

# 論 文 概 要

論文題目

TBC1D24 regulates formation of tubular recycling endosomes  
and promotes recycling of clathrin-independent cargo proteins

(TBC1D24 はチューブ様リサイクリングエンドソームの形成を制御し  
クラスリン非依存性に取り込まれる膜タンパク質のリサイクリングを促進する)

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*TBC1D24* was initially identified as a novel causative gene for familial infantile myoclonic epilepsy. *TBC1D24* has a Tre2/Bub2/Cdc16 (TBC) domain, which is a common structure found in regulators of Rab small GTPases, and a TBC-Lysin motif (LysM)-Domain catalytic (TLDC) domain, which is suggested to be involved in oxidative stress resistance. Accumulating evidence supports that *TBC1D24* is required for neuronal development and regulation of synaptic endocytic vesicle trafficking. *TBC1D24* has been shown to bind Arf6, through which *TBC1D24* regulates neuronal migration and maturation in a mouse model. Skywalker, the *Drosophila melanogaster* homologue of *TBC1D24*, exhibits *in vitro* GAP activity toward Rab35 and regulates synaptic endocytic vesicle trafficking through Rab35. However, detailed functions of *TBC1D24* in mammalian cells are still unclear.

Plasma membrane proteins internalize into cells through either clathrin-mediated endocytosis (CME) or clathrin-independent endocytosis (CIE). Following endocytosis, cargo proteins are transported to lysosomes for degradation or recycled back to the plasma membrane. Since Arf6 and Rab proteins play pivotal roles in endocytosis and following intracellular membrane trafficking of plasma membrane proteins, *TBC1D24* is likely to be involved in the regulation of these processes. In this study, I show in HeLa cells that overexpression of *TBC1D24* increases CIE cargo-containing tubular recycling endosomes (TREs) that are a hallmark of the CIE cargo trafficking pathway, while CRISPR/Cas9-based deletion of *TBC1D24* decreases this compartment. The deletion of *TBC1D24* did not affect the internalization of CIE cargo into cells, suggesting that the decrease of TREs filled with CIE cargo proteins is not due to the reduction in incoming cargo proteins into cells. By employing GFP appended with the C-terminal 20 amino acids of H-Ras (GFP-Ras-C<sup>20</sup>) as a marker for TREs, I find that the deletion of *TBC1D24* impairs formation of TREs. The impairment of TRE formation in *TBC1D24*-deleted cells led to the delay of CIE cargo recycling back to the plasma membrane. I also find that *TBC1D24* binds to the small GTPase Rab22A, the key regulator of TRE formation as well as recycling of CIE cargo proteins. Knockdown of Rab22A reversed the effect of *TBC1D24* overexpression on the trafficking of CIE cargo proteins, while the decrease of CIE cargo-specific TREs in *TBC1D24*-deleted cells was rescued by overexpression of Rab22A. These results suggest that *TBC1D24* regulates TRE formation through Rab22A. Furthermore, I find that deletion of *TBC1D24* and knockdown of Rab22A both impairs cell proliferation.

Based on the results obtained, I propose a novel function of *TBC1D24* in the CIE cargo trafficking pathway: *TBC1D24* promotes formation of CIE cargo-specific TREs through Rab22a, leading to facilitation of CIE cargo recycling. Through this function, *TBC1D24*

appears to control quantity and/or quality of the plasma membrane proteins at the cell surface, thereby contributing to the regulation of cellular functions, including cell proliferation.