

Vps34 and the Armus/TBC-2 Rab GAPs: Putting the brakes on the endosomal Rab5 and Rab7 GTPases

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ABSTRACT

Rab5 and Rab7 GTPases are key regulators of endosome maturation and lysosome fusion. They activate the class III phosphoinositide 3-kinase (PI3K) Vps34 to generate pools of phosphatidylinositol-3 phosphate [PI(3)P] on endosomes. Together PI(3)P and the GTP-bound Rabs coordinate the recruitment of endosomal regulators to drive early to late endosome maturation and ultimately lysosome fusion. Counterintuitively, loss of Vps34 results in enlarged endosomes, like those seen from expressing activated Rab GTPases. Two recent papers in the *Journal of Cell Science*, Jaber *et al.*, 2016 and Law, Seo *et al.*, 2017, demonstrate that a function of Vps34 is to inactivate the Rab5 and Rab7 GTPases via recruitment of the TBC1D2 family of Rab GTPase Activating Proteins (GAPs).

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The Rab5 and Rab7 GTPases are important regulators of early and late endosome trafficking of cargo to the lysosome for degradation (Fig. 1).¹ Like other small GTPases, Rab GTPases cycle between a GTP-bound active state and a GDP-bound inactive state, both of which are regulated by Rab Guanine nucleotide Exchange Factors (GEFs) and Rab GTPase Activating Proteins (GAPs), respectively. In the GTP-bound state, Rab5 and Rab7 localize to early and late endosomes, respectively, where they can recruit effector proteins. Both Rab5 and Rab7 activate the Vps34 PI3K to generate PI(3)P on endosomes.^{2–6} Many effectors bind to PI(3)P via PX (Phox) and FYVE (Fab1, YOTB, Vac1, EEA1) domains to coordinate aspects of endosome maturation and trafficking such as vesicle fusion.^{7–14} While Vps34 and the Rab GTPases cooperate to regulate endosome trafficking, loss of Vps34 results in enlarged endosomes,^{15,16} a phenotype that is also observed from increased Rab5 activity.¹⁷ These observations suggest a potential negative feedback loop for Rab5 and Rab7 GTPases.

Jaber *et al.* (2016) and Law, Seo *et al.* (2017) provide evidence that specific loss of Vps34 results in increased Rab GTPase activity.^{18,19} The Zong lab generated Vps34 knockout mouse embryonic fibroblasts (MEFs) that accumulate enlarged Rab7 positive endosomes.¹⁵ They found that Vps34-null MEFs have increased Rab5 and Rab7 activities using effector pull-down assays.^{18,19} In the nematode *Caenorhabditis elegans*, loss of *vps-34*, or its complex components *vps-15* and *bec-1*, led to enlarged RAB-5 and RAB-7 positive endosomes full of undegraded material.^{18,20} This phenotype had only previously been reported in animals either expressing constitutively activated RAB-5 Q78L or carrying a loss-of-function mutation in *tbc-2*, a

gene which encodes for a RAB-5 GAP.²¹ Together, these findings in mouse cells and *C. elegans* indicate that the Vps34 PI3K has a role in Rab GTPase inactivation.

The key to this novel negative feedback loop is the TBC1D2 family of Rab GAPs, whose members include the *C. elegans* TBC-2 and its mammalian homolog Armus (aka TBC1D2A and PARIS-1).^{21–24} This protein family is characterized by an N-terminal Pleckstrin Homology (PH) domain, a central coiled-coil (CC) domain and a C-terminal Tre-2/Bub2/Cdc16 (TBC)/Rab GAP domain (Fig. 1). *In vitro*, TBC-2 catalyzed GTP hydrolysis of RAB-5 and to a lesser extent RAB-7.²¹ Membrane fractionation studies from *C. elegans* extracts confirmed that *tbc-2* mutant animals have increased Rab5 activity as compared to wild-type.²⁵ Genetic and phenotypic analyses are consistent with TBC-2 functioning as a RAB-5 GAP in both the recycling and degradative pathways, but are not inconsistent with it also regulating RAB-7.^{21,24–28} Armus had Rab7 GAP activity *in vitro*,²² and overexpression of Armus in MEFs resulted in decreased Rab7 activity.²⁹ Armus may have additional functions earlier in the endosomal pathway, including a role in endosome recycling.^{22,30} TBC-2 and Armus localized to late endosomes where they could regulate Rab7 activity or, in the case of TBC-2, inactivate Rab5 to promote Rab5 to Rab7 conversion during endosome maturation (Fig. 1).^{19,21}

How are TBC-2 and Armus recruited onto endosomes? PH domains can bind phosphoinositides³¹; therefore, TBC-2 and Armus were obvious candidates for regulation by Vps34. Consistent with this hypothesis, knockdown of Vps34 resulted in a loss of TBC-2 and Armus localization to endosomes.^{18,19} While PH domains do not typically bind PI(3)P³¹, both Jaber *et al.*

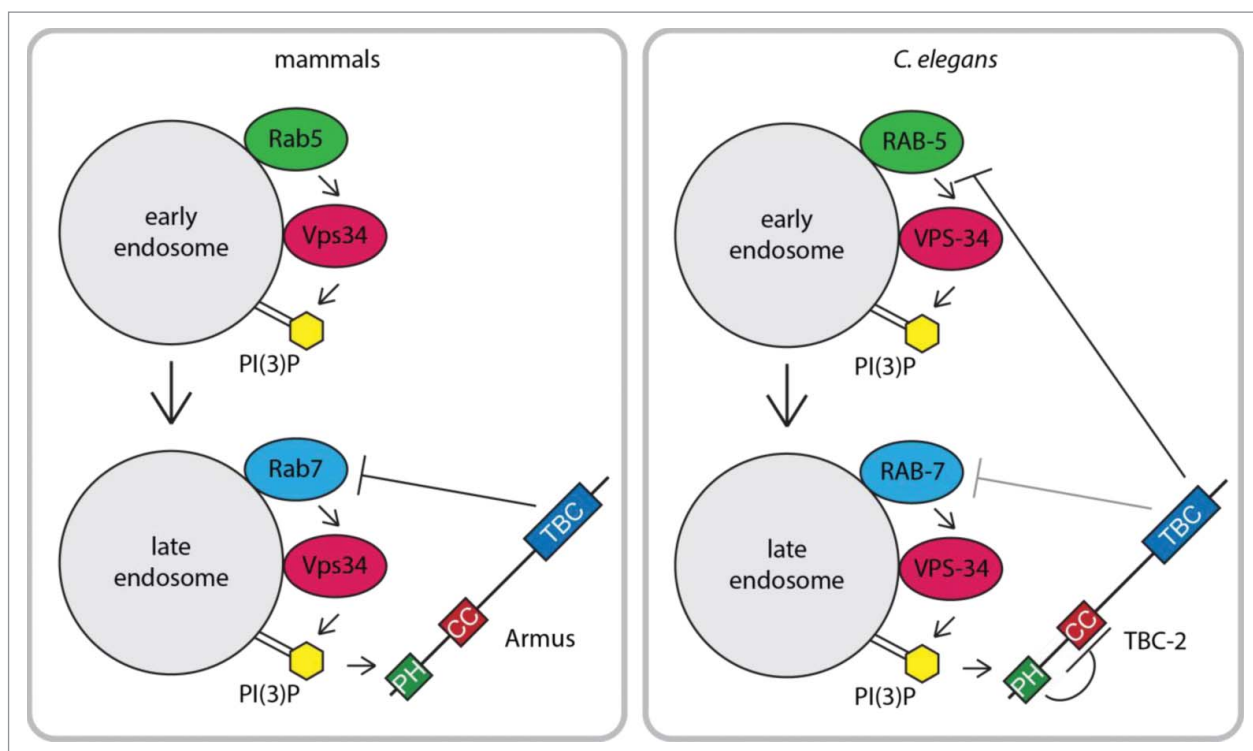


Figure 1. Model of mammalian Armus and *C. elegans* TBC-2 function and regulation during early to late endosome maturation.

(2016) and Law, Seo et al. (2017) independently found that the PH domains of TBC-2 and Armus preferentially bound to PI(3)P and PI(4)P (phosphatidylinositol-4 phosphate) using protein-lipid overlay assays.^{18,19} In collaboration with the laboratory of Guangwei Du (University of Texas Health Science Center at Houston), they demonstrated that the PH domains of both TBC-2 and Armus preferentially bound liposomes containing PI(3)P or PI(4)P over other acidic phospholipids such as phosphatidic acid.^{18,19} Together, these data suggest a direct role for Vps34-generated PI(3)P in recruiting *C. elegans* TBC-2 and mammalian Armus to endosomes as part of a negative feedback loop to inactivate Rab5 and Rab7, respectively (Fig. 1).

The plot thickens as the PH domain of TBC-2 is not required for localization to endosomes or phagosomes.^{18,24} In fact, we found that deletion of the PH domain, TBC-2(Δ PH), increased TBC-2 localization to endosomes and rescued the *tbc-2* mutant intestinal phenotype.¹⁸ However, further deletion of the CC domain region abrogated TBC-2 localization to endosomes and did not rescue the *tbc-2* mutant intestinal phenotype. These data suggest that (1) the PH domain antagonizes TBC-2 recruitment to endosomes and (2) binding of PI(3)P to the PH domain permits the CC domain region to interact with proteins and/or lipids on the endosome membrane. Interestingly, expression of Armus(Δ PH) in MEFs was more potent than full-length Armus for decreasing Rab7 activity²⁹, although we do not know if it is due to increased endosome localization as seen with TBC-2. However, the PH domain of Armus was shown to bind Armus(Δ PH), suggesting that Armus localization and/or activity is regulated by an intramolecular interaction.²⁹ We hypothesize that PI(3)P binding by the PH domain could relieve an autoinhibitory intramolecular interaction and permit TBC-2/Armus localization to endosomes.

In a second plot twist, we discovered that TBC-2(Δ PH) still required Vps34 for endosome localization.¹⁸ Therefore, Vps34 appears to have dual roles in regulating the recruitment of TBC-2 to endosomes. An outstanding question remains on what mechanism regulates TBC-2 recruitment through the region containing the CC domain. This could be through a second cryptic PI(3)P binding site, or through mediating an interaction with an endosomal protein that is itself regulated by PI(3)P or its derivative, PI(3,5)P₂. Alternatively, TBC-2 could directly interact with the Vps34 class III PI3K complex in a PI(3)P-independent manner.

In summary, Jaber et al. (2016) and Law, Seo et al. (2017) uncovered a negative feedback loop whereby activation of the class III PI3K Vps34 by the Rab5 and Rab7 GTPases leads to endosomal recruitment of the Armus and TBC-2 Rab GAPs in mammalian cells and *C. elegans*.^{18,19} These findings echo that of a previous study, which found that non-selective PI3Ks inhibitors (Wortmannin or LY294002) increased Rab5 activity on phagosomes of hamster and murine cell lines.³² Vieira et al. (2003) proposed that PI3Ks could mediate the activation and/or recruitment of a Rab5 GAP to phagosomes. We do not know if Armus and/or its paralog, TBC1D2B, have Rab5 GAP activity. If so, they could be responsible for those findings. There are still many outstanding questions to be answered. Does Vps34 dually regulate Armus and TBC1D2B as it does for TBC-2? What are the factors that recruit TBC-2 to late endosomes? Identifying the additional regulator(s) of TBC-2/Armus recruitment to endosomes should help answer some of these questions.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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