In-vivo interactor and pathway analysis of CLN3, CLN6, and CLN8 using proximal biotinylation

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Abstract

For many years, scientists have sought common, overlapping mechanisms for the proteins mutated in Batten disease with little success. Three of these proteins, CLN3, CLN6, and CLN8, are transmembrane proteins suggested to be involved with intracellular trafficking, though the relevance of these roles for neuron-specific functions remains largely unknown. Here we employ BioID to label interacting and associated proteins of these CLN proteins. Initial data was generated using a neuroblastoma cell line, validating this approach with these proteins and generating an initial interactome. To refine this interactome to CNS specific interactions, we next performed BioID in mouse cortices transduced with AAV9 expressing the CLN-BioID constructs. This proteomic screen reveals many novel interactors for these proteins, providing strong evidence of neuron-specific functions. CLN3 shows enrichment for SNARE interacting proteins, anterograde axonal transport proteins, and vesicular transport proteins. CLN6 and CLN8 interactors also have strong enrichment for proteins involved in vesicle-mediated transport, dendritic morphogenesis, axodendritic trafficking, and potential interactions with mitochondrial proteins. Combining these analyses implies a broader function held in common by these CLN proteins to direct heterotypic membrane fusion such as that involved in synaptic vesicle release. Aberrations in this vesicular trafficking would be especially detrimental to neurons with far-reaching arbors, giving a possible explanation for the neuronal etiology of Batten disease. This work gives insight to the pathways affected by CLN3, CLN6, and CLN8 proteins and may provide an understanding of the primarily neurodegenerative presentation of Batten disease pathology.

Interacting protein partners of CLN3, CLN6, and CLN8 identified by BioID

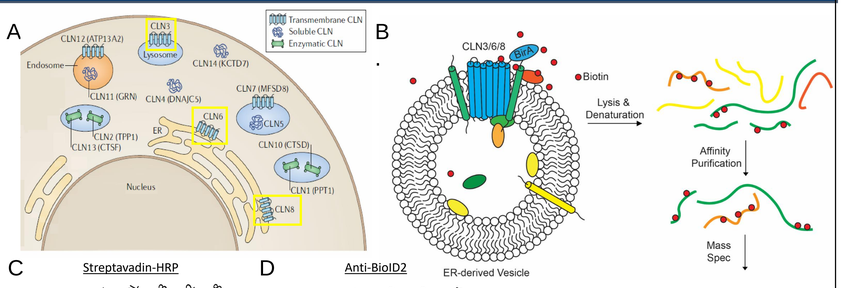


Fig 1. (A) Diagram showing predicted cellular localization of NCL proteins with proteins for BioID screen indicated by yellow boxes (adapted from Johnson et al., Nat. Neurology 2019). (B) Diagram of CLN-BioID construct and BioID workflow. BioID is a promiscuous biotin ligase which biotin labels (biotinylates) proximal protein species, allowing for streptavidin enrichment of biotinylated proteins for characterization by mass spectrometry (C) Streptavidin-HRP blot showing biotinylated species from Neuro2A neuroblast samples expressing BioID constructs. (D) BioID2 blot showing expression of BioID and CLN-BioID fusion constructs in stable Neuro2A cell lines.

Colocalization of CLN-BioID fusion proteins with secretory and lysosomal compartment markers

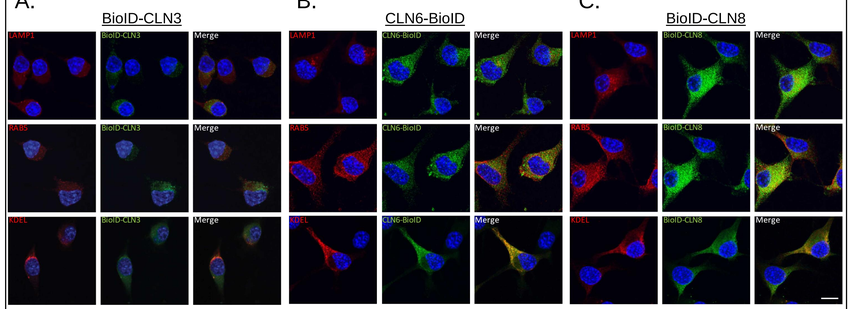
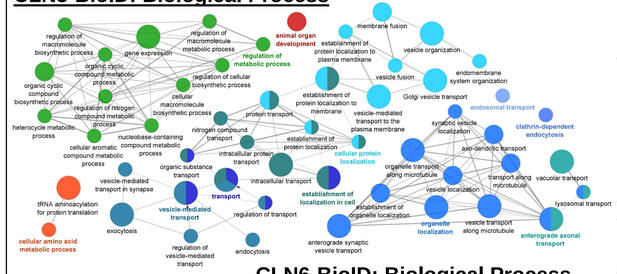


Fig 2. (A) Fluorescent immunocytochemistry imaging of Neuro2A cells expressing BioID2-CLN3 (A), CLN6-BioID2 (B), and BioID2-CLN8 (C). Green signal indicates anti-BioID2 with the localization determined by fusion to CLN protein indicated. Red signal indicates Lysosome (LAMP1), early endosome (RAB5) and endoplasmic reticulum (KDEL) localization in these cells. Merged images show colocalization between BioID2 and these compartmental markers. Scale bar indicates 10μM

Functional analysis of CLN-BioID interactors in neuroblastoma cells enriches for vesicular transport functions

CLN3-BioID: Biological Process

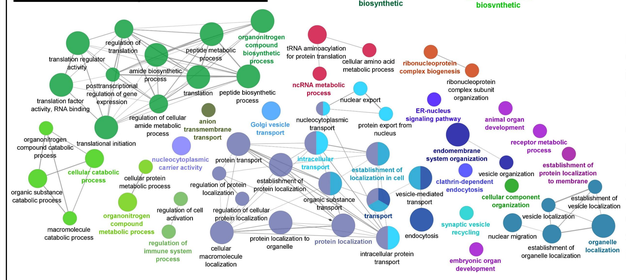


Enrichment of biotinylated protein interactors in CLN3-BioID samples show significant functional grouping of transport and vesicle sorting associated proteins. While interactions with lysosomal and endocytic molecules is shown in this interactome, there is also significant representation of axonal transport, synaptic vesicle transport, and vesicle membrane fusion proteins. These functions implicate pathways uniquely impacting neuronal health and function.

CLN6-BioID: Biological Process

CLN6-BioID interactors are highly enriched for proteins performing vesicle coating roles especially when considering intracellular vesicular secretion. Other significant functions include protein transport and localization mechanisms with many interactors performing roles in mitochondrial localization and metabolic pathways. Protein transport to these vital organelles may have an outsized role in the neuron with these cells' highly localized and dynamic metabolic demands.

CLN8: Biological Process



Interactions resulting from CLN8-BioID enrichment indicate role in intracellular transport functions. Particularly pathways determining vesicle-mediated protein localization. Interactions with proteins associated with synaptic vesicle recycling and clathrin-dependent endocytosis have specific impact on neuronal function.

Fig 3. Networks were created using the Cytoscape application ClueGO 2.5.8. Interactors for each CLN-BioID data set were determined as significant with 3-fold higher enrichment than control BioID. Significant interactors input and run to determine enriched Gene Ontology terms under the category Biological Process. Networks shown consist of significantly enriched terms reaching a $p < 0.05$ using a Bonferroni step down statistical test.

Common interactors for CLN3, CLN6, and CLN8 suggest shared functional pathways

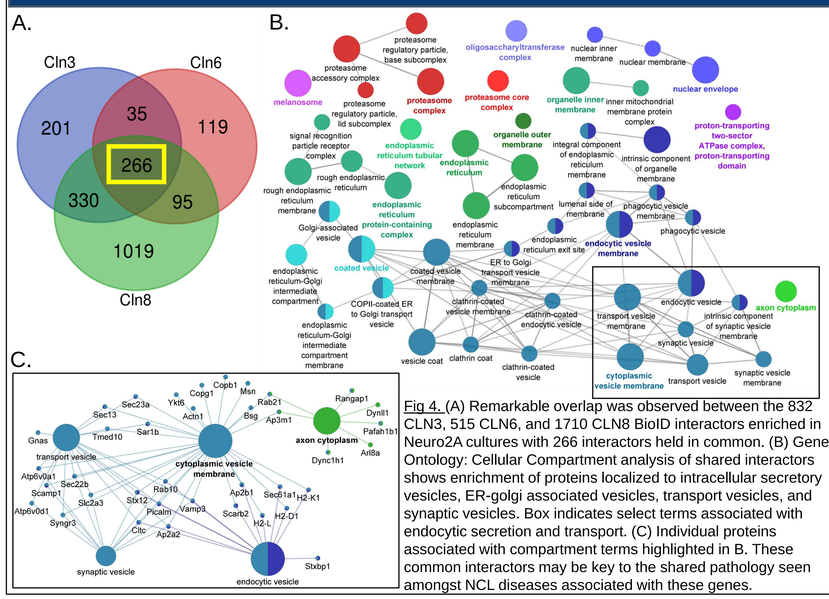
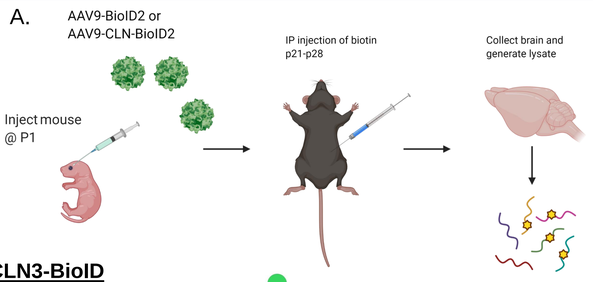


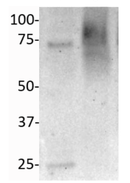
Fig 4. (A) Remarkable overlap was observed between the 832 CLN3, 515 CLN6, and 1710 CLN8 BioID interactors enriched in Neuro2A cultures with 266 interactors held in common. (B) Gene Ontology: Cellular Compartment analysis of shared interactors shows enrichment of proteins localized to intracellular secretory vesicles, ER-golgi associated vesicles, transport vesicles, and synaptic vesicles. Box indicates select terms associated with endocytic secretion and transport. (C) Individual proteins associated with compartment terms highlighted in B. These common interactors may be key to the shared pathology seen amongst NCL diseases associated with these genes.

In-vivo interactor analysis of CLN3 and CLN6 in mouse cortex enriched for neuronal transport functions



In-Vivo CLN3-BioID

B.



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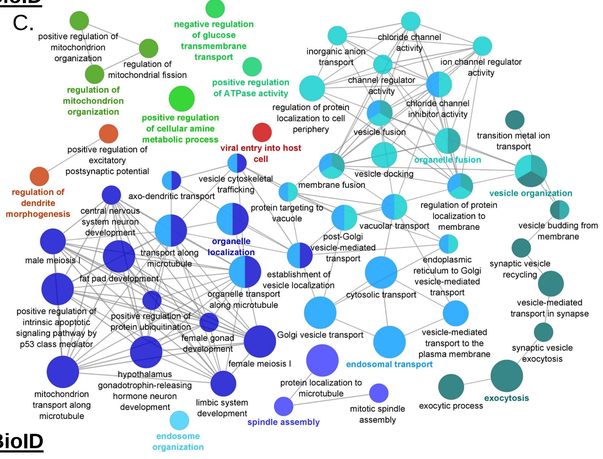
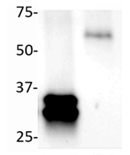


In-Vivo CLN6-BioID

D.



E.

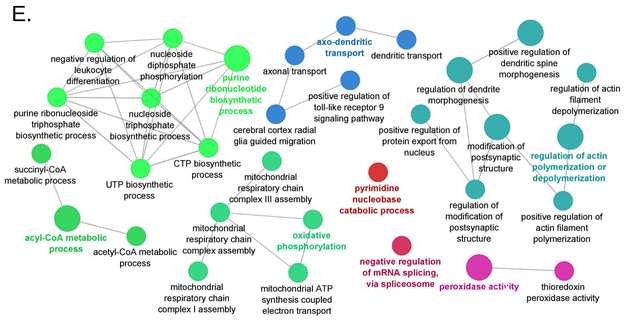


Fig 5. (A) Diagram showing AAV9-BioID administration and collection for lysate preparation. Immunoblots against BioID2 showing BioID2 only and BioID2-CLN3 fusion protein (B) or BioID2-KDEL and CLN6-BioID2 fusion protein (D) expressed in cortical lysates of AAV9 ICV injected mice. (C) Biological Process functional terms for CLN3-BioID cortical interactors are enriched for vesicular secretion and transport. (D) Functional terms for CLN6-BioID cortical interactors shows significant enrichment for neuronal arbor modification and transport as well as key mitochondrial and metabolic functions. Significant interactors for CLN-BioID data sets were determined as significant with 3-fold (CLN3-BioID) or 2-fold (CLN6-BioID) higher enrichment than control BioID.

Summary

- Using Neuro2a neuroblastoma cell lines stably expressing CLN3, CLN6, CLN8-BioID2 we have identified numerous proximal and interacting proteins with these transmembrane NCL proteins.
- These fusion proteins show colocalization with markers for secretory and lysosomal compartments.
- These expression constructs were packaged into AAV9 viral particles for intracerebroventricular (ICV) injection of P1 mice allowing for BioID labeling in mouse cortex.
- CLN3, CLN6, and CLN8 interactors all showed enrichment for secretory and transport functions including specific roles for synaptic vesicle function, vesicle fusion, and axo-dendritic transport.
- Comparing common interactors among CLN3, CLN6, and CLN8 interactors shows several overlapping interactors which are associated with secretory vesicles and synaptic vesicles.
- Studying these interactors and the pathways in which they function, especially in cortical tissue, will give key insights into the function of these proteins and will drive understanding of the primarily neuronal pathology seen in NCL patients.

Acknowledgments

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