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Small GTPase Rab12 regulates transferrin receptor degradation

Implications for a novel membrane trafficking pathway from recycling endosomes to lysosomes

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Plasma membrane receptor proteins play a key role in signal transduction and nutrient uptake, thereby controlling quality of receptor proteins is one of the most important issues in cellular logistics. After endocytosis, receptor proteins are generally delivered to lysosomes for degradation or recycled back to the plasma membrane for recycling. Transferrin receptor (TfR) is a well-known representative of recycling receptor proteins, which are traveled between plasma membrane and recycling endosomes. Although the molecular mechanism of the TfR recycling pathway has been extensively investigated in the literature, almost nothing is known about its degradation mechanism. We have recently shown that small GTPase Rab12 and its upstream activator Dennd3 regulate the constitutive degradation of TfR without modulating a conventional endocytic degradation pathway or TfR recycling pathway. Our findings suggest that Rab12 regulates membrane trafficking of TfR from recycling endosomes to lysosomes. In this addendum, we discuss the physiological significance of TfR degradation and the fate of determination of TfR (recycling or degradation).

Key words: degradation, lysosome, membrane traffic, recycling endosome, small GTPase Rab, transferrin receptor

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Discovery of a Novel Degradation Pathway of Transferrin Receptor (TfR) from Recycling Endosomes to Lysosomes

TfR and transferrin (Tf) involve maintenance of intracellular iron homeostasis.[1,2] After binding of diferric Tf (Fe_2Tf), internalized TfR is first transported to early endosomes, where iron is released from Tf-TfR complex. TfR is then transported to recycling endosomes and finally to the plasma membrane (so-called recycling pathway).[3] However, since TfR protein could be damaged during recycling process, e.g., by acidic pH in early/recycling endosomes, it seems unreasonable to use the same TfR protein forever, and we anticipated that TfR is constitutively degraded and synthesized, the same as other intracellular proteins. In contrast to the TfR recycling pathway,[4-7] however, almost nothing is known about the mechanism of TfR degradation. Recently, we found that treatment of mouse embryonic fibroblast (MEF) cells with cycloheximide, an inhibitor of protein synthesis, caused a dramatic reduction in TfR signals (**Fig. 1A**, compare left and middle parts), indicating that TfR is constitutively degraded in MEF cells.[8] On the contrary, treatment of MEF cells with bafilomycin A1, an inhibitor of V-ATPase, that blocks lysosomal degradation, caused a dramatic increase in TfR signals (**Fig. 1A**, right part), indicating that some portions of TfR protein are actually degraded by lysosomes.[8]

To unravel the molecular mechanism of TfR degradation, we recently employed a comprehensive analysis of the mammalian Rab family small GTPases[9,10] and successfully identified Rab12 as a novel regulator of TfR degradation.[8] We found that overexpression of a constitutive active mutant of Rab12 caused a reduction in the amount of TfR protein, whereas functional ablation of Rab12 by knockdown of either Rab12

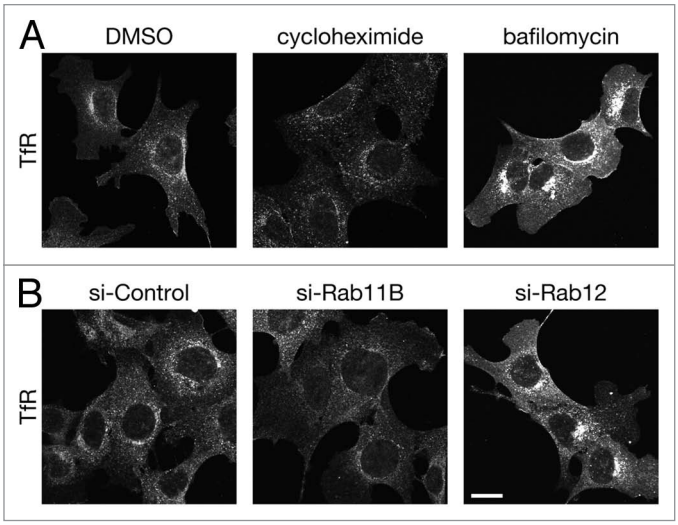

Figure 1. (A) TfR is constitutively degraded by lysosomes in MEF cells. MEF cells were treated with DMSO (left part), 50 μg/ml cycloheximide (middle part) for 4 h or 100 nM bafilomycin A1 (bafilomycin; right part) for 9 h. The cells were fixed and immunostained with anti-TfR antibody. Note that TfR signals in cycloheximide-treated and bafilomycin-treated cells were weaker and stronger, respectively, than those in control cells. (B) Rab11 and Rab12 differently regulate the trafficking of TfR protein from recycling endosomes. MEF cells transfected with control siRNA (si-Control; left part), *Rab11B* siRNA (si-Rab11; middle part) or *Rab12* siRNA (si-Rab12; right part) were immunostained with anti-TfR antibody. Note that TfR signals in Rab11B-knockdown and Rab12-knockdown cells were weaker and stronger, respectively, than those in control cells. Scale bar, 20 μm.

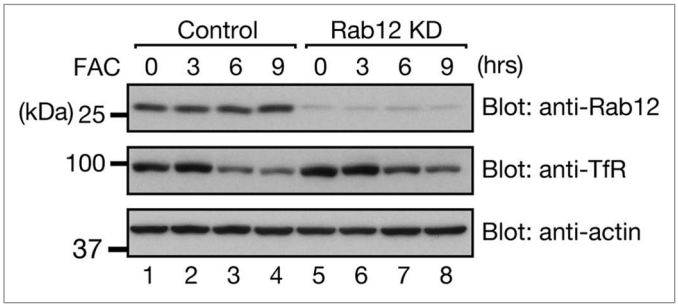

Figure 2. Rab12 is not involved in iron-induced TfR degradation. MEF cells transfected with control siRNA or *Rab12* siRNA were treated with 50 μg/ml FAC (ferric ammonium citrate, an iron supplier) for the indicated times. Cell lysates were analyzed by 10% SDS-PAGE followed by immunoblotting with anti-Rab12 antibody (top part), anti-TfR antibody (middle part), and anti-β-actin antibody (bottom part). The positions of the molecular mass markers (in kDa) are shown on the left.

itself or its upstream activator Dennd3 (i.e., guanine nucleotide exchange factor (GEF) for Rab12[11]) caused an increase in the amount of TfR protein, indicating that Rab12 functions as a positive regulator of TfR degradation. Most importantly, Rab12 knockdown has no effect on degradation of epidermal growth factor receptor (EGFR), which is known to be degraded by the conventional degradation pathway,[12-14] or TfR recycling pathway. Furthermore, Rab12 co-localizes with TfR-positive recycling endosomes and partially with lysosomes, but not with early endosomes or late endosomes/multi vesicular bodies (MVBs). These findings strongly indicated the presence of a novel membrane trafficking pathway, in which Rab12 regulates TfR protein trafficking from recycling endosomes to lysosomes.

Physiological Significance of TfR Degradation Pathway

What is the physiological significance of TfR degradation pathway? One might expect that cells actively degrade TfR protein when they are placed under iron-rich conditions. Actually, Tachiyama and coworkers very recently reported that excess iron treatment induced TfR degradation at lysosomes.[15] In contrast to our finding,[8] they also showed that ubiquitylated TfR protein is accumulated at late endosomes/MVBs in cells expressing a dominant negative mutant of SKD1/Vps4, which is required for the conventional degradation pathway,[16-19] and that ubiquitylation of TfR is increased after excess iron treatment. So far, however, there was no report on ubiquitylation of TfR under "basal" conditions, suggesting that ubiquitylation-dependent degradation of TfR may occur under "selective" conditions (e.g., excess irons). Since iron treatment also affects transcription of *TfR* gene,[20,21] we speculate that cells rapidly decrease TfR protein level both by reducing transcription of *TfR* gene and by ubiquitylation-dependent degradation of TfR protein in response to excess iron concentrations.

An interesting question is as to whether Rab12 is involved in iron-induced TfR degradation described above. The answer is probably no, because our data showed that iron-induced TfR degradation normally occurs even in Rab12 knockdown cells (**Fig. 2**). We therefore propose that two distinct TfR degradation pathways exist in cells: (1) iron-induced degradation pathway for reducing iron uptake under "selective" conditions[15] and (2) Rab12-dependent constitutive degradation pathway[8] for quality control of TfR protein under "basal" conditions (**Fig. 3**).

The Fate Determination of TfR Protein

What is the molecular determinant of the fate of TfR, i.e., recycling back to the plasma membrane or to lysosomes? One possible mechanism for the determination of the final destination of TfR protein is the mechanism regulated by two small GTPase Rabs, Rab11 and Rab12. Rab11 functions as a crucial regulator for TfR trafficking from recycling endosomes to the plasma membrane,[4-6] and knockdown of Rab11B, a major Rab11 isoform in MEF cells, caused a reduction in TfR signals (**Fig. 1B**, compare left and middle

parts). In contrast, knockdown of Rab12 in MEF cells caused an increase in TfR signals, the same as the bafilomycin A1 treatment did (**Fig. 1A and B**, right parts). Recruitment of Rab11 and Rab12 to TfR-positive recycling endosomes may be regulated by a random process. Alternatively, some sort of competition between Rab11 and Rab12 on TfR-positive recycling endosomes may occur. Further elucidation of the regulatory mechanism of their upstream regulators (e.g., Dennd3 for Rab12 and unidentified Rab11-GEF) will be necessary to determine when and where Rab11 and Rab12 are activated.

Perspectives

Two recent reports, including our own work, indicated that TfR, which is previously thought to be recycled back to the plasma membrane, undergo degradation at lysosomes under "selective" conditions (e.g., excess iron concentrations)[15] and "basal" conditions[8] (**Fig. 3**). The latter pathway is extremely interesting, because TfR protein is degraded by a novel Rab12-dependent pathway, which is likely to travel directly from recycling endosomes to lysosomes, but not by the conventional endocytic pathway (from late endosomes/MVBs to lysosomes). One important remaining question is as to whether recycling receptor proteins other than TfR (e.g., LDL receptor, TGFβ receptor and β2-adrenergic receptor) also undergo Rab12-dependent constitutive degradation. Future investigation of the degradation of other recycling receptor proteins in Rab12-knockdown cells or of the cargos of Rab12-bearing vesicles/membranes will be necessary to determine whether Rab12-dependent membrane trafficking pathway has a more general role in lysosomal degradation of recycling receptor proteins.

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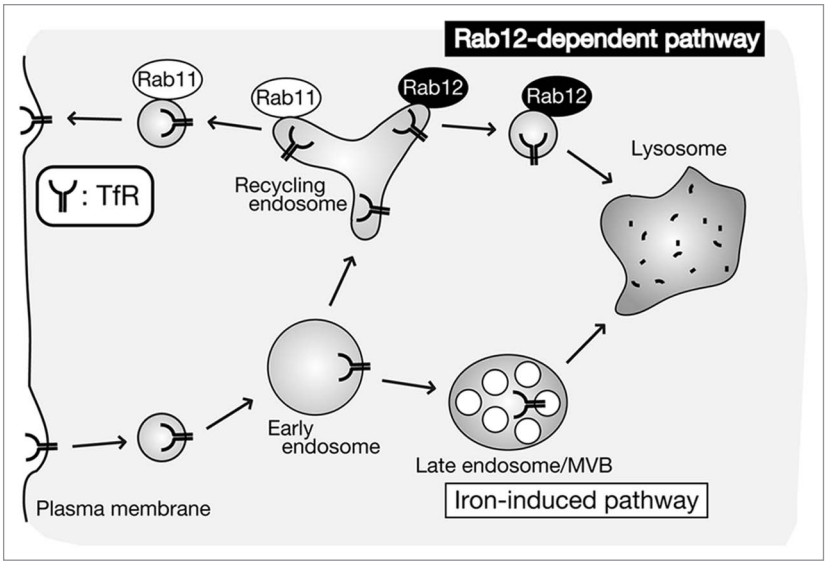

Figure 3. A model for two distinct TfR degradation pathways. Internalized TfR is recycled back to the plasma membrane through Rab11-depdenent recycling pathway or delivered to lysosomes through either iron-induced "selective" pathway[15] or Rab12-dependent "constitutive" pathway.[8] Adapted with permission from reference 8.

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