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## The Role of PI3P Phosphatases in the Regulation of Autophagy

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### Abstract

Autophagy initiation is strictly dependent on phosphatidylinositol 3-phosphate (PI3P) synthesis. PI3P production is under tight control of PI3Kinase, hVps34, in complex with Beclin-1. Mammalian cells express several PI3P phosphatases that belong to the myotubularin family. Even though some of them have been linked to serious human diseases, their cellular function is largely unknown. Two recent studies indicate that PI3P metabolism involved in autophagy initiation is further regulated by the PI3P phosphatases Jumpy and MTMR3. Additional pools of PI3P, upstream of mTOR and on the endocytic pathway, may modulate autophagy indirectly, suggesting that other PI3P phosphatases might be involved in this process. This review sums up our knowledge on PI3P phosphatases and discusses the recent progress on their role in autophagy.

### Keywords

autophagy; myotubularin; PI3P; phosphatase; Jumpy; MTMR14

### 1. Introduction

PI3P synthesis has long been recognized as one of the key events in the initiation of the autophagic process (1). The phosphorylation of phosphatidylinositol is catalyzed by the well known, type III phosphatidylinositol 3-kinase, Vps34, in complex with the autophagy proteins, Beclin-1 (Atg6) and Atg14 (2–4) (Fig. 1). This highly regulated complex (see chapter in this issue), conserved from yeast to humans, allows spatio-temporal control of autophagy initiation (5). Besides, and in contrast, to this proximal action at the early stage of autophagy, PI3P may also regulate autophagy upstream of mTOR, a negative regulator autophagy. Amino-acids have been shown to stimulate mTOR activity via Vps34 activation (6,7), therefore, this pool of PI3P could potentially inhibit autophagy, even though at the moment, evidence is lacking to support this model. Finally, PI3P plays a major role in the endocytic pathway where it promotes fusion of endosomes and receptor degradation (8). Since autophagic and endocytic pathways merge, it is likely that this third pool of PI3P modulates either directly or indirectly the last stages of autophagy (9,10).

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Although, the synthesis of PI3P and its regulation have been well studied, how PI3P is turned-over and how autophagy is turned-off once PI3P is produced, remained to be elucidated. The PI3P phosphatases were one of the likely candidates as it is known that the phosphoinositide, PI3,4,5P<sub>3</sub> and its signaling can be down-regulated by PI3,4,5P<sub>3</sub> phosphatase, PTEN. In this review we will summarize briefly the structure and functions of PI3P phosphatases (for reviews see, (11–13)) culminating in a discussion of the recently developing role of PI3P phosphatases in autophagy.

## 2. Structure and expression of PI3P phosphatases

In mammalian cells PI3P phosphatases belong to the myotubularin family (11–13). This subgroup of phosphatases contains 15 members in humans of which all have a characteristic phosphatase domain with a C×5R motif. Only 9 of them, MTM1 (also called myotubularin), MTMR1, MTMR2, MTMR3, MTMR4, MTMR6, MTMR7, MTMR8 and Jumpy (also known as MTMR14), possess an active phosphatase domain, that specifically dephosphorylates PI3P and phosphatidylinositol (3,5)-bisphosphate (PI3,5P<sub>2</sub>) at position 3 on inositol ring. However, MTMR7 seems to have preference for inositol (1,3)-bisphosphate as a soluble substrate (14). The six remaining myotubularins are inactive pseudophosphatases due to a substitution of Cys and Arg residues in their catalytic site. Besides the catalytic domain, all myotubularins, with the exception of MTMR14, contain two other protein domains, PH-GRAM (Pleckstrin Homology-Glucosyl transferases, Rab-like GTPase activators and myotubularins) and coiled-coil, that are believed to be important for lipid-protein or protein-protein interactions. Subgroups of myotubularins present additional domains such as PH and FYVE which are predicted to bind to phosphoinositides, PDZ-binding site likely to mediate protein-protein interaction and DENN in inactive myotubularins MTMR5 and MTMR13 (Fig. 1) (11,12). Myotubularins are ubiquitously expressed, however, they can be enriched in certain tissues. For instance, MTM1 and Jumpy are highly expressed in skeletal muscles whereas MTMR2 is abundant in neurons and Schwann cells (15–19).

*S. cerevisiae* genome encodes only one myotubularin, Ymr1p. However, yeast expresses two additional unrelated phosphatases, members of the synaptojanin-like family, Sjl2p and Sjl3p that account also for PI3P dephosphorylation (20). Only *ymr1Δ sjl3Δ* and *ymr1Δ sjl3Δ* double mutants show a significant increase in PI3P level, suggesting that these phosphatases may have redundant function in yeast (20).

## 3. PI3P phosphatases and diseases

Four of the fifteen PI3P phosphatases present in mammals have been linked to serious human diseases. MTM1 defects cause X-linked myotubular myopathy (XLMTM) also known as centronuclear myopathy, a severe congenital disorder affecting the physiology of skeletal muscle fibers and characterized by centrally localized nuclei and hypotonia (12,13). More than two hundred different mutations, truncations and missenses, in *MTM1* have been reported in XLMTM patients that result in loss or decrease of MTM1 level (21). A severe case of centronuclear myopathy was observed in one patient with an inactive MTM1 suggesting that XLMTM is likely due to defect in PI3P metabolism (21). *MTM1* knockout mice studies indicate that myotubularin is essential for muscle growth and maintenance but not for myogenesis (22). Histopathological analyses of *MTM1*-deficient mice reveal skeletal hypotrophic myofibers with numerous vacuoles (22). However, ultrastructural analyses show that these vacuoles do not seem to contain cellular debris (22).

Recently, another gene encoding for a PI3P phosphatase, *jumpy*, has been identified as mutated in some patients with centronuclear myopathy (15). The mutations lead to a reduced or total loss of phosphatase activity and they are likely the basis for the disease as observed for MTM1

(15). Indeed, a recent study reports that *MTMR14*<sup>-/-</sup> mice display muscle weakness and fatigue similar to patients with centronuclear myopathy (16).

*MTMR2* and *MTMR13* have been found mutated in two forms of Charcot-Marie-Tooth (CMT) Type 4B disease, an autosomal recessive disorder of the peripheral nervous system characterized by nerve demyelination and myelin outfoldings (13,21). CMT type 4B1 is caused by missense or deletion mutations in *MTMR2* gene that result in *MTMR2* loss of function. In addition to CMT4B1, one patient manifests azoospermia suggesting that *MTMR2* also plays a role in testis. *MTMR2*-deficient mice develop CMT4B1-like neuropathy and azoospermia (21). Interestingly, inactive myotubularin *MTMR5*, which interacts with *MTMR2*, has been shown to be essential for spermatogenesis in mice (13). Cell type-specific gene knockout indicates that loss of *MTMR2* in Schwann cells but not in neurons lead to CMT4B1-like neuropathy. CMT type 4B2 is due to mutations in *MTMR13* gene which encodes a pseudophosphatase (12,13). *MTMR13*-deficient mice manifest myelin outfoldings in peripheral nerves very similar to ones found in CMT4B2 (13). It has been suggested that *MTMR2* might form two complexes, one with *MTMR5*, in Sertoli or germ cells, involved in spermatogenesis and a second one with *MTMR13*, in Schwann cells, important in myelin homeostasis (13).

#### 4. Regulation and cellular functions of PI3P phosphatases

One important feature of myotubularins is their ability to form homo- and heterodimers (23) (Table 1). These interactions often lead to an enhancement of phosphatase activity or to regulation of myotubularin intracellular localization. For instance, *MTMR9*, an inactive phosphatase, binds to both *MTMR6* and *MTMR7* resulting in a significant increase of their activities (14,24) whereas *MTMR13* binds and activates *MTMR2* (25). Myotubularin coiled-coil domains play a major role in oligomer formation (14,26). Generally, these interactions take place between active and inactive myotubularins (23), however, it is interesting to note that *MTMR3* and *MTMR4*, two active phosphatases also form a heterodimer, which has been postulated to play a role in their redistribution within cells (23). Importantly, phosphatase activity does not seem to be required for myotubularin interactions (23,27). Several other myotubularin interacting proteins have been identified and they are summarized in Table 1.

In addition to being regulated by other myotubularins, *MTM1*, *MTMR3* and *MTMR6* have been shown to be activated by phosphatidylinositol 5-phosphate (PI5P), the product of PI3,5P<sub>2</sub> dephosphorylation (27). PI5P binds to PH-GRAM, a domain important for *MTM1* and *MTMR3* phosphatase activities, and for *MTMR2*, *MTMR3* and *MTMR6* association to intracellular membranes (27–30). PI5P is a specific allosteric activator of *MTM1* that promotes oligomerization of *MTM1* into a heptamer (27).

For the most part, the biological functions of myotubularins are poorly understood. When overexpressed most myotubularins promote a decrease in PI3P levels on endosomal compartments (23). Exogenously expressed myotubularins are found in cytosol and on intracellular structures that display different partners depending on the myotubularin investigated, suggesting that each myotubularin is targeted to a specific intracellular compartment where it dephosphorylates a definite pool of PI3P (23). A recent siRNA screen identified several myotubularins, *MTMR1*, *MTMR6*, *MTMR7*, *MTMR8* and *MTMR12*, as being important for cellular survival, yet, the molecular mechanisms behind this effect remain to be clarified (31). Since both are associated with debilitating human illnesses, *MTM1* and *MTMR2* have been the most studied. *MTM1* is present on ruffles at the plasma membrane, on early endosomes and partially on late endosomes where they regulate intracellular trafficking of Glut-4 and EGF receptor, respectively (32–35). In muscle fibers lacking *MTM1*, the structure of T-tubules, invaginations originating from the plasma membrane, is impaired,

supporting the idea of MTM1 being involved in membrane traffic (36,37). MTMR2 localizes on Rab7 positive compartment and similar to MTM1 its depletion results in reduction of EGFR degradation (33). In Schwann cells MTMR2 is believed to play an important role in membrane homeostasis at the plasma membrane during the process of myelination (38). Interestingly, MTMR2 binds to type III PI3K regulatory subunit, hVps15 (p150), which results in inhibition of phosphatase activity and in diminution of hVps34-Rab7 interaction important for PI3P synthesis (33) (Table 1). A similar binding was also observed between MTM1 and hVps15 (35). Finally, a recent study reports that in muscle fibers, MTMR14 regulates  $Ca^{2+}$  homeostasis by dephosphorylating PI3,5P<sub>2</sub>, an activator of  $Ca^{2+}$  release channel ryanodine receptor 1 (16).

## 5. PI3P phosphatases and autophagy

The majority of PI3P phosphatase functions described so far take place along the endocytic pathway. Given that PI3P plays a central role in autophagy, we wondered whether PI3P phosphatase(s) were also involved in regulating this pathway. A recent siRNA-based screen identified three PI3P phosphatases, Jumpy, MTMR6 and MTMR7, that affect level of autophagic marker LC3-II (39).

Jumpy-depleted mammalian cells show an increase in number of autophagic vacuoles in nutrient-rich and starvation conditions as well as an upregulation of long-lived protein proteolysis (39). In contrast cells overexpressing Jumpy but not MTM1 or MTMR2, fail to degrade autophagic substrate p62 leading to accumulation of protein aggregates (39). Jumpy colocalizes with LC3 positive compartments in particular with isolation membranes marked with Atg12 and Atg16 indicating that this phosphatase acts directly, and at an early stage of autophagic process (39). Importantly, Jumpy regulates Atg9 intracellular distribution, a PI3P-dependent event important for initiating autophagy (39–41). In yeast, Atg9 retrieval from autophagosomes requires the PI3P-binding protein Atg18 (41). Interestingly, Jumpy depletion in mammalian cells increases WIPI-1 (Atg18 ortholog) on membranes (39) suggesting that Jumpy dephosphorylates a pool of PI3P involved in WIPI-1 binding and Atg9 retrieval. In the future it would be important to elucidate the molecular mechanisms involved in Jumpy targeting to autophagic vacuoles and its regulation. In addition to increasing autophagy initiation, Jumpy depletion also promotes maturation of autophagosomes and LC3-II degradation (39). At the moment is unclear, whether this later effect is coupled to the initiation event or whether Jumpy acts at two different stages of the pathway. Inactive Jumpy variant, R336Q, found in one patient with centronuclear myopathy, is unable to inhibit autophagy suggesting that deregulation of autophagic pathway might be an important contributing factor to the disease (39).

In macrophages, MTMR6 and MTMR7 knockdown increase LC3-II level suggesting that additional phosphatases besides Jumpy may be important in regulating autophagy, however their mechanism of action remains to be investigated (39). MTMR6 is known to prevent activation of  $Ca^{2+}$ -activated K<sup>+</sup> channel, KCa3.1, which results in decreased  $Ca^{2+}$  influx during T-cell activation (42). Even though the role of  $Ca^{2+}$  in autophagy is still controversial (43–45), it is possible that MTMR6 modulates indirectly the autophagic response through change in  $Ca^{2+}$  homeostasis (Fig. 2).

Walker and al., 2001 reported that an inactive form of MTMR3, mutant C413S, is present inside compartment resembling autophagosomes in Hela cells (46). More recently, MTMR3 was reported to regulate constitutive autophagy initiation and autophagosome size in epithelial cells (47). However, MTMR3 knockdown does not affect steady-state level of LC3-II in macrophages (39). One possible explanation for this apparent discrepancy is that MTMR3 and Jumpy might play a redundant role during constitutive autophagy depending on cell types and

levels of expression. Yet, given that Jumpy lacks most of MTMR3 domains, their modes of regulation and recruitment are likely to be different.

PI3P is known to be a substrate for PIP5-kinase, Fab1 (also named PIKfyve), an enzyme that generates phosphoinositide PI3,5P<sub>2</sub> (Fig. 1). Although PI3,5P<sub>2</sub> does not appear to be important for autophagy in yeast, several studies indicate that PI3,5P<sub>2</sub> plays a key role in maturation of autophagosomes in mammals and flies (9,10,48). Mice deficient for Fig4 and Vac14, two regulatory proteins required for PI3,5P<sub>2</sub> synthesis, accumulate p62 in Lamp-2 positive compartments in neurons and astrocytes (48). Chemical inhibition of PIKfyve activity was shown to block LC3-II turn-over, and in drosophila, with impaired Fab1 function, an accumulation of autolysosomes was observed (9,10). Since myotubularins are also efficient PI3,5P<sub>2</sub> phosphatases, one can speculate that they might also be involved in late stages of autophagy.

Dephosphorylation of PI3,5P<sub>2</sub> by myotubularins gives rise to PI5P (Fig. 1). In particular, cells overexpressing MTM1 show an increase in the amount of PI5P which was not observed in cells expressing an inactive mutant D278A (49). This rare phospholipid, PI5P, has been found to activate the Akt pathway, though its mechanism of action remains elusive (50). It is important to note that MTMR2 overexpression also leads to sustained Akt activation (51). Thus, myotubularins may potentially activate mTOR via generation of PI5P and consequently block autophagy (Fig. 2). On the other hand, as we mentioned in the introduction, amino-acid-mediated PI3P synthesis induces mTOR activation (6,7). Therefore, if any myotubularin acts on this PI3P pool, it might affect mTOR activity and therefore autophagic response (Fig. 2). Anyhow, it would be of interest to investigate whether myotubularins play a role in regulating Akt/mTOR pathway and hence autophagy.

## 6. Conclusions and perspectives

The discovery of the PI3P phosphatases, Jumpy and more recently, MTMR3, as regulators of autophagy, shed some light on an unappreciated regulatory mechanism of the autophagic process. The initiation of autophagy is not only controlled by hVps34/Beclin-1-mediated synthesis of PI3P but also by its turn-over via action of specific phosphatases. These findings add Jumpy and MTMR3 to the list of potential therapeutic targets that could be used to modulate autophagy in the context of human diseases. The recent work on PI3P phosphatases and autophagy also indicate that other myotubularins could be implicated in this process. Elucidating their biological functions will undoubtedly improve our understanding of autophagy pathway and its regulation.

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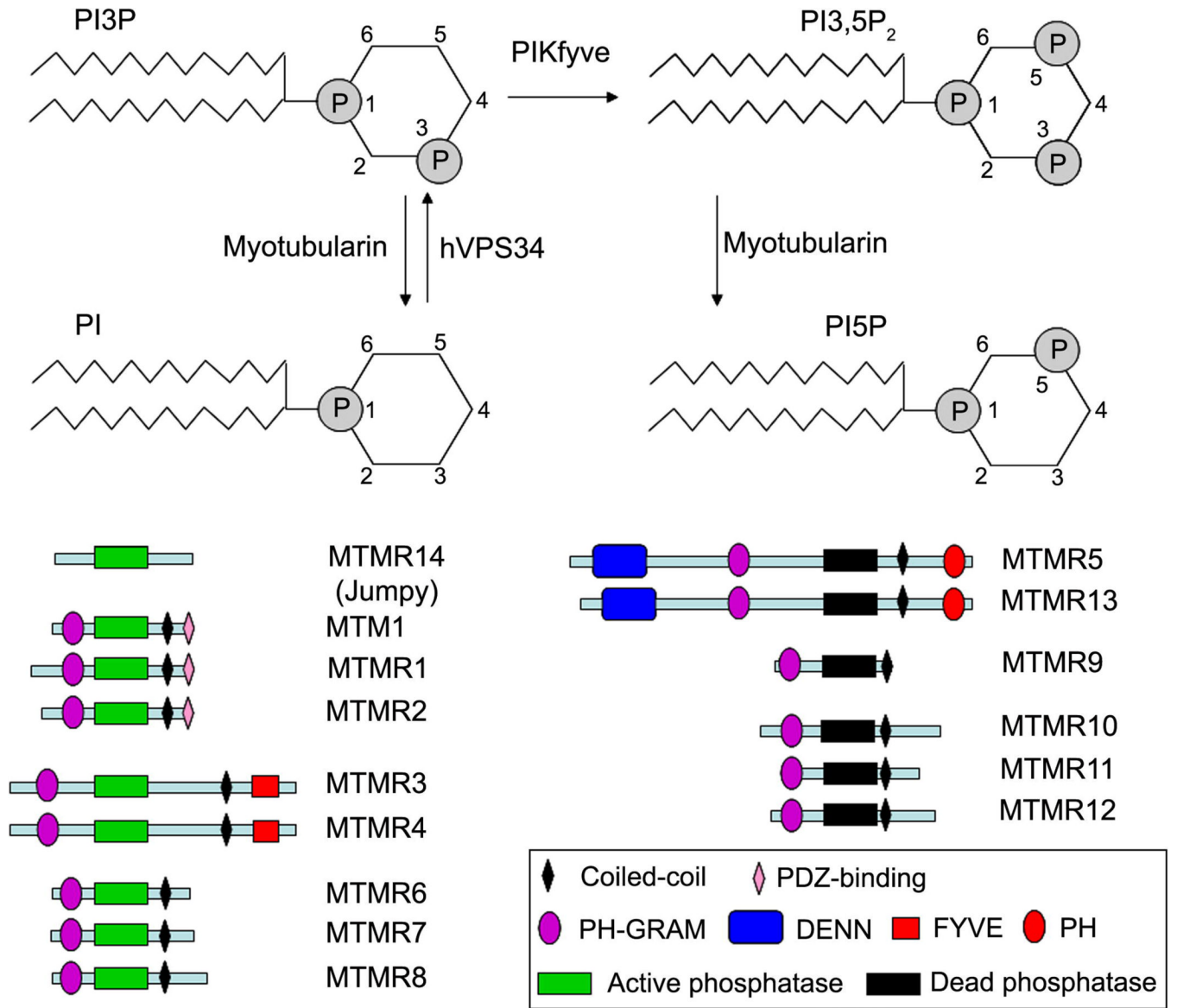
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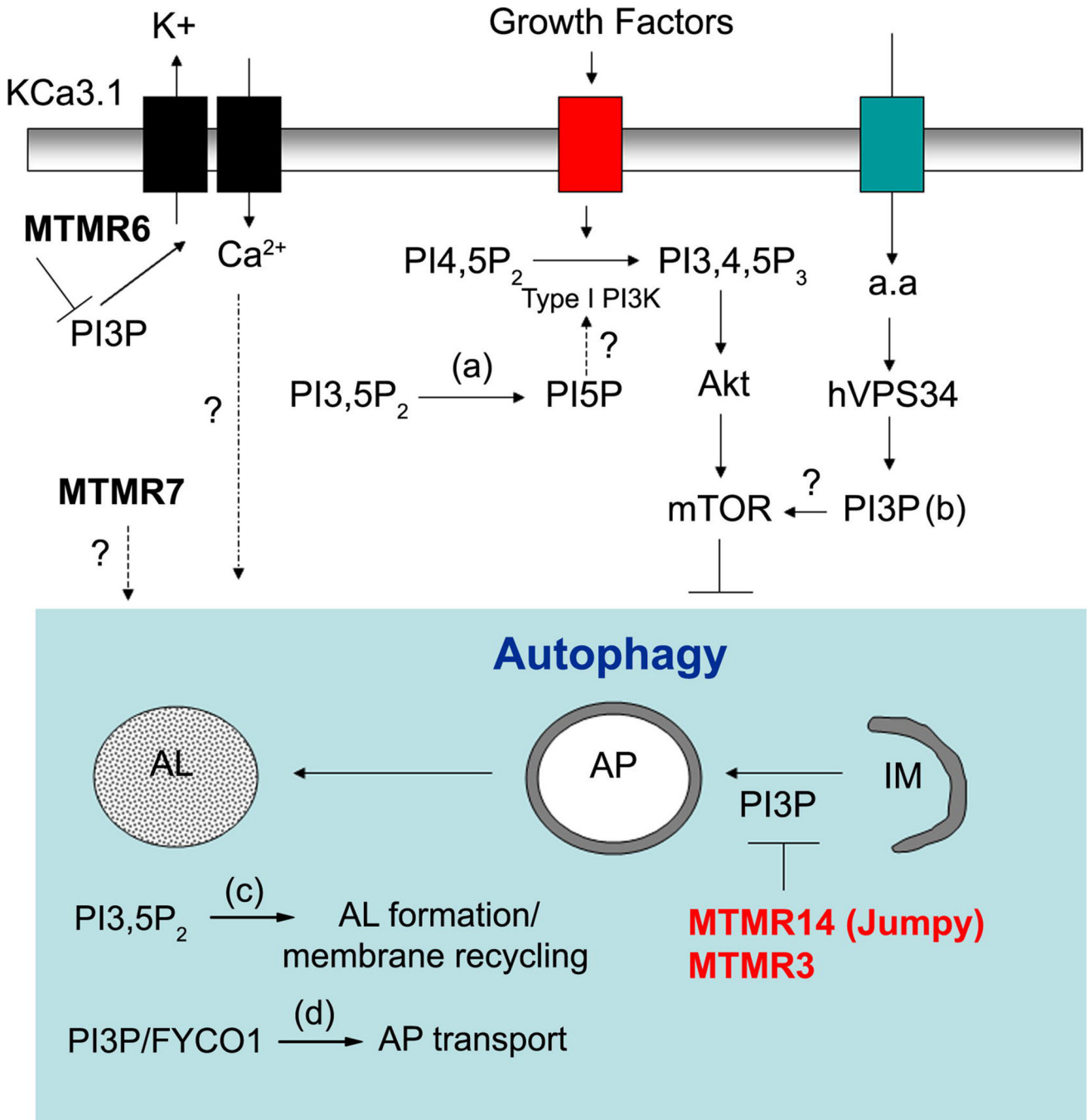
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**Figure 1. Myotubularin family: activity and different members with their domains**  
 hVps34 catalyzes phosphatidylinositol (PI) phosphorylation at position 3 on inositol ring to form phosphatidylinositol 3-phosphate (PI3P). PI3P can be further phosphorylated by PYKfyve to generate PI3,5P<sub>2</sub>. Myotubularins remove 3-phosphate on PI3P and PI3,5P<sub>2</sub> resulting in generation of PI and PI5P, respectively. Nine active and six inactive phosphatases are present in humans. See text for domain description.



**Figure 2. Intracellular PI3P pools and PI3P phosphatases in autophagy regulation**  
 MTMR14 and MTMR3 inhibit autophagy by dephosphorylating PI3P involved in autophagosome formation. MTMR6 and MTMR7 depletions increase LC3-II via unknown mechanisms. Since MTMR6 regulates KCa3.1, we propose that MTMR6 might modulate autophagy by acting on Ca<sup>2+</sup> influx. We speculate that some PI3P phosphatases might regulate autophagy by dephosphorylating pools of PI3P and/or PI3,5P<sub>2</sub> involved in Akt and mTOR activation (a and b), autolysosome formation/membrane recycling (c) and autophagosome transport (d) (see text and (52)). AP: autophagosome; AL: autolysosome. a.a.:amino-acids. In red: PI3P phosphatases inhibiting autophagy. IM: isolation membrane; In bold: PI3P

phosphatases whose knockdown affect LC3-II level. Question marks indicate that the role of these pathways in regulating autophagy is speculative and remains to be investigated.

**Table 1**

## Protein Interacting partners of active PI3P phosphatases

Myotubularins	Interacting partners	Effects or Functions	References
MTMR1	Myotubularins: 1, R10, R12	- MTM1 activity increase with MTM1 oligomerization - MTM1 redistribution with MTMR12	(23,27,53)
	p150 (hVps34)	Unknown	(35)
MTMR1	Unknown	-	-
MTMR2	Myotubularins: R2,R5, R10, R12, R13	- MTMR2 activity increase with MTMR13 - MTMR2 redistribution with MTMR5	(23,25,26, 53,54)
	p150 (hVps34)	- MTMR2 activity decrease - Indirect PI3P inhibition	(33)
	Neurofilament light chain, Disk large 1 Dlg1/SAP97	Myelin outfoldings regulation with Dlg1	(38,55,56)
	Nonreceptor protein tyrosine kinase c-Src	Possibly cell adhesion regulation	(57)
MTMR3	Myotubularins: R3, R4	MTMR3 redistribution with MTMR4	(23)
MTMR4	Myotubularins: R3, R4	MTMR4 redistribution with MTMR3	(23)
	Ubiquitin ligase Need4	Unknown	(58)
MTMR6	Myotubularins: R9	MTMR6 activity increase MTMR6 stability increase	(23,24)
	Ca <sup>2+</sup> -activated K <sup>+</sup> channel (K(Ca)3.1)	Indirect K(Ca)3.1 inhibition	(42)
MTMR7	Myotubularins: R9	MTMR7 activity increase	(14,23)
MTMR8	Myotubularins: R8, R9	Unknown	(23)
MTMR14	Unknown	-	-