Determining the Place of AQP-3B in the Wnt/CA2+ Noncanonical Pathway

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During Xenopus laevis gastrulation, convergent extension is required for the mesoderm to extend into the embryo and shape the embryonic body plan. Recent results from our lab suggest that the inhibition of aquaporin3b (aqp3b) prevents convergent extension of the mesoderm and that aqp3b acts through noncanonical Wnt signaling. Wnt signaling is a key signal pathway for embryo and tissue development and is composed of the canonical and the noncanonical pathways. Our lab has shown that aqp3b acts through a specific noncanonical pathway, the Wnt/CA2+ pathway, and acts upstream of the cytoplasmic Wnt signaling pathway member Disheveled (Dsh). Frizzled7 (Fz7) is a membrane receptor in the noncanonical Wnt/CA2+ pathway which also acts upstream of Disheveled. Our question for this project is whether aqp3b acts upstream or downstream of Fz7? When Fz7 was present, protein kinase C fused to green fluorescent protein (PKC-GFP) attached to the cell membrane but when Fz7 was absent, PKC-GFP remained freely in the cytoplasm. This served as our control injections: PKC-GFP + fz7 or PKC-GFP alone, respectively. The experimental injections included a morpholino (MO), small oligonucleotide that inhibited aqp3b expression, or a control MO which did not inhibit aqp3b. We have shown that the MO has kept PKC-GFP localized to the cytoplasm despite the presence of Fz7, while the control MO is allowing PKC-GFP membrane attachment. These data demonstrate that aqp3b acts downstream of the Fz7 receptor in the noncanonical Wnt signal cascade.