

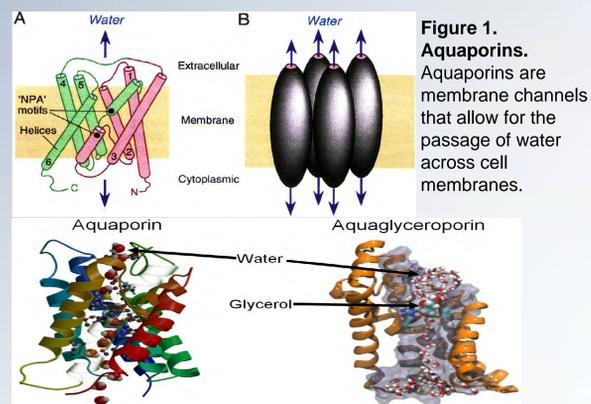
# How Aqp3b Influence Convergent Extension Through Noncanonical Wnt Signaling

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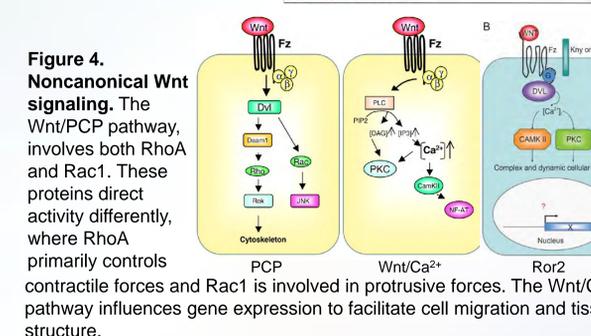
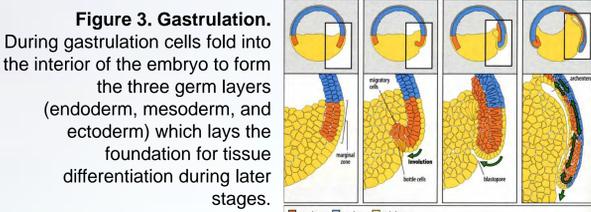
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## INTRODUCTION

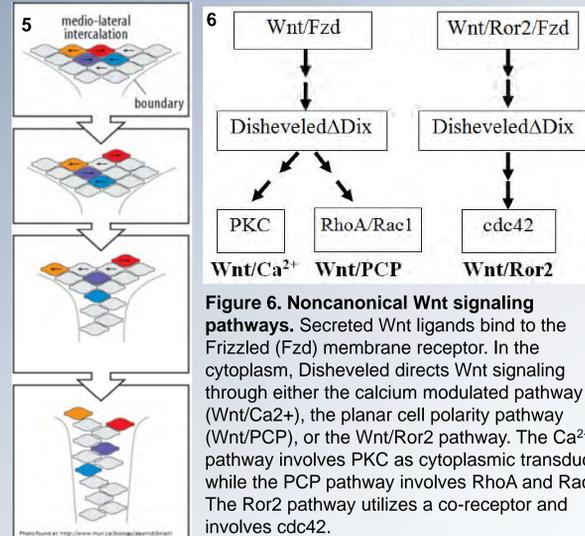
Aquaporin-3b, Aqp3b, is an aquaglyceroporin, a membrane bound water channel, that is present during gastrulation and other stages of early embryonic development (Figures 1 and 2). Gastrulation organizes cells into germ layers, which will later form different body tissues. Convergent extension cell movements are critical in driving gastrulation. During convergent extension, cells fold into the embryo, then merge to form the long body axis (Figure 5). These cell movements are regulated by noncanonical Wnt signaling, an intercellular signaling pathway that controls the patterning, migration, and polarity of tissues. In previous experiments, when Aqp3b is inhibited using a morpholino oligonucleotide, convergent extension does not occur properly, suggesting a link between Aqp3b and noncanonical Wnt signaling. In order to assay these defects, we use the Keller tissue explanting method to observe convergent extension (Figure 11). Our goal is to determine which parts of the Wnt signaling pathway are influenced by Aqp3b. We conducted rescue experiments by inhibiting Aqp3b and injecting an RNA or DNA construct of several proteins involved in Wnt signaling. We have shown that Aqp3b is involved in noncanonical Wnt signaling, as demonstrated by successful rescue using Dvl1 Dlx and Dvl2 Dlx. Aqp3b appears to act through the Wnt/Ca<sup>2+</sup> subpathway, since PKC is able to rescue the defects caused by the inhibition of Aqp3b. In conclusion, I have demonstrated that the ability of Aqp3b to influence convergent extension is dependent on noncanonical Wnt signaling, specifically the Wnt/Ca<sup>2+</sup> subpathway.



**Figure 2. Aquaporins and Aquaglyceroporins.** Aquaporins allow only the passage of water. Aquaglyceroporins have a slightly wider pore, which allows for the passage of glycerol in addition to water. Both exclude ions. Aqp3b is an aquaglyceroporin.

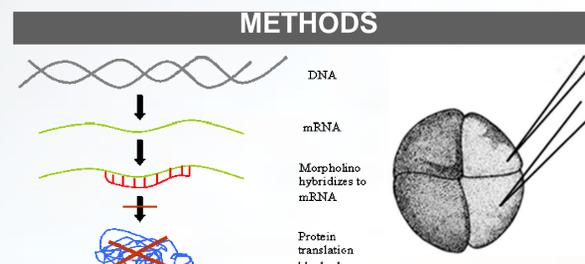


**Figure 4. Noncanonical Wnt signaling.** The Wnt/PCP pathway, involves both RhoA and Rac1. These proteins direct activity differently, where RhoA primarily controls contractile forces and Rac1 is involved in protrusive forces. The Wnt/Ca<sup>2+</sup> pathway influences gene expression to facilitate cell migration and tissue structure.

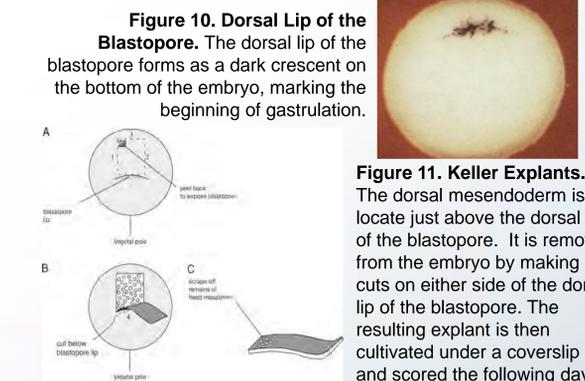


**Figure 5. Convergent Extension.** Cells merge (intercalate) to form a long column of cells that migrate into the embryo's interior. A short wide section of cells forms a long, narrow column of cells. Early in development, this process helps drive gastrulation and elongation of the body axis.

**Figure 7. Xenopus laevis.** Xenopus laevis frogs are a well-established model for the study of early embryonic development, as they are large and develop outside the body at room temperature.



**Figure 8. Morpholino Oligonucleotides.** The Aqp3b morpholino oligonucleotide (MO) binds to the aqp3b mRNA by complementary base pairing. The 5'UTR aqp3bMO binds in the 5'UTR of aqp3b and blocks ribosome progression, which prevents translation of the aqp3b mRNA. The morpholino backbone differs from the DNA phosphodiester backbone, protecting it from degradation.

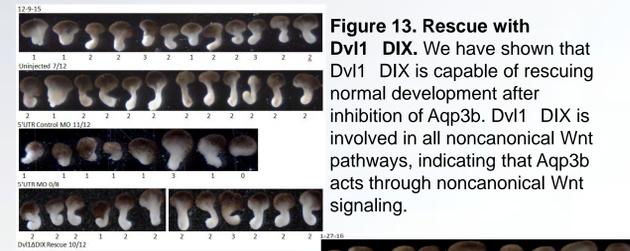


**Figure 10. Dorsal Lip of the Blastopore.** The dorsal lip of the blastopore forms as a dark crescent on the bottom of the embryo, marking the beginning of gastrulation.

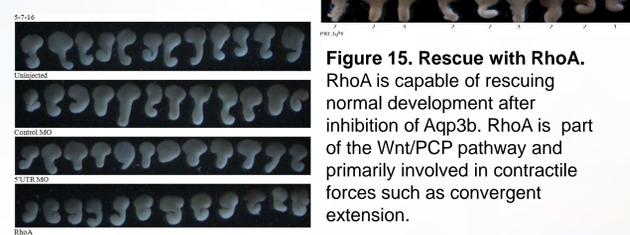
**Figure 11. Keller Explants.** The dorsal mesendoderm is located just above the dorsal lip of the blastopore. It is removed from the embryo by making cuts on either side of the dorsal lip of the blastopore. The resulting explant is then cultivated under a coverslip and scored the following day.

**Figure 12. Explant Scoring.** The explants form long narrow protrusions by convergent extension cell movements. When convergent extension is inhibited, the protrusion does not form (0 or 1). When only convergence is inhibited, the protrusion is wide (3).

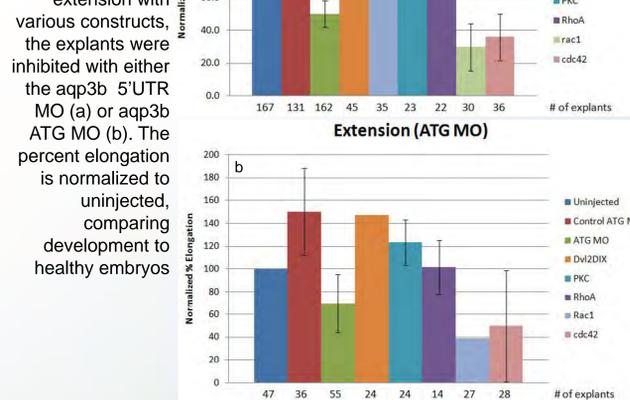
## RESULTS



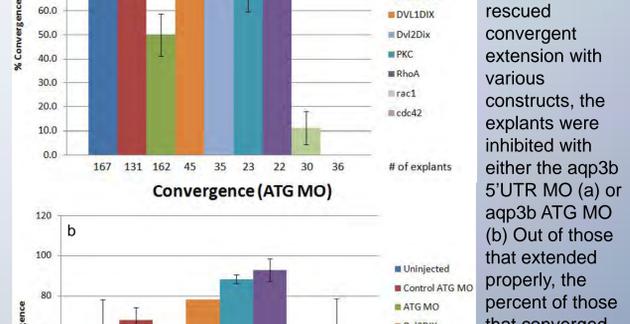
**Figure 13. Rescue with Dvl1 Dlx.** We have shown that Dvl1 Dlx is capable of rescuing normal development after inhibition of Aqp3b. Dvl1 Dlx is involved in all noncanonical Wnt pathways, indicating that Aqp3b acts through noncanonical Wnt signaling.



**Figure 14. Rescue with PKC.** PKC is a component of the Wnt/Ca<sup>2+</sup> pathway. We have shown that rescuing with PKC restores normal convergent extension activity. Aqp3b influences the Wnt/Ca<sup>2+</sup> pathway



**Figure 15. Rescue with RhoA.** RhoA is capable of rescuing normal development after inhibition of Aqp3b. RhoA is part of the Wnt/PCP pathway and primarily involved in contractile forces such as convergent extension.



**Figure 16. Extension.** In explants with inhibited aqp3b expression, we rescued convergent extension with various constructs, the explants were inhibited with either the aqp3b 5'UTR MO (a) or aqp3b ATG MO (b). The percent elongation is normalized to uninjected, comparing development to healthy embryos

## SIGNIFICANCE

To quote Louis Wolpert, "it is not birth, marriage or death, but gastrulation, which is truly the most important time in your life". Defects in embryonic development that occur during gastrulation are often lethal and convergent extension is a fundamental process during gastrulation. Convergent extension is important in many other developmental stages, not just during gastrulation. During neurulation, convergent extension aids in the closure of the neural tube by directing cells to migrate medially and intercalate to form a long, narrow neural tube. When convergent extension is defective during this process, the neural tube does not close properly (Wallingford 2002). The resulting neural tube closure defects include spina bifida and anencephaly (Figures 18 and 19). Neural tube closure defects occur in about 1 in 2000 babies born in the United States. Later in development, convergent extension is important, for example, in the formation of the heart. Here, we use convergent extension that occurs during Xenopus gastrulation as a paradigm for all these other convergent extension events during embryonic development.



**Figure 18. Spina Bifida.** If the neural tube fails to close at the caudal end, it may lead to spina bifida. Severe cases create a cyst-like structure, called the myelomeningocele, on the back, as spinal nerves protrude from the spinal canal, filling with cerebrospinal fluid.

**Figure 19. Anencephaly.** Anencephaly is the condition where the cerebrum of the brain does not form due to improper closure of the rostral neural tube. Some reflexes may be functional if a brain stem has developed, but infants are typically still-born or die within hours or days following birth.

## CONCLUSION

- We have shown that when Aqp3b is inhibited with a morpholino oligonucleotide, convergent extension in gastrulation is adversely affected. Thus, Aqp3b is required for convergent extension movements of cells.
- Dvl1 Dlx restores normal convergent extension after inhibition of Aqp3b, which indicates that Aqp3b acts through noncanonical Wnt signaling.
- PKC rescues normal development after inhibition of Aqp3b, demonstrating that Aqp3b influences the Wnt/Ca<sup>2+</sup> pathway
- RhoA is capable of rescuing convergent extension defects, showing that Aqp3b may play a part in Wnt/PCP

Our results suggest that aquaporins engage in specific signaling during convergent extension. This is a significant departure from the general picture of aquaporins, which have mainly been considered passive pores that allow bidirectional passage of water through cell membranes, depending on the osmotic gradient. Thus, this continued research may ultimately have a very significant impact on understanding the role of aquaporins in cellular processes, such as convergent extension and cell migration.

## FUTURE WORK

- We will continue Rescue experiments involving Rac1 (Another component within the Wnt/PCP pathway) and cdc42 (Wnt/Ror2). As well as working with the ATG Morpholino Oligonucleotide

## ACKNOWLEDGMENTS

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