Two-pore channels (TPCs) are ancient ion channels that reside within acidic organelles, such as endosomes and lysosomes (1). TPCs are the proposed target of the second messenger nicotinic acid adenine dinucleotide phosphate (NAADP), which mobilizes Ca\(^{2+}\) from “acidic Ca\(^{2+}\) stores” (2). However, fundamental aspects of TPC biology—how they activate and their native ionic permeability (3–8)—remain controversial (reviewed in references 9 and 10).

Photolabeling studies using a radioactive NAADP-derived probe (5N\(_3\)-[\(^{32}\)P]NAADP) have suggested that NAADP may not directly bind to TPCs but may stimulate channel activity via small NAADP-binding proteins (∼23 kDa) associated with the TPC complex (11–14). One piece of evidence supporting this idea is the preservation of photolabeling in TPC knockout mice (11). For example, as shown in Fig. 1A, NAADP-specific labeling at 23 kDa is apparent in the knockout TPC\(^{-/-}\) mouse sample and selectively displaced by unlabeled NAADP, with affinity similar to that of wild-type mice. These data indicate that TPCs are unlikely the direct targets of NAADP.

In a recently published study (15), Ruas and colleagues challenged this interpretation. Their data suggest the existence of a mouse TPC1 variant (TPC1B) with a truncated NH\(_2\)-terminal domain compared to the previously studied TPC1 isoform (TPC1A). If this TPC1B variant were to be prevalently expressed, or if gene trap skipping occurred in the previously used TPC1 null line (11), Western blotting data using 5N\(_3\)-[\(^{32}\)P]NAADP showing specific photolabeling of an apparent molecular mass of ∼23 kDa candidate NAADP binding protein in mouse pancreatic samples from a matched littermate mouse (TPC1\(^{+/+}\) [left]) and a TPC knockout mouse (TPC\(^{-/-}\) [right]; Tpcn1\(^{-/-}\) ) (Fig. 4B in reference 15). Further detail is available in reference 11. (B) Schematic of TPC1 membrane topology to highlight the COOH-terminal epitope (red) present in wild-type TPC1 and the putative NH\(_2\)-terminal truncation of TPC1B (the missing sequence is shown with a black line). (C and D) Western blot analysis of liver samples from wild-type (+/+) and Tpcn1\(^{-/-}\) mice described in reference 11 using an antibody raised to the C terminus of TPC1 (ab80961; Abcam). (D) Samples were treated with peptide-N-glycosidase F (PNGase F) (right) to remove N-linked oligosaccharides as described in reference 17. Note the absence of major immunoreactive bands (solid arrowheads) in the transgenic mice. Bands present in both samples (open arrowheads) are likely nonspecific, but we cannot rule out the possibility of severely truncated, nonfunctional TPC1 variants that escape inactivation. Numbers at the left of the gels are molecular masses (in kilodaltons).

FIG 1 TPC1 protein is absent in transgenic TPC1 mice. (A) Photolabeling data using 5N\(_3\)-[\(^{32}\)P]NAADP showing specific photolabeling of an ∼23-kDa candidate NAADP binding protein in mouse pancreatic samples from a matched littermate mouse (TPC1\(^{+/+}\) [left]) and a TPC knockout mouse (TPC\(^{-/-}\) [right]; Tpcn1\(^{-/-}\) ). Further detail is available in reference 11. (B) Schematic of TPC1 membrane topology to highlight the COOH-terminal epitope (red) present in wild-type TPC1 and the putative NH\(_2\)-terminal truncation of TPC1B (the missing sequence is shown with a black line). (C and D) Western blot analysis of liver samples from wild-type (+/+) and Tpcn1\(^{-/-}\) mice described in reference 11 using an antibody raised to the C terminus of TPC1 (ab80961; Abcam). (D) Samples were treated with peptide-N-glycosidase F (PNGase F) (right) to remove N-linked oligosaccharides as described in reference 17. Note the absence of major immunoreactive bands (solid arrowheads) in the transgenic mice. Bands present in both samples (open arrowheads) are likely nonspecific, but we cannot rule out the possibility of severely truncated, nonfunctional TPC1 variants that escape inactivation. Numbers at the left of the gels are molecular masses (in kilodaltons).

Citation


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TPC1 Knockout Knocks Out TPC1

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TWO-PORE CHANNELS (TPCS) ARE ANCIENT ION CHANNELS THAT RESIDE WITHIN ACIDIC ORGANELLES, SUCH AS ENDOSOMES AND LYSOSONES (1). TPCs ARE THE PROPOSED TARGET OF THE SECOND MESSENGER NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE (NAADP), WHICH MOBILIZES CA\(^{2+}\) FROM “ACIDIC CA\(^{2+}\) STORES” (2). HOWEVER, FUNDAMENTAL ASPECTS OF TPC BIOLOGY—HOW THEY ACTIVATE AND THEIR NATIVE IONIC PERMEABILITY (3–8)—REMAIN CONTROVERSIAL (REVIEWED IN REFERENCES 9 AND 10).

PHOTOLABELING STUDIES USING A RADIOACTIVE NAADP-DERIVED PROBE (5N\(_3\)-[\(^{32}\)P]NAADP) HAVE SUGGESTED THAT NAADP MAY NOT DIRECTLY BIND TO TPCs BUT MAY STIMULATE CHANNEL ACTIVITY VIA SMALL NAADP-BINDING PROTEINS (∼23 kDA) ASSOCIATED WITH THE TPC COMPLEX (11–14). ONE PIECE OF EVIDENCE SUPPORTING THIS IDEA IS THE PRESERVATION OF PHOTOLABELING IN TPC KNOCKOUT MICE (11). FOR EXAMPLE, AS SHOWN IN FIG. 1A, NAADP-SPECIFIC LABELING AT 23 kDA IS APPARENT IN THE KNOCKOUT TPC\(^{-/-}\) MOUSE SAMPLE AND SELECTIVELY DISPLACED BY UNLABELED NAADP, WITH AFFINITY SIMILAR TO THAT OF WILD-TYPE MICE. THESE DATA INDICATE THAT TPCs ARE UNLIKELY THE DIRECT TARGETS OF NAADP.

IN A RECENTLY PUBLISHED STUDY (15), RUAS AND COLLEAGUES CHALLENGED THIS INTERPRETATION. THEIR DATA SUGGEST THE EXISTENCE OF A MOUSE TPC1 VARIANT (TPC1B) WITH A TRUNCATED NH\(_2\)-TERMINAL DOMAIN COMPARED TO THE PREVIOUSLY STUDIED TPC1 ISOFORM (TPC1A). IF THIS TPC1B VARIANT WERE TO BE PREVALENTLY EXPRESSED, OR IF GENE TRAP SKIPPING OCCURRED IN THE PREVIOUSLY USED TPC1 NULL LINE (11), WESTERN BLOTTING DATA USING 5N\(_3\)-[\(^{32}\)P]NAADP SHOWING SPECIFIC PHOTOLABELING OF AN APPARENT MOLECULAR MASS OF ∼23 kDA CANDIDATE NAADP BINDING PROTEIN IN MOUSE PANCREATIC SAMPLES FROM A MATCHED LITTERMATE MOUSE (TPC1\(^{+/+}\) [LEFT]) AND A TPC KNOCKOUT MOUSE (TPC\(^{-/-}\) [RIGHT]; TPcNh1\(^{-/-}\) ) (FIG. 4B IN REFERENCE 15). FURTHER DETAIL IS AVAILABLE IN REFERENCE 11. (B) SCHEMATIC OF TPC1 MEMBRANE TOPOLOGY TO HIGHLIGHT THE COOH-TERMINAL EPITOPE (RED) PRESENT IN WILD-TYPE TPC1 AND THE PUTATIVE NH\(_2\)-TERMINAL TRUNCATION OF TPC1B (THE MISSING SEQUENCE IS SHOWN WITH A BLACK LINE). (C AND D) WESTERN BLOT ANALYSIS OF LIVER SAMPLES FROM WILD-TYPE (+/+) AND TPCn1\(^{-/-}\) MICE DESCRIBED IN REFERENCE 11 USING AN ANTIBODY RAISED TO THE C TERMINUS OF TPC1 (AB80961; ALCAM). (D) SAMPLES WERE TREATED WITH Peptide-N-Glycosidase F (PNGase F) (RIGHT) TO REMOVE N-LINKED OLIGOSACCHARIDES AS DESCRIBED IN REFERENCE 17. NOTE THE ABSENCE OF MAJOR IMMUNOREACTIVE BANDS (SOLID ARROWHEADS) IN THE TRANSGENIC MICE. BANDS PRESENT IN BOTH SAMPLES (OPEN ARROWHEADS) ARE LIKELY NONSPECIFIC, BUT WE CANNOT RULE OUT THE POSSIBILITY OF SEVERELY TRUNCATED, NONFUNCTIONAL TPC1 VARIANTS THAT ESCAPE INACTIVATION. NUMBERS AT THE LEFT OF THE GELS ARE MOLECULAR MASSES (IN KILOCALTONS).
scores prior conclusions (11–13). Repetition of our published photolabeling experiments with Tpcn1\textsuperscript{LIXKO-471} samples characterized by Ruas and colleagues (15) is expected to yield a similar result. Comparison of whole animal phenotypes between different TPC1\textsuperscript{−/−} strains will require further investigation.

In conclusion, the Tpcn1\textsuperscript{LIXKO-471} mouse line can be employed by the community as a Tpcn1\textsuperscript{−/−} sample.

REFERENCES


