Learning from Nature: Comparative Biology of Tissue Regeneration and Aging

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Poster Abstracts

Alphabetical by presenting author

Utilization of the auxin-degradation system to eliminate P granules in *C. elegans*

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In *C.elegans*, germ granules, known as P granules, play a critical role in maintaining germline pluripotency. However, much remains unknown about how P granules carry out this function. One limitation has been the ability to deplete P granules completely due to redundancy in p granule proteins. Recently our lab has tried to circumvent this issue by using the auxin-degradation system, originally identified in plants, to degrade essential P granule proteins in *C. elegans*. Normally, in plants, Auxin combines with TIR1 to degrade any proteins with a degron sequence. For our research, we obtained a *C.elegans* strain with *Tir1* under the control of a germline promoter. Then, using CRISPR, we engineered degron tags to the critical P granule genes *Pgl1*, *Pgl2* and *Pgl3*. In these worms, exposure to auxin will trigger P granule degradation. This new system will now enable us to better study how P granules help maintain pluripotency.

Axolotl tails as a model for regenerative angiogenesis

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Angiogenesis, the formation of new vessels from pre-existing vessels, is critical for vertebrate tissue growth and repair, but less is known about its role in epimorphic regeneration. Here, we use chemical genetic approaches to modify vascular formation during tail regeneration in albino axolotls. Whole mount *in vivo* imaging, cardiac perfusion

of contrast agent, and histology were used to evaluate the morphological consequences of disrupting the Vascular Endothelial Growth Factor (VEGF) signaling pathway. Early administration of VEGFR inhibitor during regeneration diminishes vascular density but does not prevent the initial regeneration of other tissues. The results form the basis for a model system in which to manipulate angiogenesis during regeneration with temporal control of VEGFR function.

Epicardium activation of *mmp13a* stimulates cardiomyocyte proliferation during adult zebrafish heart regeneration

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Heart muscle regeneration is a complex process involving heterogeneous signals from the extracellular space that influence cardiomyocyte behavior. Matrix metalloproteinases (MMPs) are endopeptidases critical for cellular remodeling and stimulating signal transduction between different cell types. We examine the role of *mmp13a* during adult zebrafish heart regeneration following either ventricular resection or ablation by cryoinjury. Post-injury, *mmp13a* mRNA and protein levels are strongly upregulated within the epicardium, coinciding with phases of cardiomyocyte proliferation. Following resection, expression of *mmp13a* transcripts was inversely correlated with microRNA-101a levels, a previously identified microRNA required for cardiomyocyte proliferation and scar tissue resolution. Importantly, *mmp13a* mRNA contains predicted binding sites for microRNA-101a, suggesting its activity is regulated in part by posttranslational silencing. Pharmacological inhibition of *mmp13a* resulted in defective epicardium migration during wound healing and suppressed cardiomyocyte proliferation. Thus, *mmp13a* activation in nonmyocyte cells potentially controls the proliferative activity of cardiomyocytes during heart regeneration in adult vertebrates.

MiR-101 directs cross-talk between the epicardium and cardiomyocytes during zebrafish heart regeneration

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Heart disease is a major cause of morbidity and mortality worldwide, in large part because humans replace damaged myocardium with non-functional scar tissue. By contrast, zebrafish possess the remarkable ability to completely regenerate heart muscle without residual scar. Using the zebrafish as a model to elucidate the genetic circuitry

controlling regeneration, we have identified downregulation of the microRNA (miR) miR-101 and concurrent upregulation of Cox2, a recognized miR-101 target of miR-101, at 3 days-post-amputation (dpa) in the injured heart. Functional studies demonstrate that either amplified levels of miR-101, or selective inhibition of Cox2, suppresses cardiomyocyte proliferation at 3dpa. Moreover, enzyme-linked immune-sorbent assays reveal an inverse relationship between miR-101 expression and concentrations of the Cox2 product prostaglandin E2, a proliferation-enhancing signaling molecule. Interestingly, both miR-101 and Cox2 are preferentially expressed in the epicardium, suggesting that during the early stages of heart regeneration, miR-101 directs crosstalk between tissues to promote cardiomyocyte proliferation.

Elucidating the role of FGFR-4 in skeletal muscle homeostasis and regeneration

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Fibroblast growth factor receptor 4 (FGFR-4) is specifically expressed in skeletal muscle development and adult muscle stem cells (satellite cells). However, its exact role in skeletal muscle remains unknown. Our aim is to assess the effects of *Fgfr4* deletion in a ubiquitous versus a conditional knock out model to dissect the muscle-specific role of FGFR-4. Analysis of skeletal muscle in *Fgfr4* knockout mice show loss of muscle mass, increase of scapular brown adipose tissue and changes in lipid compositions in the muscle, highlighting its metabolic importance. Cardiotoxin injury model in this knockout have failed to recapitulate the regeneration defect reported previously. Additionally, we have developed a conditional inducible deletion model that selectively knocks out *Fgfr4* in satellite cells. RNA-seq analysis of these satellite cells may elucidate the signaling role of FGFR-4. Understanding these external signals may provide us with new tools to enhance regeneration in muscle ageing and disease.

Deciphering the wound repair strategy: cell growth vs division

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Cells can either grow in size or divide during tissue repair. Using *Drosophila* as a model, we previously discovered that wounds heal by inducing differentiated cells to grow by becoming polyploid. The molecular mechanism limiting cell division and promoting polyploid growth however remains poorly understood. Here we found that injury to adult fly epithelium causes cells to enter S phase through activation of Yki-dependent cell cycle genes, including *myc, cycE,* and *e2f1*. Cells then fail to express mitotic cyclins and constitutively express the ubiquitin ligase Fzr, which targets the mitotic cyclins for proteolytic degradation. As result, cells grow instead of dividing during wound repair similar to the mechanism known to regulate developmentally programmed polyploidy. Inducing ectopic expression of mitotic cyclins in the fly epithelium appears to be detrimental to wound repair, suggesting that cells opt for polyploid growth as a means to promote repair in the absence of cell division.

Early Life Chronic Stress Programs the Immune System via Epigenetic and Circadian Regulation

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Early life chronic stress increases disease risk in adulthood, possibly due to the effects of chronically elevated cortisol on the developing immune system. We have shown that zebrafish embryos treated continuously with 1 μM cortisol for the first five days of development up-regulate pro-inflammatory genes and give rise to adults that aberrantly regulate those genes. To identify genetic loci epigenetically affected by the cortisol treatment, we performed ATAC-seq on blood cells from adults derived from cortisol-treated embryos versus untreated controls. This analysis showed that early life cortisol treatment differentially affects chromatin in the vicinity of genes involved in glucocoticoid receptor (GR) signaling, immune system function, hematopoiesis, and the circadian clock. Adults derived from cortisol-treated embryos also display aberrant circadian rhythms and gene expression. We therefore hypothesize that chronic GR activation during early development perturbs adult immunoregulation by modifying chromatin in the vicinity of key immunoregulatory genes and/or by perturbing circadian regulation.

Molecular mechanisms that generate muscle fibre type diversity during vertebrate evolution.

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Vertebrate skeletal muscle is composed of a complex array of muscle fibres that differ in physiological type, size, and organisation, depending on mode of locomotion. In the zebrafish (*Danio rerio*, teleost), axial muscle formation includes the formation of distinct epaxial and hypaxial muscle groups separated by a horizontal myoseptum. Muscle pioneer cells (MP, slow muscle fibre precursors) are medially aligned to this myoseptum in each myomere, of which they define structure and function. Signalling factors including Hedgehogs, BMPs, and FGFs, function in regulating high expression levels of the transcription factor *engrailed2a*, the earliest marker for MPs. It is unclear how evolutionarily broad this MP developmental model stands amongst vertebrates, therefore understanding molecular signals involved in muscle fibre type determination within vertebrate model (zebrafish) and non-model basal gnathostome species (Epaulette shark (*Hemiscyllium ocellatum*, elasmobranch), and Elephant shark (*Callorhinchus milii*, holocephalan)), will provide evolutionary insight into vertebrate muscle development and diversity.

Cardiac growth and coronary vessels development in a giant danio (*D. cf Aequipinnatus*) heart

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The giant danio (GD) heart displays robust cardiac regenerative response to injury. However little is known about its cardiac development and growth. We studied growth parameters of GDs from embryonic segmentation to 10 months post-fertilization using light, transmission, scanning, and serial block face-scanning electron microcopy. We documented that cardiovascular development, growth and maturation proceed along well defined dynamic patterns and conserved milestones. Like the zebrafish, the coronary vasculature emerged in the late larval stage. The switch from an avascular to vascularized myocardium was concomitant with the formation of a junctional region occupied by a spatially organized fibroblasts network. This is the first comprehensive study of the cardiac growth and maturation in the GD heart that may serve as an important baseline for comparative cardiac biology studies.

Elucidating the effects of aging on muscle

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Progressive loss of muscle with age (sarcopenia) negatively impacts health. Since targeting muscle has the potential to delay age-related functional decline, it's important to elucidate mechanisms underlying muscle aging. We use zebrafish displaying accelerated aging to study the first consequences of aging on muscle structure/function. S*pinster* mutant zebrafish express biomarkers of aging in muscle during embryonic stages and are an ideal model for studying the initiating events of muscle decline with age.

Skeletal muscle consists of myofibers that attach to surrounding laminin-rich basement membranes (BMs), which is critical for muscle structure/function. We're interested in understanding how aging affects muscle-BM interactions. We hypothesize that potentiating muscle-BM adhesion may treat and/or prevent sarcopenia. Preliminary data have identified signaling networks that regulate laminin expression in aging muscle. Knowledge of the genetic changes that contribute to the onset of aging in muscle may provide new therapies to prolong muscle function in aging and disease.

Epidermal damage as underlying cause of paclitaxel-induced peripheral neuropathy

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Paclitaxel is a microtubule (MT)-stabilizing chemotherapeutic agent that, despite its effectiveness, also damages the axons of peripheral sensory neurons. Mammalian studies suggest that neuron-intrinsic damage may be a cause of axon degeneration, but no direct evidence has yet been established. We assessed MT stabilization in live zebrafish using

the MT cap-end protein EB3:GFP to visualize MT dynamics and with an antibody detecting detyrosinated MTs. This revealed that paclitaxel preferentially stabilizes MTs in keratinocytes, consistent with our previous findings that paclitaxel preferentially damages the epidermis. We previously also showed that epidermal damage is mediated by upregulation of MMP-13, a collagenase degrading primarily basement membrane (BM) proteins. Consistently, we now find that paclitaxel treatment causes defects in the epidermal BM, which is traversed by unmyelinated sensory axons. MMP-13 upregulation appears to be caused by oxidative stress formation in the skin. Future studies will assess the molecular mechanisms underlying paclitaxel-dependent MMP-13 regulation.

PQN-75 is secreted from the pharyngeal gland cells of *C. elegans* and is dispensable for germline development

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C. elegans P granules are dynamic RNPs that regulate translation in the germline. Disordered phenylalanine-glycine (FG) repeats within P granules create a phase that extends the size exclusion properties of the nuclear pore complex into the cytoplasm. A mis-sense allele of *pqn-75*, which encodes a novel protein with both polyQ and FG peptide repeats, was isolated in a mutagenesis screen for factors that affect P-granule assembly; however, the P-granule phenotype was later attributed to a non-sense mutation in a linked gene. Using CRISPR, we tagged endogenous *pqn-75* with GFP and found no evidence of germline expression. PQN-75 expression was found to be in all G1 and G2 pharyngeal gland cells and associated excretion ducts, thought to aid in digestion, lubrication of bacteria or pharyngeal lumen, cuticle formation, and disassociation during larval stage transition periods. Here we report our progress to understand the consequence of unchecked development in *pqn-75* mutants.

***Girardia dorotocephala* transcriptome sequence, assembly, and validation through characterization of *piwi* homologs and stem cell progeny markers**

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Planarian flatworms are popular models for the study of regeneration and stem cell biology *in vivo*. Technical advances and increased availability of genetic information have fueled the discovery of molecules responsible for stem cell pluripotency and regeneration in flatworms. Unfortunately, most of the planarian research performed worldwide utilizes species that are not natural habitants of North America, which limits their availability to newcomer laboratories and impedes their distribution for educational activities. In order to circumvent this limitation and increase the genetic information available for comparative

studies, we sequenced the transcriptome of *Girardia dorotocephala*, a planarian species pandemic and commercially available in North America. Over 250 million paired sequence reads were obtained by RNA-seq from intact clonal individuals, regenerating fragments, as well as freshly excised auricles, and used for *de novo* assembly. The resulting transcriptome draft was validated through functional analysis of genetic markers of stem cells and their progeny in *G. dorotocephala*. Akin to orthologs in other planarian species, *G. dorotocephala Piwi1* (*GdPiwi1*) was found to be a robust marker of the planarian stem cell population and *GdPiwi2* an essential component for stem cell-driven regeneration. Identification of *G. dorotocephala* homologs of the early stem cell descendent marker PROG-1, revealed a family of lysine-rich proteins expressed during epithelial cell differentiation. Sequences from the transcriptome draft of our clonal line were found to be 98% to 99% identical to nucleotide sequences from populations of *G. dorotocephala* with different chromosomal number, demonstrating strong conservation regardless of karyotype evolution. Altogether, this work establishes *G. dorotocephala* as a viable and accessible option for analysis of gene function in North America.

CRISPR-Based, Germline Specific Protein Overexpression and Visualization in *C. elegans*

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Currently, systems of protein overexpression do not exist for the *C. elegans* germline. Traditionally, transgenic expression in the germline utilizes extrachromosomal arrays that become silenced. Our goal is to provide a more targeted method that consolidates effective overexpression and fluorescence of desired proteins in the germline of *C. elegans*. GLH-1, which is expressed abundantly in the germline, is linked via bicistronic spacer to achieve co-expression with a target protein and GFP in our base plasmid PDU92, allowing for germline exclusive protein overexpression and fluorescence. Red and blue plasmid variants are currently in production to allow for greater versatility in imaging and expressing multiple proteins simultaneously. All alternate color plasmids produced from the PDU92 base retain the same restriction sites surrounding the target protein for overexpression. Culmination of the project will result in a toolkit of three vibrantly colored, easily customizable plasmids that over-express a target protein exclusive to the germline.

Let-7 regulation of TNFα activity stimulates heart tissue regeneration in adult zebrafish

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In this study, we defined the role of the highly upregulated microRNA let-7i, in the context of adult zebrafish heart regeneration. Depletion of let-7 function using antisense oligonuecleotides at the onset of injury resulted in defective epicardium migration and attenuated cardiomyocyte proliferation indices. Conversely, increased let-7i activity with

a heat-inducible transgenic strain, *Tg(hsp70:let-7ipre)*, enhanced cardiomyocyte proliferation indices by ~30% when compared with heat-treated control animals. Fluorescence-activated cell sorting of cardiac tissues revealed let-7i is enriched by ~3-fold within epicardial cells compared to other cell types of the heart. RNA-sequencing experiments identified components of the TNFα pro-inflammatory signaling pathway as direct let-7 target genes. Importantly, co-inhibition of let-7i and TNFα rescued defects in epicardium migration and cardiomyocyte proliferation. Thus, our zebrafish data indicate that injury-induced upregulation of let-7i is critical for dampening the inflammatory response during heart regeneration.

Some cardiac and somatic parameters in zebrafish as tools for the evaluation of cardiovascular function

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Cardiovascular disease has a major impact on global public health. To date, copious research has got a great advance to elucidate the pathophysiological mechanisms of cardiovascular diseases and to evaluate therapeutic options. Several animal models are widely used to achieve these goals, and zebrafish have emerged as an efficient model that can be used in different ways to help in this area. The majority number body of research is centered to the cardiovascular development and pathology of zebrafish embryos and larvae. However, less research has been developed on adult zebrafish specimens. In this study, we evaluated a method to obtain and to evaluate morphometric parameters of adult zebrafish. We used these data to calculate additional parameters, such as body mass index, condition factor and cardiac somatic index. This method and its results can be used as reference for future studies, no only, in cardiovascular disease, but also in heart regeneration that aim to evaluate the pathophysiological aspects of the zebrafish cardiovascular system.