Mosaic Analysis of Precursors of Nerve, Bone and Vasculature in Caudal Body and Fin of Zebrafish

Madeline Fitzhugh, Emily Kim, Linda Kim, William Mati, Tahir Razzaq, Maya Roytman, Tom Scott
Dharm Sodha, Leah Grcevich, Grace Manske, Ashley Doina, Paula R. Martin, and F. Bryan Pickett
Department of Biology, Loyola University Chicago

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Abstract

This project focuses on the specific developmental origin and formation of pre-osteoblasts, vascular cells and sensory neurons in the caudal region and tail of zebrafish. Our objective is to generate genetically marked clones using transposon microinjections and to analyze fluorescent protein markers in deep body and tail cell populations. If clones are shared between the fin and deep body it is suggestive that these cells share a common progenitor cell. Zebrafish fins can serve as a model for human organogenesis because conserved genes and cell types are found in human limbs.

Introduction

Cells giving rise to bone, neurons and vessels are generally thought of a being pluripotential stem cells. These types of stem cells produce a limited set of final cell types in the body, but often can give rise to a number of different tissues. In the zebrafish tail the origin of the stem cells giving rise to these tissues is not known, although experiments designed to determine the developmental lineage of cells using postgenomic and high throughput gene expression or genomic state techniques suggest that common mother cells do exist for different tissues. In the caudal or posterior region of the body there are two anatomical compartments that are immediately adjacent to each other, the caudal region of the body (containing part of the spine, body wall muscle blocks and the caudal skeleton) and the caudal fin, a specialized structure containing bone, skin, blood vasculature and nerves which is used for forward motion through swimming. Both regions produce shared tissue types (i.e. nerves, bone and vessels) but the developmental origin of these cells is unclear. If these cells arise from a shared stem cell population it should be possible to identify related progeny cells in both compartments. Additionally the regenerative potential of these cell types in fins is only partially explored in the literature. In our experiments we hope to both determine if shared stem cells exist in both the caudal body and the fin, and to test the developmental potential of cells using clone/mosaic analysis.

Experimental Approach

Cell lines that are genetically labeled with different fluorescent protein genes in animals that are otherwise wild-type are called genetic mosaics. These labelled cell lineages are useful in tracing the descendants of stem cells, because all of the cellular offspring of these labelled cells should share the same label. Cells that share the same origin are referred to as cell lineages.
In this study we are microinjecting a specially built transposon and transposase mRNA into one cell newly fertilized zygotes. As this embryo undergoes cell division transposons and transposase mRNA are randomly assorted to daughter cells. Cells that happen to get a combination of the transposon and enough mRNA to make an active amount of transposase will integrate the transposon into the fish genome. This new "minigene" will transcribe and translate Green Fluorescent Protein which will cause the cell and its descendants to produce green cytoplasm that can be seen in all descendant cells. To chart these "clones of cells" we use Laser Confocal Microscopy to view living fish larvae. These images are analyzed after capture to establish the tissues that include the clone. For regeneration studies on cell lineages we anesthetize mosaic animals and clip the end of their caudal fin. We then chart the clone by allowing the fin to regenerate, We chart the fin by imaging the fin both before and after clipping, and we can compare the two images to evaluate the regeneration potential of the labelled cells. Thus with this experiment we can both establish the developmental lineage history of cells in the body and tail, and also assess the regenerative potential of the same lineages. The transposon we are using for this experiment is diagramed below. Tol2: binding site for transpose, ef1alpha: constitutive promoter, EGFP: Green Fluorescent Protein..TER transcription terminator

Figure 1.
Schematic representation of transposable element.

Methods & Results, Conclusions

Microinjection

Newly fertilized, single to two cell embryos were injected with a mix of transposase mRNA and the Tol2 (Kawakami) derived mini-transposon ef1α:EGFP. We injected embryos at the 1 to 2 cell stage when the embryonic cells still shared a syncytium with the major yolk cell, as per normal protocols. Embryos were stored in a petri dish filled with water supplemented with Instant Ocean Marine Salts at 28.5 ˚C following injection. Embryos were hatched from their chorions and allowed to develop in our nursery incubator. Larval and juvenile fish were maintained in the incubator prior to imaging or fin clipping and were fed using lab grown rotifers as a live food source.

Imaging, Clone Scoring and Fin Clipping

All imaging of larval fish was done using the Fluoview 1000 confocal microscope and the Olympus Epifluorescence Dissecting Microscope. Clone charting was done when bone joints were seen in the fin ray bones (lepidotrichia) which occurred over the first 14 days of development. All imaging was done on anesthetized whole larvae or on freshly dissected tails. A subpopulation of mosaic fish were chosen for regeneration studies based on the extent of mosaicism in the caudal body and caudal fin. Juvenile fish with good bone development and clear evidence of fin mosaicism were anesthetized, their fins were imaged and the fin tip was clipped with a sharp razor blade. Fish were allowed to recover after clipping and were housed individually in incubators to monitor their health. No adverse effects of fin clipping were seen and no procedure associated mortality occurred. Clipped fins were allowed to regenerate over the next two weeks and regenerated fins were imaged after fish were sacrificed by anesthetic overdose.

Figure 2: EGFP Mosaics

Developmental snapshots of the developing fin of a mosaic larval fish. Anterior to the left in all images. The BW series shows white light imaging of the fin for anatomical context. The GFP series shows lineages of cells that share fluorescent label and were thus derived from labeled progenitor (stem) cells. The labeled clones of cell are stable over time because transposons are integrated into the DNA in their nuclei.

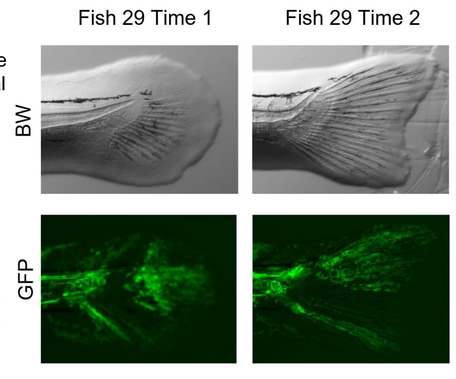
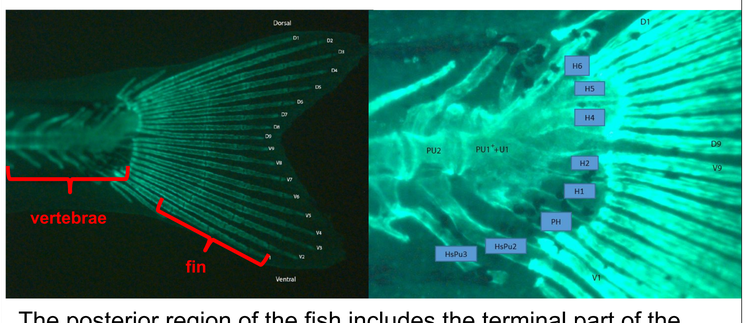
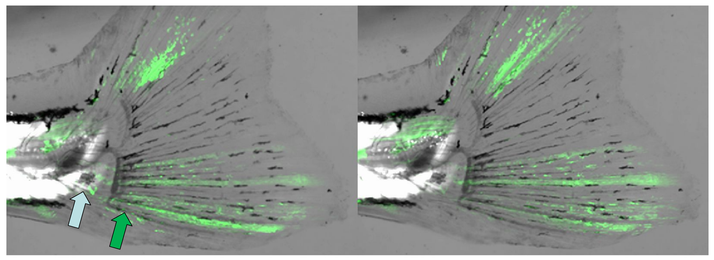


Figure 3: Anatomy of the Caudal Skeleton



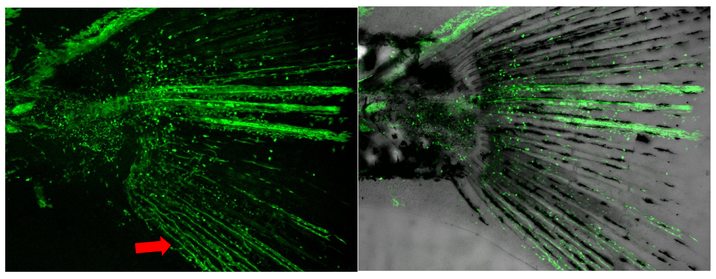
The posterior region of the fish includes the terminal part of the postcranial body, composed of vertebrae and muscle, and the fin, which is a specialized organ composed of bone, nerve, vasculature and skin. Between the vertebrae and the fin are bony elements called the caudal skeleton (shown under the blue text boxes in the image above, right), which articulate both with the vertebrae in the spinal column and the bony elements in the fin called the fin ray lepidotrichia. All the bone in the tails shown above is labeled with EGFP whose expression is driven by the bone specific gene Osterix/Sp7. Thus all fluorescent signal is associated with bones, and these animals are not mosaics as is the case with our experimental animals.

Figure 4: Identification of Shared Stem Cells in Bone

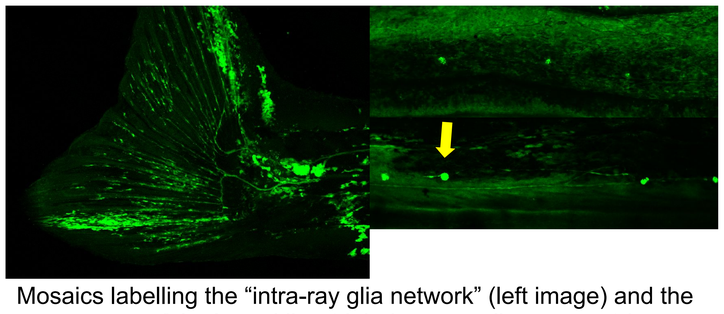


Although we are still collecting data across many mosaic fish, some patterns of clone sharing are beginning to emerge. We commonly see fluorescent signal in caudal skeleton bones (blue arrow) and in adjacent fin ray lepidotrichial bone (green arrow) indicating that a common bone precursor cell produced offspring giving rise to endochondral and membrane bone. It is possible that the cells in the caudal skeleton are doing direct replacement of cartilage with bone, although at this point we have not independently confirmed that these cells are undergoing direct ossification. As we accumulate more examples we will be able to model the developmental potential of the shared precursor cells for both skeletal and lepidotrichial bone.

Figure 5/6: Identification of Vessel and Nerve Mosaics

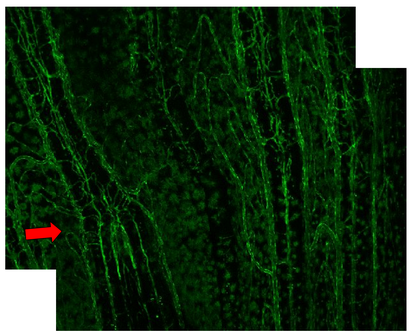


Blood vessel mosaics have been identified in a number of our mosaic larvae, distinguished by the "railroad track" pattern (red arrow) seen within the fins. These clones show a tendency to share vessels between different fin rays, although we also see that the vessels of the entire fin tend not to be developed from a single precursor cell,, as we see that regions of the fin vessels are labeled but all the vessels in the fin are not labeled. We do not yet have complete enough data to say whether or not deeper vessels in the tail (the cardinal vein and the dorsal aorta running running underneath the spinal column) share common cells with the vessels in the tail, although we do have some preliminary data suggesting that the entire vasculature of the tail buds off of the major veins and arteries of the body. However because of the patchy nature of the vessel clones we see in the fin these buds must be built from several independent cells in a non lineage driven fashion.



Mosaics labelling the "intra-ray glia network" (left image) and the neuromasts of the lateral line (right image, yellow arrows) have been identified in a number of our mosaics. We tend to see extensive arrays of nerves labeling, suggesting that we are able to trace many of these neurons back to a small number of founding cells, however we also see some diffuse patches of neurons labeling in some animals, suggesting that the founder cell number for these structures is either variable or that we are labeling an even earlier stem cell that is responsible for most of the neurons in the body. Since we often get extensive clones in skin and brain, it is possible that early labeling of a broad tissue like neural crest or neurectoderm might give rise to these mosaics.

Figure 7:Regeneration and Conclusion



Preliminary studies in regenerating our clipped fin mosaics shows evidence that all the cell types we have been able to label have considerable regenerative potential. In Figure 7 we show a vessel clone regeneration where the vessels of two fin rays have combined to regenerate a single fin ray vascular network (red arrow) , indicating that the vessel do not recognize the fin rays as developmental compartments that must be respected before and after clipping. This suggests that during regeneration there can be significant mixing of cell types and that vessels at least can reorganize into novel architectures after amputation. As we advance our experiments in the future we anticipate further defining the developmental potential and lineage of diverse bone, nerve and vascular tissue in the fin.

Conclusion: Our mosaic analysis identifies likely shared stem cells in bone, vessel and nerve between the body of the fish and the fin. This suggests that common pluripotential stem cells contributing specific tissue fates to different regions of the body are able to be localized and preserved for processes as distinct as the production of skeletal and fin bone. Although our work is at an early stage we are optimistic that as we accumulate a large number of clones we should be able to quantitate relationships between different cells and different regions of the body, to identify shared cells.

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Acknowledgements

A special thank you to Dr. and Mrs. Pickett for their unwavering support and mentoring throughout our research. We would like to thank The Mulcahy Family, The Biology Summer Fellowship, and the Provost Fellowship for support and resources. We are also grateful for the Biology Department and LUROP/Center for Engaged Learning at Loyola University Chicago for their continuous support to undergraduate research.

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