Chapter 13
The TRPML3 Channel: From Gene to Function

Konrad Noben-Trauth

Abstract TRPML3 is a transient receptor potential (TRP) channel that is encoded by the mucolipin 3 gene (*MCOLN3*), a member of the small mucolipin gene family. *Mcoln3* shows a broad expression pattern in embryonic and adult tissues that includes differentiated cells of skin and inner ear. Dominant mutant alleles of murine *Mcoln3* cause embryonic lethality, pigmentation defects and deafness. The TRPML3 protein features a six-transmembrane topology and functions as a Ca^{2+} permeable inward rectifying cation channel that is open at sub-physiological pH and closes as the extracytosolic pH becomes more acidic. TRPML3 localizes to the plasmamembrane and to early- and late-endosomes as well as lysosomes. Recent advances suggest that TRPML3 may regulate the acidification of early endosomes, hence playing a critical role in the endocytic pathway.

Mucolipins constitute a small gene family, mucolipin 1–3, which are named after the gene (*MCOLN1*) underlying the lysosomal storage disorder mucolipidosis type 4 [1]. In this review I will focus on different biological aspects of the *Mcoln3* gene and its protein product TRPML3.

13.1 Genomics of *Mcoln3*

TRPML3 is a transient-receptor-potential (TRP) channel that is encoded by the mucolipin-3 gene (*MCOLN3*). In humans, the gene (reference sequence NM_018298) is located on the short arm of chromosome 1 (p22.3) at base pair positions 85, 490, 242–85, 514, 169 (Genome Reference Consortium; release h37). The coding sequence of human *MCOLN3* is represented by 12 exons that give rise to an mRNA with an open reading frame of 1,659 nucleotides. *MCOLN3* orthologs are present in most vertebrate species for which a genomic sequence is available and

K. Noben-Trauth (✉)
Section on Neurogenetics, Laboratory of Molecular Biology, National Institute on Deafness and Other Communication Disorders, Rockville, MD 20850, USA
e-mail: nobentk@nidcd.nih.gov

M.S. Islam (ed.), *Transient Receptor Potential Channels*, Advances in Experimental Medicine and Biology 704, DOI 10.1007/978-94-007-0265-3_13,

homologs are also found in *Drosophila*, *C. elegans*, sea urchin and lower organisms such as Hydra, Dictyostelium, Schistosoma and Leishmania [2] (see Chapter 12).

The mouse *Mcoln3* gene (reference sequence NM_134160) is located on the distal end of chromosome 3 (qH2) at base pair position 145, 784, 755–145, 803, 610 (NCBI build37). It encodes a protein of 553 amino acids (reference sequence NP_598921) with a predicted molecular weight of 64 kDa. Human and mouse TRPML3 share 91% sequence identity with most of the discordant amino acids being located at the N-terminus and the first extracellular/luminal loop. Secondary structure analysis predicts an integral transmembrane topology with six helices, and cytosolic amino-(aa 1–63) and carboxy-(aa 502–553) termini. The protein sequence spanning the third to the sixth transmembrane domain (aa 341–501) contains an ion transport motif (PF00520) and a transient-receptor-potential-like motif (PS50272) (Fig. 13.1a and Table 13.1).

Mcoln3 shows a broad expression pattern as demonstrated by RT-PCR and is expressed in all major tissues, albeit at different levels [3]. RNA in situ hybridization on mouse inner ear sections revealed a similar expression profile showing strong expression in cochlear and vestibular sensory hair cells, marginal cells of the stria vascularis, Reissner's membrane, and weaker expression in cells of the spiral limbus and Hensen's and Claudius' cells [4]. Immunohistochemistry demonstrated that TRPML3 is expressed in native skin cells at the bulb region of hair follicles coinciding with the location of melanocyte markers (HMB45 and tyrosinase) and is also expressed in the adult mouse melanocyte cell line melan-a2 [5]. Western blot analyses of protein extracts from adult mouse inner ear and the melan-a2 cell line

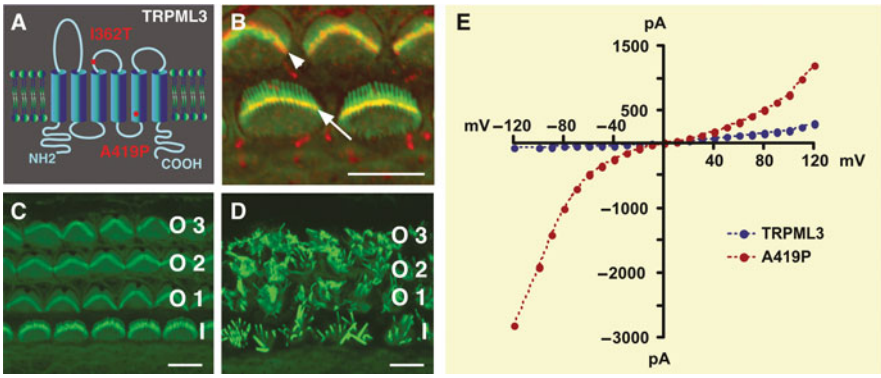

Fig. 13.1 (**a**) Schematic diagram of the TRPML3 channel showing the six-transmembrane topology, cytosolic amino-(NH2) and carboxy-(COOH) termini, and the location of the isoleucine to threonine (I362T) and alanine to proline (A419P) mutations (*red dots*). (**b**) Confocal image showing immuno labeling of TRPML3 at the base of the stereociliary hair bundle of inner (*arrow*) and outer hair cells (*arrowhead*). Stereocilia were counterstained with phalloidin (*green*). Scale bar = 5 μm. (**c**), (**d**) Confocal images of stereocilia of wildtype (**c**) and Va^J/Va^J (**d**) inner (I) and outer (O1-3) hair cells. Note the normal orientation and structure of the hair bundle in (**c**) and the disorganized splayed appearance in Va^J mutants. Scale bars = 5 μm. (**e**) Schematic diagram of the voltage/current relationship of wildtype and mutant (A419P) TRPML3. mV, milliVolt; pA, picoAmpere; from [4]

Table 13.1 Characteristics of *Mcoln3*/TRPML3

	Human	Mouse	References
Chromosomal location	1p22.3	3qH2	[11]
Physical location	85,490–85,514 Kb	145,784–145,803 Kb	GRC
Gene symbol	*MCOLN3* (NM_018298)	*Mcoln3* (NM_134160)	[24]
Protein name	TRPML3 (NP_060768)	TRPML3 (NP_598921)	NCBI
Mutant alleles	Unknown	Varitint-waddler, *Va* and *Va*^{J}	[11]
Expression		Cochlea, vestibule, many other tissues	[4] [3, 5]
	Normal channel	*Mutant channel (A419P)*	
Gating mechanism	Opens at positive voltage	Constitutively active	[4–6, 17, 18]
	Removal/addition of Na^+	Inwardly rectifying	[18]
I/V function	Inwardly rectifying	Inwardly rectifying	[4, 5, 17, 18]
Conductance	50 pS	50 pS	[4]
Ion selectivity	$Ca^{2+} >> Na^+ \geq K^+ > Cs^+$, Sr^{2+}, Ba^{2+}, and Mg^{2+}	Ca^{2+} Na^+ K^+ Mg^{2+}	[4, 5, 17, 18]
Channel inhibition	H^+ (pH 4.6)		[23]
Cellular localization	Stereocilia		[6]
	Plasma membrane, vesicles	Plasma membrane, vesicles	[21, 22]
Cellular function	Membrane trafficking,	Reduced mechanotransduction	[6]
	Endocytic pathway	Hair cell degeneration	[12, 15]
	Autophagy	Apoptosis	[4, 17]

identified a single band with a molecular weight of ~68 kDa and ~59 kDa respectively [6]. On the sub-cellular level, TRPML3 was localized to vesicular cytoplasmic structures and the plasma membrane of stereocilia of auditory sensory hair cells. High-resolution immuno-confocal and electron microscopy refined the TRPML3 location to the ankle-link region at the base of developing hair cell stereocilia (Fig. 13.1b) [6].

13.2 Normal and Mutant Alleles of *Mcoln3*

Spontaneous and induced mutant variants of mucolipin genes have been identified in different species and model organisms. Mutations in human *MCOLN1* underlie an autosomal recessive lysosomal storage disorder known as mucolipidosis type IV (OMIM 252650), which is characterized by psychomotor retardation and abnormalities affecting the cornea and photoreceptor layer in the eye [1, 7]. Induced loss-of-function mutations in the mucolipin homolog CUP-5 in *C. elegans*

disrupt the biogenesis of lysosomes, leading to an accumulation of large vacuoles in many cell types, increased cell death, and embryonic lethality [8–10]. In the mouse, a spontaneous dominant gain-of-function mutation in *Mcoln3* causes hearing loss, vestibular dysfunction, pigmentation defects and embryonic lethality in the Varitint-waddler (*Va*) mutant [11–13]. No mutant allele for *Mcoln2* has yet been identified.

The *Va* mutation was first described by Cloudman and Bunker in 1945. This mutation is semidominant and heterozygotes can be distinguished from homozygotes by their lower degree of coat color variegation and greater embryonic viability. Mice of both *Va/+* and *Va/Va* genotypes are deaf and display nervous, choreic head movements together with erratic circling behavior [13]. A second spontaneous mutation occurred in the *Mcoln3* locus in a cross segregating for *Va* and coincidentally occurred *in cis*, that is, on the same parental chromosome that carried the *Va* mutation. This second site mutation, named Va^J, has an ameliorating effect on *Va* such that it partially restores embryonic viability, coat color variegation, hearing loss and choreic vestibular appearance [14].

The physiology and anatomy of the *Va* and Va^J cochlea have been studied in great detail [12, 15, 16]. The earliest phenotypic anomalies are seen in late *Va/+* embryos at gestation day 17.5, which have severely disorganized stereociliary hair bundles in the outer and inner hair cells. By postnatal day 11, most stereociliary hair bundles have become splayed in both *Va* and Va^J mutants; this is accompanied by some hair cell loss (Fig. 13.1c, d) [11]. In early postnatal and young adult mutant mice, an underpigmented stria vascularis, degeneration of outer and inner cochlear sensory hair cells, as well as degeneration of the spiral ganglion, leads to an abnormally low endocochlear potential, a loss of compound action potentials and absent auditory-evoked brain stem responses [12, 15]. All of these phenotypes are expressed in neonatal hetero- and homozygous mutants resulting in congenital deafness.

The mechanism underlying the spotted and dilute coat color in *Va* and Va^J is not precisely known, but it likely consists of a developmental defect during melanoblast differentiation and migration. A cellular defect at the level of the differentiating neural crest may also underlie the embryonic lethality in *Va* homozygotes. The embryonic lethality in Va^J homozygotes is tightly controlled by the genetic background; on the C57BL/6 J background Va^J homozygotes are lethal, whereas they survive and develop normally on a C3HeB/FeJ background [16].

A positional cloning approach aimed at identifying the *Va* locus revealed two mutations in the *Mcoln3* gene. The *Va* allele harbors a c.1,255 G to C transversion in exon 10, causing an alanine to proline substitution at amino acid position 419 (Ala419Pro). This substitution is located in the fifth predicted transmembrane domain, which is part of the ion pore region. The Va^J allele carries a c.1,085 T to C transition in exon 8, which changes the isoleucine residue at position 362 to a threonine (Ile362Thr) in the second extracellular/luminal loop. As expected from the origin and ameliorating effect of Va^J, the Va^J allele also has the Ala419Pro mutation [11].

13.3 Molecular Physiological Function of TRPML3

Heterologously-expressed wild-type TRPML3 is largely inactive at normal negative resting membrane potentials, exhibiting only small negative currents [4, 5, 17, 18], but shows a strong outward rectifying current (300 pA) when the membrane potential is shifted to positive voltages (+120 mV) [4]. TRPML3, however, can be gated at normal resting potentials by a brief depletion, then addition of Na^+ in the incubation solution, which generates a large inward rectifying current (8 nA at –100 mV) that slowly inactivates [18]. The TRPML3 channel is highly permeable to Ca^{2+}, Na^+ and K^+ and, to a lesser extent, to Cs^+, Sr^{2+}, Ba^{2+}, and Mg^{2+} [4, 5, 18]. The activity of the channel can be further modulated by decreasing the extracytosolic pH from 7.4 to 6.4, at which pH 50% of the activity is inhibited [18]. Three histidine residues (His252, His273 and His283) residing in the large first extracytosolic loop are critical for this regulation.

In contrast to wild-type TRPML3, the isoform mutated in the *Va* allele, here referred to as TRPML3(A419P), shows a strong constitutively-active inward-rectifying current at normal negative resting potentials [4, 5, 17, 18]. TRPML3(A419P) heterologously expressed in either LLC-PK1-Cl4 or HEK293 cells exhibits an inward current of ~3 nA at –120 mV that escapes regulation by extracytosolic Na^+ and does not inactivate. The A419P mutation does not alter the channel's pore properties; the mutant channel displays reversal potentials, ion permeabilities, and conductances (~50 pS) similar to that of the native channel. Interestingly, although the I362T mutation attenuates the *Va* phenotype on the organismal level, no effect was measured on the molecular level; the TRPML3(A419P/I362T) mutant showed the same physiological properties as the TRPML3(A419P) isoform (Fig. 13.1d) [4, 5, 17, 18].

Introduction of proline and glycine into an alpha helix has a destabilizing effect on the conformation for both chemical and biophysical reasons. The Ala>Pro substitution in *Va* occurs in the fifth alpha helical transmembrane domain. Amino acid substitution scans showed that A419G has a similar constitutive effect as the A419P mutation (spontaneous activity and minimal inactivation), whereas A419V had 20% of the normal inward rectifying activity, but behaved otherwise similarly to the wild-type channel. This suggests that the *Va* mutation introduces a conformational change, most likely a kink or swivel, in the transmembrane alpha helix, locking the channel in an open state [4, 5, 17, 18].

In heterologous expression systems, transient expression of TRPML3(A419P) results in cell death from permanent influx of extracellular Ca^{2+}, as demonstrated by Ca^{2+} imaging and expression of apoptotic markers such as annexin V and staining with propidium iodide. Transient expression of TRPML3(I362T) alone has no apoptotic effect. Biotinylation assays showed that surface expression of mutant TRPML3 was significantly less in TRPML3(A419P/I362T) transfected cells than in TRPML3(A419P) expressing cells [4, 5, 17, 18]. Hence, the reduced plasma membrane localization of the TRPML3(A419P/I362T) isoform in vivo could account for the attenuated Va^J phenotype.

The effect of the TRPML3(A419P/I362T) mutant channel in vivo was studied in detail in mouse cochlear hair cells [6]. These sensory cells express an ordered array of staggered elongated microvilli (so-called stereocilia) at their apical surface. These stereocilia respond to minute mechanical (i.e. acoustic) stimulation with nanometer-scale deflections, upon which, yet-unknown mechano-sensitive transducer channels open to initiate the signal transduction cascade. The hearing impairment in *Va* and Va^J mutants, the localization of TRPML3 near the base of developing stereocilia as well as the disrupted structure of the stereociliary hair bundle suggested defects in mechanosensation. Indeed, mechanical stimulation of postnatal mutant cochlear hair cells *ex vivo* revealed reduced and absent transducer currents in Va^J/+ and Va^J/Va^J respectively [6]. However, this raises the question of whether TRPML3 is directly linked to the mechanotransduction process, or whether the reduced transducer currents are secondary to the gain-of-function mutation. The lack of TRPML3 labeling in mature stereocilia seems to argue against a direct role of TRPML3 in mechanotransduction.

Consistent with the in vitro experiments, using the TRPML3(A419P/I362T) mutant, hair cells of Va^J/Va^J mutants showed an inwardly-rectifying leak current (1.6 nA at –100 mV). The demonstrated apoptotic effect of this constitutive conductance in vitro explains the hair cell and melanocyte loss in the Va^J cochlea [12, 15].

13.4 TRPML3 and Its Role in the Endocytic Pathway

The cell biological function of the TRPML proteins is best understood for TRPML1. *MCOLN1* encodes two di-leucine motifs that target TRPML1 primarily to vesicles at the late endosome-lysosome stage [19, 20]. Recent studies suggest that TRPML3 also plays a role in the endocytic pathway, although at a different point. The endocytic pathway is part of the larger intracellular vesicular traffic system, and consists of mostly uni-directional routes, highly organized and regulated, which involve early- and late endosomes, and lysosomes. The main function of this pathway is to engulf, degrade and recycle fluid, molecules and other bioparticles and, as such, is a major component of every eukaryotic cell. A series of transfection experiments using different cell lines (HeLa, HEK293, CL4 and the human epithelia cell line ARPE19) showed that heterologously-expressed TRPML3 co-localizes with markers (Hrs, EEA1, dextran) of early and late endosomes [4, 21, 22]. In addition, adenovirus-mediated overexpression of tagged TRPML3, as well as siRNA-mediated down-regulation of endogenous TRPML3, affected trafficking of the native EGF receptor, EGFR, along the endosomal pathway [21, 22]. Consistent with these findings, biochemical subcellular fractionation experiments localized transiently-expressed TRPML3 to endosomes and lysosomes [22]. Furthermore, TRPML3-overexpressing cells had higher endosomal pH compared to non-transfected cells [21].

Autophagy, or autophagocytosis, is the degradation of intra-cellular components as part of a regular recycling and clearance process or as result of cellular

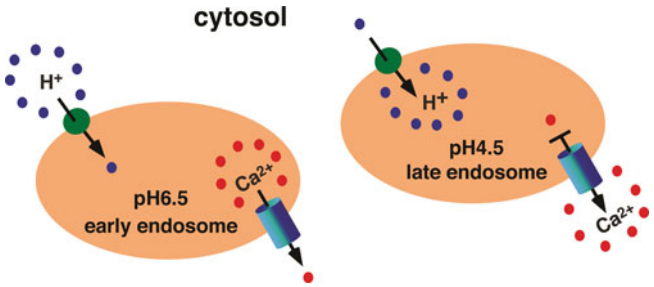

Fig. 13.2 Schematic diagram of suggested function of TRPML3 in acidification and differentiation of endosomes [21]. At pH6.5 TRPML3 releases Ca^{2+} (*red dots*) from the lumen of the early endosome into the cytosol. As H^+ (*blue dots*) enter the endosome, the lumen becomes more acidic, upon which the TRPML3 channel closes promoting differentiation into late endosomes

stress. The autophagic vesicle or autophagosome may fuse with lysosomes to form autolysosomes or with late endosomes to become amphisomes. Overexpression of TRPML3 resulted in a significant accumulation of amphisomes blocking the further maturation into autolysosomes [21].

Localization of TRPML3 to the upper and less acidic (pH 6.0) vesicular components of the endocytic pathway is consistent with the maximal activity of the TRPML3 channel being at a slightly acidic 6–6.5 pH [23]. It was proposed that TRPML3 under slightly acidic conditions, extrudes Ca^{2+} from the lumen of the vesicle into the surrounding cytosol; as Ca^{2+} exits the endosome, H^+ enters, acidifying the vesicle, thereby closing the TRPML3 channel and leading the vesicle to become a late endosome (pH 4–4.5) (Fig. 13.2) [21].

13.5 Open Questions – Future Directions

In recent years, excellent progress has been made in elucidating the molecular and cellular function of TRPML3. This work has generated exciting hypotheses and opened up promising avenues for further experimentation. On the molecular level, it will be important to identify the components that interact with TRPML3. Further precise in situ measurements of TRPML3 activity in early and late endosomes, combined with high-resolution live imaging, will provide further insights into a TRPML3 gating mechanism, and the role of TRPML3 in both in endosome differentiation and membrane trafficking. In addition, analyses of tissue-specific loss-of-function alleles of TRPML3, in combination with other members of the TRPML family, will provide first-class evidence as to the precise and indispensable role of TRPML3 in vivo, in particular during embryogenesis, in the migration and differentiation of melanocytes, and in the vestibular and cochlear development. Lastly, it will be important to identify pathological human alleles that may exist either in the form of rare and highly-penetrant alleles or as hypomorphic modifiers of *Trpml1* and/or *Trpml2*, which will aid in improved diagnostics and therapeutics.

Acknowledgments I thank Rosa Puertollano and Jamie García-Añoveros for discussions and comments on the manuscript. Supported by the Intramural Research Program at the National Institute on Deafness and Other Communication Disorders.

References

1. Bargal R, Avidan N, Ben-Asher E, Olender Z, Zeigler M, Frumkin A, Raas-Rothschild A, Glusman G, Lancet D, Bach G (2000) Identification of the gene causing mucolipidosis type IV. Nat Genet 26:118–123
2. Chenik M, Douagi F, Achour YB, Khalef NB, Ouakad M, Louzir H, Dellagi K (2005) Characterization of two different mucolipin-like genes from Leishmania major. Parasitol Res 98:5–13
3. Cuajungco MP, Samie MA (2008) The varitint-waddler mouse phenotypes and the TRPML3 ion channel mutation: cause and consequence. Pflugers Arch 457:463–473
4. Nagata K, Zheng L, Madathany T, Castiglioni AJ, Bartles JR, Garcia-Anoveros J (2008) The varitint-waddler (Va) deafness mutation in TRPML3 generates constitutive, inward rectifying currents and causes cell degeneration. Proc Natl Acad Sci USA 105:353–358
5. Xu H, Delling M, Li L, Dong X, Clapham DE (2007) Activating mutation in a mucolipin transient receptor potential channel leads to melanocyte loss in varitint-waddler mice. Proc Natl Acad Sci USA 104:18321–18326
6. van Aken AF, Atiba-Davies M, Marcotti W, Goodyear RJ, Bryant JE, Richardson GP, Noben-Trauth K, Kros CJ (2008) TRPML3 mutations cause impaired mechano-electrical transduction and depolarization by an inward-rectifier cation current in auditory hair cells of varitint-waddler mice. J Physiol 586:5403–5418
7. Sun M, Goldin E, Stahl S, Falardeau JL, Kennedy JC, Acierno JS Jr., Bove C, Kaneski CR, Nagle J, Bromley MC, Colman M, Schiffmann R, Slaugenhaupt SA (2000) Mucolipidosis type IV is caused by mutations in a gene encoding a novel transient receptor potential channel. Hum Mol Genet 9:2471–2478
8. Fares H, Greenwald I (2001) Regulation of endocytosis by CUP-5, the Caenorhabditis elegans mucolipin-1 homolog. Nat Genet 28:64–68
9. Hersh BM, Hartwieg E, Horvitz HR (2002) The Caenorhabditis elegans mucolipin-like gene cup-5 is essential for viability and regulates lysosomes in multiple cell types. Proc Natl Acad Sci USA 99:4355–4360
10. Treusch S, Knuth S, Slaugenhaupt SA, Goldin E, Grant BD, Fares H (2004) Caenorhabditis elegans functional orthologue of human protein h-mucolipin-1 is required for lysosome biogenesis. Proc Natl Acad Sci USA 101:4483–4488
11. Di Palma F, Belyantseva IA, Kim HJ, Vogt TF, Kachar B, Noben-Trauth K (2002) Mutations in *Mcoln3* associated with deafness and pigmentation defects in varitint-waddler (*Va*) mice. Proc Natl Acad Sci USA 99:14994–14999
12. Cable J, Steel KP (1998) Combined cochleo-saccular and neuroepithelial abnormalities in the Varitint-waddler-J (Va^J) mouse. Hear Res 123:125–136
13. Cloudman AM, Bunker LE (1945) The varitint-waddler mouse. J Hered 36:258–263
14. Lane PW (1969) VaJ – varitint-waddler-jackson. Mouse News Lett 41:32
15. Deol MS (1954) The anomalies of the labyrinth of the mutants varitint-waddler, shaker-2 and jerker in the mouse. J Genet 52:562–588
16. Kim HJ, Jackson T, Noben-Trauth K (2003) Genetic analyses of the mouse deafness mutations varitint-waddler (Va) and jerker (Espnje). J Assoc Res Otolaryngol 4:83–90
17. Grimm C, Cuajungco MP, van Aken AF, Schnee M, Jors S, Kros CJ, Ricci AJ, Heller S (2007) A helix-breaking mutation in TRPML3 leads to constitutive activity underlying deafness in the varitint-waddler mouse. Proc Natl Acad Sci USA 104:19583–19588
18. Kim HJ, Li Q, Tjon-Kon-Sang S, So I, Kiselyov K, Muallem S (2007) Gain-of-function mutation in TRPML3 causes the mouse Varitint-Waddler phenotype. J Biol Chem 282: 36138–36142

19. Kiselyov K, Chen J, Rbaibi Y, Oberdick D, Tjon-Kon-Sang S, Shcheynikov N, Muallem S, Soyombo A (2005) TRP-ML1 is a lysosomal monovalent cation channel that undergoes proteolytic cleavage. J Biol Chem 280:43218–43223
20. Vergarajauregui S, Puertollano R (2006) Two di-leucine motifs regulate trafficking of mucolipin-1 to lysosomes. Traffic 7:337–353
21. Martina JA, Lelouvier B, Puertollano R (2009) The calcium channel mucolipin-3 is a novel regulator of trafficking along the endosomal pathway. Traffic 10:1143–1156
22. Kim HJ, Soyombo AA, Tjon-Kon-Sang S, So I, Muallem S (2009) The Ca(2+) channel TRPML3 regulates membrane trafficking and autophagy. Traffic 10:1157–1167
23. Kim HJ, Li Q, Tjon-Kon-Sang S, So I, Kiselyov K, Soyombo AA, Muallem S (2008) A novel mode of TRPML3 regulation by extracytosolic pH absent in the varitint-waddler phenotype. EMBO J 27:1197–1205
24. MGD (2010) Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, ME. World Wide Web (URL: http://www.informatics.jax.org)