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**Research Experiences for Teachers**

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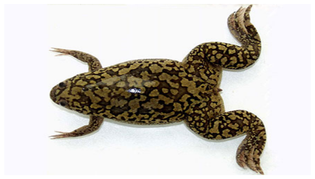
# *The Characterization of TAO3K*

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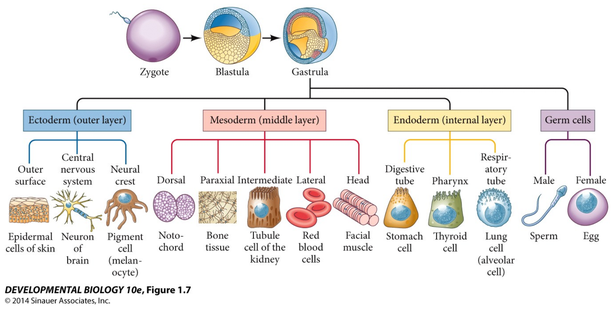
## Introduction

- Embryonic development requires a delicate balance between cell migration and changes in cell identity, which lead to the formation of new tissues and tissue boundaries. The ability of cells to detach from their neighbors, migrate, and establish new contacts is known as 'cell sorting'
- During gastrulation, cell sorting mechanisms and boundary formation help to establish the three primary germ layers: ectoderm, mesoderm, and endoderm. Mesodermal tissue further subdivides into two distinct populations: axial and paraxial mesoderm, which gives rise to the notochord and somites, respectively. The notochord is an essential signaling center and serves as a flexible skeletal rod that supports the body of embryonic chordate animals. Whereas, somites give rise to muscle, bone, and dermal tissues.




***DEVELOPMENTAL BIOLOGY 10e*, Figure 1.7**
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- During notochord morphogenesis, axial mesoderm converges towards the midline of the embryo, where it forms a distinct boundary (the notochord sheath) to separate it from the adjacent paraxial mesoderm (somites). When mesodermal cell sorting is misregulated, the notochord and somites fail to form (see **Figure 1**).

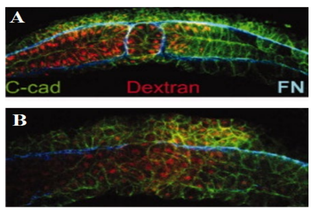



**Figure 1: Notochord morphogenesis in *Xenopus laevis***
**A.** During gastrulation, the axial and paraxial mesoderm separate into two distinct tissues. These tissues form boundaries by secreting extracellular matrix proteins (fibronection (FN), blue). **B.** When cell sorting is perturbed, the axial and paraxial mesoderm fails to separate and no tissue boundaries are formed. Images are from a transversely bisected embryo. Dorsal is up. C-cadherin (green) marks the membrane of all embryonic cells. Image reproduced from Yoder and Gumbiner, 2011.

- Cell sorting is implicitly influenced by the differential expression of cell adhesion molecules, such as cadherins, protocadherins, and eph/ephrins (Fagotto, 2014). Additionally, cell sorting (and gastrulation in general) is further regulated by a number of signaling pathways, including the Wnt, FGF, TGF-β, and BMP pathways. As cells cross the dorsal lip during gastrulation, they receive these instructive signals, which results in changes in cell adhesion, ultimately providing the mechanism for cell sorting (**Figure 2**)

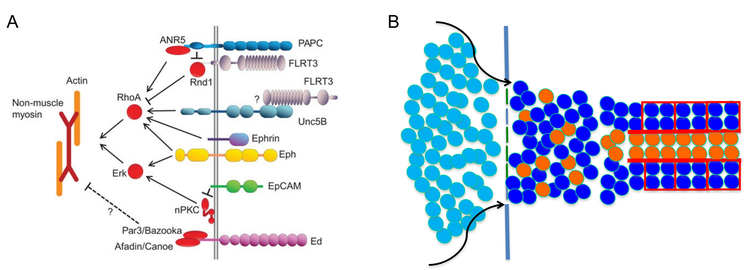
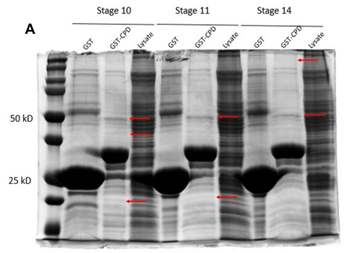



**Figure 2: Model of cellular movements during early gastrulation**
A. Diagram showing potential adhesion molecules involved in cell sorting. (Cadherins not showing) B. Model of cell movement sorting in involuted mesoderm. As mesodermal cells (light blue) involute across the dorsal lip (green dotted line) they receive instructional cues leading to changes in cell fate. Since all dorsal mesoderm is experiencing the same cues, 'microchanges' must occur that allow the axial mesoderm (orange) to separate from the paraxial mesoderm (dark blue). These 'microchanges' are likely the result of differential expression of cell adhesion molecules

## How TAO3K Found



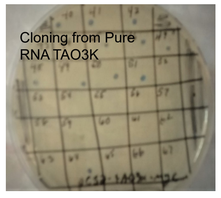

B

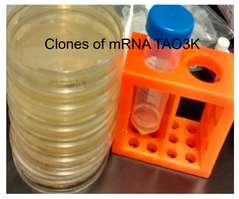
| |
|---|
| casein kinase II subunit alpha [Xenopus laevis] |
| casein kinase 2, alpha prime polypeptide [Xenopus laevis] |
| developmentally-regulated GTP-binding protein 1 [Xenopus laevis] |
| ★ GTP-binding protein 10 [Xenopus laevis] |
| rho GTPase-activating protein 32 [Xenopus laevis] |
| SRSF protein kinase 1 [Xenopus laevis] |
| serine/threonine-protein kinase TAO3 [Xenopus laevis] |
| roundabout guidance receptor 3 precursor [Xenopus laevis] |
| sorting nexin family member 27 [Xenopus laevis] |
| MGC82073 protein [Xenopus laevis] |
| vacuolar protein sorting 4 homolog B [Xenopus laevis] |

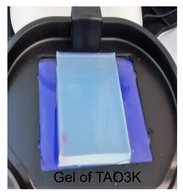
In order to see AXPC function, we identified the proteins that interact and elucidate the cytoplasmic tail. Figure 3: A. shows Coomassi stained gel showing potential interacting proteins. Red arrows show the list identified proteins sequenced. GSTi control and CPD cytoplasmic domain tail B. shows the list of candidates an (green star) GTP-binding protein 10 has been submitted for publication.

### TAO3K

- Purpose of lab was to characterize TAO Kinase expression and function during development.
- TAO3K will activate Mitogen-activated proteins(MAP) pathways which can phosphorylate the p38-activating kinases necessary for translation of mRNA.
- Techniques that were used to approach the experiment were: Cloning with Restriction Digest and PCR, and Morpholino

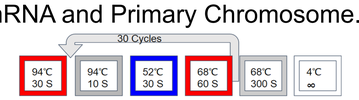



### Cloning Procedures

- Ligation and Transformation protocol, restriction digest to isolate mRNA and Primary Chromosome Replication to get pure DNA of TAO3K bands expressed on during electrophoresis on gel
- Phosphorylation process using Phosphatase binders to extract DNA on binding site and check for band using electrophoresis
- PCR need reaction primers forward and reverse, buffer, and template
- Isolate TAO3K out of gel with PCR
- Restriction digest to isolate mRNA and Primary Chromosome.
- Temperatures to run digest




### Morpholino Knockdown

Restriction enzyme cuts at a specific restricted site to look at insert

- Used to modify gene expression
- Binds to mRNA and blocks translations and correct orientation.

## Conclusions

Results of lab activities

- This is what we expected to see in figure A but instead no bands were expressed due to either low concentrations of TAO3K DNA cloned and or failure of pure sample of mRNA made in the previous lab.

figure A

A. shows second column bright band
B. Shows no band only vector

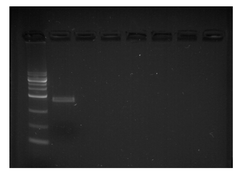
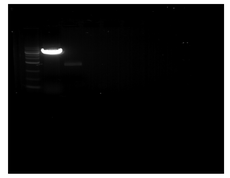
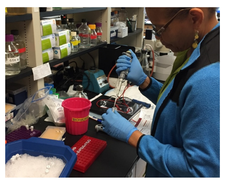


figure B

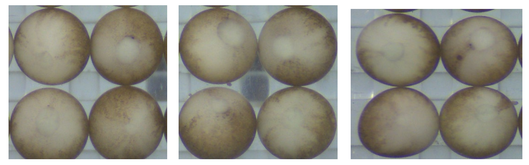


- TAO3K cells where expressed in digestion ligation and RT-PCR and stored for cloning process and clones needed to be purified but very little bands occurred.

Restriction Digestion



Morpholino Knockdown




Injection of Embryos


## Acknowledgements

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## References


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