CLN5 and CLN3 function as a complex to regulate endosome-to-TGN trafficking

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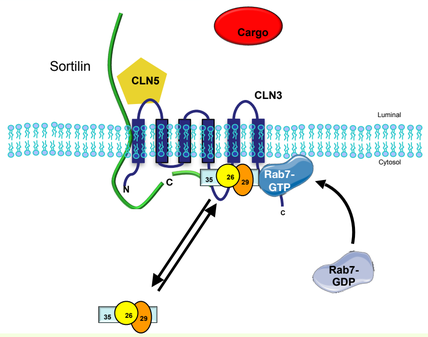
INTRODUCTION



Mutations in the *CLN5* and *CLN3* genes are the cause of a rare neurodegenerative disorder called Batten disease. CLN5 is a soluble endolysosomal protein, while CLN3 is an integral membrane protein localized to the same compartment. Previously we have shown that both CLN5 and CLN3 are required for the recruitment of retromer to endosomal membranes. However, how a soluble lysosomal protein (CLN5) can modulate recruitment of a protein in the cytosol is not known. Studies have shown that CLN3 can interact with other CLN proteins including CLN5, however the molecular function of this interaction has not been shown.

Retromer is a protein complex that mediates the endosome-to-trans Golgi Network (TGN) trafficking of the lysosomal sorting receptor sortilin, under the regulation of the small GTPase Rab7A. GTP bound Rab7 is localized to late endosomes and regulates the spatiotemporal recruitment of retromer.

In this work, we demonstrate a role for CLN5/CLN3 as a complex on the sorting of sortilin and the effects of CLN5 disease-causing mutations on this function.

RESULTS

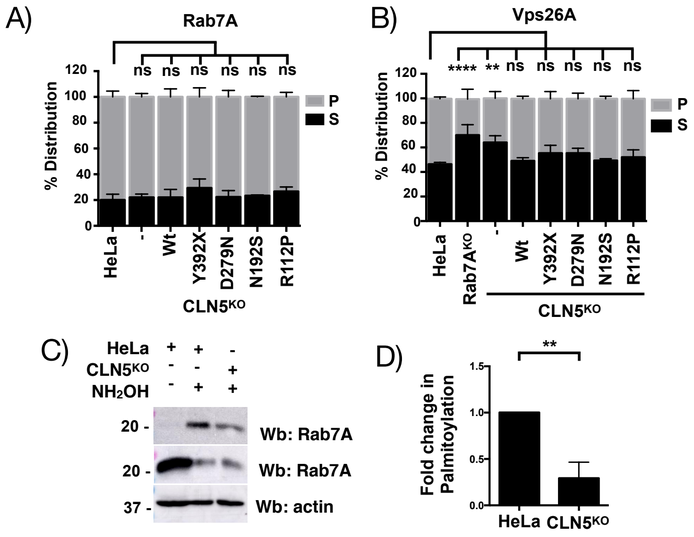


Figure 1. Rab7A palmitoylation is reduced in CLN5^{KO} HeLa cells affecting retromer recruitment.

Quantification of 3 separate membrane isolation assay experiments for Rab7A (A) and Vps26A (B) distribution. (C) Whole cell lysate from wild-type and CLN5^{KO} HeLa cells were subjected to Acyl-RAC analysis to determine the palmitoylation status of Rab7A. NH_2OH: hydroxylamine, PD: pull-down (D) Quantification of 3 separate Acyl-RAC assay experiments.

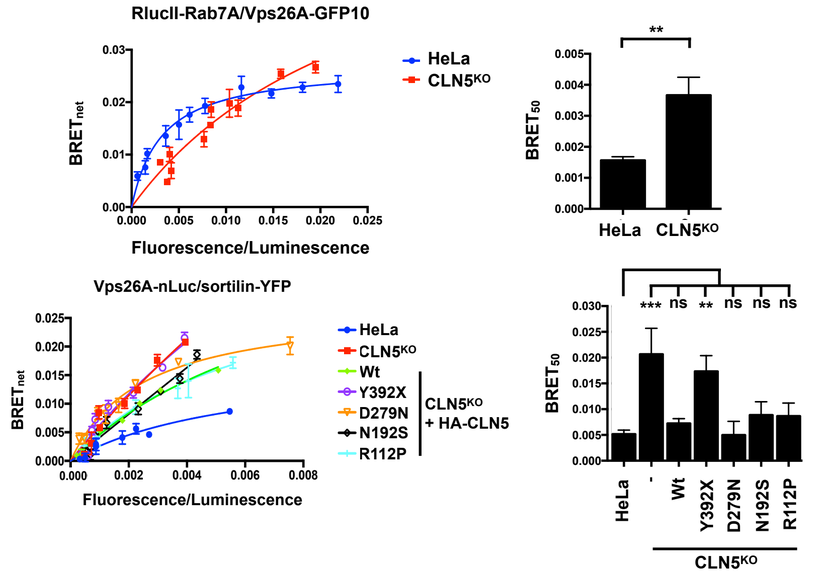


Figure 2. CLN5 is required for efficient retromer interactions

To generate BRET titration curves, wild-type and CLN5^{KO} HeLa cells were transfected with a constant amount of RlucII-Rab7A and increasing amounts of Vps26A-GFP10 (**A**), wild-type, CLN5^{KO} and CLN5^{KO} HeLa rescued with wild-type HA-CLN5 or harbouring a disease-causing mutation were transfected with a constant amount of Vps26A-nLuc and increasing amounts of sortilin-YFP (**C**). BRET signals are plotted as a function of the ratio between the GFP10 fluorescence over RlucII luminescence, YFP fluorescence over nLuc. (**B, D**) $BRET_{50}$ was extrapolated from 3 independent experiments.

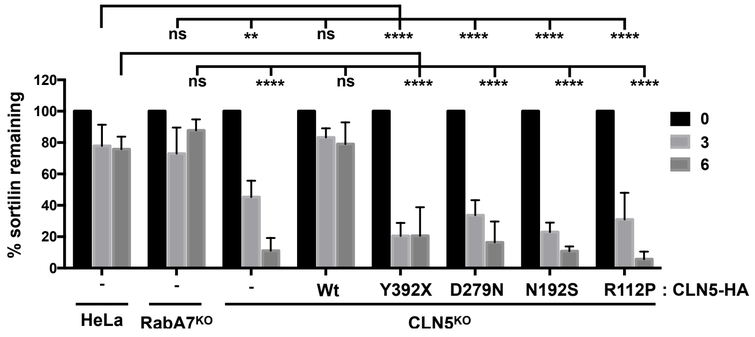


Figure 3. The lysosomal sorting receptor sortilin is degraded in CLN5^{KO} HeLa cells.

(**A**) Wild-type, Rab7A^{KO}, CLN5^{KO}, and CLN5^{KO} HeLa cells expressing CLN5-HA, CLN5^{Y392X}-HA, CLN5^{N192S}-HA, CLN5^{D279N}-HA or CLN5^{R112P}-HA were treated with 50 µg/ml of cycloheximide in serum free media for the indicated times. Whole cell lysate was run on a SDS-PAGE and Western blotting (Wb) was performed with anti-sortilin, anti-actin and anti-HA antibodies. (**B**) Quantification of 3 independent experiments.

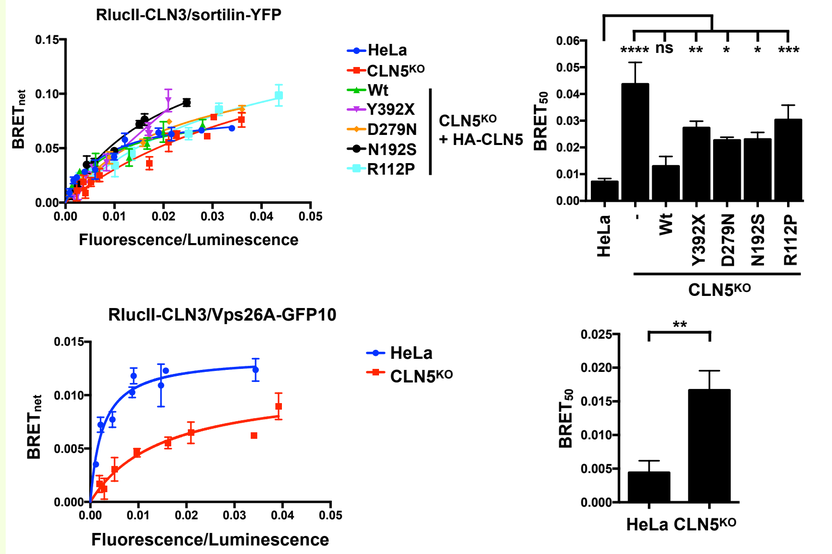


Figure 4.CLN5 modulates CLN3 interactions

Wild-type and CLN5^{KO} HeLa cells were transfected with a constant amount of RlucII-CLN3 and increasing amounts of Vps26A-GFP10 (A), wild-type, CLN5^{KO} and CLN5^{KO} HeLa rescued with wild-type HA-CLN5 or harbouring a disease-causing mutation were transfected with a constant amount of RlucII-CLN3 and increasing amounts of sortilin-YFP (B) to generate BRET titration curves. BRET signals are plotted as a function of the ratio between the GFP10 or YFP fluorescence over RlucII luminescence. (**D**) Quantification of 3 independent experiments.

SUMMARY

CLN5 regulates retromer function by modulating;

- **Rab7 palmitoylation for its membrane recruitment**
- **the key interactions between CLN3, Rab7A, retromer, and sortilin.**

Therefore, CLN5/ CLN3 molecular complex serves as endosomal switch regulating the itinerary of the lysosomal sorting receptors.

ACKNOWLEDGEMENT

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Abstract: Mutations in the CLN5 and CLN3 genes are the cause of a rare neurodegenerative disorder called neuronal ceroid lipofuscinosis, more commonly referred to as Batten disease. CLN5 is a soluble endolysosomal protein, while CLN3 is an integral membrane protein localized to the same compartment. Previously we have shown that both CLN5 and CLN3 are required for the recruitment of retromer to endosomal membranes. However, how a soluble lysosomal protein (CLN5) can modulate recruitment of a protein in the cytosol is not known. Furthermore, the impact of disease-causing mutations in CLN5 on this process have not been studied. Retromer is a protein complex that mediates the endosome-to-trans Golgi Network (TGN) trafficking of the lysosomal sorting receptor sortilin, under the regulation of the small GTPase Rab7A. GTP bound Rab7 is localized to late endosomes and regulates the spatiotemporal recruitment of retromer, the degradation of integral membrane proteins such as epidermal growth factor (EGF) receptor (EGFR), lysosomal positioning, and also participates in autophagosome-lysosome fusion through HOPS complex, thereby regulating autophagy. Our previous work demonstrated that silencing of CLN5 using siRNA resulted in the degradation of sortilin as a result of inefficient retromer membrane recruitment. Recently, we have shown that CLN3 is also required in the endosome-to-TGN trafficking of the lysosomal sorting receptors. Studies have shown that CLN3 can interact with other CLN proteins including CLN5, however the molecular function of this interaction is not known. In this work, we engineered a CLN5 knockout HeLa cell line using CRIPSR/Cas9. We showed that CLN5 is required for the palmitoylation of Rab7A, which is required for the efficient recruitment and function of retromer. We found that sortilin is degraded in CLN5 knockout HeLa cells. CLN3 is also required for the endosome-to-TGN sorting of sortilin by interacting with sortilin, a step required for the sortilin/retromer interaction. The CLN3/sortilin interaction was disrupted in CLN5 knockout HeLa cells. This suggests that CLN5 and CLN3 function as a complex at endosomes to coordinate the sorting of sortilin. In summary, we demonstrate a role for CLN5/CLN3 as a complex on the sorting of sortilin and the effects of CLN5 disease-causing mutations on this function.