Tuberous sclerosis complex (TSC) is a rare genetic disorder that results in the formation of benign tumors throughout the body. When these tumors become too large or interrupt function of vital organs, including the kidney, this can result in a significant decrease in quality of life, and even death [1,2]. TSC is caused by a mutation in TSC2, a tumor suppressor gene [1], which also plays a role in nitrogen metabolism [3], though this function is still unclear. In the clinic, nitrogen levels are measured using a blood urea nitrogen (BUN) test, yet TSC patients do not normally undergo BUN screening to detect kidney dysfunction [4]. The goal is to investigate *the molecular* *function of TSC2 in nitrogen metabolism in the kidney*.

My **primary goal** is to determine the role of TSC2 in nitrogen metabolism. I will use *Schizosaccharomyces pombe* as a model organism because nitrogen metabolism can be easily assayed and the *S. pombe* tsc2+ homolog affects gene expression under nitrogen starvation [3]. Furthermore, *R. norvegicus* will be used because it has similar renal physiology to humans, and thus a BUN test may reflect human urea nitrogen levels, particularly as they develop TSC-associated renal tumors [2]. My **hypothesis** is that TSC2 has a role in nitrogen metabolism in the kidney. My **long-term goal** is to determine if administration of a blood urea nitrogen test may be useful in early detection of TSC-associated renal tumors, so that treatment may be administered before complications occur.

**Aim 1: Identify domains in the TSC2 gene important for nitrogen metabolism.**

**Hypothesis**: I hypothesize that specific domains in TSC2 play a role in nitrogen metabolism, particularly in amino acid transport.

**Approach**: I will use BLAST to identify TSC2 homologs in humans and other species, followed by multiple sequence alignment with Clustal Omega to identify highly conserved amino acids. Then I will use CRISPR/Cas9 to create deletion mutants in *S. pombe*. Next, I will grow these mutants in nitrogen-rich and nitrogen-deprived environments, and screen for growth on leucine rich and deficient media (decreased leucine uptake is a Δtsc2+ phenotype) [3]. Then I will use CRISPR/Cas9 to create mutants in *R. norvegicus,* perform a BUN test, and histologically identify any tumors in the kidney.

**Rationale**: Conserved regions are likely to indicate domains in the gene that are necessary for nitrogen metabolism, which will confirm the role of TSC2 in nitrogen metabolism.

**Aim 2: Identify differentially expressed transcripts in tsc2+ mutants.**

**Hypothesis**: I hypothesize that tsc2+ regulates nitrogen metabolism by gene expression responsible for amino acid transport.

**Approach:** I will use RNA-seq on wild-type and Δtsc2+ *S. pombe* under nitrogen-rich and nitrogen-deprived conditions to identify differences in transcript levels. Then I will use Gene Ontology to identify clusters of genes that are lost when tsc2+ is mutated. These gene clusters will then be mutated using CRISPR/Cas9 to create a new cohort of *S. pombe* mutants. I will grow these mutants in nitrogen-rich and nitrogen-deprived environments, and screen for growth on leucine rich and deficient media [3]. Then I will use CRISPR/Cas9 to create mutants in *R. norvegicus,* perform a BUN test, and histologically identify any tumors in the kidney.

**Rationale**: TSC2 is likely involved in regulating nitrogen metabolism and genes associated with amino acid transport in the kidney. Identifying differences in transcript levels will demonstrate the role of nitrogen on TSC2 in gene regulation.

**Aim 3: Identify proteins that interact with TSC2 in nitrogen metabolism in the kidney.**

**Hypothesis**: I hypothesize that TSC2 interacts with other proteins that act in nitrogen metabolism in the kidney.

**Approach:** I will use a library-based yeast-two-hybrid assay between the TSC2 domains identified in aim 1 and the proteins encoded by the transcripts identified in aim 2, as well as any proteins yielded by literature search. I will do this in both nitrogen-rich and nitrogen-deficient conditions. Then I will create mutants with CRISPR/Cas9 and grow these mutants in nitrogen-rich and nitrogen-deprived environments, and screen for growth on leucine rich and deficient media [3]. Finally, I will use CRISPR/Cas9 to create mutants in *R. norvegicus,* perform a BUN test, and histologically identify any tumors in the kidney.

**Rationale:** Identifying interacting proteins may elucidate the role of TSC2 in nitrogen metabolism in the kidney.

Kidney tumors in TSC may be fatal if the tumors interfere with organ function. Blood urea nitrogen tests may be a non-invasive method to detect TSC-associated renal tumors when treatment is more likely to be successful. Additionally, by learning about the role of TSC2 in nitrogen metabolism, this may help to derive new treatments for all renal tumors.

**References**

1. Orlova, K. A., Crino, P. B. (2010). The tuberous sclerosis complex. *Annals of the New York Academy of Sciences, 1184*, 87–105. http://doi.org/10.1111/j.1749-6632.2009.05117.x

2. Huang, J., & Manning, B. D. (2008). The TSC1–TSC2 complex: a molecular switchboard controlling cell growth. *The Biochemical Journal, 412*(2), 179–190. http://doi.org/10.1042/BJ20080281

3. Nakase Y, Fukuda K, Chikashige Y, Tsutsumi C (2006). A defect in protein farnesylation suppresses a loss of Schizosaccharomyces pombe tsc2+, a homolog of the human gene predisposing to tuberous sclerosis complex. *Genetics 173*(2):569-78. http://doi.org/10.1534/genetics.106.056895

4. Mayo Clinic Staff (2016). Blood urea nitrogen (BUN) test. *Mayo Clinic, Patient Care & Health Information.* http://www.mayoclinic.org/tests-procedures/blood-urea-nitrogen/home/ovc-20211239