Nephrogenic diabetes insipidus(NDI) is a disorder caused by the inability of cells in the kidney to respond to vasopressin, leading to excessive dilute urine production and chronic thirst1. One gene implicated in causing NDI is aquaporin-2 (*AQP2*), necessary for increasing solute concentration of urine in the collecting duct of the kidney2,3. Activation of the vasopressin receptor promotes movement of vesicles containing AQP2 to the plasma membrane, and prior studies have shown that AQP2 proteins with altered phosphorylation sites at the C terminus do not reach excretory vesicles 4,5. However, *it is not clear how phosphorylation of AQP2 interacts with the endomembrane system*.

 The **objective** of this study will be to determine what proteins interact with AQP2 when S264 is phosphorylated within a mouse model. I **hypothesize** that AQP2 with S264 phosphorylated will have heightened interactions with proteins associated with exocytosis. This hypothesis is based on a former study that suggested phosphorylated S264 counteracts the endocytosis associated with phosphorylated S2616. The **long-term goal** of this research is to determine how phosphorylation of serine residues at the C terminus of AQP2 affects interactions with other cellular components.

Aim 1: **Evaluate conservation of S261 and S264 across homologs of AQP2**

**Approach:** Homologs of AQP2 will be identified based on reciprocal BLAST7. Functional conservation of the serines will be evaluated based on relative position and sequence context in protein alignments of the C terminus of the homologs.

**Hypothesis:** I hypothesize that the pairing of S261 and S264 will only be well conserved within organisms that mobilize AQP2 homologs from intracellular stores.

**Rationale:** Evaluating conservation of these two residues will provide insight into the functional conservation of the interaction of the residues across evolutionary time.

Aim 2: **Determine effects of phosphorylated S264 on retention of AQP2 in the plasma membrane**

**Approach:** CRISPR/Cas9 will be used within mouse cells to form two cell populations: one in which only a turboGFP tag is attached to the C terminus of *AQP2* and another turboGFP tagged population in which S264 is replaced with adjacent glutamate residues to simulate constitutive phosphorylation.8,9

**Hypothesis:** I hypothesize that cells in which *AQP2* has substitutions mimicking constitutive S264 phosphorylation will be retained within the membrane longer than *AQP2* with only a turboGFP tag added.

**Rationale:** Performing this CRISPR mediated mutation of *AQP2* will confirm the role of phosphorylated S264 in keeping *AQP2* at the plasma membrane.

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