IOWA STATE UNIVERSITY
Department of Genetics, Development, and Cell Biology

Honors Poster Presentation
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Actin Dynamics During Endothelial Tubulogenesis

Purpose

Endothelial tubulogenesis is the formation of functional blood vessels. The purpose of this research is to understand how actin mediates this process. Actin forms microfilaments that are dynamic structures associated with focal adhesions, cell to cell junctions, and the apical membrane. Actin acts as structural units to provide the force generation that drives morphogenesis. To observe the function of actin and actin-associated structures, transgenic gene lines were characterized. These transgenic gene lines express *LifeAct*, *Moesin*, the *Moesin-Actin-Binding-Domain*, *PH-Plc*, and *Alpha-Catenin* fused in frame to a fluorescent protein. These genes allow visualization of actin and actin-based structures during vascular formation. This formation will be visualized using fluorescent proteins RFP and GFP. These proteins will act as markers to monitor the process of endothelial tubulogenesis in zebrafish using confocal microscopy. The zebrafish will express fluorescents using the GAL4-UAS system. This system activates the expression of our gene of interest with fluorescents. *LifeAct* is a short motif that links the fluorescent protein to actin. This allows for the visualization of actin during blood vessel morphogenesis throughout the endothelial cells. In contrast, *Moesin* was specifically used to visualize the linkages of the actin cytoskeleton and the plasma membrane. *Moesin-Actin-Binding-Domain* is similar to *Moesin* because it also binds to actin, but lacks a membrane binding domain. *PH-Plc* reflects where *Moesin* actually is during vessel morphogenesis. *Alpha-Catenin* serves as a linking protein that is associated with cellular junctions allowing co-localization of actin at adherens junctions. By observing these transgenic gene lines, we can observe how actin contributes to endothelial tubulogenesis.

Procedure and Methods

- The first step involves creating a plasmid to flank the gene of interest. The plasmid has transposon flanking arms, which allows the plasmid to integrate the desired cargo into the genome and express it in the organism.
- Each gene will be paired with either the fluorescent protein RFP or GFP. This will allow us to see the expression of our desired gene by fluorescents.
- Fluorescents will be expressed in our gene lines by using the GAL4-UAS system. GAL4-VP16 will be used with the gene *Fli1b.* This allows this system to be expressed in the vascular system. Then the UAS will control the expression of the target gene with the fluorescent protein.
- When the plasmid is complete, the next step involves injecting this plasmid with the gene of interest into zebrafish embryos at the one-cell stage.
- After the injection, the embryos need to be screened for fluorescents. This is be done under a fluorescent microscope. Then these embryos will be left to grow until endothelial tubulogenesis occurs.
- The zebrafish will then be imaged using a confocal microscope to observe the vascular formation.

Figure 1: Embryo Injection

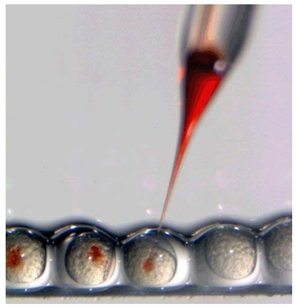
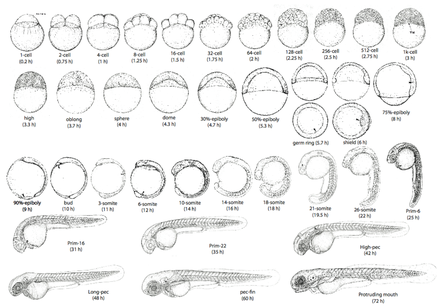


Figure 2: Zebrafish Lifecycle



GAL4/VP16 Amplifies Signals of Tagged Alleles

Some alleles do not have high enough expression thresholds or broad enough expression patterns to be visualized with simple fluorescent protein knock-in. To counteract this, the GAL4-UAS system was used to optimize and amplify the signal of tagged transgenic gene lines.

Figure 3: GAL4/UAS System

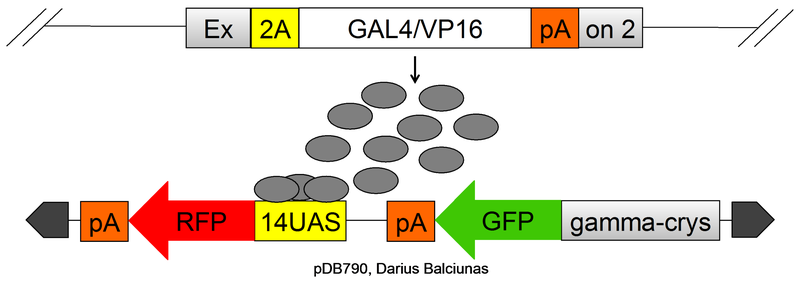


Figure 3, strategy for amplifying signal of tagged alleles using the GAL4/UAS strategy.

Figure 4: *Moesin-Actin-Binding Domain* eGFP

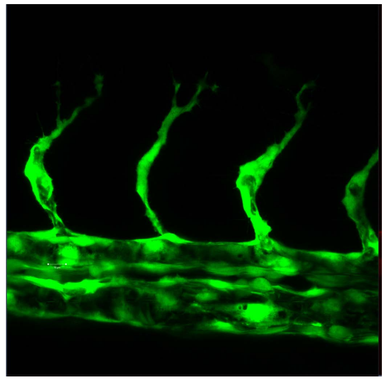


Figure 5: *LifeAct* Tag RFP

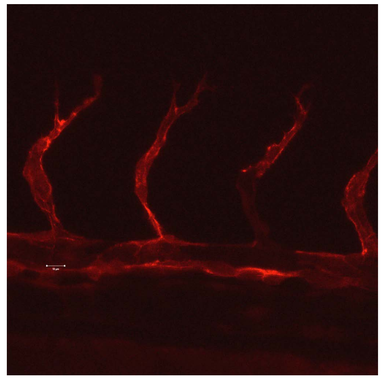


Figure 4, confocal image of *Moesin-Actin-Binding-Domain* eGFP in zebrafish. The *Moesin-Actin-Binding-Domain* eGFP shows green fluorescents of the actin cytoskeleton linkages forming lumen vasculature from the dorsal aorta.

Figure 5, confocal image of *LifeAct* Tag RFP in zebrafish. *LifeAct* Tag RFP shows the fluorescents of actin forming the vasculature of the zebrafish. The intersegmental vessel is full of actin based on the expression of RFP.

Figure 6: *Alpha-Catenin* eGFP

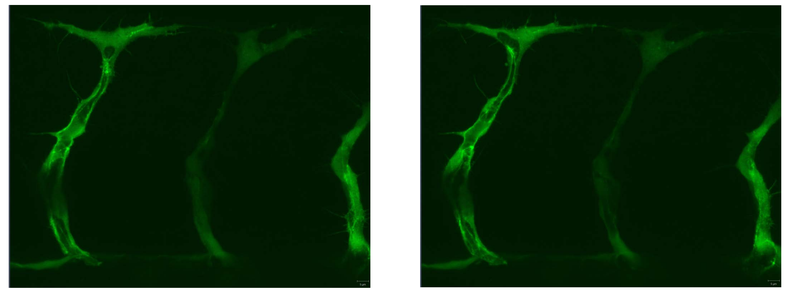


Figure 6, confocal image of *Alpha-Catenin* eGFP. Both images show co-localization of actin at adherens junctions. These images show how actin co-localizes to act as structural units to drive vascular morphogenesis.

Figure 7: *PH-Plc* eGFP

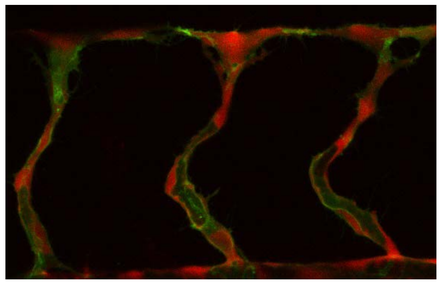


Figure 8: *Moesin* eGFP

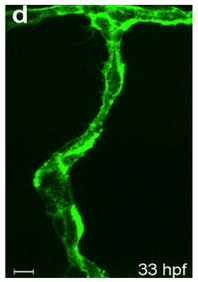


Figure 7, confocal image of *PH-Plc* eGFP in zebrafish. This image shows where *Moesin* actually is during vessel morphogenesis. It reflects where actin cytoskeleton linkages are based on fluorescents.

Figure 8, confocal image of *Moesin* eGFP in zebrafish. This image shows the linkages of the actin cytoskeleton in the intersegmental vessel. Based on the degree of fluorescents, there are many actin cytoskeleton linkages occurring in the vessel.

Conclusion

The purpose of this research is to understand how actin mediates the process of Endothelial tubulogenesis. Actin was observed by looking at transgenic gene lines that express *LifeAct*, *Moesin*, *Moesin-Actin-Binding-Domain*, *PH-Plc*, and *Alpha-Catenin.* Based on the results of vasculature formation, actin is likely associated with important processes of endothelial tubulogenesis. The transgenic gene lines *LifeAct*, *Moesin*, *Moesin-Actin-Binding Domain*, *PH-Plc*, and *Alpha-catenin* showed evidence that actin is very dynamic during vasculature formation. *LifeAct* showed that actin is present during blood vessel morphogenesis. The *Moesin-Actin-Binding-Domain*, *PH-Plc*, and *Moesin* showed how actin linkages were used for vasculature formation. *Alpha-Catenin* showed the co-localization of actin at adherens junctions. By observing these transgenic gene lines, we are beginning to understand the mechanisms that drive endothelial tube formation.

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