Griscelli Syndrome Type II (GS) is a genetic disorder where patients have silvery skin and hair and immunodeficiency.[1,2] The disease has an autosomal recessive inheritance pattern. Type II is the most common form of GS and immunodeficiency is what sets it apart from the other types.[3] Partial albinism means that patients have silvery hair due to pigment being trapped in the hair follicles in big clumps. Similarly, in melanin producing cells the pigment gets trapped and is never exported. Type II is caused by mutations in the gene *RAB27A*. The protein encoded by this gene is a GTPase and is involved with the export of cellular products to the outside of the cell.[3] Failure to export pigment explains the observed abnormalities in pigment. GS patients have normal lymphocyte counts and yet are unable to fight off infections.[1,2] The link between *RAB27A* and immunodeficiency is that when the lymphocytes are unable to export components of the lysosome they are unable to fight off infections.[3] The current treatment is a bone marrow transplant to extend lifespan beyond a few years. Still unknown is why GS has very specific phenotypes when expression through the body is currently understood to have little tissue specificity.

My **objective** is to clarify the specific role of *RAB27A* in its transport pathway and why loss of function is particularly disastrous in specific tissues. I **hypothesize** that asymptomatic tissue in individuals with *RAB27A* mutations are utilizing other transport proteins to bypass the non-functioning *RAB27A* protein. Mice will be used as a model organism for experiments because they have previous use in studies on RAB27A, the phenotype is easy to observe, and the mouse copy of *RAB27A* has already been mutated in lines for studies.[5] In mice the RAB27A mutant phenotype is referred to as ashen because the mice have silvery fur due to the albinism associated with GS. The **long-term goal** is to uncover mechanisms for successful exocytosis in the absence of functioning *RAB27A*.

Aim Number 1 – Identify essential amino acid differences for maintenance of RAB27A function.
Approach: A library of siRNAs targeting Rab27A transcripts will be used on wild type mice a mutagenic screen will be conducted. The mutagenized mice will be visually assayed for the ashen phenotype to identify mutants lacking functional RAB27A proteins. The ashen mice will then have their genomes sequenced to determine the nature of the mutation that resulted in the phenotype and which amino acids were affected. Rationale: Specific amino acids critical to the function of RAB27A can be identified and in doing so can give insights into where and how the protein is interacting with other molecules in its cellular environment. Hypothesis: Single nucleotide polymorphisms in key parts of the sequence of Rab27A will lead to loss of function mutations.

Aim Number 2 – Identify differently transcribed genes that are implicated in exocytosis failure.
Approach: Using RNA-Seq transcription will be compared between wild type and ashen mice to seek out genes that differentially expressed in the mutant. Differentially expressed genes will then be sorted using gene ontology (GO) to identify those specifically relevant to exocytosis. CRISPR will then be used to knock out differentially expressed genes in WT mice and the resulting mutants will be screened for phenotypes similar to the ashen phenotype to confirm these genes role in exocytosis. Rationale: Differently expressed genes in ashen mutants vs wild type mice can be identified giving insights as to if functioning RAB27A is involved in the regulation of any other genes. Hypothesis: Genes differentially expressed in ashen mutants will show tissue specific expression involving hair and the immune system.

Aim Number 3 – Identify proteins interacting with RAB27A as part of the exocytosis pathway.
Approach: Tagged proteins will be used in both WT and ashen mutant mice to compare interacting proteins and look for differences. Differently interacting proteins will be sorted using GO to identify if any are involved in exocytosis. I will then compare differently interacting proteins with the current STRING interaction network to see if any new protein interactions are discovered. Rationale: Differently interacting proteins can be discovered and expand the known network of RAB27A protein interactions to further understand the transport mechanism RAB27A participates in. Hypothesis: Differentially interacting proteins in mutant RAB27A networks will help identify a specific transport mechanism that will help explain why the phenotypes of GS are so specific.

References

1 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.

2 Klein, Christoph, et al. “Partial Albinism with Immunodeficiency (Griscelli Syndrome).” *The Journal of Pediatrics*, vol. 125, no. 6, 1994, pp. 886–895., doi:10.1016/s0022-3476(05)82003-7.

3 Ménasché, Gaël, et al. “Mutations in RAB27A Cause Griscelli Syndrome Associated with Haemophagocytic Syndrome.” *Nature Genetics*, vol. 25, no. 2, 2000, pp. 173–176., doi:10.1038/76024.

4 Ostrowski, Matias, et al. “Rab27a And Rab27b Control Different Steps of the Exosome Secretion Pathway.” *Nature Cell Biology*, vol. 12, no. 1, 2009, pp. 19–30., doi:10.1038/ncb2000.

5 Wilson, S. M., et al. “A Mutation in Rab27a Causes the Vesicle Transport Defects Observed in Ashen Mice.” *Proceedings of the National Academy of Sciences*, vol. 97, no. 14, 2000, pp. 7933–7938., doi:10.1073/pnas.140212797.

6 Wu, Xufeng S., et al. “Melanophilin and Myosin Va Track the Microtubule plus End on EB1.” *The Journal of Cell Biology*, vol. 171, no. 2, 2005, pp. 201–207., doi:10.1083/jcb.200503028.