ROLE OF TRPML AND TPC CHANNELS IN ENDO-LYSOSOMAL CATION HOMEOSTASIS

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LTS, lysosomal targeting sequence; ML IV, mucolipidosis type IV; TRP, transient receptor potential
ABSTRACT

The transient receptor potential (TRP) channels TRPML1, TRPML2 and TRPML3 (also called mucolipins 1-3 or MCOLN1-3) are non-selective cation channels. Mutations in the Trpml1 gene cause mucolipidosis type IV in humans with clinical features including psychomotor retardation, corneal clouding, and retinal degeneration while mutations in the Trpml3 gene cause deafness, circling behaviour, and coat color dilution in mice. No disease causing mutations are reported for the Trpml2 gene. Like TRPML channels which are expressed in the endo-lysosomal pathway, TPC channels (two-pore channels), namely TPC1, TPC2, and TPC3 are found in intracellular organelles, in particular in endosomes and lysosomes. Both, TRPML and TPC channels may function as calcium/cation release channels in endosomes, lysosomes and lysosome related organelles with TRPML1 being activated by PI(3,5)P₂ and regulated by pH, and TPC channels being activated by NAADP in a calcium and pH dependent manner. They may also be involved in endo-lysosomal transport and fusion processes, e.g. as an intracellular calcium source. Currently however, it still remains quite elusive what exactly the physiological role(s) of TRPML and TPC channels are, and whether TRPML channels are purely endo-lysosomal ion channels or whether they may also be functionally active at the plasma membrane in-vivo.
INTRODUCTION

The lysosome received its name in 1955. Christian de Duve, the discoverer of the lysosome (de Duve et al., 1955) and also the peroxisome (de Duve & Baudhuin, 1966), chose the term ‘lysosome’, ‘Greek for digestive body’, for a class of granules that he found to be rich in hydrolytic enzymes, namely acid phosphatase, ribonuclease, deoxyribonuclease, cathepsin and β-glucuronidase (de Duve et al., 1955). Today we know that lysosomes contain more than 60 different degradative enzymes that can hydrolyze proteins, DNA, RNA, polysaccharides, and lipids (Saftig & Klumperman, 2009; Schröder et al., 2010).

Most of the lysosomal enzymes are acid hydrolases, which work best at the acidic pH (about 4.5) that is maintained within lysosomes but not at the neutral pH (about 7.2) characteristic of the rest of the cytosol. The requirement of these lysosomal hydrolases for acidic pH provides an effective protection against uncontrolled digestion of the contents of the cytosol. To maintain their acidic luminal pH, lysosomes must actively concentrate protons. This is accomplished by a proton pump (V-type H⁺ ATPase) in the lysosomal membrane, which actively transports protons into the lysosome from the cytosol. This pumping requires energy in the form of ATP hydrolysis, since it maintains approximately a 100 to 1000-fold higher proton concentration inside the lysosome.

Mutations in the genes that encode lysosomal enzymes are responsible for more than 30 different human genetic diseases, which are called lysosomal storage diseases or disorders (LSD) because undegraded material accumulates within the lysosomes of affected individuals.
Lysosomal storage disorders

LSDs are usually grouped biochemically by the accumulated metabolite. Subgroups include mucopolysaccharidoses, sphingolipidoses and mucolipidoses (for review see e.g. Vellodi, 2005 or Cox & Cachón-González, 2012).

While mucopolysaccharidoses are inherited deficiencies of enzymes involved in glycosaminoglycan breakdown (for review see e.g. Coutinho et al., 2012) sphingolipidoses are caused by malfunction of enzymes that are involved in the breakdown of sphingolipids (for review see e.g. Ozkara, 2004 or Eckhardt et al., 2010). Gaucher disease is the most common sphingolipidosis. Others include Tay-Sachs, Sandhoff, Fabry, or Krabbe disease.

Mucolipidoses are divided into four different types. Mucolipidosis type I (ML I) or sialidosis results from a deficiency in one of the digestive enzymes known as sialidase. Mucolipidosis types II and III (ML II and ML III) result from a deficiency of the enzyme N-acetylglucosamine-1-phosphotransferase (for review see e.g. Dierks et al., 2009). ML II is a particularly severe form of ML that resembles one of the mucopolysaccharidoses called Hurler syndrome. In contrast, ML III produces less severe symptoms and progresses more slowly, probably because the deficient enzyme retains some of its activity.

ML IV is an autosomal recessive LSD characterized by severe psychomotor retardation and ophthalmologic abnormalities including corneal opacity, retinal degeneration and strabismus. Storage bodies of lipids and water-soluble substances are seen by electron microscopy in almost every cell type of the patients. Most patients are unable to speak or walk independently. All patients have constitutive achlorhydria associated with a secondary elevation of serum gastrin levels. ML IV is found at relatively high frequency among Ashkenazi Jews. In 1974 Berman and colleagues (Berman et al., 1974) reported an Ashkenazi Jewish infant with congenital corneal
clouding and abnormal systemic storage bodies. Lysosomal hydrolases were normal. The disorder was characterized as a mucolipidosis (mucolipidosis type IV) because electron microscopy showed lysosomal storage of lipids together with water-soluble granulated substances.

The identification of mutations in the \textit{Trpml1} gene as causative for ML IV

Twenty-five years later, in 1999, Slaugenhaupt et al. mapped by linkage analysis the ML IV locus to chromosome 19p13.3-p13.2 (Slaugenhaupt et al., 1999). Finally, in 2000 Bargal et al. identified in an Arab-Druze ML IV patient a homozygous 1048C-T transition in exon 8 of the \textit{Trpml1} gene encoding the TRP (transient receptor potential) cation channel TRPML1, resulting in an arg321-to-ter (R321X) mutation (Fig. 1). The parents were first cousins and carried the same unique haplotype. Likewise in 2000 Sun et al. identified compound heterozygosity for a 3-bp deletion eliminating codon 408 of the \textit{Trpml1} gene in an Ashkenazi Jewish ML IV patient (Sun et al., 2000). In addition, in a non-Ashkenazi Jewish ML IV patient they found compound heterozygosity for 2 mutations in the \textit{Trpml1} gene: a 1209G-T transition resulting in an asp362-to-tyr (D362Y) substitution and a 429C-T transition resulting in an arg102-to-ter (R102X) termination codon (Fig. 1). In 2005, Bach et al. reported three TRPML1 mutations in ML IV patients including a 1207C-T transition resulting in an arg403-to-cys (R403C) substitution and a 235C-T transition resulting in an gln79-to-ter (Q79X) termination codon (Fig. 1). In 2009, Tüysüz et al. reported a Turkish patient who, in addition to the typical neurological and visceral characteristics of ML IV, also demonstrated defects in the posterior limb of internal capsule by MRI, micrognathia and clinodactyly of the fifth fingers. Direct sequencing of his DNA revealed a homozygous 1364C-T (S456L) mutation in TRPML1 (Fig. 1), which was heterozygous in both consanguineous parents.

Today, at least 15 different ML IV causing mutations, most of them point mutations, have been identified throughout the \textit{Trpml1} gene (summarized in Fig. 1).
Similar to the human phenotype, Trpml1 knockout mice display inclusion bodies, enlarged vacuoles, psychomotor defects and retinal degeneration (Venugopal et al., 2007). In addition, Chandra et al. reported that Trpml1 knockout mice have significant impairments in basal and histamine-stimulated gastric acid secretion. Histologic and ultrastructural analyses revealed that Trpml1−/− parietal cells are enlarged and have multivesicular and multi-lamellated lysosomes. Loss of Trpml1 causes reduced levels and mislocalization of the gastric proton pump and alters the secretory canaliculi, causing hypochlorhydria and hypergastrinemia (Chandra et al., 2011). In summary, the reported Trpml1 knockouts exhibit many clinical and cellular features of the human disease.

**Structural and functional aspects of TRPML channels**

TRPML channels were originally called MCOLN channels or mucolipins. Cloning of MCOLN1 also led to the identification of two additional genes, both located on human chromosome 1, MCOLN2 and MCOLN3 (Bargal et al., 2000). Due to structural and sequence similarities with TRP (transient receptor potential) cation channels, MCOLN channels were later named TRPML channels, i.e. TRPML1, TRPML2, and TRPML3.

There are 28 mammalian TRP (transient receptor potential) channels known today. TRP channels are mostly non-selective cation channels containing 6 transmembrane domains (TMD) with the pore between TMD5 and TMD6. TRP channels are generally subdivided, based on sequence similarities, into TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystic), and TRPML (mucolipin) channels (for excellent recent reviews see e.g. Gees et al., 2010 or Nilius & Owsianik, 2011).

Functional characterization of TRPML channels has recently gained momentum with the investigation of the constitutively active inwardly rectifying TRPML3 varitint-waddler mutant isoforms Va (A419P) and VaJ (A419P+I362T) (Fig. 1) and their TRPML1
and TRPML2 counterparts (Grimm et al., 2007; Kim et al., 2007; Nagata et al., 2007; Xu et al., 2007; Dong et al., 2009; Samie et al., 2009; Lev et al., 2010; Grimm et al., 2010; Kim et al., 2010). Since then, other constitutively active mutant isoforms, e.g. the sodium and proton insensitive TRPML3(H283A) mutant isoform, have been identified and characterized (Kim et al., 2008).

TRPML3 Va and VaJ mutations cause deafness, circling behaviour, and coat color dilution in mice due to severe calcium overload in cells natively expressing the channel, e.g. hair cells of the inner ear and melanocytes. The calcium overload can be rescued by coexpressing a plasma membrane ATPase such as PMCA2 (Grimm et al., 2009). Similarly, Lev et al. have demonstrated rescue effects for the TRPML2 Va equivalent isoform when coexpressing PMCA2 (Lev et al., 2010). In addition to the effect of A419P, which is located in the pore forming transmembrane domain 5 (TMD5) on channel activity, it could be demonstrated that other residues within TMD5 of TRPML3 result in the same gain-of-function phenotype when mutated to proline, e.g. M413P, R414P, F415P, C416P, C417P, C418P, G4125P, or C429P. Equivalent mutations in TRPML1 showed similar effects (Grimm et al., 2007; Dong et al., 2009). Likewise it was found that proline mutations at equivalent positions in other TRP channels, not only TRPML channels (Grimm et al., 2007; Dong et al., 2009; Samie et al., 2009) but also TRPV5 and TRPV6 which share a comparably high sequence similarity with TRPML channels in the pore region, have an impact on channel activity (Grimm et al., 2007; Lee et al., 2010). In contrast, more distantly related TRP channels such as TRPC6, TRPV2 or TRPM2 were not affected by such mutations (Grimm et al., 2007).

Based on the Va phenotype, TRPML3 was proposed to play a role in hearing and balance and the channel was put forward as potential candidate for the hair cell mechanoelectrical transduction channel (van Aken et al., 2008). However, as reported by Jörs et al. Trpml3 knockout mice show no circling behaviour, head-bobbing, or
waddling (Jörs et al., 2010). Also, in Rotarod tests no significant differences between knockout mice and control mice were detected. Furthermore, they have normal Preyer's reflexes and in auditory brainstem response (ABR) measurements no hearing threshold differences between knockout mice and controls were detectable. In summary, the data did not indicate that inactivation of Trpml3 leads to hearing or balance defects. Another Trpml3 knockout mouse, generated by Castiglioni et al. was likewise not reported with a hearing phenotype. Expression of TRPML3 in the inner ear was however confirmed and investigated in more detail (Castiglioni et al., 2011). Castiglioni et al. used in situ hybridization, quantitative RT-qPCR, and immunohistochemistry with several antisera raised against TRPML3 to determine the expression and subcellular distribution of TRPML3 in the inner ear. They used the Trpml3 knockout tissues to distinguish TRPML3-specific from nonspecific immunoreactivities and found that TRPML3 localizes to vesicles of hair cells and strial marginal cells but not to stereociliary ankle links or pillar cells. The exact role of TRPML3 in the inner ear remains however unclear.

While the introduction of prolines in TMD5 of TRPML channels causes a gain of function (constitutive activity), ML IV causing mutations generally appear to render TRPML1 non-functional. Dong et al. demonstrated that ML IV mutant isoforms such as T232P, D362Y, F465L, or R403C (Fig. 1; Table I) in combination with the Va equivalent mutation in TRPML1, V432P, abolishes the constitutive activity of TRPML1(V432P) (Dong et al., 2008). One exception was F408Δ which still showed some constitutive activity in combination with TRPML1(V432P). In accordance with this, patients with the F408Δ mutation in TRPML1 reportedly show a very mild ML IV phenotype (Raychowdhury et al., 2004).

Furthermore, Dong et al. found that TRPML1 and TRPML2 channels, but not TRPML3 are permeable for iron and concluded, by showing that cytosolic Fe^{2+}
deficiency is concurrent with intralysosomal iron overload in ML IV cells, that TRPML1 has a critical role in cellular iron homeostasis.

In addition they found that the constitutive activity of TRPML1 and TRPML2 Va isoforms can be potentiated by low pH while the activity of TRPML3 Va is inhibited by low pH (Xu et al., 2007; Dong et al., 2008; Dong et al., 2009). The latter finding is in accordance with results obtained by Kim et al. with wild-type TRPML3, i.e. block of TRPML3 channel activity by low extracellular pH (Kim et al., 2008).

In contrast, Raychowdhury et al. reported an inhibitory effect of low extracellular pH on TRPML1 wild-type activity. Raychowdhury et al. also reported that wild-type TRPML1 is outwardly rectifying when measured in endosomal vesicles reconstituted in a lipid bilayer system (Raychowdhury et al., 2004). Kiselyov and colleagues who reported that a fraction of TRPML1 reaches the plasma membrane when overexpressed in HEK293 cells likewise detected outwardly rectifying currents in whole-cell patch-clamp experiments (Kiselyov et al., 2005; Soyombo et al., 2005).

Dong et al. however showed inwardly rectifying currents for wild-type TRPML channels when activated with PI(3,5)P₂, the major functions of which are in membrane and protein trafficking, and in pH control in the endosome-lysosome axis (Michell et al., 2006). These measurements by Dong et al. were performed as whole-lysosome patch-clamp experiments (Dong et al., 2010). Likewise, it was found that TRPML3 activated by small chemical compounds such as SF-21 (4-chloro-N-(2-morpholin-4-ylcyclohexyl)benzenesulfonamide or SF-81 (4,6-di-methyl-3-(2-methylphenyl)sulfonyl-1-propan-2-yl-pyridin-2-one), identified in a recent high-throughput screening, is inwardly rectifying when overexpressed in HEK293 cells (Grimm et al., 2010; Yamaguchi & Muallem, 2010; Saldanha et al., 2011; Table I). When activated by low extracellular sodium (Grimm et al., 2010) or by sodium deprivation followed by rapid sodium re-addition (Kim et al., 2008) wild-type TRPML3 also shows inward rectification similar to
TRPML Va isoforms. The reason(s) for the different findings regarding TRPML channel rectification remain unclear.

Likewise unclear is how exactly protons and/or sodium regulate TRPML and TRPML3 channel activity in particular. One interesting feature common to all TRPML channels is the conspicuously long extracellular (extracytosolic) loop between transmembrane domains TMD1 and TMD2 which may play different physiological roles in the three TRPML channels (Fig. 1). In TRPML1, this loop appears to contain a serine-lipase motif (GXSXG motif, aa 108-112 in human TRPML1) (Bargal et al., 2000; Akoh et al., 2004) and a proline rich domain (PRD, aa 197-205 in human TRPML1) which are however both not conserved in TRPML2 and TRPML3 (Fig. 1). In TRPML3, the loop has recently been found to contain a proton regulatory domain. Kim et al. have demonstrated that regulation by extracellular pH is completely lost in the constitutively active H283A mutant isoform of TRPML3 which is also insensitive to extracellular sodium. Based on these findings they suggested that the proton regulatory domain in the first extracellular loop (two further histidine residues in the same loop, H252 and H273 appear to also have an effect on pH regulation when mutated) influences the orientation of TMD5 and that binding of protons to H283 exerts a long-range conformational change affecting pore opening (Kim et al., 2008). Due to its unusual length a functional interaction between distinct residues of the first extracellular loop and either the second extracellular loop, between TMD3 and TMD4, or the pore loop, or both may be an intriguing scenario. Among the 20 amino acids, at least 12 (especially charged amino acids: Asp, Glu, Arg, and Lys) can form hydrogen bonds with their side chains. Hydrogen bonds e.g. between His-Glu or His-Asp are a common element in many catalytic sites (Tanaka et al., 1998; Lau et al., 1999). Gao et al. showed in site-directed mutagenesis studies that the closely linked residues E13 and H278 in A(2A) adenosine receptors are involved in ligand binding and sodium modulation (Gao et al., 2000). Like
in a channel selectivity filter, proper geometry and energetics may play an important role in precisely positioning the extracellular loop(s) and thus blocking the TRPML3 pore entrance. Increased extracellular pH and/or absence of extracellular sodium may disrupt this interaction (Fig. 2).

The identified small molecule activators of TRPML channels (Grimm et al., 2010) could act in a similar manner by disrupting the interaction between the two extracellular loops and/or between the first extracellular loop and the pore loop, and may thus lock the channel in an open state. Many of the identified small molecule agonists are sulfonamides and sulfonamides are among the chemical fragments which are able to form hydrogen bonds with several amino acids (Chan et al., 2010), thus enabling them to interfere with potential amino acid interactions in TRPML channels (Fig. 2).

For the future characterization of endogenous TRPML1, TRPML2, and TRPML3 currents it will be important to further assess TRPML isoform selectivity of these compounds using e.g. whole-lysosome patch-clamp techniques and if necessary increase isoform selectivity by chemical modifications. While some of the compounds activate only TRPML3 at concentrations up to 10 \( \mu \)M (Grimm et al., 2010), Shen et al. showed recently that compound SF-51 (2-[2-oxo-2-(2,2,4-trimethylquinolin-1-yl)ethyl]isoindole-1,3-dione) also activates TRPML1 at concentrations of 30 \( \mu \)M. When SF-51 was chemically modified (ML-SA1) it could activate all three TRPML channels at a concentration of 10 \( \mu \)M (Shen et al., 2012). Thus, further effort will be necessary to design TRPML1 or TRPML2 selective compounds. Ligand binding assays and site-directed mutagenesis studies may help to identify activator/ligand binding sites for the future development of more potent and selective pharmacological tools. Chemical modifications may ultimately be applied to design pharmacological tools to restore TRPML1 channel function in the case of loss-of-function mutations causing ML IV. A good example for such a strategy is the CFTR channel. Here, compounds
(benzothiophenes, phenylglycines and sulfonamides) have been developed which are able to correct the defective gating e.g. of ΔF508-CFTR. Other small molecules have been developed that correct its defective cellular processing (van Goor et al., 2011). An interesting compound in this context is VX-770 which has advanced to phase III clinical trials in CF patients (Amaral, 2011; Ashlock & Olson, 2011). Some of the ML IV causing TRPML1 mutations lead to early sequence termination resulting in variants that are lacking the pore domain, hence this strategy may not be applicable in those cases. Nevertheless, in cases of mislocalization or defective gating, the approach may be promising for the treatment of ML IV. However, in contrast to CFTR which is localized in the plasma membrane, TRPML1’s localization in the lysosomal membrane may further hamper compound development.

In lysosomal storage diseases such as Fabry or Gaucher ERT (enzyme replacement therapy) has been applied with some success (Lachmann, 2010; Lachmann, 2011). While intravenous ERT appears to be efficacious at ameliorating non-central nervous system pathology in several LSDs, there is little evidence supporting its use for neurological disease, suggested to be due to rapid clearance via the spleen and liver and the poor ability of enzyme to penetrate the blood-brain barrier (Hemsley & Hopwood, 2011). Neurodegeneration and/or dysmyelination are the hallmark of roughly 70% of LSDs. Gene therapy represents a promising approach for the treatment of CNS manifestations in LSDs, as it has the potential to provide a permanent source of the deficient enzyme, either by direct injection of vectors or by transplantation of gene-corrected cells. In case of ML IV with its severe neurological manifestations the latter option may thus be an alternative to the small molecule therapeutic approach discussed above.

Another unsolved problem from a functional point of view is the question whether TRPML channels are purely endo-lysosomal or intracellular ion channels in-vivo.
TRPML1 at least appears to localize almost exclusively to endo-lysosomal vesicles, both in-vivo and in-vitro, most likely due to the presence of endo-lysosomal targeting sequences (LTS), i.e. dileucine motifs (D/EXXXLL/I) in the N- and C-terminus, respectively (aa 11-16 and aa 573-578 in human TRPML1). If these motifs are cleaved or mutated to alanine (LL/AA) TRPML1 is predominantly found in the plasma membrane (Vergara & Puertollano, 2006; Grimm et al., 2010). However, such motifs are absent in TRPML3, and TRPML2 contains only one such motif in the C-terminus which is not fully conserved in primates including human. Nevertheless, TRPML2 and TRPML3 appear to localize both to the plasma membrane as well as intracellular vesicles in-vitro (Karacsonyi et al., 2007; Kim et al., 2008; Kim et al., 2010; Grimm et al., 2010). This may point to different subcellular functions and physiological roles and possibly to dual functions at the plasma membrane and in intracellular compartments. Dual functional roles have been demonstrated for several other TRP channels which localize both to the plasma membrane and intracellular structures, e.g. TRPM1, TRPM2, TRPM7, TRPM8, TRPV2, TRPV5, or TRPP2 (for review see e.g. Patel & Docampo, 2009; Dong et al., 2010; Gees et al., 2010; Abe & Puertollano, 2011; Tsiokas et al., 2007).

In the overexpression system it appears that TRPML2 and TRPML3 are also functionally active at the plasma membrane (Kim et al., 2008; Kim et al., 2010; Grimm et al., 2010; Saldanha et al., 2011). However, when small molecule agonists shown to activate TRPML3 in-vitro, were applied to detect endogenous TRPML3 currents, TRPML3 activation was either weak (e.g. in human skin melanocytes) or undetectable (e.g. in rat cochlear inner ear hair cells) (Grimm et al., 2010). One explanation for this observation may be heteromultimerization with TRPML1. TRPML channels are able to heteromultimerize with each other. This has been shown by different groups (Curcio-Morelli et al., 2010; Grimm et al., 2010; Venkatachalam et al., 2006; Zeevi et al., 2009; Zeevi et al., 2010). In addition, in-vitro TRPML1/TRPML3 coexpression experiments
indicated that TRPML1 is able to suppress TRPML3 activation by small molecule agonists (Grimm et al., 2010). In-vivo, TRPML1 may likewise control TRPML3 and possibly also TRPML2 surface expression and tightly regulate the amount of functional channel at the plasma membrane by retaining the majority of protein in intracellular vesicles. The degree of heteromultimerization and intracellular retention may vary depending on expression levels. Whether this is indeed the case in-vivo needs to be further clarified.

Towards a physiological role of TRPML and TPC channels

In addition to PI(3,5)P2 mentioned above, TRPML1 was also reported to be activated by nicotinic acid adenine dinucleotide phosphate (NAADP) (Zhang et al., 2007). NAADP is an endogenous activator of two-pore channels (TPCs), a family of novel intracellular ion channels containing 12 transmembrane domains and predicted to form dimers (Fig. 1) (Brailoiu et al., 2009; Calcraft et al., 2009; Galione, 2011; Rietdorff et al., 2011; Schieder et al., 2010; Zhu et al., 2009; Zong et al., 2009;). Interestingly, TRPML1 and to some extent TRPML3 co-immunoprecipitate with TPC1 and TPC2 channels but do reportedly not interact with each other functionally (Yamaguchi et al., 2011). The authors concluded that although TRPMLs and TPCs are present in the same organelles and can physically interact with each other, they function as independent organellar ion channels. Yamaguchi et al. found no evidence of TRPML1 in regulating NAADP responses.

Release of stored calcium by calcium-mobilizing messengers such as inositol trisphosphate, cyclic ADP-ribose, and NAADP is a ubiquitous mechanism for effecting changes in cytosolic calcium (Berridge et al., 2000). Inositol trisphosphate receptors and ryanodine receptors are well-defined ER calcium channels that open in response to receptor-mediated generation of inositol trisphosphate and cyclic ADP-ribose, respectively (Berridge et al., 2000). However, the molecular identity of the channel(s)
involved in NAADP mediated calcium release remained elusive until recently. Several groups have now demonstrated that TPC channels are endo-lysosomal proteins which are activated by NAADP (Brailoiu et al., 2009; Calcraft et al., 2009; Galione et al., 2010; Galione, 2011; Schieder et al., 2010; Zhu et al., 2009; Zong et al., 2009). Knockdown of wild-type TPC channels or overexpression of pore mutants inhibited NAADP responses and pancreatic β-cells from Tpc2 knockout mice have been reported to be NAADP insensitive (Calcraft et al., 2009). TPCs recapitulate many features of endogenous NAADP-sensitive calcium channels, thus providing strong evidence for their role as NAADP targets. However, whether NAADP binds directly to TPCs or activates TPCs indirectly remains unclear. Lin-Moshier et al. and Walseth et al. have recently suggested, based on photolabeling data that an accessory component within a larger TPC complex may be responsible for binding NAADP (Lin-Moshier et al., 2011; Walseth et al., 2011). In contrast, Calcraft et al. had concluded previously, based on ligand competition assays that NAADP binds directly to TPC2. Calcraft et al. emphasized that it could not be excluded that interactions with accessory proteins may be necessary for NAADP binding to TPC2, however that such proteins would have to associate with TPC2 tightly in order to explain their binding results (Calcraft et al., 2009). Future studies will have to clarify how exactly NAADP activates TPC channels.

While TPC1 and TPC2 are functionally expressed in humans and rodents, Tpc3 is a pseudogene in humans and some primates, and the gene is completely missing in mice and rats (Zhu et al., 2009). By analyzing the sequence data from ten primate species, Cai & Patel have recently determined the degeneration process of the Tpc3 gene (Cai & Patel, 2010). They showed that degeneration of Tpc3 likely began in the common ancestors of Apes and Old World monkeys through a conserved inactivating mutation, followed by additional deleterious mutations resulting in the generation of a Tpc3 pseudogene in the descendant catarrhine lineage. Located at a chromosome
recombination hot spot, catarrhine Tpc3 pseudogenes underwent a series of lineage-specific rearrangements, including exon deletion and duplication. In contrast, they identified near full-length Tpc3 sequences in New World monkeys and Prosimians. The evolutionary path that has led to the deletion of Tpc3 in mice and rats remains unknown. The fact that Tpc3 but also Tpc1 or Tpc2 are partially or fully absent in some species may point to functional redundancy of Tpc genes (Zhu et al., 2009).

There are no disease-related (point) mutations known for human or rodent TPC channels. However, Sulem et al. have recently postulated a link between human TPC2 mutations and pigmentation (Sulem et al., 2008). Four SNPs on 11q13.2 showed association with blond versus brown hair color in an Icelandic discovery sample that reached genome-wide significance. These SNPs are located within a single LD (linkage disequilibrium) block that overlaps with only one gene, TPC2. All of the observed association with blond versus brown hair could be explained by two of the coding SNPs: rs35264875 (encoding M484L) and rs3829241 (encoding G734E) (Sulem et al., 2008; Sturm, 2009) (Fig. 1). These findings point to a potential role of TPC2 channels in melanosomes which are lysosome related organelles (LRO).

Lysosomes are derived from late endosomes (LEs). While early endosomes (EEs) usually have a luminal pH of about 6, LEs have a luminal pH of approximately 5.5 and finally lysosomes have a pH of about 4.5. Likewise, the melanosomal pH varies during melanosome maturation. However, while premelanosomes have a luminal pH of about 5, mature melanosomes have a pH of about 6.8. The optimum for tyrosinase activity, a key enzyme of melanin synthesis is at pH 6.5-7.0 (Schallreuter et al., 2007). Ancans et al. reported that melanin production in Caucasian melanocytes is suppressed by low melanosomal pH and that the ratio of eumelanin/phaeomelanin production and maturation rate of melanosomes can be regulated by melanosomal pH (Ancans et al.,
Thus, melanosomal pH appears to be highly critical in determining the pigmentation phenotype (Ito & Wakamatsu, 2011).

Besides the melanosomal proton concentration, calcium also appears to be highly critical for melanogenesis (Schallreuter et al., 2007). It is therefore tempting to speculate that pH-regulated calcium permeable ion channels such as TRPML and TPC channels may be important regulators and sensors of endo-lysosomal and/or melanosomal proton and calcium levels (Pitt et al., 2010; Lloyd-Evans & Platt, 2011; Abe & Puertollano, 2011). Since TRPML channels have also been shown to be permeable for other cations, not only sodium or potassium but also iron or zinc (Dong et al., 2008; Eichelsdoerfer et al., 2010), they may have a more general role in controlling cation/heavy metal homeostasis in lysosomes and LROs such as endosomes and melanosomes (Lelouvier & Puertollano, 2011; Kiselyov et al., 2011). Furthermore, it is currently unclear which channels provide the calcium that is necessary for intracellular vesicle fusion processes (either homotypic or heterotypic fusion), e.g. between endosomes, endosomes and lysosomes or phagosomes and lysosomes. In this context, it will be important to investigate the protein-protein-interaction networks of TRPML as well as TPC channels in more detail to better understand and appreciate functional and physiological roles of these channels in their respective environments.

**AUTHORSHIP CONTRIBUTIONS**

CG, SH, CW-S and MB wrote the manuscript.
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pore channels are functionally independent organellar ion channels. *J. Biol. Chem.* 286:22934-22942


### TABLE I

<table>
<thead>
<tr>
<th>Family</th>
<th>TRPML1</th>
<th>TRPML2</th>
<th>TRPML3</th>
<th>TPC1</th>
<th>TPC2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td>MCOLN1</td>
<td>MCOLN2</td>
<td>MCOLN3</td>
<td>888 (816)</td>
<td>752</td>
</tr>
<tr>
<td><strong>Synonyms</strong></td>
<td></td>
<td></td>
<td></td>
<td>817</td>
<td>731</td>
</tr>
<tr>
<td><strong>Length (aa) Hs</strong></td>
<td>580</td>
<td>566</td>
<td>553</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Length (aa) Mm</strong></td>
<td>566 (538)</td>
<td>553</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seq motifs</strong></td>
<td>Serine-ipease; Lysosomal targeting seq. (N- and C-terminal); Proline rich domain (PRD)</td>
<td>Lysosomal targeting seq. (N-terminal in Mm)</td>
<td>N.D.</td>
<td>Legume lectins beta-chain signature; Lysosomal targeting seq. (N-terminal)</td>
<td></td>
</tr>
<tr>
<td><strong>Localization</strong></td>
<td>Lysosomes; LRO?</td>
<td>PM (in-vitro); lysosomes?; LRO?; endosomes</td>
<td>PM (in-vitro); (early)late endosomes; melanosomes?</td>
<td>Endosomes</td>
<td>Lysosomes; LRO? melanosomes?</td>
</tr>
<tr>
<td><strong>Tissue distribution</strong></td>
<td>Ubiquitous</td>
<td>Thymus, spleen, kidney, trachea, liver, lung, colon, tests, thyroid, inner ear?, lymphocyte B-cells</td>
<td>Hair cells of the inner ear, organ of cort, utricle, srm vacuulars, (sk) melanocytes, kidney, lung, liver, olaffatory bulb, nasal cavity, thymus, colon, trachea, brain?</td>
<td>Ubiquitous</td>
<td>Ubiquitous</td>
</tr>
<tr>
<td><strong>Activators</strong></td>
<td>NAADP?; Pi(3,5)P?; ML-SA1; SF-22; SF-51 (30 µM)</td>
<td>Pi(3,5)P?; ML-SA1; SF-21; SF-41; SF-81</td>
<td>Pi(3,5)P?; ML-SA1; SN-1; SN-2; SF-11; SF-21; SF-22; SF-23; SF-24; SF-31; SF-32; SF-33; SF-41; SF-51; SF61; SF-71; SF-81</td>
<td>NAADP</td>
<td>NAADP</td>
</tr>
<tr>
<td><strong>Inhibitors</strong></td>
<td>Sphingomyelins (SMs)</td>
<td>N.D.</td>
<td>Low pH and high extracellular Na⁺</td>
<td>Ned-19</td>
<td>Ned-19</td>
</tr>
<tr>
<td><strong>Regulators</strong></td>
<td>Low pH, Smases and sphingosine potentiates</td>
<td>Low pH potentiates</td>
<td>Low extracellular Na⁺ potentiates activation by chemical compounds</td>
<td>N.D.</td>
<td>Low pH and Ca²⁺ potentiates?</td>
</tr>
<tr>
<td><strong>Disease mutations or polymorphisms associated with a phenotype</strong></td>
<td>Mucolipidosis type IV (ML IV) is associated with mutations in HsTRPML1; symptoms include severe psychomotor retardation and retinal degeneration</td>
<td>N.D.</td>
<td>Deafness, circling behaviour, head bobbing and coat color dilution is associated with mutations in MmTRPML3 (Varitint-wadder mutations Va and Va⁺)</td>
<td>N.D.</td>
<td>Polymorphisms in HsTPC2 are associated with blond versus brown hair</td>
</tr>
<tr>
<td><strong>Gain-of-function mutants</strong></td>
<td>V432P</td>
<td>A396P (Mm)</td>
<td>Va (A419P) and Va⁺ (A419P+362T)</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>Disease-associated loss-of-function mutants</strong></td>
<td>e.g. T232P, D362Y, F465L, R403C, F408Δ</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td><strong>Knockout mouse models</strong></td>
<td>Knockout mice display enlarged vacuoles, psychomotor defects and retinal degeneration</td>
<td>Knockout mice display no auditory or vestibular phenotype and no coat color dilution</td>
<td>Overexpression increases endosomal pH; Endosomal pH regulation and cation homeostasis?; Vesicle fusion and transport?</td>
<td>N.D.</td>
<td>Pancreatic beta-cells from TPC2 knockout mice are NAADP insensitive</td>
</tr>
<tr>
<td><strong>Functions</strong></td>
<td>Role in sorting/transport in late endocytic pathway; Regulates lysosomal lipid and cholesterol trafficking; Endo-lyosomal pH regulation and cation/heavy metal (iron) homeostasis?; NAADP receptor?</td>
<td>Endo-lysosomal pH regulation and cation homeostasis?; Vesicle fusion and transport?</td>
<td>Overexpression increases endosomal pH; Endosomal pH regulation and cation homeostasis?; Vesicle fusion and transport?</td>
<td>NAADP receptor complex?; Vesicle fusion and transport?; Endo-lysosomal pH and Ca²⁺ regulation?</td>
<td>NAADP receptor complex?; Vesicle fusion and transport?; Endo-lysosomal pH and Ca²⁺ regulation?</td>
</tr>
<tr>
<td><strong>Interacting proteins</strong></td>
<td>TRPML2, TRPML3, TPC1?, TPC2?, LAPTMs; Hsp40; Hsc70</td>
<td>TRPML1, TRPML3, Hsc70?</td>
<td>TRPML1, TRPML2, TPC1?, TPC2, Hsc70?</td>
<td>TRPML1, TRPML3</td>
<td>TRPML1, TRPML3</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. **TRPML and TPC channel cartoons.** Upper panel: Cartoon of human TRPML1 displaying the estimated positions of mutations causing ML IV (red) as well as the estimated positions of the two LTS (lysosomal targeting sequence), the predicted serine-lipase motif (GXSXG), and the PRD (proline rich domain). Cartoons of murine TRPML2 (which in contrast to human TRPML2 contains a classical LTS (D/EXXXLL/I) in the C-terminus) and murine TRPML3 which does not contain LTS. The estimated positions of the mutations in murine TRPML3 which cause the varitint-waddler phenotypes Va (A419P) and VaJ (A419P+I362T) and which result in deafness, circling behaviour, and coat color dilution in mice are highlighted in red. The mutation causing constitutive activity with sodium and proton insensitivity (H283A) is depicted in light green. TRPML channels are predicted to form tetramers (4x). Lower panel: Cartoons showing the human TPC1 and TPC2 (two-pore channel) isoforms which are predicted to form dimers (2x). TPC1 contains a legume lectins beta-chain signature (LLBCS) shortly after TMD6 marked in orange (aa 316-322 LAVVFDT, D is predicted to bind calcium and manganese). Estimated positions of the two polymorphisms in human TPC2 associated with blond versus brown phenotype are highlighted in red.

Figure 2. **Hypothetical model of TRPML3 inhibition by low pH and sodium, and activation by small molecules.** Left: Cartoon displaying the TRPML3 channel tetramer with the large extracellular loop between TMD1 and TMD2. Right: Hypothetical change in conformation when TRPML3 is exposed to high extracellular sodium or low pH or both. Center top: Chemical formulae of SF-21 (4-chloro-N-(2-morpholin-4-yl-cyclohexyl)benzenesulfonamide, SF-41 (1-(2,4-dimethylphenyl)-4-piperidin-1-yl-sulfonyl
piperazine), and SF-81 (4,6-di-methyl-3-(2-methylphenyl)sulfonyl-1-propan-2-yl-pyridin-2-one) which are activators of both TRPML2 and TRPML3.