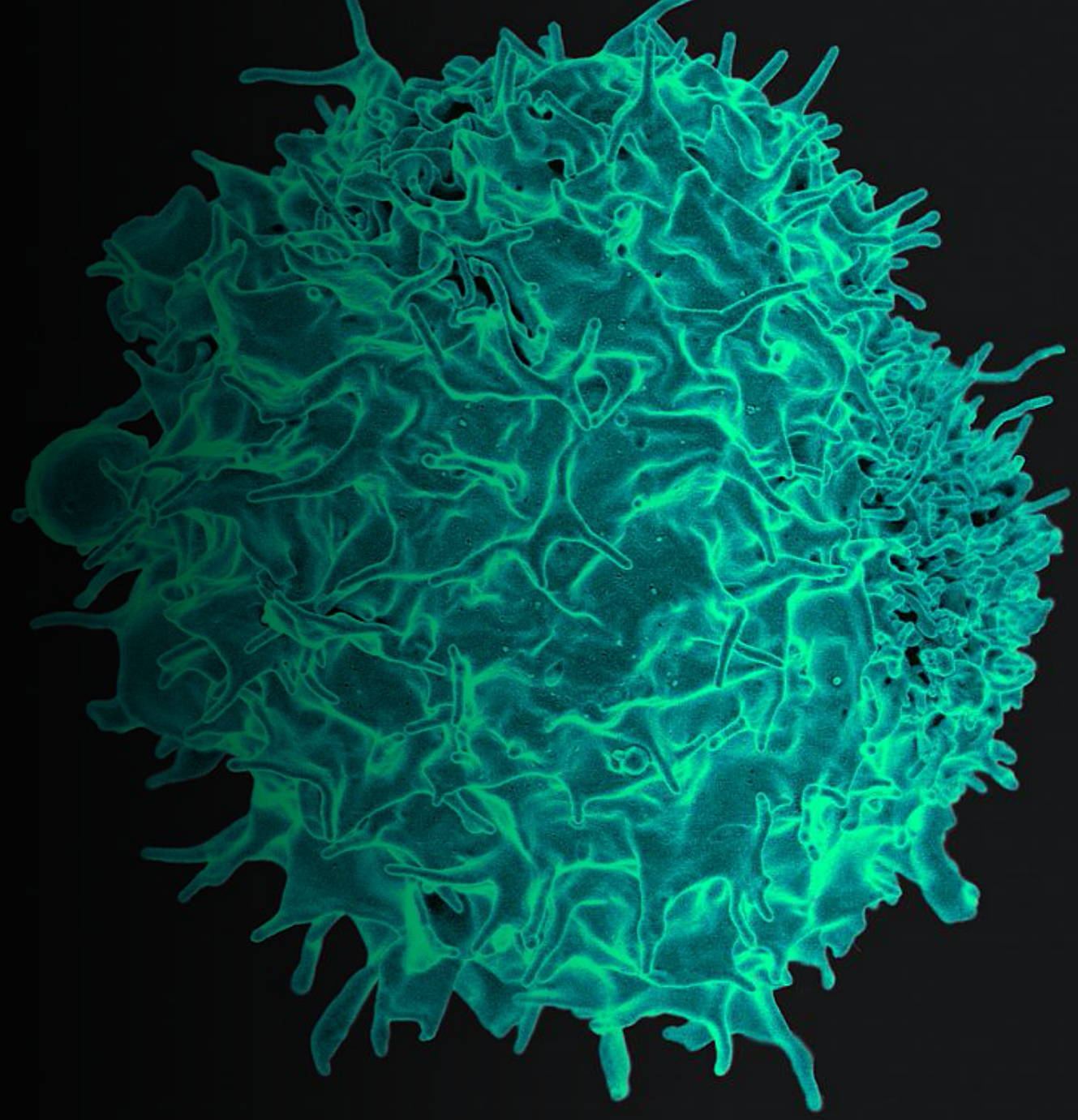
WISCONSIN
UNIVERSITY OF WISCONSIN-MADISON

A Syndrome Associating Partial Albinism and Immunodeficiency

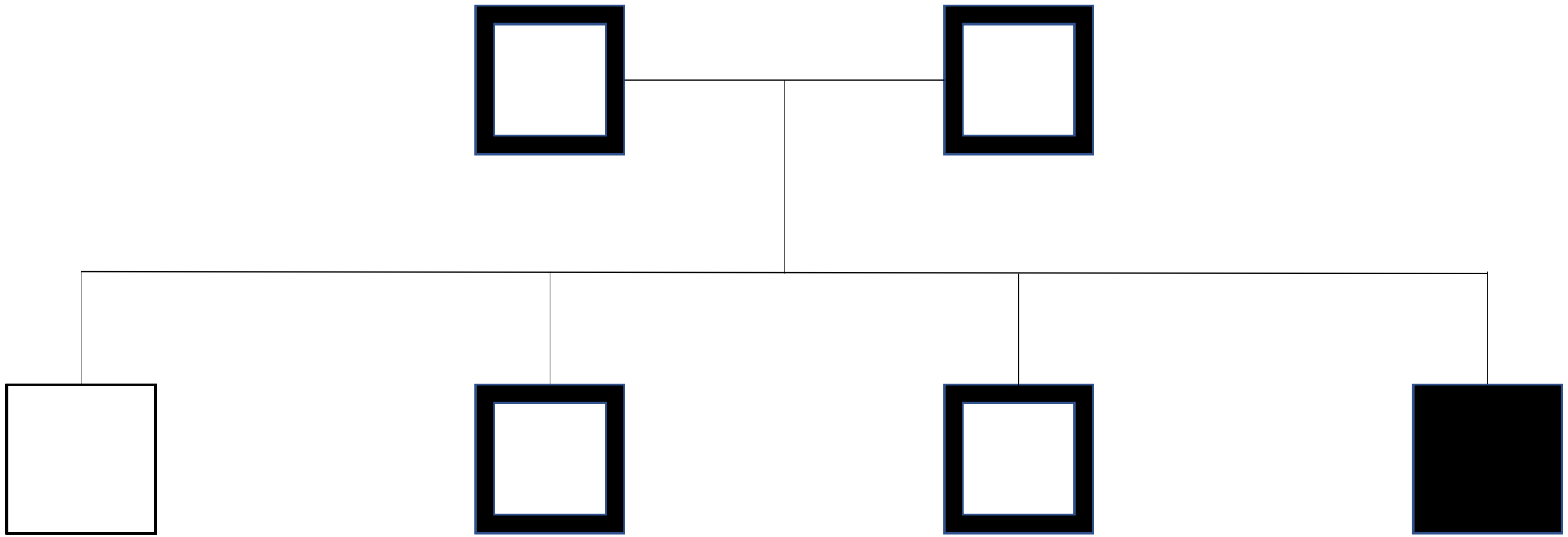
Griscelli Syndrome and the *RAB27A* Gene

Presented By Gus Hermberg

For Genetics 564

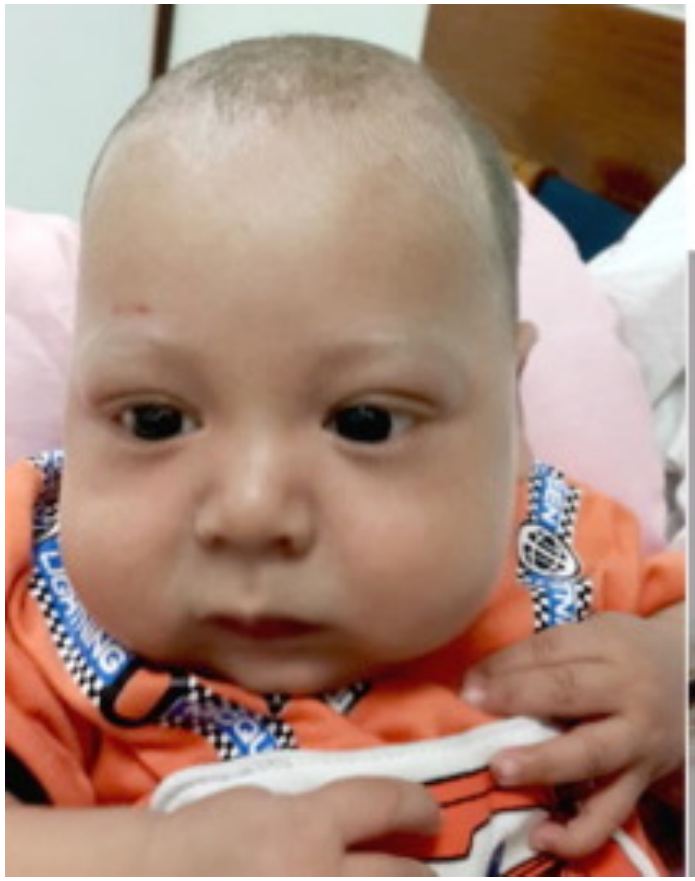


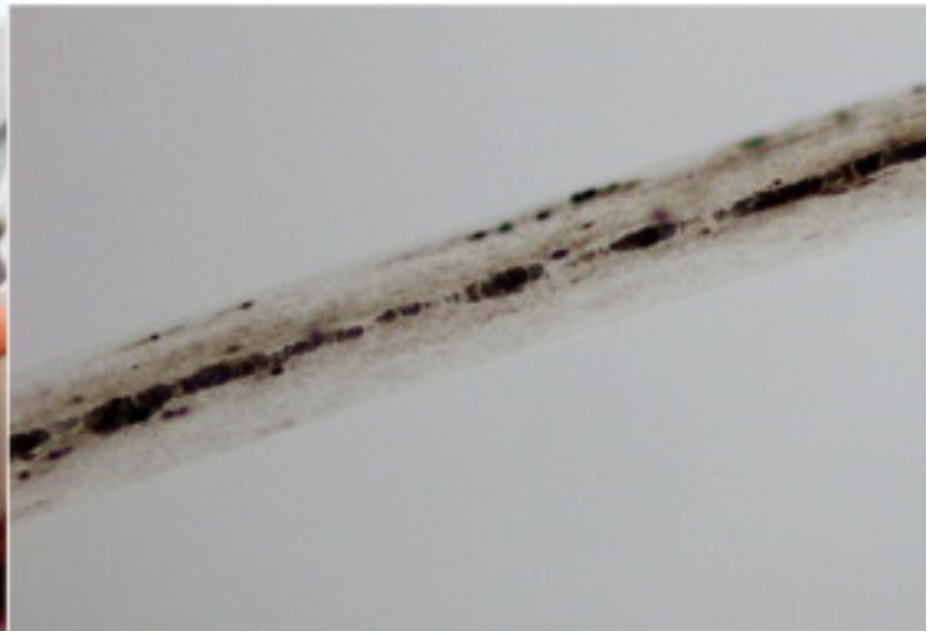
What is Griscelli Syndrome (GS)?



GS is an extremely rare genetic disease that is inherited autosomal recessively.

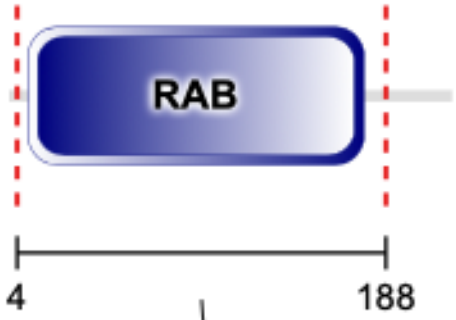
What is partial albinism?

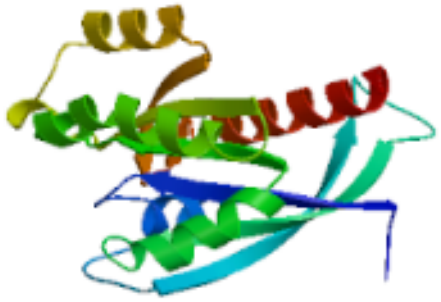




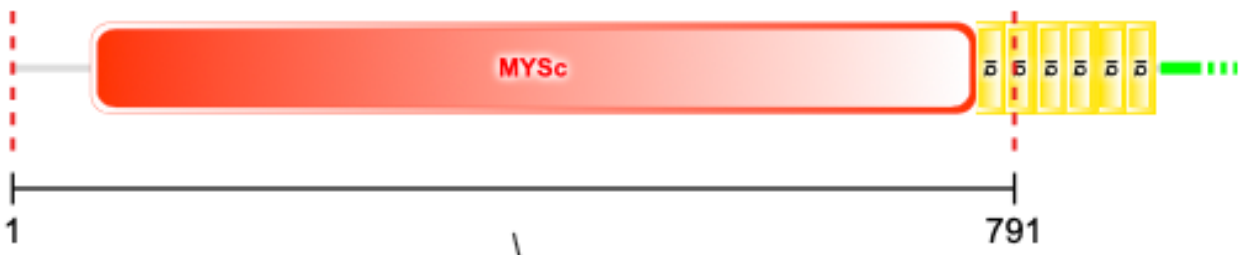
Partial albinism is a loss of pigmentation leading to silvery hair with pigment trapped inside the follicle.

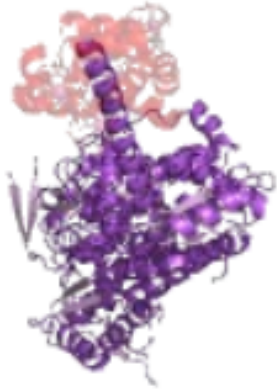
Type II is caused by mutations in the Rab27A Gene





Type 2 involves the gene *RAB27A*





Type 1 involves the gene *MYO5A*

The types of Griscelli Syndrome are associated with mutations in different genes.

What is *RAB27A*?

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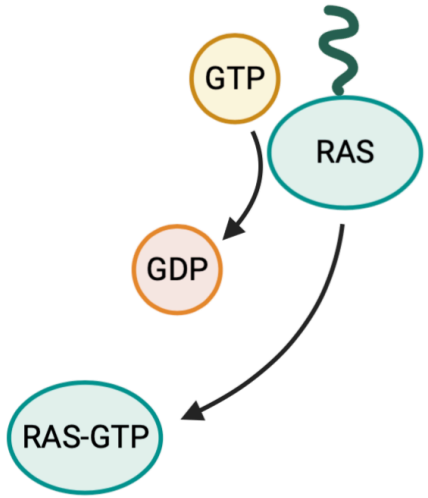
Molecular Function

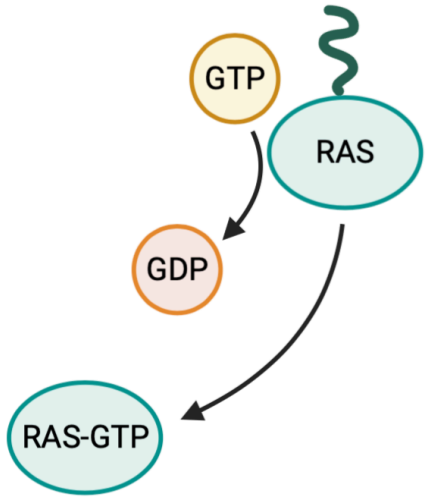


It is a GTPase and becomes active when GTP is bound.

What is *RAB27A*?

Human

Molecular Function



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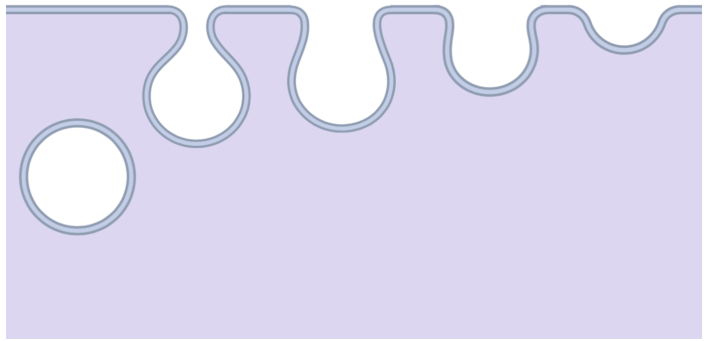
Biological Process

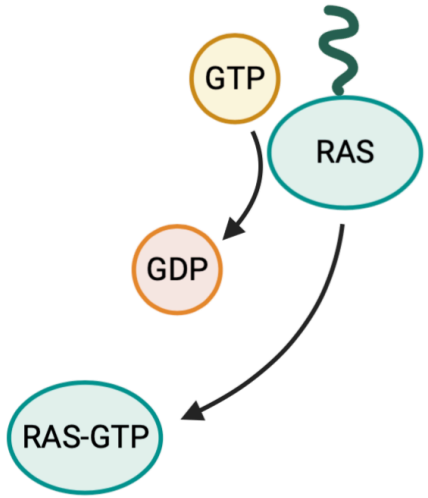


It’s involved in exocytosis and the transport of cellular products.

What is *RAB27A*?

Human

Molecular Function



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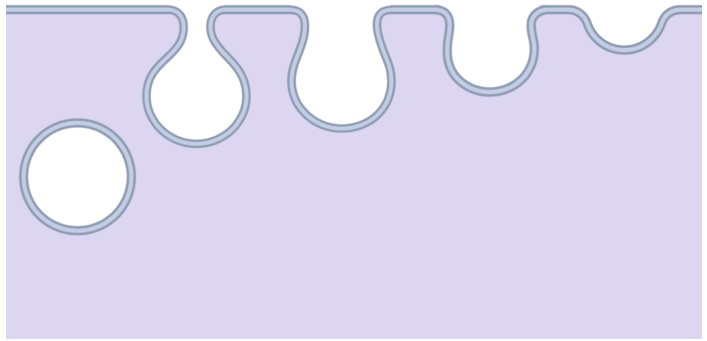

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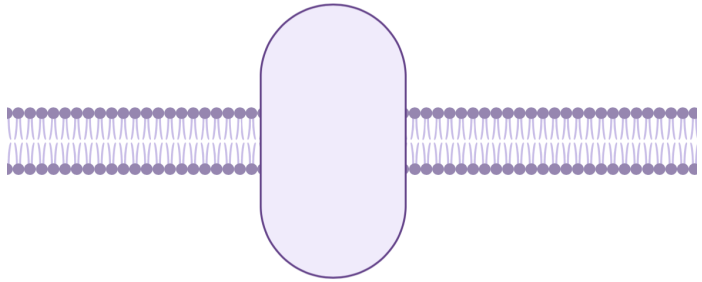
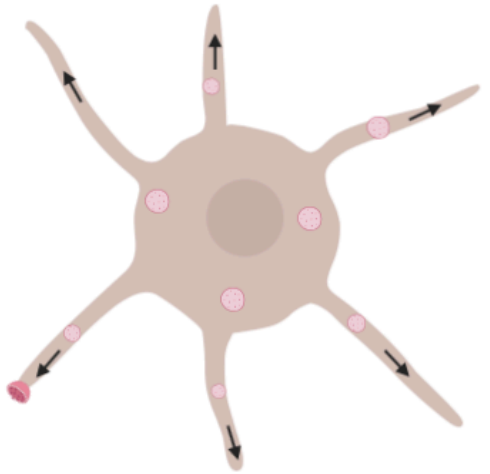
Cellular Component

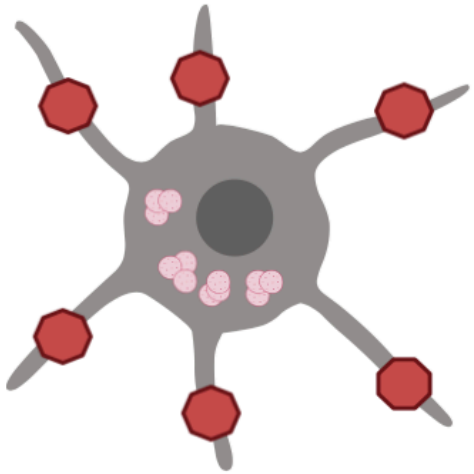


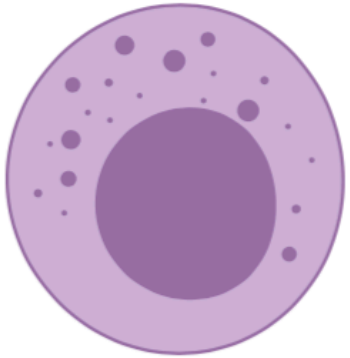
It is bound to the membrane.

How are the symptoms connected?

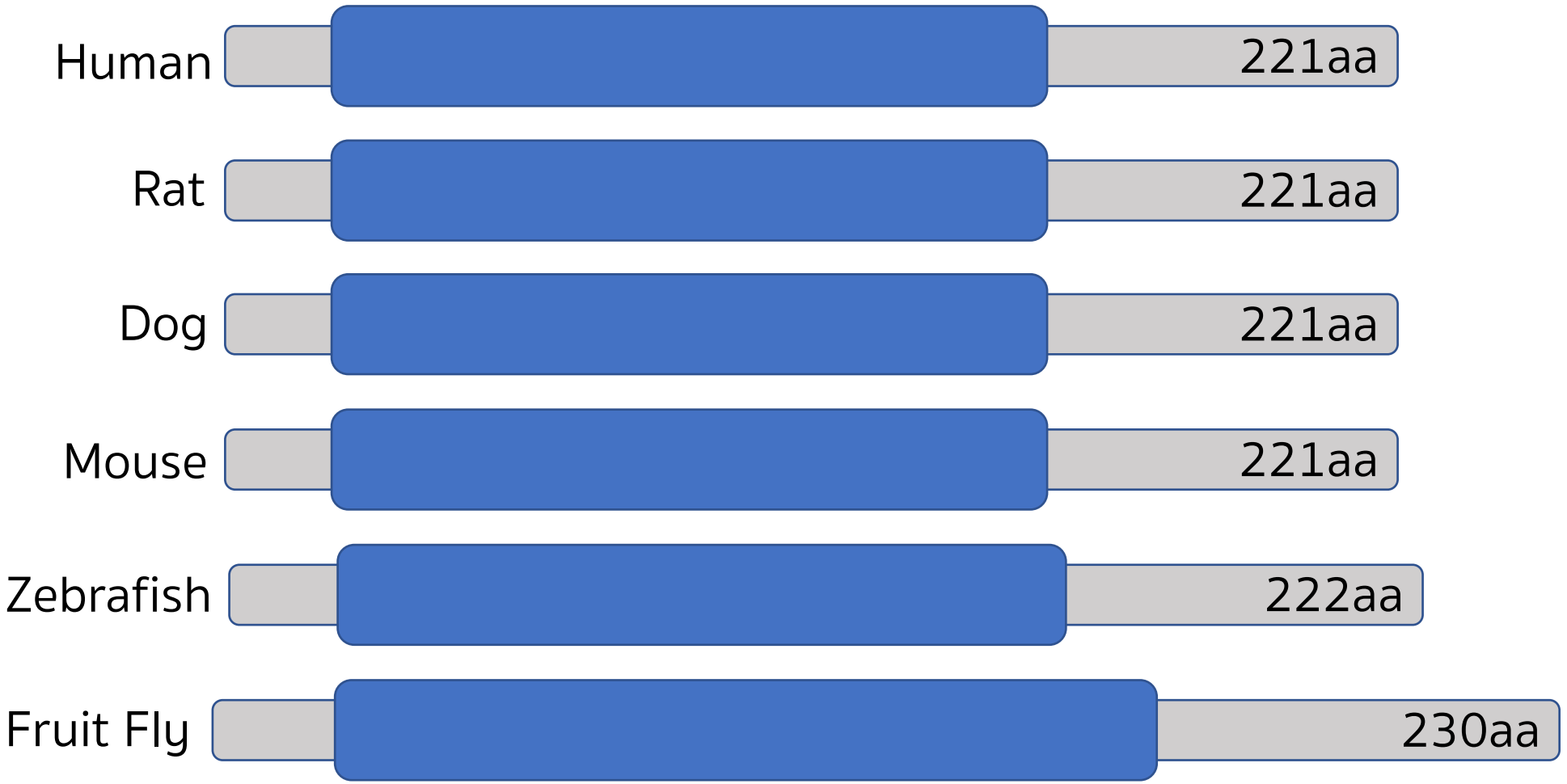
Albinism and immunodeficiency are both the result of failed exocytosis.





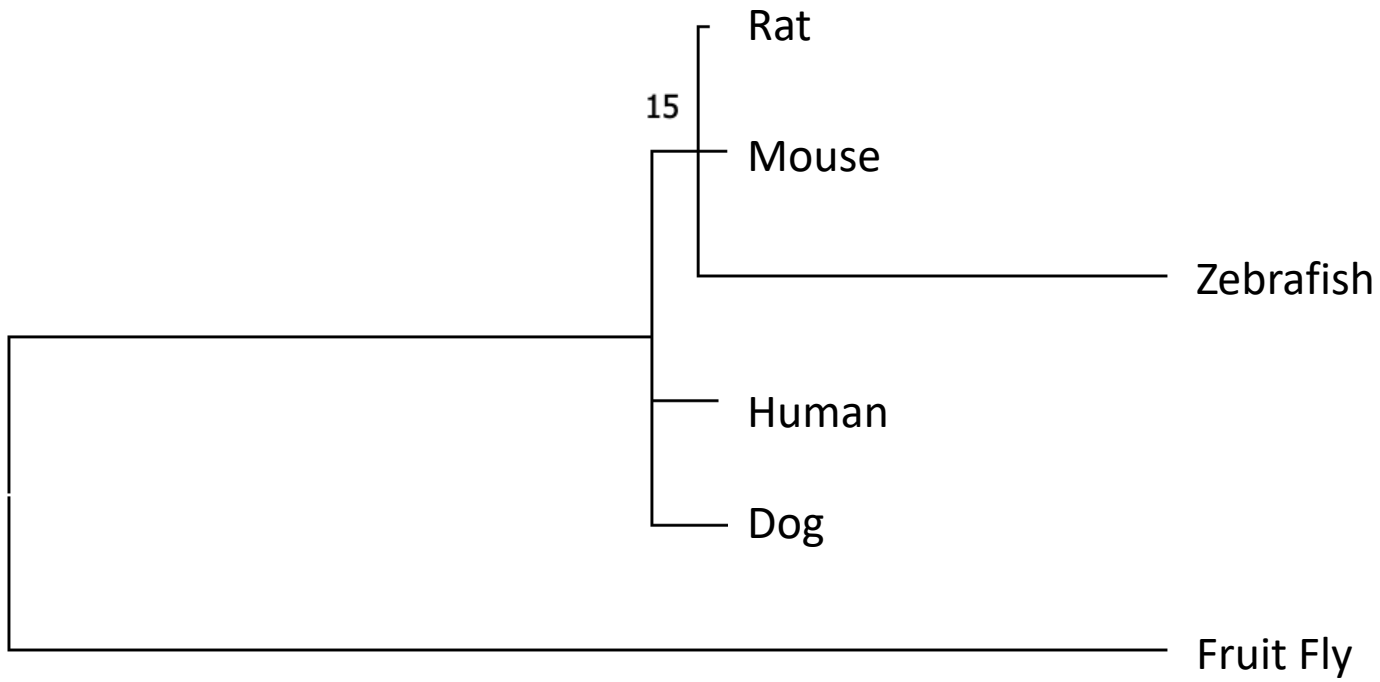


How well conserved is *RAB27A* among species?



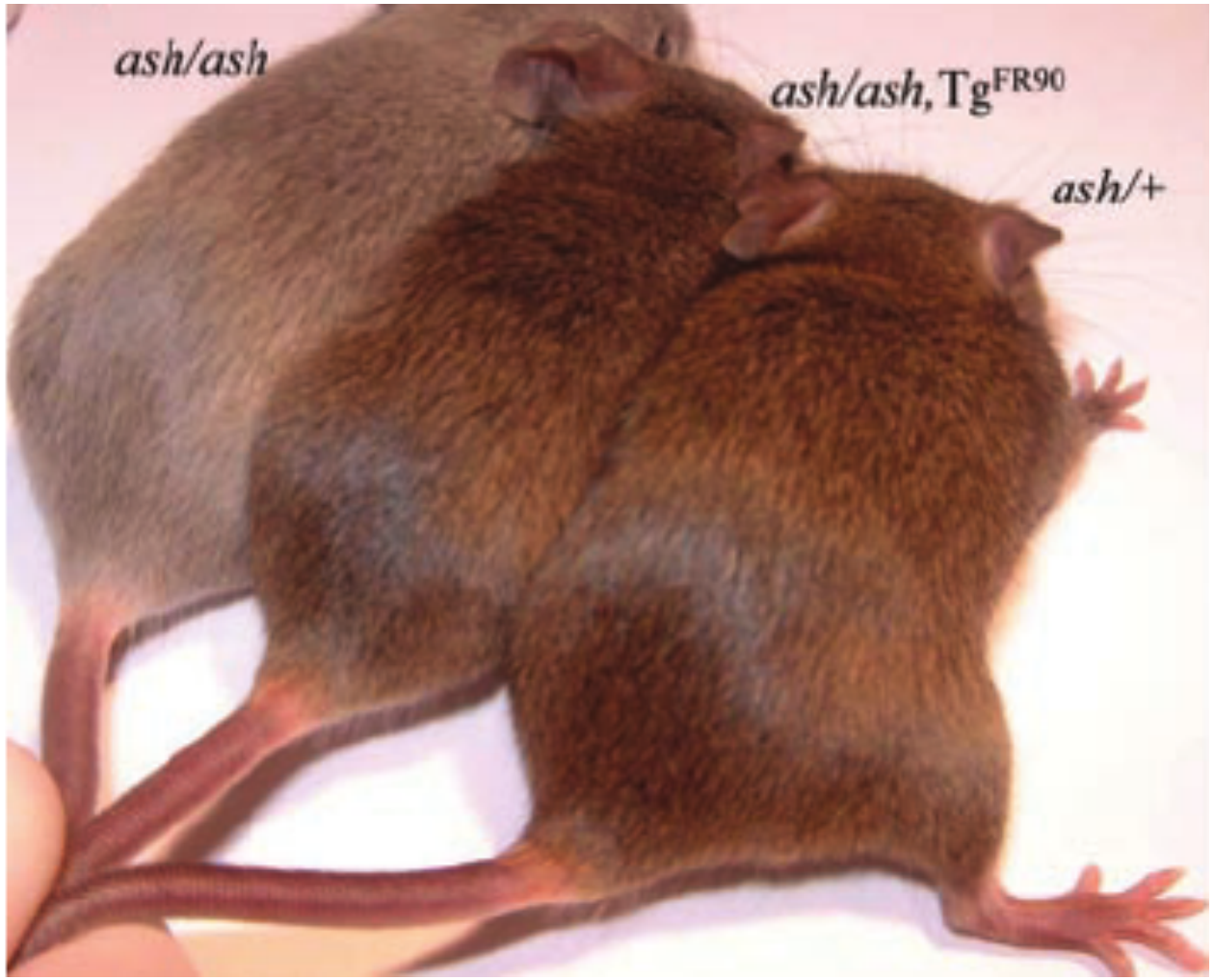
The simple architecture is extremely well conserved.

What are the phylogenetic relationships?



The zebrafish protein is closely related to mammals which is interesting.

Why use mice?

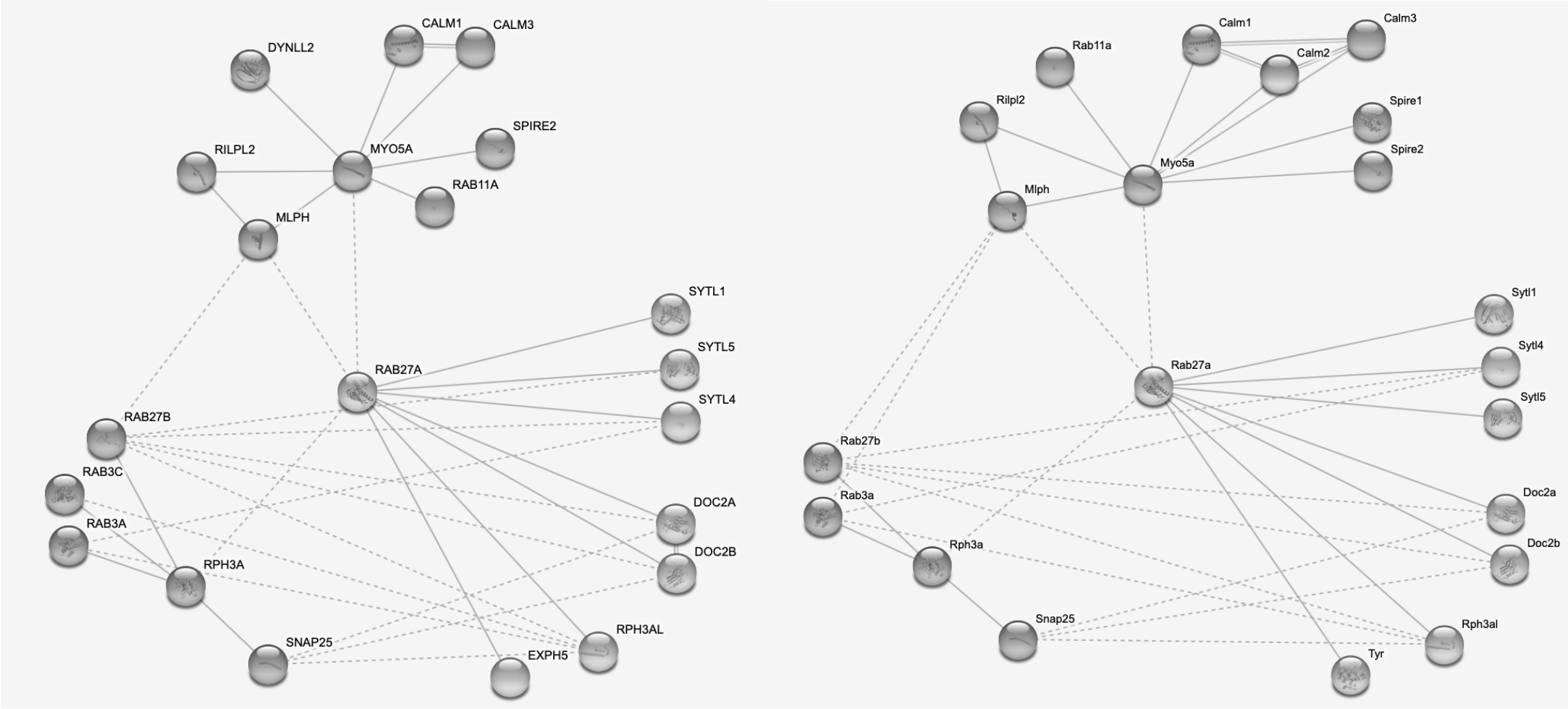


The silvery coat of mutants makes them easy to identify visually.

Why use mice?

Human

Mouse

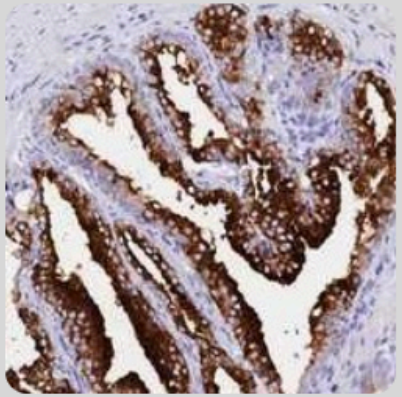


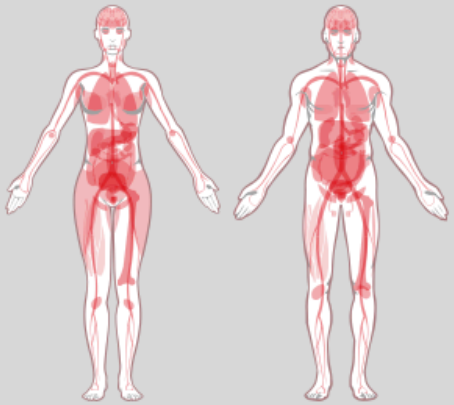
Human and mouse protein interaction networks are very similar.

What is the gap in knowledge?

THE HUMAN PROTEIN ATLAS

TISSUE ATLAS[i]





Tissue specificity (RNA)[i]	Low tissue specificity
Tissue distribution (RNA)[i]	Detected in all
Protein expression[i]	Cytoplasmic expression in most tissues, including immune cells.

Why are the symptoms of GS so distinct if *RAB27A* is expressed with such low tissue specificity?

How do we seek to address this gap?

Aim 1

Use a mutant screen to identify which amino acids are essential to protein function.

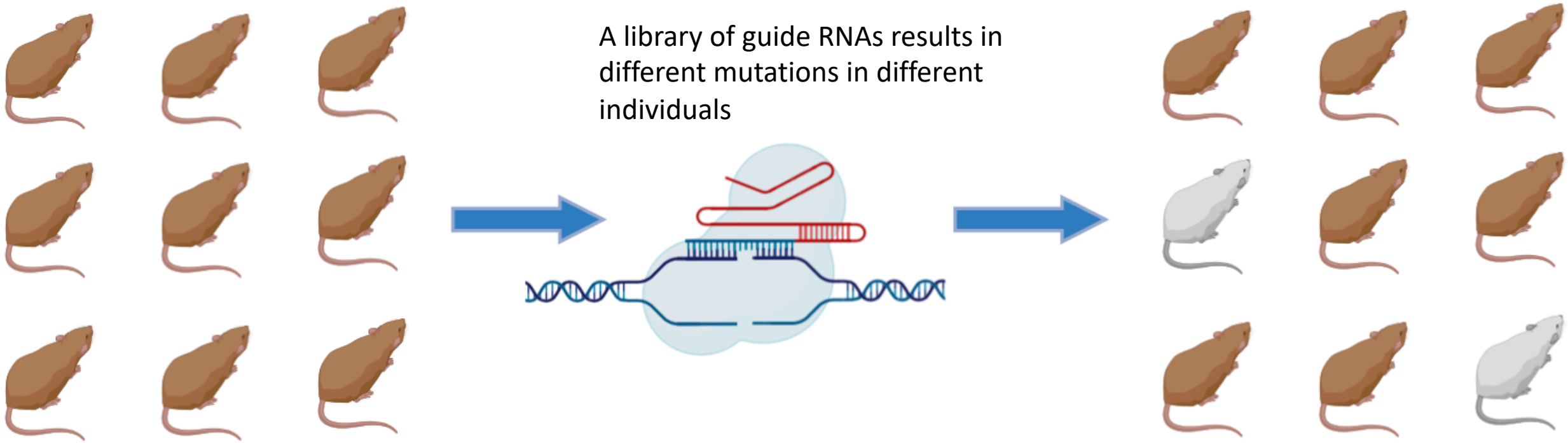
Aim 2

Use RNA-seq to identify genes that are transcribed differently in *RAB27A* mutants.

Aim 3

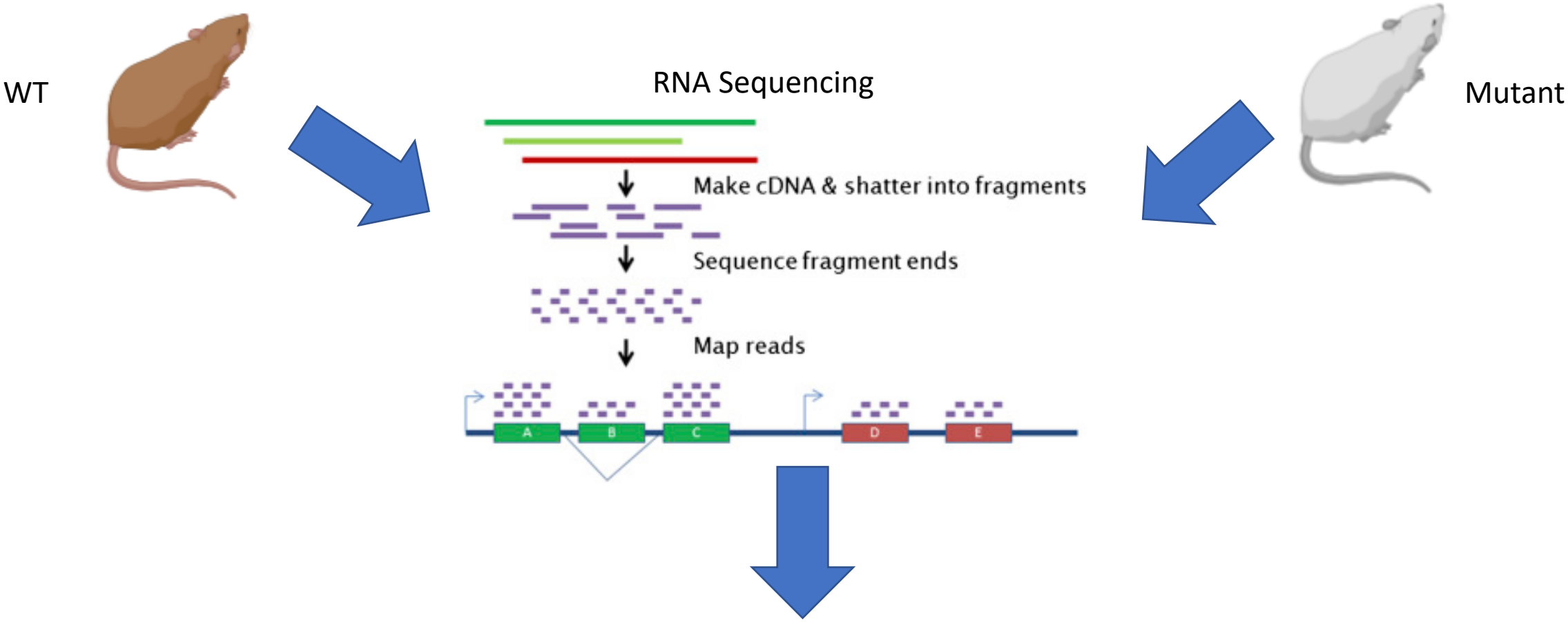
Use BioID to identify proteins that interact differently in *RAB27A mutants.*

Hypothesis 1: Mutations in specific amino acids will result in loss of function.



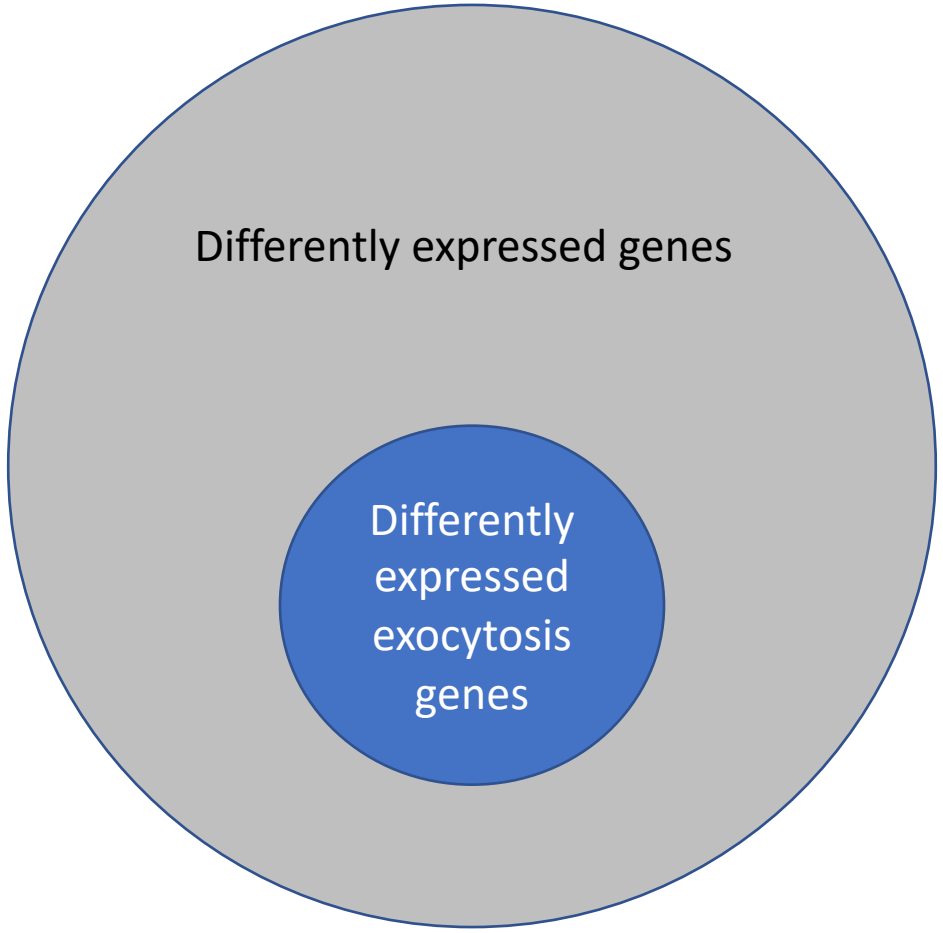
Sequencing the mutants will give evidence as to which sites, and their associated reactivity, are essential for protein function.

Hypothesis 2: Genes expressed differently in mutants will show tissue specific patterns.



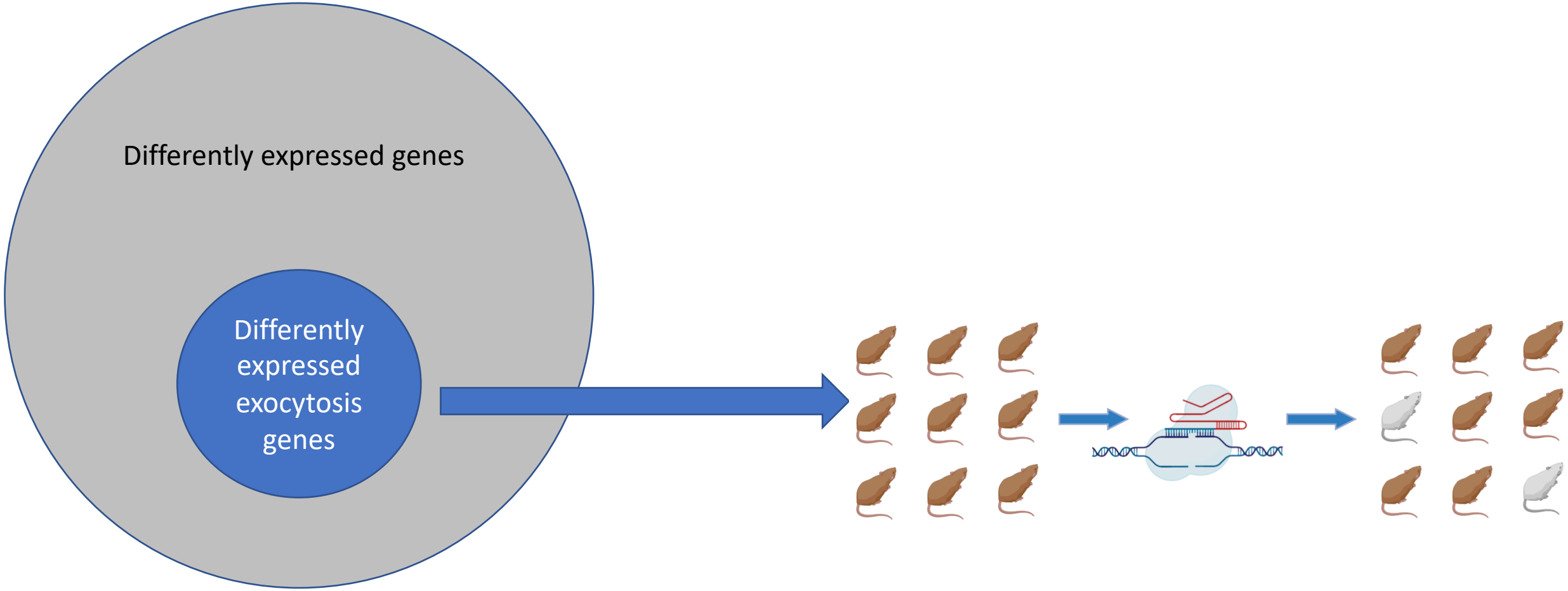
Compare Expression Profiles

Hypothesis 2: Genes expressed differently in mutants will show tissue specific patterns.



Sort differently expressed genes using gene ontology to find those involved in exocytosis.

Hypothesis 2: Genes expressed differently in mutants will show tissue specific patterns.

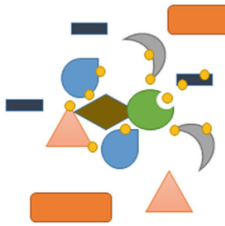


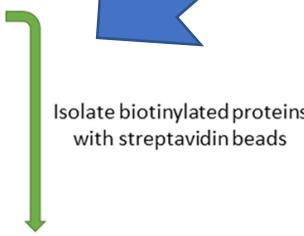
CRISPR knockout of differently expressed genes and then a visual assay for the ashen phenotype to confirm role of those genes in GS

Hypothesis 3: Proteins interacting differently in mutants will show tissue specific patterns.

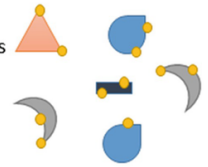
WT
Functional
RAB27A as POI

Add excess biotin to
promote proximity-based biotinylation

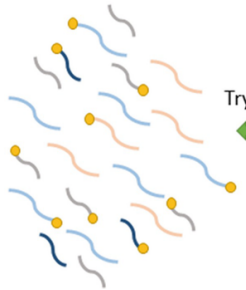




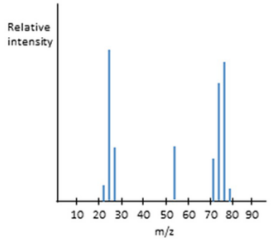
Isolate biotinylated proteins
with streptavidin beads



Trypsin-digest proteins



Perform MS analysis

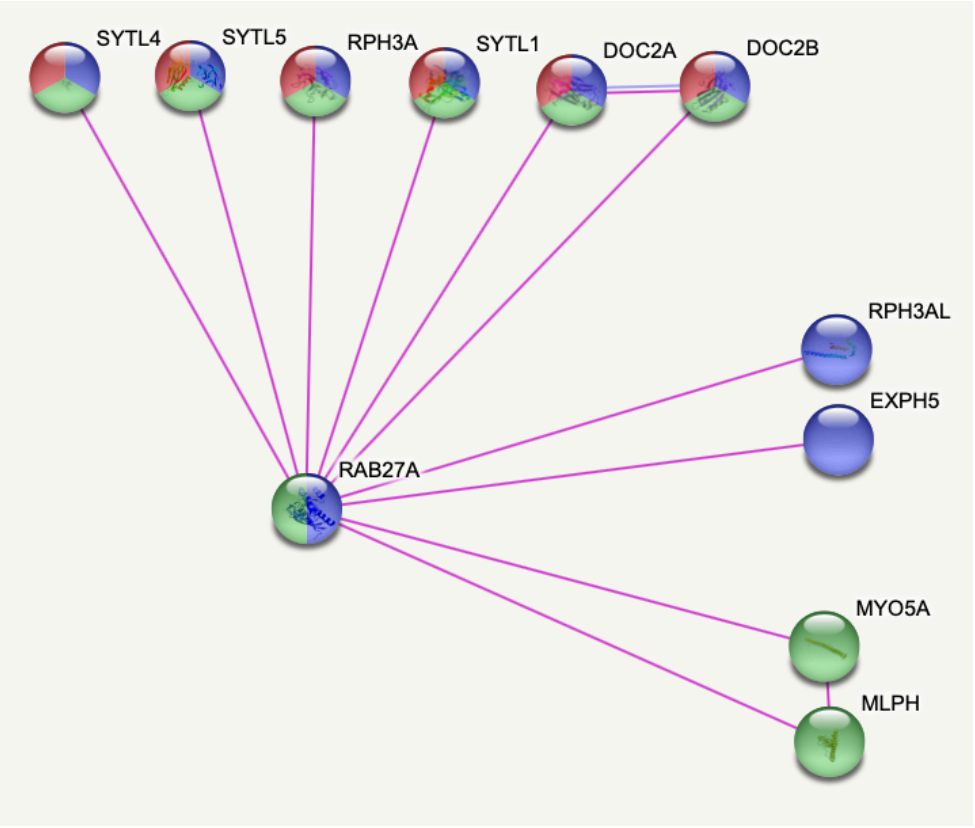


Mutant
Non-Functional
RAB27A as POI

Compare biotinylated proteins to compare protein interactions

Hypothesis 3

Human



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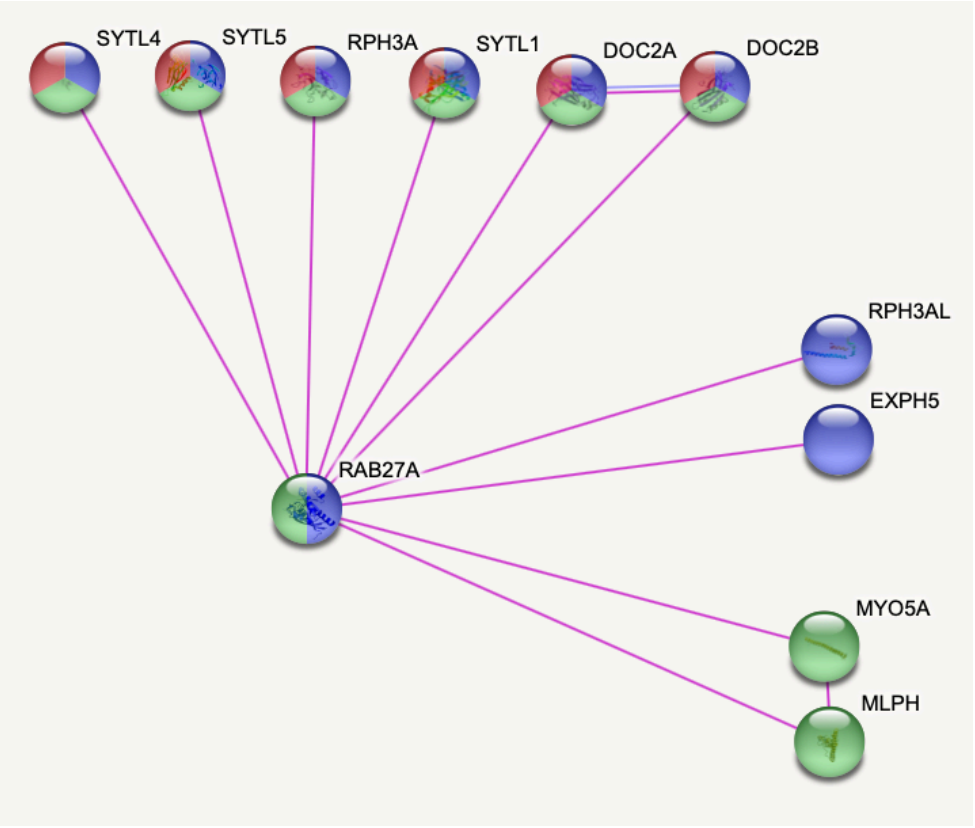


Biological Process

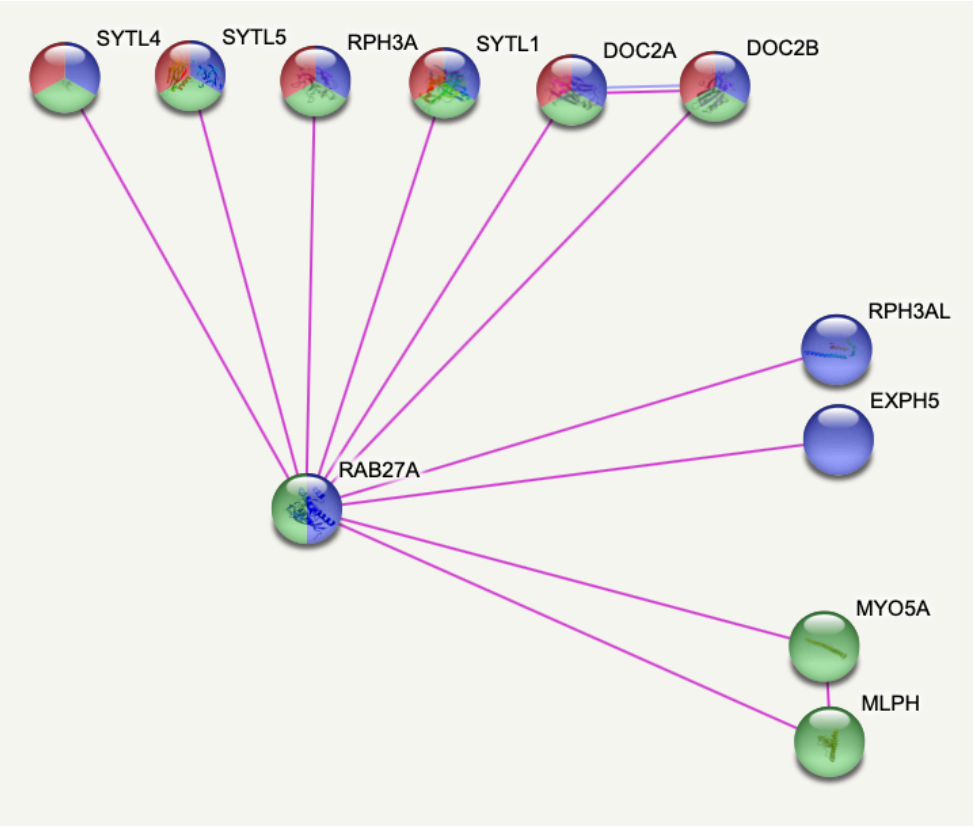
Calcium ion regulated exocytosis

Establishing vesicle localization

Regulation of exocytosis

Hypothesis 3

Human



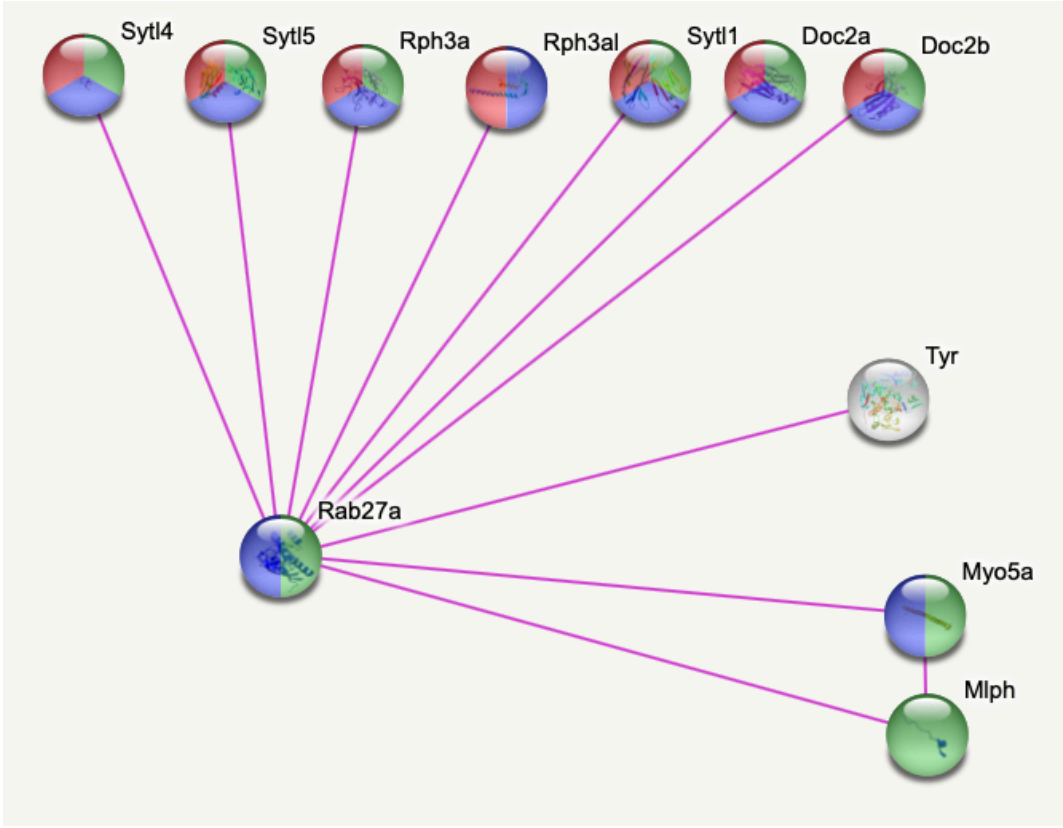
Biological Process

Calcium ion regulated exocytosis

Establishing vesicle localization

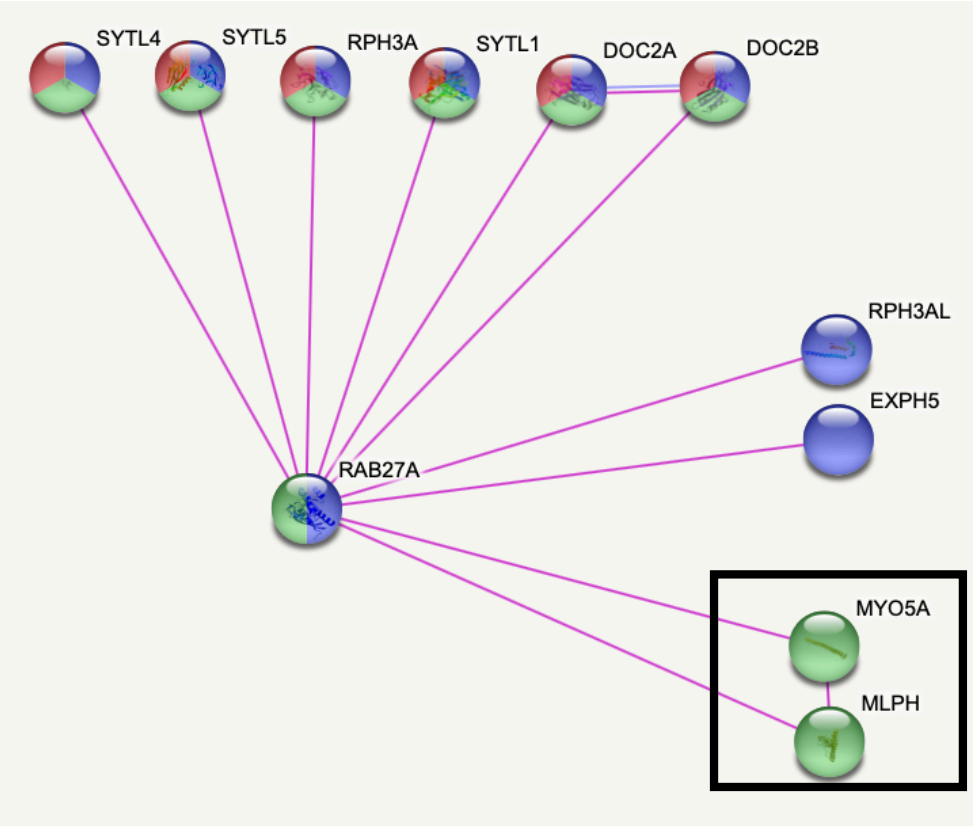
Regulation of exocytosis

Mouse



Hypothesis 3

Human



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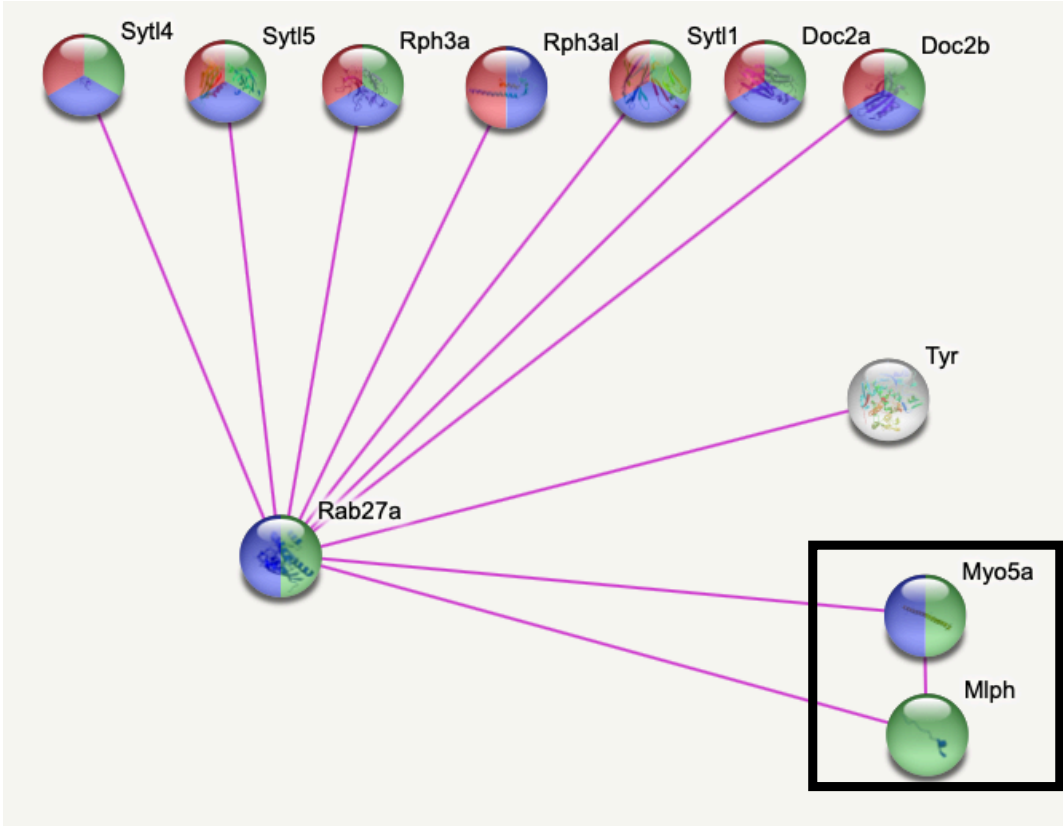

Does RAB27A still form its complex with MYO5A and MLPH in mutants?

Does it form a complex with any new proteins in mutants?

In Summary

In the genome we seek to discover what specific mutations result in loss of function.

In the epigenome we seek to discover what genes have altered expression in mutants.

In the proteome we seek to discover what proteins have altered interactions in mutants.

Future Directions

In patients with bone marrow transplants, are there interactions between the host and donated cells?

How can researching *RAB27A* help further personalized medicine to help other extremely rare diseases?

References

Griscelli, Claude, et al. “A Syndrome Associating Partial Albinism and Immunodeficiency.” *The American Journal of Medicine*, vol. 65, no. 4, 1978, pp. 691–702., doi:10.1016/0002-9343(78)90858-6.

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Images

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Slide 3: https://string-db.org

Slide 4: https://biorender.com

Slide 11: EGFP-Rab27a-rescues-the-phenotype-of-ashen-mice-A-Photograph-showing-one-homozygous.png

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