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Perspectives in Pharmacology

Role of TRPML and Two-Pore Channels in Endolysosomal Cation Homeostasis

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ABSTRACT

The transient receptor potential (TRP) channels TRPML1, TRPML2, and TRPML3 (also called mucolipins 1–3 or MCOLN1–3) are nonselective cation channels. Mutations in the *Trpml1* gene cause mucolipidosis type IV in humans with clinical features including psychomotor retardation, corneal clouding, and retinal degeneration, whereas mutations in the *Trpml3* gene cause deafness, circling behavior, and coat color dilution in mice. No disease-causing mutations are reported for the *Trpml2* gene. Like TRPML channels, which are expressed in the endolysosomal pathway, two-pore channels (TPCs), namely TPC1, TPC2, and TPC3, are found in intracellular organelles, in particular in endosomes and lysosomes. Both TRPML channels and TPCs may function as calcium/cation release channels in endosomes, lysosomes, and lysosome-related organelles with TRPMLs being activated by phosphatidylinositol 3,5-bisphosphate and regulated by pH and TPCs being activated by nicotinic acid adenine dinucleotide phosphate in a calcium- and pH-dependent manner. They may also be involved in endolysosomal transport and fusion processes, e.g., as intracellular calcium sources. Currently, however, the exact physiological roles of TRPML channels and TPCs remain quite elusive, and whether TRPML channels are purely endolysosomal ion channels or whether they may also be functionally active at the plasma membrane in vivo remains to be determined.

Introduction

The lysosome received its name in 1955. Christian de Duve, the discoverer of the lysosome (de Duve et al., 1955) and the peroxisome (de Duve and Baudhuin, 1966), chose the term "lysosome," which is Greek for digestive body, for a class of granules 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 and Klumperman, 2009; Schröder et al., 2010).

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Most of the lysosomal enzymes are acid hydrolases, which work best at acidic pH (approximately 4.5) that is maintained within lysosomes but not at neutral pH (approximately 7.2), which is 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, because 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 dis-

ABBREVIATIONS: LSD, lysosomal storage disease; CFTR, cystic fibrosis transmembrane conductance regulator; LRO, lysosome-related organelle; LTS, lysosomal-targeting sequence; ML, mucolipidosis; ML IV, ML type IV; MCOLN, mucolipin; NAADP, nicotinic acid adenine dinucleotide phosphate; $PI(3,5)P_2$, phosphatidylinositol 3,5-bisphosphate; PRD, proline-rich domain; SNP, single-nucleotide polymorphism; TMD, transmembrane domain; TPC, two-pore channel; TRP, transient receptor potential; SF-21, 4-chloro-*N*-(2-morpholin-4-yl-cyclohexyl)benzenesulfonamide; SF-41, 1-(2,4-dimethylphenyl)-4-piperidin-1-yl-sulfonyl piperazine; SF-51, 2-[2-oxo-2-(2,2,4-trimethylquinolin-1-yl)ethyl]isoindole-1,3-dione; SF-81, 4,6-di-methyl-3-(2-methylphenyl)sulfonyl-1-propan-2-yl-pyridin-2-one; ML-SA1, 2-[2-oxo-2-(2,2,4-trimethyl-3,4-dihydroquinolin-1-yl)ethyl]isoindole-1, 3-dione; VX-770, *N*-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide.

eases, which are called lysosomal storage diseases (LSDs) 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 (Vellodi, 2005; Cox and Cachón-González, 2012).

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

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

ML type IV (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. 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 *Trpml1* Gene as Causative for ML IV

Twenty-five years later, Slaugenhaupt et al. (1999) mapped by linkage analysis the ML IV locus to chromosome 19p13.3-p13.2. Finally, Bargal et al. (2000) identified in an Arab-Druze patient with ML IV a homozygous 1048C-T transition in exon 8 of the *Trpml1* gene encoding the transient receptor potential (TRP) 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, Sun et al. (2000) identified compound heterozygosity for a three-base pair deletion eliminating codon 408 of the *Trpml1* gene in an Ashkenazi Jewish patient with ML IV. In addition, in a non-Ashkenazi Jewish patient with ML IV they found compound heterozygosity for two mutations in the *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). Bach et al. (2005) reported three TRPML1 mutations in patients with ML IV, including a 1207C-T transition resulting in an Arg403-to-Cys (R403C) substitution and a 235C-T transition resulting in a Gln79-to-Ter (Q79X) termination codon (Fig. 1). Tüysüz et al. (2009) reported a Turkish patient, who, in addition to the typical neurological and visceral characteristics of ML IV, demonstrated defects in the posterior limb of internal capsule by magnetic resonance imaging, micrognathia, and clinodactyly of the fifth fingers. Direct

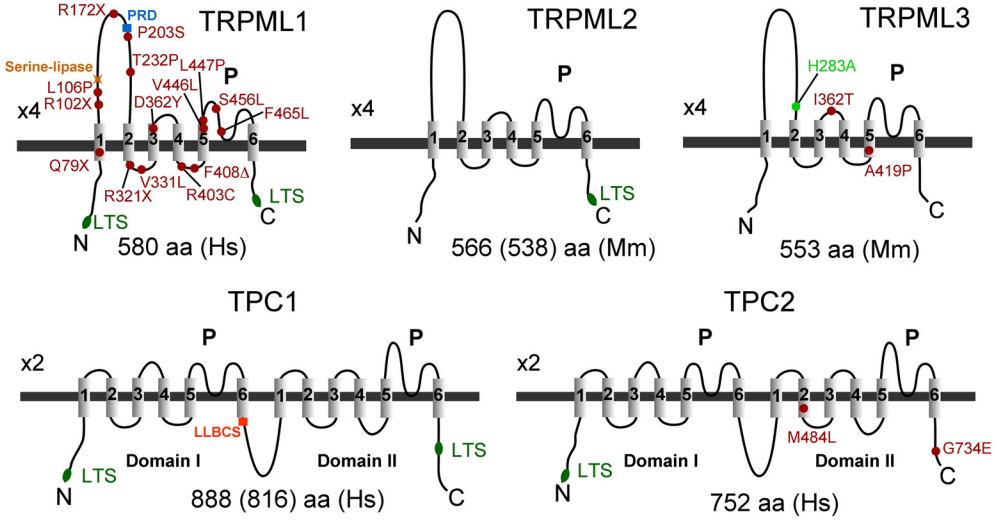

Fig. 1. TRPML channel and TPC cartoons. Top left, cartoon of human TRPML1 displaying the estimated positions of mutations causing ML IV (red) and the estimated positions of the two LTSs, the predicted serine-lipase motif (GXSXG) and the PRD. Top, center and right, cartoons of murine TRPML2 (center), which in contrast to human TRPML2 contains a classic LTS (D/EXXXLL/I) in the C terminus, and murine TRPML3 (right), which does not contain LTS. The estimated positions of the mutations in murine TRPML3, which cause the varitint-waddler phenotypes Va (A419P) and Va^{J} (A419P+I362T) and result in deafness, circling behavior, 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 (4×). Bottom, cartoons showing the human TPC1 (left) and TPC2 (right) isoforms that are predicted to form dimers (2×). TPC1 contains a legume lectin β-chain signature (LLBCS) shortly after TMD6 marked in orange (amino acids 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.

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 *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. (2011) 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 multilamellated 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). Because of structural and sequence similarities with TRP cation channels, MCOLN channels were later named TRPML channels, i.e., TRPML1, TRPML2, and TRPML3.

There are 28 mammalian TRP channels known today. TRP channels are mostly nonselective cation channels containing six transmembrane domains (TMDs) 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 (Gees et al., 2010; Nilius and Owsianik, 2011).

Functional characterization of TRPML channels has gained momentum with the investigation of the constitutively active inwardly rectifying TRPML3 varitint-waddler mutant isoforms Va (A419P) and Va^J (A419P + I362T) (Fig. 1) and their TRPML1 and TRPML2 counterparts (Grimm et al., 2007, 2010; Kim et al., 2007, 2010; Xu et al., 2007; Nagata et al., 2008; Dong et al., 2009; Samie et al., 2009; Lev 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 Va^J mutations cause deafness, circling behavior, and coat color dilution in mice because of 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 plasma membrane calcium-ATPase 2 (Grimm et al., 2009). Similarly, Lev et al. (2010) have demonstrated rescue effects for the TRPML2 Va equivalent isoform when coexpressing plasma membrane calcium-ATPase 2. In addition to the effect of A419P, which is located in the pore forming 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, and 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. (2010), *Trpml3* knockout mice show no circling behavior, head bobbing, or waddling. In addition, 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 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. (2011), likewise was 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 reverse transcription-polymerase chain reaction, 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 unclear, however.

Although the introduction of prolines in TMD5 of TRPML channels causes a gain of function (constitutive activity), ML IV-causing mutations generally seem to render TRPML1 nonfunctional. Dong et al. (2008) demonstrated that ML IV mutant isoforms such as T232P, D362Y, F465L, or R403C (Fig. 1; Table 1) in combination with the Va equivalent mutation in TRPML1, V432P, abolish the constitutive activity of TRPML1(V432P). 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 they 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, the constitutive activity of TRPML1 and TRPML2 Va isoforms can be potentiated by low pH, whereas the activity of TRPML3 Va is inhibited by low pH (Xu et al., 2007; Dong et al., 2008, 2009). The latter finding is in accor-

TABLE 1
Summary of TPC and TRPML channel characteristics

Family Name	TRP			TPC	
	TRPML1	TRPML2	TRPML3	TPC1	TPC2
Synonyms	MCOLN1	MCOLN2	MCOLN3	TPCN1	TPCN2
Length (aa) Hs	580	566	553	888 (816)	752
Length (aa) Mm	580	566 (538)	553	817	731
Seq motifs	Serine-lipase; lysosomal targeting sequence (N and C terminal); PRD	Lysosomal targeting sequence (N terminal in Mm)	N.D.	Legume lectins β-chain signature; lysosomal targeting sequence (N and C terminal)	Lysosomal targeting sequence (N terminal)
Localization	Lysosomes; LRO?	PM (in vitro); lysosomes?; LRO?; endosomes	PM (in vitro); (early/late) endosomes; melanosomes?	Endosomes	Lysosomes; LRO? melanosomes?
Tissue distribution	Ubiquitous	Thymus, spleen, kidney, trachea, liver, lung, colon, testis, thyroid, inner ear?, lymphocyte B cells	Hair cells of the inner ear, organ of corti, utricle, stria vascularis, (skin) melanocytes, kidney, lung, liver, olfactory bulb, nasal cavity, thymus, colon, trachea, brain?	Ubiquitous	Ubiquitous
Activators	NAADP?; $PI(3,5)P_2$; ML-SA1; SF-22; SF-51 (30 μM)	$PI(3,5)P_2$; ML-SA1; SF-21; SF-41; SF-81	$PI(3,5)P_2$; 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; SF-61; SF-71; SF-81	NAADP	NAADP
Inhibitors	Sphingomyelins	N.D.	Low pH and high extracellular Na^+	Ned-19	Ned-19
Regulators	Low pH, Smases and sphingosine potentiate	Low pH potentiates	Low extracellular Na^+ potentiates activation by chemical compounds	N.D.	Low pH and Ca^{2+} potentiate?
Disease mutations or polymorphisms associated with a phenotype	ML IV is associated with mutations in HsTRPML1; symptoms include severe psychomotor retardation and retinal degeneration	N.D.	Deafness, circling behavior, head bobbing, and coat color dilution is associated with mutations in MmTRPML3 (Varitint-waddler mutations Va and Va^J)	N.D.	Polymorphisms in HsTPC2 are associated with blond versus brown hair
Gain-of-function mutants	V432P	A396P (Mm)	Va (A419P) and Va^J (A419P + I362T)	N.D.	N.D.
Disease-associated loss-of-function mutants	T232P, D362Y, F465L, R403C, F408Δ	N.D.	N.D.	N.D.	N.D.
Knockout mouse models	Knockout mice display enlarged vacuoles, psychomotor defects, and retinal degeneration	N.D.	Knockout mice display no auditory or vestibular phenotype and no coat color dilution	N.D.	Pancreatic β-cells from TPC2 knockout mice are NAADP insensitive
Functions	Role in sorting/transport in late endocytic pathway; regulates lysosomal lipid and cholesterol trafficking; endolysosomal pH regulation and cation/heavy metal (iron) homeostasis?; NAADP receptor?	Endolysosomal pH regulation and cation homeostasis?; vesicle fusion and transport?	Overexpression increases endosomal pH; endosomal pH regulation and cation homeostasis?; vesicle fusion and transport?	NAADP receptor complex?; vesicle fusion and transport?; endolysosomal pH and Ca^{2+} regulation?	NAADP receptor complex?; vesicle fusion and transport?; endolysosomal pH and Ca^{2+} regulation?
Interacting proteins	TRPML2, TRPML3, TPC1?, TPC2; LAPTMs; Hsp40; Hsc70	TRPML1, TRPML3, Hsc70?	TRPML1, TRPML2, TPC1?, TPC2, Hsc70?	TRPML1, TRPML3	TRPML1, TRPML3

N.D., not determined; PM, plasma membrane; SF-11, (3-(4-chlorophenyl)-5-methyl-4-[2-(4-methylphenyl)sulfonylpyrazol-3-yl]-1,2-oxazole); SN-1, (*N*-tert-butyl-3-(3-tert-butyl-1-methyl-7-oxo-4*H*-pyrazolo[4,3-d]pyrimidin-5-yl)-4 ethoxybenzenesulfonamide); SF-22, (5-chloro-*N*-(2-piperidin-1-ylphenyl)thiophene-2-sulfonamide); SF-31, (1-(4-ethoxynaphthalen-1-yl)sulfonylazepane); SF-23, (5-chloro-*N*-(2-morpholin-4-ylphenyl)thiophene-2-sulfonamide); SF-32, (1-(4-ethoxy-2,3-dimethylphenyl)sulfonylpiperidine); SF-24, (4-methyl-*N*-(2-phenylphenyl)benzenesulfonamide); SF-33, (5-chloro-*N*,*N*-diethyl-4-methyl-2-propoxybenzenesulfonamide); SF-61, (4-(2-methoxyphenyl)spiro[3,4-dihydropyrazole-5,8′-6,7-dihydro-5*H*-benzo[7]annulene]-9′-one); SF-71, ([2-tert-butyl-5-methyl-4-(4-methylphenyl)sulfonylpyrazol-3-yl]butanoate); SN-2, (5-mesityl-3-oxa-4-azatricyclo[5.2.1.0~2,6~]dec-4-ene).

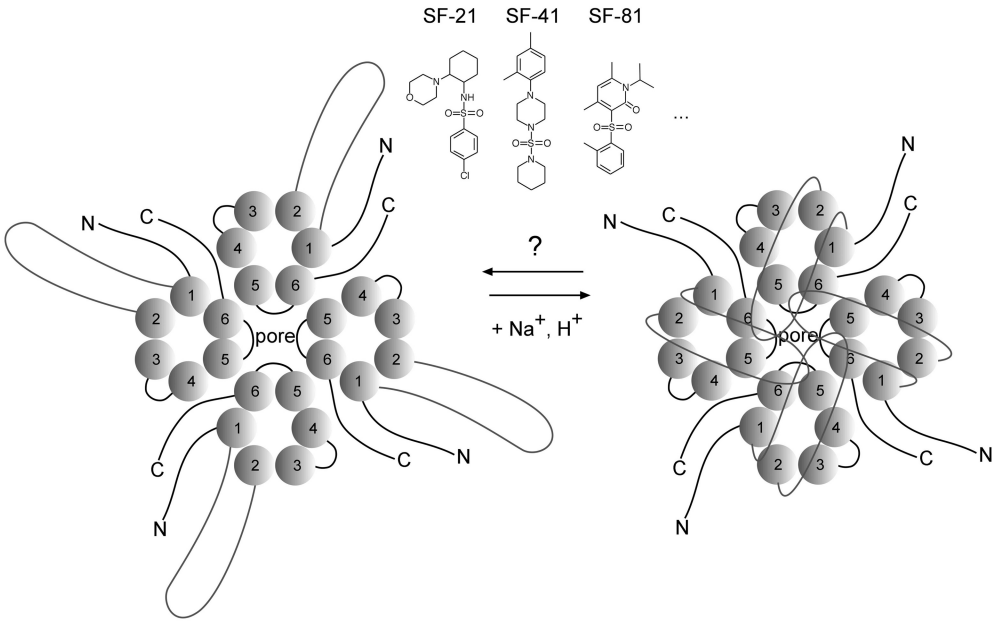

Fig. 2. Hypothetical model of TRPML3 inhibition by low pH and sodium, and activation by small molecules. Left, cartoon displaying the TRPML3 channel tetramer with the large extracellular loop between TMD1 and TMD2. Right, hypothetical change in conformation when TRPML3 is exposed to high extracellular sodium or low pH or both. Chemical formulae of SF-21, SF-41 (1-(2,4-dimethylphenyl)-4-piperidin-1-yl-sulfonyl piperazine), and SF-81, which are activators of both TRPML2 and TRPML3, are shown on top.

dance with results obtained by Kim et al. (2008) with wild-type TRPML3, i.e., block of TRPML3 channel activity by low extracellular pH.

In contrast, Raychowdhury et al. (2004) reported an inhibitory effect of low extracellular pH on TRPML1 wild-type activity. They also reported that wild-type TRPML1 is outwardly rectifying when measured in endosomal vesicles reconstituted in a lipid bilayer system. Kiselyov and colleagues, who reported that a fraction of TRPML1 reaches the plasma membrane when overexpressed in human embryonic kidney 293 cells, likewise detected outwardly rectifying currents in whole-cell patch-clamp experiments (Kiselyov et al., 2005; Soyombo et al., 2005).

Dong et al. (2010a,b), however, showed inwardly rectifying currents for wild-type TRPML channels when activated with $PI(3,5)P_2$, 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. Likewise, it was found that TRPML3 activated by small chemical compounds such as 4-chloro-*N*-(2-morpholin-4-yl-cyclohexyl)benzenesulfonamide (SF-21) or 4,6-dimethyl-3-(2-methylphenyl)sulfonyl-1-propan-2-yl-pyridin-2-one (SF-81), identified in high-throughput screening, is inwardly rectifying when overexpressed in human embryonic kidney 293 cells (Grimm et al., 2010; Yamaguchi and Muallem, 2010; Saldanha et al., 2011; Table 1). When activated by low extracellular sodium (Grimm et al., 2010) or sodium deprivation followed by rapid sodium readdition (Kim et al., 2008) wild-type TRPML3 also shows inward rectification similar to TRPML Va isoforms. The reasons 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 TMD1 and TMD2, which may play different physiological roles in the three TRPML channels (Fig. 1). In TRPML1, this loop seems to contain a serine-lipase motif (GXSXG motif; amino acids 108–112 in human TRPML1) (Bargal et al., 2000; Akoh et al., 2004) and a proline-rich domain (PRD; amino acids 197–205 in human TRPML1), both of which, however, are 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. (2008) 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, seem to also have an effect on pH regulation when mutated) influences the orientation of TMD5 and binding of protons to H283 exerts a long-range conformational change affecting pore opening (Kim et al., 2008). Because of its unusual length, a functional interaction between distinct residues of the first extracellular loop and either the second extracellular loop, between TMD3 and TMD4, 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 and Bruice, 1999). Gao et al. (2000) 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. As in a channel selectivity filter, proper geometry and energetics may play an important role in precisely positioning the extracellular loops, thus blocking the TRPML3 pore entrance. Increased extracellular pH and/or the 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 that 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 by using whole-lysosome patch-clamp techniques and, if necessary, increasing isoform selectivity by chemical modifications. Although some of the compounds activate only TRPML3 at concentrations up to 10 μM (Grimm et al., 2010), Shen et al. (2012) showed recently that the compound 2-[2-oxo-2-(2,2,4-trimethylquinolin-1-yl)ethyl]isoindole-1,3-dione (SF-51) also activates TRPML1 at concentrations of 30 μM. When SF-51 was chemically modified (ML-SA1) it could activate all three TRPML channels at a concentration of 10 μM. 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 that 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 *N*-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide (VX-770), which has advanced to phase III clinical trials in patients with cystic fibrosis (Amaral, 2011; Ashlock and 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, enzyme replacement therapy has been applied with some success (Lachmann, 2010, 2011). Although intravenous enzyme replacement therapy seems to be efficacious at ameliorating noncentral nervous system pathology in several LSDs, there is little evidence supporting its use for neurological disease, possibly because of rapid clearance via the spleen and liver and the poor ability of enzyme to penetrate the blood-brain barrier (Hemsley and Hopwood, 2011). Neurodegeneration and/or dysmyelination are the hallmark of approximately 70% of LSDs. Gene therapy represents a promising approach for the treatment of central nervous system manifestations in LSDs, because it has the potential to provide a permanent source of the deficient enzyme, either by direct injection of vectors or transplantation of gene-corrected cells. In the 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 of whether TRPML channels are purely endolysosomal or intracellular ion channels in vivo. TRPML1 at least seems to localize almost exclusively to endolysosomal vesicles, both in vivo and in vitro, most likely because of the presence of endolysosomal targeting sequences (LTSs), i.e., dileucine motifs (D/EXXXLL/I) in the N and C terminus, respectively (amino acids 11–16 and 573–578 in human TRPML1). If these motifs are cleaved or mutated to alanine (LL/AA) TRPML1 is found predominantly in the plasma membrane (Vergarajauregui and 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 seem to localize to both the plasma membrane and intracellular vesicles in vitro (Karacsonyi et al., 2007; Kim et al., 2008, 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 that localize both to the plasma membrane and intracellular structures, e.g., TRPM1, TRPM2, TRPM7, TRPM8, TRPV2, TRPV5, and TRPP2 (Tsiokas et al., 2007; Patel and Docampo, 2009; Dong et al., 2010b; Gees et al., 2010; Abe and Puertollano, 2011).

In the overexpression system it seems that TRPML2 and TRPML3 are also functionally active at the plasma membrane (Kim et al., 2008, 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 (Venkatachalam et al., 2006; Zeevi et al., 2009, 2010; Curcio-Morelli et al., 2010; Grimm 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.

Toward a Physiological Role for TRPML Channels and TPCs

In addition to $PI(3,5)P_2$ mentioned above, TRPML1 was reported to be activated by nicotinic acid adenine dinucleotide phosphate (NAADP) (Zhang et al., 2007). NAADP is an endogenous activator of 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; Zong et al., 2009; Schieder et al., 2010; Zhu et al., 2010; Galione, 2011; Rietdorf et al., 2011;). It is noteworthy that TRPML1 and, to some extent, TRPML3 coimmunopre-

cipitate with TPC1 and TPC2 but reportedly do not interact with each other functionally (Yamaguchi et al., 2011). The authors (Yamaguchi et al., 2011) 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 endoplasmic reticulum 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 channels involved in NAADP-mediated calcium release remained elusive until recently. Several groups have now demonstrated that TPCs are endolysosomal proteins that are activated by NAADP (Brailoiu et al., 2009; Calcraft et al., 2009; Zong et al., 2009; Galione et al., 2010, 2011; Schieder et al., 2010; Zhu et al., 2010). Knockdown of wild-type TPCs 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. (2012) and Walseth et al. (2012) have recently suggested, based on photolabeling data, that an accessory component within a larger TPC complex may be responsible for binding NAADP. In contrast, Calcraft et al. (2009) 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, such proteins would have to associate with TPC2 tightly to explain their binding results. Future studies will have to clarify how exactly NAADP activates TPCs.

Although 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., 2010). By analyzing the sequence data from 10 primate species, Cai and Patel (2010) have determined the degeneration process of the *Tpc3* gene. 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, Cai and Patel (2010) 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 the functional redundancy of *Tpc* genes (Zhu et al., 2010).

There are no disease-related (point) mutations known for human or rodent TPCs. However, Sulem et al. (2008) have postulated a link between human TPC2 mutations and pigmentation. Four SNPs on 11q13.2 showed association with blond versus brown hair color in an Icelandic discovery sample that reached genomewide significance. These SNPs are located within a single linkage disequilibrium block that overlaps with only one gene, *TPC2*. All of the observed associations 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 that are lysosome-related organelles (LROs).

Lysosomes are derived from late endosomes. Whereas early endosomes usually have a luminal pH of approximately 6, late endosomes have a luminal pH of approximately 5.5, and lysosomes have a pH of approximately 4.5. Likewise, the melanosomal pH varies during melanosome maturation. However, whereas premelanosomes have a luminal pH of approximately 5, mature melanosomes have a pH of approximately 6.8. The optimum for tyrosinase activity, a key enzyme of melanin synthesis, is at pH 6.5 to 7.0 (Schallreuter et al., 2008). Ancans et al. (2001) reported that melanin production in white melanocytes is suppressed by low melanosomal pH and the ratio of eumelanin/phaeomelanin production and maturation rate of melanosomes can be regulated by melanosomal pH. Thus, melanosomal pH seems to be highly critical in determining the pigmentation phenotype (Ito and Wakamatsu, 2011).

In addition to the melanosomal proton concentration, calcium seems 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 TPCs may be important regulators and sensors of endolysosomal and/or melanosomal proton and calcium levels (Pitt et al., 2010; Abe and Puertollano, 2011; Lloyd-Evans and Platt, 2011). Because 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 lysosome-related organelles such as endosomes and melanosomes (Kiselyov et al., 2011; Lelouvier and Puertollano, 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 channels and TPCs in more detail to better understand and appreciate the functional and physiological roles of these channels in their respective environments.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Grimm, Hassan, Wahl-Schott, and Biel.

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