Teaming with NAADP

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Abstract

NAADP is a potent Ca^{2+}-mobilizing messenger linked to numerous Ca^{2+}-dependent effects across the natural world. On 18 February 2021, delegates attended a virtual symposium to discuss studies of the mechanisms of action of NAADP, including the work of Gunaratne *et al*. and Roggenkamp *et al*. in this issue of *Science Signaling*.

Introduction

The Ca^{2+}-mobilizing properties of NAADP (nicotinic acid adenine dinucleotide phosphate) were first identified by Lee and Aarhus more than two decades ago in experiments with the humble sea urchin egg (*1*). Since then, the actions of NAADP have been extended to many cell types; however, the molecular mechanism underpinning its activity has frustrated many over the years. On Feb 18th, 2021, ~90 delegates gathered online at a virtual symposium entitled "Special Session on NAADP Signaling" covering advances in our understanding of NAADP action. This virtual symposium was a new addition to the webinar program of the European Calcium Society (https://gbiomed.kuleuven.be/apps/lmcs/ecs/index.php). The discussion was based around "Team Talks" in which the virtual podium was shared by two speakers. All of the talks provided insights into the molecular identity of the elusive NAADP receptor.

Much evidence implicates endo-lysosomal, two-pore channels (TPCs) as targets of NAADP (*2*). These channels reside on acidic organelles, such as lysosomes, and appear to switch their ion selectivity in an agonist-dependent manner (*3*). Although TPCs are essential for NAADP-mediated Ca^{2+} release in many cells, photoaffinity labelling studies with probes developed by Timothy Walseth and colleagues indicate that TPCs do not bind NAADP. Rather, NAADP appears to bind to smaller proteins that associate with TPCs (*4*). Ryanodine receptors (RyRs) have also been proposed as NAADP targets, but again, the effects of NAADP are thought to be indirect.

The first session of the symposium, chaired by Yu Yuan (University College London, UK), began with a Team Talk by Antony Galione and Margarida Ruas (University of Oxford, UK). The Galione team has a long-standing interest in NAADP and provided the initial evidence showing that NAADP releases Ca^{2+} from acidic organelles (*5*). The team outlined their ongoing proteomic efforts to isolate NAADP-binding proteins from both mammalian preparations and "gold standard" sea urchin eggs. One clever approach capitalized on a fortuitous finding that anti-TPC3 antibodies equally capable of immunoprecipitating TPC3 differ in their ability to recover NAADP-binding sites, thereby focusing efforts on differential interactors.

The next Team Talk was by Jiusheng Yan and Xin Guan (University of Texas, USA). The Yan team are newcomers to the NAADP field. Through the use of immobilized NAADP and TPC proteomics, they singled out LSM12 as an NAADP- and TPC-interacting partner in HEK 293 cells. Recombinant LSM12 bound to NAADP, but not NADP, with high affinity. Knockout of LSM12 in cells inhibited the interaction of TPCs with immobilized NAADP and reduced NAADP-induced Ca^{2+} signals mediated by both expressed and endogenous TPCs. Knockout of LSM12 also prevented NAADP-induced currents by TPCs re-routed to the plasma

membrane. Although little is known about LSM12, other members of the LSM protein family regulate RNA trafficking through their LSM domain. This domain (in particular, a short, 5–amino acid residue peptide within it) is crucial for the interaction of LSM12 with TPCs and rendering them sensitive to NAADP (*6*).

The second session chaired by Martina Gregori (University College London, UK) featured Team Talks from Jonathan Marchant and Gihan Gunaratne (Medical College of Wisconsin, USA) and Andreas Guse and Björn-Philipp Diercks (University Medical Center Hamburg-Eppendorf, Germany). Both teams converged on JPT2 as an NAADP receptor. The USA-based team identified human red blood cells as an abundant source of NAADP-binding sites. Using a novel bifunctional NAADP photoprobe, they isolated a single protein from enriched lysates and identified it as JPT2. Recombinant JPT2 selectively bound to NAADP with nanomolar affinity. Endogenous JPT2 co-immunoprecipitated with TPC1, and knockdown studies revealed it to be essential for NAADP-induced Ca^{2+} release in U2OS cells. This is similar to the requirement for TPC1 in other cancer cells (*7*). Previous work by the group had implicated TPCs in the endocytic transit of the MERS coronavirus, adding to a body of literature implicating TPCs in endo-lysosomal membrane trafficking. Data showing that both TPCs and JPT2 were required for the trafficking of SARS-CoV-2 provided a timely functional readout for JPT2 given the ongoing COVID-19 pandemic (*8*).

The Germany-based team identified JPT2 from purification studies with Jurkat cells, a human $CD4^+$ T cell line. Recombinant JPT2 bound to NAADP, albeit weakly (with micromolar affinity). Previous work by this team implicated RyRs in Ca^{2+} signals evoked by NAADP and T cell receptor (TCR) engagement (*9*). Here, JPT2 partly colocalized and co-immunoprecipitated with RyRs. CRISPR-based knockout of JPT2 in Jurkat cells and primary

effector T cells delayed Ca^{2+} signals evoked by antigen similarly to pharmacological antagonism of NAADP in wild-type cells. Together, these data are consistent with a working model in which JPT2 confers NAADP sensitivity to RyRs and suggest that the complex is functionally recruited by NAADP formed early during TCR activation (*10*).

Team Talks were used flexibly by the speakers. Formats included a short introduction by one of the investigators, with the bulk of the talk delivered by the other, through to a fifty-fifty split and back-and-forth between the team members. Team Talks provided an effective means to increase the exposure of junior scientists and to fully reflect the diversity of the community, thereby adding value to the symposium. As research efforts become necessarily more inter-disciplined, we anticipate that this format could be mobilized to bring together collaborating teams. There was a general "round table" discussion at the end of the meeting between all of the speakers and participants, which continued for some time after the formal close.

So, what did we learn? The exciting convergence by two teams on JPT2 as an NAADP receptor boosts confidence and provides a clear way forward to interrogate NAADP signaling at the molecular level. There is little in the published literature on this protein, although its links to cancer are consistent with known roles for TPCs in this pathology. The identification of LSM12 as an NAADP receptor is equally exciting but demands independent verification and an understanding of the relationship between LSM12 and JPT2. Do LSM12 and JPT2 work together to confer NAADP sensitivity? Parallel assaying of the two receptors by the various groups would be welcome. Another issue that requires scrutiny is the identification of the channels targeted by these proteins. Both LSM12 and JPT2 reportedly interact with TPCs. Do both also interact with RyRs in T cells, where RyRs may play a primary role in NAADP action? What are the molecular determinants of such interactions? Might all the players be part of a

NAADP signaling complex? For sure, answers to these questions will be forthcoming. In sum, teaming with NAADP left the field teeming with excitement.

References and Notes

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