

Chapter 19

TRPML Channels and Mucopolipidosis Type IV

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Abstract The transient receptor potential (TRP) mucopolipin channels TRPML1, TRPML2, and TRPML3 are non-selective cation channels predominantly found in the endolysosomal system. Mutations in the *TRPML1* gene (also known as *MCOLN1*) cause mucopolipidosis type IV in humans manifested by psychomotor abnormalities, corneal clouding, retinal degeneration, and progressive neurodegeneration. The recent identification of small compound chemical activators for TRPML channels has opened up the possibility to study TRPML mutant and wild-type isoforms both in vitro and in vivo in more detail. These compounds will permit further investigation on the functional roles of TRPML channels in the endolysosomal system. Ultimately, these drugs or their derivatives could be used to design selective pharmacological tools to gate and rescue specific loss-of-function point mutations in TRPML1 that cause mucopolipidosis type IV. Recently discovered TRPML channel interaction partners may serve as alternative pharmacological targets for the treatment of mucopolipidosis type IV.

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19.1 Introduction

Lysosomes are important cell organelles involved in the breakdown of proteins, lipids, and other molecules. These intracellular vesicles have been implicated not only in endolysosomal storage disorders (LSDs) such as mucopolysaccharidoses or sphingolipidoses but also in several neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Endolysosomal organelles contain a plethora of different receptors, ion channels, and transporters that are crucial for their normal functions. Ion homeostasis, regulation of distinct proton concentrations in different organelles and regulation of heavy metal concentrations such as copper, zinc, or iron are essential not only for lysosomes and endosomes but also for lysosome-related organelles (LROs) such as melanosomes, lytic granules, or platelet-dense granules. It has been known for a while that acidification of lysosomes and LROs is primarily regulated by ATP-driven proton pumps (vacuolar H⁺-ATPases). However, it is still largely unclear how the fine-tuning of luminal pH in early endosomes (pH 6–7), late endosomes (pH 5–6) and lysosomes (pH 4–5) or different LROs is achieved. Likewise, the mechanisms underlying the regulation of typical intraluminal cation concentrations of sodium, potassium or calcium, and different heavy metal cation concentrations are largely unexplored. For example, luminal pH as well as cation concentrations need to be readjusted after fusion events between intracellular vesicles and after endocytosis or phagocytosis. Similarly, the luminal pH needs to be controlled during maturation processes of endolysosomal organelles or LROs like melanosomes (Gerasimenko et al. 1998; Lelouvier and Puetollano 2011; Morgan et al. 2011; Ancans et al. 2001). Calcium levels in vesicles after endocytic or phagocytic events reach millimolar values; however, micromolar concentrations are re-established in a matter of minutes. The proteins which control these processes remain unknown.

Likewise, the role of calcium on endolysosomal fusion processes and how endolysosomal membrane fusion is regulated remain to be elucidated (Pryor and Luzio 2009; Luzio et al. 2007, 2010; Mindell 2012). In analogy to fusion processes between the plasma membrane and synaptic vesicles which use voltage-gated calcium channels (N- or P/Q-type calcium channels) as their calcium source, endolysosomal fusion processes (Südhof and Rizo 2011; Südhof 2012) appear to be calcium dependent as well and require the presence and interaction of distinct SNARE (soluble N-ethylmaleimide-sensitive-factor attachment receptor) proteins (Jahn and Scheller 2006; Luzio et al. 2010). For example, homotypic fusion processes, between late endosomes, or heterotypic fusion processes, between endosomes and lysosomes, or phagosomes and lysosomes involve different SNARE proteins such as syntaxins, Vamp, or vti1 isoforms (Prekeris et al. 1998, 1999; Brandhorst et al. 2006; Luzio et al. 2007, 2010; Pattu et al. 2011; Fig. 19.1). Finally, the participation of specific ion channels in endolysosomal trafficking processes also needs further investigation. Members of the TRP family of non-selective cation channels, in particular, the mucopolysaccharidoses or TRPML channels (TRPML1-3) are expressed in the endolysosomal system. Essential contributions

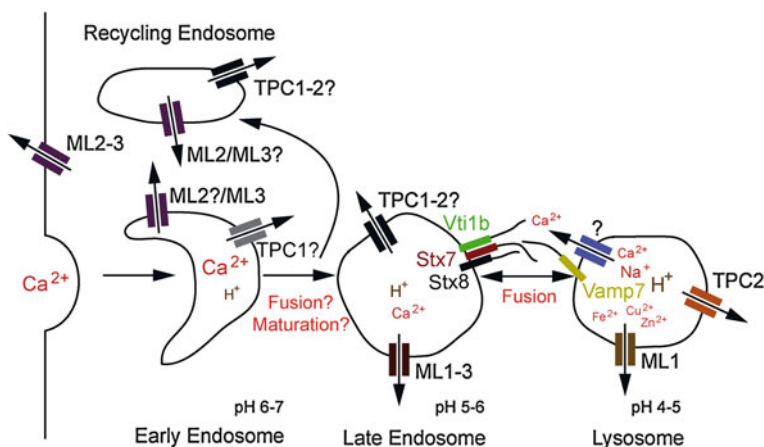


Fig. 19.1 Cartoon displaying putative subcellular localizations of TRPMLs and TPCs. While TPC2 and TRPML1 are predominantly found in lysosomes and possibly late endosomes, TRPML2, TRPML3, and TPC1 appear to be predominant in early endosomes, late endosomes, or recycling endosomes as well as the plasma membrane (TRPML2, TRPML3). Stx7 (Syntaxin7), Stx8 (Syntaxin8), Vti1b, and Vamp7 form a SNARE complex during fusion processes between late endosomes and lysosomes

of these ion channels to some of the aforementioned processes have recently been postulated (Dong et al. 2010b; Lelouvier and Puertollano 2011; Abe and Puertollano 2011; Scott and Gruenberg 2011; Shen et al. 2012; Grimm et al. 2012a).

Since their discovery by Christian de Duve in the 1950s (de Duve et al. 1955), it was found 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). 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 endolysosomal system of affected individuals.

19.2 Mucopolipidosis Type IV: A Lysosomal Storage Disorder Caused by Mutations in TRPML1

Mucopolipidosis type IV (ML IV) is an autosomal recessive LSD characterized by severe neurological problems, progressive neurodegeneration, and ophthalmologic abnormalities such as corneal opacity, retinal degeneration, and strabismus. Most patients are unable to speak or walk independently. Motor and mental retardation can however also be mild in some cases. All patients have constitutive

achlorhydria associated with a secondary elevation of serum gastrin levels. Storage bodies of lipids and water-soluble substances are seen by electron microscopy in almost every cell type of the patients. Iron deficiency anemia is another phenotypic feature of this condition.

Eventually, it was discovered that mutations in the *TRPML1* gene are causative for ML IV (Sun et al. 2000; Bargal et al. 2000). TRPML channels were originally called mucolipins (MCOLN). Cloning of *MCOLN1* 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 channels, MCOLN channels were later named TRPML channels, i.e., TRPML1, TRPML2, and TRPML3 (for recent reviews on the TRP channel family see, e.g., Gees et al. 2010 or Nilius and Owsianik 2011). Today, a plethora of different ML IV causing mutations have been identified throughout the *TRPML1* gene (reviewed in more detail elsewhere, e.g., Altarescu et al. 2002; Bach 2005; Bach et al. 2005; Grimm et al. 2012a). There are ML IV-causing mutations which lead to non-functional protein, mislocalized protein, or short versions of the protein due to early stop codons. Besides those, there are also ML IV causing mutations found within intron regions of *TRPML1* which occur, as most ML IV mutations with very high prevalence in people with Ashkenazi Jewish ancestry. One such mutation, c.406-2A > G, changes a nucleotide in a region of the gene known as intron 3. This mutation, which is a splice-site mutation, introduces a premature stop signal. Another example with high prevalence is g.511_6943del, a mutation which deletes a large amount of DNA near the beginning of the *TRPML1* gene. Both of these mutations result in the production of abnormally short, non-functional proteins.

19.3 Mucolipidosis Type IV Mouse Models

Similar to the human phenotype, *Trpml1*^{-/-} knockout mice display inclusion bodies, enlarged vacuoles, psychomotor defects, and retinal degeneration (Venugopal et al. 2007). Venugopal et al. also described a gastric phenotype in *Trpml1*^{-/-} knockout mice with remarkable similarities to what has been described in ML IV patients, including elevated gastrin. Chandra et al. (2011) also reported that *Trpml1*^{-/-} knockout mice have significant impairments in basal and histamine-stimulated gastric acid secretion. Specifically, the 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 histologic and ultrastructural analyses, Chandra et al. found that *Trpml1*^{-/-} parietal cells are enlarged and have multivesicular and multilamellar lysosomes.

Mostly normal at birth, it was found that 3-month-old *Trpml1*^{-/-} knockout mice start to show weakness and progressive neurological deficits. The lifespan of *Trpml1*^{-/-} knockout mice is decreased (Venugopal et al. 2007). Retinal degeneration was found to be dramatic and similar to that described in patients. Mice

show defects in the outer nuclear layer, the outer plexiform layer, and the inner nuclear layer (Venugopal et al. 2007). Micsenyi et al. (2009) characterized the neuropathology of *Trpml1*^{-/-} knockout mice in more detail and found evidence of ganglioside accumulation, including increases in GM2, GM3, and GD3, and redistribution of GM1, found throughout the central nervous system (CNS). In contrast, cholesterol accumulation in the CNS was not as prominent as it is in other LSDs such as NPC1. Furthermore, evidence of reduced myelination in cerebral and cerebellar white matter tracts and accumulation of autofluorescent material throughout the brain were found (Micsenyi et al. 2009). Curcio-Morelli and colleagues reported that macroautophagy is defective in *Trpml1*^{-/-} knockout mice and that LC3-II, the lipidated form of the microtubule-associated protein (LC3), which associates with autophagosomal membranes is strongly increased in neuronal cultures of *Trpml1*^{-/-} knockout mice (Venugopal et al. 2009).

Thus, in summary, *Trpml1*^{-/-} knockout mice appear to exhibit many clinical and cellular features of the human disease.

19.4 Electrophysiological and Functional Properties of TRPML Wild-Type and Mutant Channels

Functional characterization of TRPML channels has gained momentum with the electrophysiological characterization of the constitutively active inwardly rectifying TRPML3 variant-waddler mutant isoforms Va (A419P) and Va^J (A419P + I362T) and their TRPML1 and TRPML2 counterparts (Grimm et al. 2007, 2010; Kim et al. 2007, 2010; Nagata et al. 2008; Xu et al. 2007; Dong et al. 2009; Samie et al. 2009; Lev et al. 2010). TRPML3 Va and Va^J mutations cause deafness, circling behavior, 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. Equivalent mutations in TRPML1 and TRPML2 showed similar calcium overload effects upon heterologous expression in cultured cells (Grimm et al. 2007, 2009; Dong et al. 2009; Lev et al. 2010).

While the introduction of proline residues 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. (2008) demonstrated that ML IV mutant isoforms such as T232P, D362Y, F465L, or R403C in combination with the Va equivalent mutation in TRPML1, known as TRPML1(V432P), abolish the constitutive activity of TRPML1(V432P) (Dong et al. 2008). One exception was the F408 Δ deletion mutant which still showed some constitutive activity in combination with TRPML1(V432P). In accordance with this result, patients with the F408 Δ deletion in TRPML1 reportedly show a very mild ML IV phenotype (Raychowdhury et al. 2004).

Dong et al. (2008) further demonstrated both TRPML1 and TRPML2 channels, but not TRPML3 to be permeable to iron. The authors concluded, by showing that

cytosolic Fe^{2+} deficiency is coincident with intralysosomal iron overload in ML IV cells, that TRPML1 may play 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, 2009). The latter finding is in accordance with the results obtained by Kim et al. (2008) with wild-type TRPML3, i.e., block of TRPML3 channel activity by low extracellular pH (Kim et al. 2008).

19.5 Identification of TRPML Channel Activators: A Perspective for the Development of Pharmacological Tools for Therapeutic Use

Dong and colleagues (2010a) also found that wild-type TRPML channels can be activated with $\text{PI}(3,5)\text{P}_2$. These measurements were performed as whole-lysosome patch-clamp experiments using vacuolin-1 to increase the size of endolysosomes as described previously (Dong et al. 2010a; Schieder et al. 2010a, b). Currents elicited with $\text{PI}(3,5)\text{P}_2$ showed inward rectification. In accordance with these results, TRPML3 was found to be inwardly rectifying when activated by small chemical compounds, identified in a recent high-throughput screening (Grimm et al. 2010, 2012b; Yamaguchi and Muallem 2010; Saldanha et al. 2011). The identified candidate compounds were inactive against other TRP channels tested, including members of the TRPC, TRPV, TRPM, TRPA, and TRPN subfamilies such as TRPC3, TRPV2, TRPM2, TRPA1, and TRPN1. It was further found that the identified candidate compounds are inactive against a plethora of other targets (generally more than 500; for further information see <http://pubchem.ncbi.nlm.nih.gov>; AID: 1448, 1525, 1526, 1562, 2719, 1809, and 2694).

While some of the compounds were found to activate only TRPML3 (Grimm et al. 2010), others also activate TRPML1 and/or TRPML2. Compound SF-22 (5-chloro-*N*-(2-piperidin-1-ylphenyl)-thiophene-2-sulfonamide, EC_{50} for TRPML3 = 900 nM), for example, was found to activate TRPML3 and TRPML1 but not TRPML2 at concentrations up to 10 μM (Grimm et al. 2010). Compounds SF-21 (4-chloro-*N*-(2-morpholin-4-ylcyclohexyl)-benzenesulfonamide, EC_{50} for TRPML3 = 860 nM), SF-41 (1-(2,4-dimethylphenyl)-4-piperidin-1-ylsulfonyl-piperazine, EC_{50} for TRPML3 = 1.44 μM), and SF-81 (4,6-di-methyl-3-(2-methylphenyl)-sulfonyl-1-propan-2-ylpyridin-2-one, EC_{50} for TRPML3 = 2.45 μM) activate TRPML2 and TRPML3 at a concentration of 10 μM (Grimm et al. 2012b). Compound SF-51 (2-[2-oxo-2-(2,2,4-trimethylquinolin-1-yl)-ethyl]isoindole-1,3-dione, EC_{50} for TRPML3 = 1.47 μM) activates TRPML1 at concentrations beyond 30 μM . A chemically modified version of SF-51 (2-[2-oxo-2-(2,2,4-trimethyl-3,4-dihydroquinolin-1(2H)-yl)ethyl]-1H-isoindole-1,3(2H)-dione; ML-SA1) was shown to activate all three TRPML isoforms at a concentration of 10 μM (Shen et al. 2012).

In addition to these dose-dependent cross reactions within the TRPML subfamily, it should be noted that some of the compounds do show other off-target effects, e.g., ML-SA1 (but not SF-51) inhibits TRPC4 with an IC_{50} of 4.2 μ M (confirmation dose–response assay), SF-11 may potentiate/activate the calcium-activated chloride channel TMEM16A (primary screening) and may also inhibit the two-pore domain potassium channel KCNK9 (primary screening); finally, SF-21 (EC_{50} for TRPML3 = 860 nM) activates the CRF-binding protein, albeit with a much higher EC_{50} (13.7 μ M; confirmation dose–response assay) (for further information see <http://pubchem.ncbi.nlm.nih.gov>; AID: 1448, 1525, 1526, 1562, 2719, 1809, and 2694).

In summary, while there are selective TRPML3 compounds available, it will be necessary for the future characterization of endogenous TRPML1 and TRPML2 channel properties, and for potential therapeutic use, to further increase selectivity for these TRPML isoforms. This may be achieved by either chemical modifications of the existing lead compounds or by additional high-throughput initiatives.

Compounds with improved selectivity profile may ultimately be applied 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 cystic fibrosis transmembrane conductance regulator (CFTR) channel. Here, compounds (benzothiofenenes, phenylglycines, and sulfonamides) have been developed which are able to correct the defective gating of $\Delta F508$ -CFTR (Amaral 2011; Ashlock and Olson 2011). Other small molecules have been developed that correct its defective cellular processing (van Goor et al. 2011). For example, VX-770 (Ivacaftor, N-(2,4-Di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide), is now commercially available under the trade name Kalydeco[®] for the treatment of cystic fibrosis patients carrying the G551D mutation in the CFTR gene. Some of the ML IV-causing TRPML1 point mutations lead to early sequence termination resulting in protein variants that are lacking the pore domain, other mutations (e.g., in intron regions) result in abnormally short, non-functional protein or prevent the protein from being produced as outlined above. Hence, this strategy may not be applicable to those cases. Nevertheless, in cases of defective TRPML1 channel gating, the approach may be promising for the treatment of ML IV.

19.6 Gene Therapy: A Treatment Option for Retinal Degeneration

Gene therapy may represent an alternative approach especially for the treatment of ML IV mutations which result in a complete loss of the TRPML1 protein, as it has the potential to theoretically provide a permanent source of the deficient protein, either by direct injection of vectors or by transplantation of gene-corrected cells (for recent reviews on LSDs and gene therapy see, e.g., Byrne et al. 2012; Parenti et al. 2012; van Gelder et al. 2012). A gene therapy approach restricted, e.g., to the

retina is something that has been successfully done with other ion channels (see, e.g., Michalakis et al. 2012; Koch et al. 2012) and thus may well be a promising approach to treat retinal degeneration in ML IV patients effectively.

19.7 TRPML1 and Other Lysosomal Storage Disorders

Interestingly, there appears to be a link between TRPML1 dysfunction and other LSDs. Shen et al. (2012) demonstrated that increasing TRPML1 activity in Niemann-Pick type C cells is sufficient to restore normal lysosomal trafficking and prevent cholesterol accumulation. It thus appears that TRPML1 channel, e.g., by controlling calcium-dependent lysosomal trafficking, is the entry point of various lysosomal storage diseases, and may therefore represent a promising pharmacological target for the treatment of several lysosomal storage disorders (Weiss 2012).

A potentially more global role for TRPML1 in cellular clearance is supported by a study by Medina et al. (2011), demonstrating that the transcription factor EB (TFEB) regulates lysosomal exocytosis both by inducing the release of intracellular Ca^{2+} through its target gene *TRPML1* and by increasing the population of lysosomes ready to fuse with the plasma membrane. Moreover, Medina et al. (2011) demonstrated that the induction of lysosomal exocytosis by TFEB promotes cellular clearance in pathological conditions such as lysosomal storage diseases. TFEB-mediated increase of intracellular Ca^{2+} was blocked by transient silencing of TRPML1. Consistently, Ca^{2+} levels were not affected by TFEB overexpression in human ML IV cells that carry loss-of-function mutations of TRPML1 (Medina et al. 2011). Thus, pharmacological induction of TFEB may be a promising approach for the treatment of LSDs other than ML IV.

19.8 Heteromultimerization of TRPML1 with Related Ion Channels

TRPML channels are able to heteromultimerize with each other. This has been shown by several groups (Venkatachalam et al. 2006; Zeevi et al. 2009; Curcio-Morelli et al. 2010; Grimm et al. 2010; Zeevi et al. 2010). To what extent such heteromultimerizations occur in vivo and how they may affect physiological processes is not fully understood. Zeevi et al. (2010) have recently suggested that TRPMLs heteromultimerize with each other to regulate cell viability and starvation-induced autophagy, a process that mediates macromolecular and organellar turnover under cell starvation conditions. Although TRPMLs have been shown to reside mostly within different intracellular organelles (Karacsonyi et al. 2007; Martina et al. 2009; Vergarajauregui and Puertollano 2006; Fig. 19.1), Zeevi et al. (2009) found that endogenous TRPML channels partially colocalize with each

other, in a subset of intracellular vesicles. In addition, TRPML2 and TRPML3 traffic, in part, to lysosomes (Karacsonyi et al. 2007; Kim et al. 2009; Martina et al. 2009; Zeevi et al. 2009) where they may like TRPML1 regulate lysosomal function(s). Indeed, it was found that gene-specific knockdown of TRPML2 or TRPML3 leads to lysosomal inclusions reminiscent of those found in MLIV patient TRPML1^{-/-} cells (Zeevi et al. 2009).

Recent evidence also suggests that TRPML channels can interact with two-pore channels (TPCs), a family of novel endolysosomal ion channels containing 12 transmembrane domains and predicted to form dimers (Yamaguchi et al. 2011). TRPML1 and TRPML3 (albeit to a lesser extent) were shown to co-immunoprecipitate with TPC1 and TPC2. In addition, a comparison of the amino acid sequences of the TRP channels with TPC1 and TPC2 (Fig. 19.2) suggests some homology between TRPML channels and TPCs when the pore regions only are compared (TMD5-pore-TMD6). However, so far no functional consequences of TRPML-TPC interactions have been found (Yamaguchi et al. 2011) and thus it was concluded that although TRPMLs and TPCs are present in the same organelles

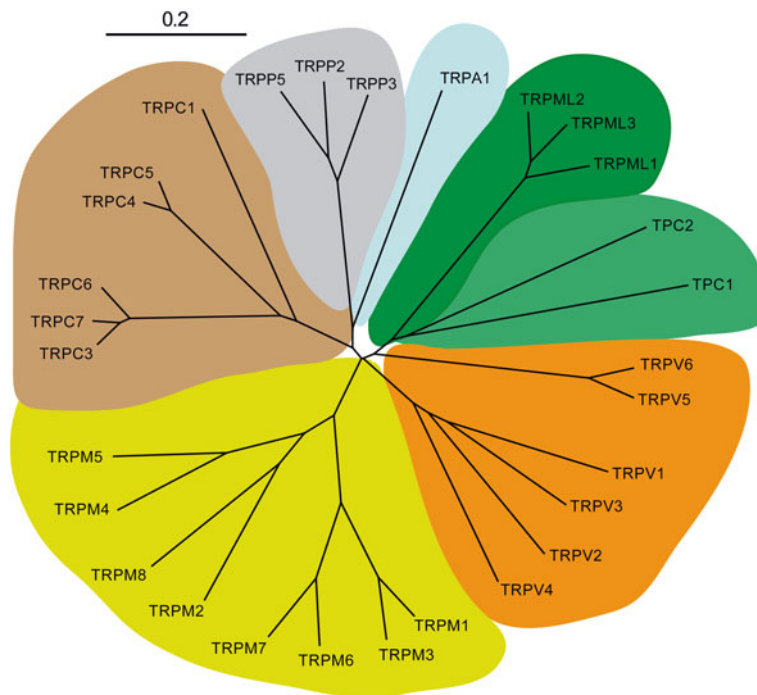


Fig. 19.2 Phylogenetic analysis of the pore regions of TRPs and TPCs. Phylogenetic analysis of human TRP channels and human TPCs based on amino acid alignments of the respective pore regions (TMD5-pore-TMD6 for TRPs; for TPCs the respective regions in domain II were used). The alignment was done using DNAMAN software (Lynnon Corporation, Pointe-Claire, Quebec, Canada); the phylogenetic tree was plotted using NJPlot software (<http://pbil.univ-lyon1.fr/software/njplot.html>)

and can physically interact with each other, they may function as independent organellar ion channels (Yamaguchi et al. 2011).

TPCs have initially been put forward as Nicotinic acid adenine dinucleotide phosphate (NAADP)-stimulated endolysosomal calcium release channels by several groups (Brailoiu et al. 2009, 2010; Calcraft et al. 2009; Zong et al. 2009; Pitt et al. 2010; Ruas et al. 2010; Schieder et al. 2010a, b; Tugba Durlu-Zhu et al. 2010; Ogunbayo et al. 2011; Rybalchenko et al. 2012; Lin-Moshier et al. 2012; Walseth et al. 2012). Quite in contrast to these findings, Wang et al. have recently provided whole-lysosome patch-clamp data suggesting that NAADP does not activate TPCs, but is activated by PI(3,5)P₂ (phosphatidylinositol 3,5-bisphosphate) instead (Wang et al. 2012). The same group has shown previously that TRPML channels are also activated by PI(3,5)P₂ (Dong et al. 2010a; Shen et al. 2012; Zhang et al. 2012). The major functions of PI(3,5)P₂ are in membrane and protein trafficking, and in pH control of the endosome-lysosome axis (Michell et al. 2006). It remains to be further established under what physiological circumstances TPCs and TRPMLs are activated by PI(3,5)P₂, whether they are activated simultaneously within the same organelle or independently of each other, and why such a large number of PI(3,5)P₂ activated cation channels may be required in the endolysosomal system. A functional connection between TPCs and TRPMLs and a physiological relevance of such a cross talk for ML IV cannot be ruled out at this time without additional research.

19.9 Other Interactors that Influence TRPML1 Channels

The endolysosomal membrane contains a myriad of proteins that regulate its function, its transport, fusion/fission events, pH, and ionic contents. Indeed, several protein interactors that appear to influence the role of TRPML1 protein in cells have been reported. For example, it was found that TRPML1 binds the apoptosis-linked gene 2 (ALG-2) protein, a penta-EF hand known to bind calcium ions (Vergarajauregui et al. 2009). ALG-2 binds to the N-terminus region of TRPML1 in a calcium-dependent manner; however, the role of the interaction between the two proteins is not clear. It is believed that binding of ALG-2 may alter the channel activity of TRPML1 relevant to fusion/fission events. Another idea is that ALG-2 might regulate the membrane trafficking of TRPML1. Lastly, it was suggested, for which the authors favored, that ALG-2 might act as a scaffold to recruit other protein interactors involved in fission/fusion events. In 2011, the same authors have reported that TRPML1 interacts with lysosome-associated protein transmembrane (LAPTMs) proteins (Vergarajauregui et al. 2011). LAPTMs are associated with the transport of molecules in the endolysosomal compartments. It was reported that overexpression of LAPTMs proteins results in enlarged lysosomes, but their depletion via RNA interference produces a similar phenotypic defect observed in ML IV (Vergarajauregui et al. 2011). The authors suggested that the binding of TRPML1 with LAPTMs proteins might aid the function of

lysosomes in transporting specific molecules (e.g., provide energy for LAPTMs-dependent transport via TRPML1-induced ionic gradients). With the knowledge that TRPML proteins form functional heteromeric channels, it would be interesting to study if any of these newly discovered binding partners directly affect the gating properties of heteromers compared to homomers.

19.10 Conclusion

Research on ML IV has come a long way from its first description in the 1970s (Berman et al. 1974) to the discovery of its genetic cause (Sun et al. 2000; Bargal et al. 2000), the generation of ML IV mouse models (Venugopal et al. 2007; Curcio-Morelli et al. 2010; Micsenyi et al. 2009; Chandra et al. 2011), the electrophysiological characterization of TRPML1 as a non-selective cation channel, its gating and permeation properties, and finally the availability of first chemical tools to modulate its gating (Kim et al. 2008; Dong et al. 2010a; Grimm et al. 2010, 2012b; Shen et al. 2012; Zhang et al. 2012). After more than 30 years of research, it seems about time to move to the next level and start to develop effective treatments for ML IV.

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