Determining the Place of Aqp3b in Noncanonical Wnt Signaling

Oscar Machado, Dr. Jennifer Forecki, and Dr. Christa Merzdorf
Department of Cell Biology and Neuroscience, Montana State University, Bozeman, MT

ABSTRACT

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 signaling pathway for embryo and tissue development. There are two types of Wnt signaling pathways, the canonical and the noncanonical pathways. There are three separate branches to noncanonical Wnt signaling. Our lab has shown that *aqp3b* acts through the noncanonical Wnt/Ca2+ pathway and that it acts upstream of the cytoplasmic Wnt signaling pathway member Disheveled (Dsh). The Frizzled7 (fzd7) membrane receptor is part of the noncanonical Wnt/Ca2+ pathway and also acts upstream of Disheveled (Dsh). I will test, whether in this signaling cascade, *aqp3b* acts upstream or downstream of fzd7. Thus, I will test whether fzd7 activates *aqp3b*, if *aqp3b* activates Fzd7, or if *aqp3b* is bypassed and fzd7 activates disheveled. When fzd7 is active, GFP-labeled protein kinase C (PKC-GFP) relocates from the cytoplasm to the plasma membrane. Thus, I will inject either PKC-GFP alone, PKC-GFP + fzd7, or PKC-GFP + fzd7 +*aqp3b*MO (morpholino oligonucleotide, which inhibits aqp3b) into two-cell *Xenopus* embryos and examine under a fluorescence microscope whether the PKC is bound to the membrane (active Wnt signaling: PKC + fzd7 injection or if aqp3b acts upstream of fzd7) or remains in the cytoplasm (no Wnt signaling: PKC injected alone or if aqp3b acts downstream of fzd7). With this procedure the place of *aqp3b* within the Wnt/Ca2+ signaling pathway will be determined.

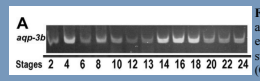


Figure 1. *aqp3b* is an aquaglyceroporin that is expressed at all embryonic stages in *Xenopus laevis* (Cornish et al., 2009).

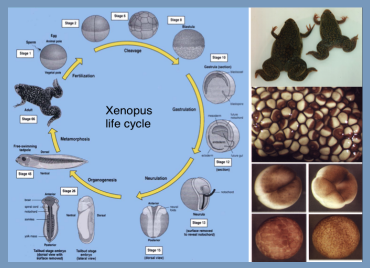


Figure 2. Life cycle of Xenopus laevis. Xenopus is an excellent model organism for research because they lay hundreds of embryos at one time and can be induced to lay year round. Also the embryos are large and develop outside the mother, thus allowing the visualization of its development.

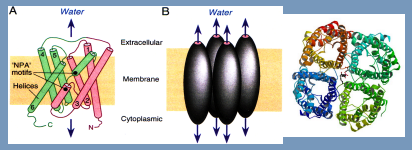


Figure 3. Structure and Function of Aquaporin. The structure of an aquaporin allows water to permeate through the cell membrane. Four proteins form a tetramer in the cell membrane, each with a water channel. Water passes through aquaporin channels passively and bidirectionally, depending on the osmotic gradient.

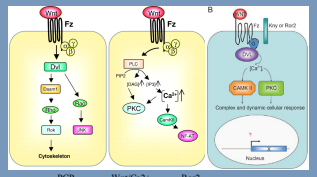


Figure 4. Noncanonical Wnt signaling. All three noncanonical Wnt Pathway Signal Cascades are shown. The Wnt/Ca2+ Pathway pertains to PKC and thus is the pathway of interest for this research project. Results in the lab have shown that *aqp3b* acts through the noncanonical Wnt/Ca2+ pathway.

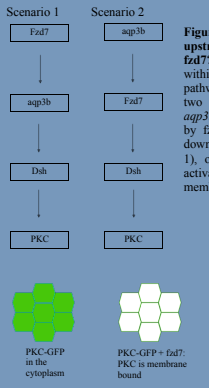


Figure 5. Does aqp3b act upstream or downstream of fzd7? The position of *aqp3b* within the noncanonical Wnt pathway is not known. There are two possible placements for *aqp3b:* either *aqp3b* is activated by fzd7 to activate the rest of downstream members (scenario 1), or *aqp3b* activates fzd7 to activate other downstream members (scenario 2).

Figure 6. Expected Localization of PKC. When PKC-GFP is injected into the embryos, there is no fzd7 Wnt receptor to activate Wnt signaling and to allow PKC to bind to the membrane. Thus, PKC-GFP remains in the cytoplasm. When PKC-GFP + fzd7 are injected, there is a Wnt receptor which activates the pathway and enables PKC to bind to the membrane. When PKC-GFP + fzd7 + aqp3b MO are injected, fzd7 could activate *aqp3b*, *aqp3b* could activate fzd7, or *aqp3b* could be bypassed and fzd7 could activate Disheveled.

METHODS



Figure 7. Injections. In order to determine, whether aqp3b acts upstream or downstream of fzd7, embryos will be injected with PKC-GFP mRNA only, in which PKC is expected to remain in the cytoplasm due to an absence of fzd7 (Wnt receptor). Then PKC-GFP + fzd7 mRNAs will be injected into the embryos, in which the PKC is expected to localize to the plasma membrane due to the presence of fzd7. Lastly, *aqp3b* expression will be inhibited with morpholino oligonucleotides (MO) that prevent the *aqp3b* mRNA from being translated.

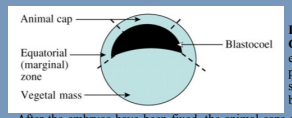


Figure 8. Animal Caps. The injected embryos are fixed in a paraformaldehyde solution at the early blastula phase.

After the embryos have been fixed, the animal caps are removed carefully with a 22 gauge needle. The removed animal caps will be antibody stained then placed upside down on microscope slides. Fluorescence microscopy will be used to analyze where the GFP-labeled PKC is located: in the cytoplasm or localized to the cell membrane. With this technique, the scenarios in Figure 5 can be distinguished.

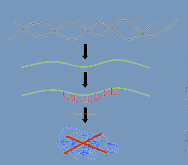


Figure 9. Morpholino Oligonucleotides. Later in this project an *aqp3b* morpholino will be used in order to inhibit the function of *aqp3b*. The *aqp3b* morpholino oligonucleotide (MO) binds to the 5' UTR of the *aqp3b* mRNA which prevents ribosomes from translating the *aqp3b* mRNA.

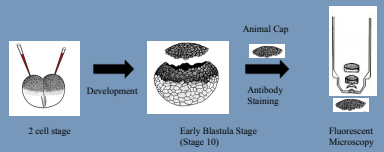


Figure 10. Project Schematic. At 2-cell stage, both cells are injected with one of the three injection mixes (Figure 7). Embryos are left to develop to the early blastula stage, before embryos are fixed in paraformaldehyde. The animal caps are then excised. Animal caps are then subjected to fluorescent anti-GFP antibody staining in order to amplify their fluorescence. The animal caps are placed upside-down on a microscope slide and fluorescence microscopy is used to determine where the fluorescence is localized.

RESULTS

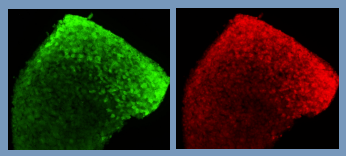


Figure 11. Excised Animal Cap Viewed with Two Different Fluorescence Channels. A red tracer (Ruby Dextran) is used in order to confirm that the observed GFP fluorescence falls into the injected area. The Ruby Dextran image (right) has the same fluorescent pattern as the GFP image (left). This overlap shows that that the fluorescence resulted from the injection and not from auto-fluorescence in the cells.

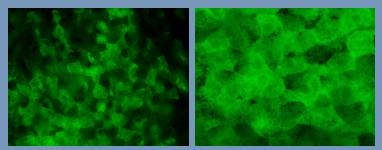


Figure 12. PKC-GFP. This animal cap was injected with PKC-GFP mRNA. Fluorescently tagged PKC (amplified with fluorescent anti-GFP antibody staining) is distributed in the cytoplasm. This image is consistent with our expectation that when there is an absence of fzd7 the downstream members specifically PKC is not activated, thus leading to cytoplasmic fluorescence.

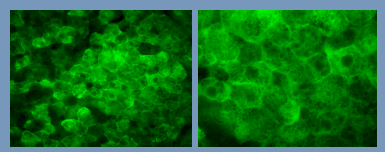


Figure 13. PKC-GFP + Fzd7. This animal cap was injected with PKC-GFP + Fzd7 mRNAs. The animal cap shows fluorescence at the membranes of the cells, indicating that PKC was activated in the presence of fzd7. This is consistent with our expectation that the PKC would be localized at the membranes because the presence of fzd7 allows for the activation of PKC.

SIGNIFICANCE

Defects that occur during gastrulation of embryos can lead to rare and severe conditions such as anencephaly and spina bifida. Both conditions occur due to defects in neural tube closure. The neural tube is the embryonic precursor to the brain and spinal cord. Anencephaly is a condition when the rostral portion of the neural tube does not close properly, leading to an infant with an underdeveloped cerebrum and incomplete skull. The affected infant will usually be stillborn or survive just a few hours after birth. A condition that can arise from the improper closure of the caudal neural tube is spina bifida. In severe cases a cyst-like protrusion filled with cerebrospinal fluid is formed. Usually paralysis of the legs and lack of bladder control result. Neural tube defects occur in 1/2000 human live births.

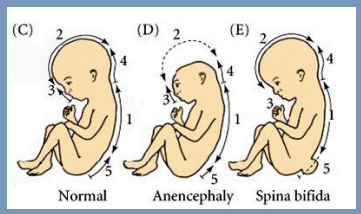


Figure 14. Spina Bifida and Anencephaly. Defects in embryology especially during neurulation can lead to conditions such as anencephaly and spina bifida. The numbers indicate the order and direction in which the neural tube closes in humans.

CONCLUSION

- We have shown that in the absence of fzd7, PKC is distributed in the cytoplasm.
- When fzd7 is present, PKC is localized to the cellular membranes.
- We still need to inject more PKC-GFP mRNA with and without fzd7 to ensure that our observed results remain consistent.
- We have yet to inject the PKC-GFP + fzd7 with the aqp3b morpholino. We expect to see fluorescence either in the cytoplasm or at the membrane. If there is fluorescence in the cytoplasm, aqp3b could then be downstream of fzd7 (Scenario 1, Figure 5) or aqp3b is upstream of fzd7 as depicted in Scenario 2 in Figure 5.

FUTURE WORK

- We will continue to inject embryos with PKC-GFP mRNA with and without fzd7, in order to finish establishing this assay.
- Also in the future we will inject the PKC-GFP + Fzd7 mRNA with the aqp3b morpholino. This will allow us to determine where in the pathway aqp3b is located.

ACKNOWLEDGMENTS

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