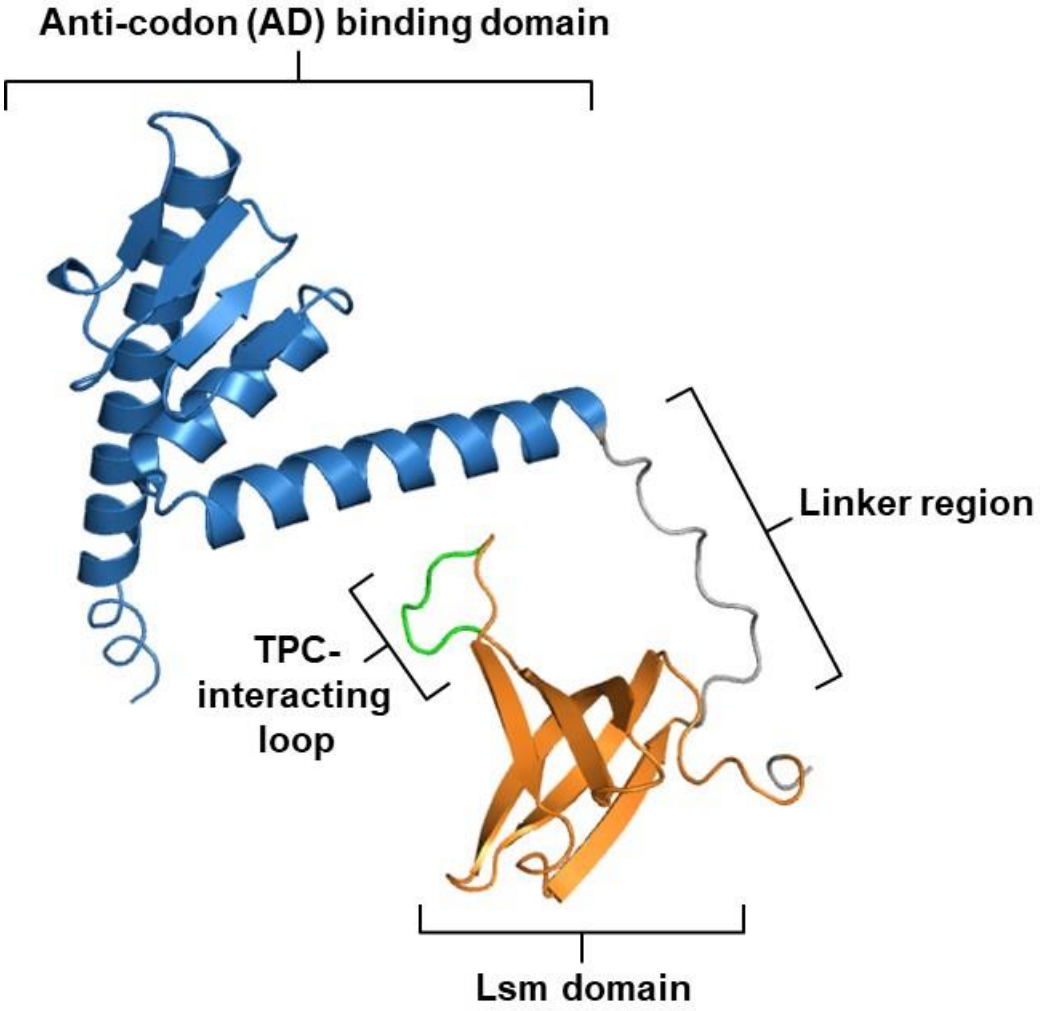

Figure 1. NAADP receptors find form. Cartoon representation of the human Lsm12 protein modelled by AlphaFold 2.0 (https://alphafold.ebi.ac.uk/entry/Q3MHD2). In contrast, JPT2 is predicted to be a disordered protein by the same method (not shown).