Nephrogenic diabetes insipidus(NDI) is a disorder caused by the inability of cells in the kidney to respond to antidiuretic hormone(vasopressin)1. Lack of response to vasopressin leads to excessive dilute urine production and chronic thirst. One gene implicated in causing NDI is aquaporin-2 (*AQP2*), necessary for concentrating urine in the collecting duct of the kidney prior to urine moving to the bladder2,3. Under normal physiological conditions, vasopressin binding to its receptor in the collecting duct leads to movement of vesicles containing AQP2 to the plasma membrane and increased transcription of the *AQP2* gene1 .Prior studies have shown that *AQP2* alleles with altered phosphorylation sites prevent AQP2 from reaching excretory vesicles 4,5.However, *it is not clear how phosphorylation of AQP2 interacts with the endomembrane system*. Identification of interactions that allow AQP2 movement within the endomembrane system may facilitate the development of therapeutics for patients with *AQP2* related NDI.

The **long-term goal** of this research program is to determine how phosphorylation of serine residues at the C terminus of AQP2 affects interactions with other cellular components. Of the serine residues at the C terminus of AQP2, the serine whose role is least clearly understood is S2646. The **objective** of this study will be to determine what proteins interact with AQP2 when S264 is phosphorylated within a mouse model. Mice will be used for this study due to the high sequence conservation of *AQP2* between humans and mice and the availability of multiple mouse models of NDI7. I **hypothesize** that AQP2 with S264 phosphorylated will have heightened interactions with proteins associated with exocytosis. A prior study has supported the phosphorylation of S264 as counteracting the effects of phosphorylated S261, which is associated with endocytosis8. Interaction with exocytosis related proteins would therefore counteract the effects of phosphorylated S261.

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

**Approach:** Homologues of AQP2 will be identified in non-human organisms based on reciprocal BLAST. The C termini of the homologues will be aligned, and conservation of the pair of serines will be evaluated based on positioning of the serines relative to each other and the sequence context around the serines.

**Hypothesis:** I hypothesize that the pairing of S261 and S264 will only be well conserved within organisms that mobilize AQP2 homologues from intracellular stores in response to external signals.

**Rationale:** Conservation of both S261 and S264 in similar sequence contexts in other organisms would allow for functional conservation of the effects of phosphorylated residues on each other. Organisms in which the aquaporin homologues are constitutively found within the cell membrane will not have selective pressure on modulating intracellular trafficking functions, and thus are less likely to have sequence conservation at these residues.

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