The role of immediate early genes in the activation of jun-1 and fos-1 in the planarian wound response.

SURG | Natural Sciences and Engineering (NSE) | *Tags: Lab-based*

This cover page is meant to focus your reading of the sample proposal, summarizing important aspects of proposal writing that the author did well or could have improved. ***Review the following sections before reading the sample****. The proposal is also annotated throughout to highlight key elements of the proposal's structure and content.*

Proposal Strengths	Areas for Improvement
The introduction and background follows a logical flow from a broad topic to the specific issues this proposal aims to address. New concepts are presented one sentence at a time, and jargon is defined in simple terms throughout.	The description of the analysis and research output would be further solidified by including how the results will be deemed significant, or what sort of magnitude of "reduction" would indicate the expected result.
The criteria for the study subject (in this case 10 genes) is clearly stated and justified in terms of how it relates to addressing the research aims.	While an aim/objective statement is present, rephrasing to create or including an explicit research question could help to strengthen the proposal.
The researcher addresses and justifies both how data will be collected and how the data will be analyzed. There is a clear description of how the output will help to answer the research questions/aims.	
The proposal uses a clear timeline to show how the 8 weeks will be spent.	

Other Key Features to Take Note Of
In biology research it is important to justify the organism you are using in your study. Often this takes the form of explaining why something is a good "model organism" within the topic/methods you plan to use.

Intro moves from broad topic to specific issue

Animals can heal minor injuries, but severe wounds result in production of scar tissue lacking the functionality of the lost tissue (1). However, some animals can replace lost organs or tissues in a specialized wound healing process called regeneration. Regeneration requires a population of undifferentiated cells that differentiate to replace lost tissue as well as complex signaling mechanisms to direct synthesis of lost structures (2). Many of the identified genetic regeneration pathways are highly conserved in humans, suggesting that a better understanding of regeneration in these species could lead to groundbreaking enhancements in wound healing in humans. The planarian Schmidtea mediterranea, a small freshwater worm with vast regenerative capability, has been developed as a strong model organism to investigate the genetic basis of this process (3). Two planarian genes with human oncogene homologs, jun-1 and fos-1, are expressed in the planarian injury response and promote regeneration; but how planarians regulate these genes is poorly understood (4). My project focuses on elucidating the role that immediate early genes, genes that are expressed rapidly after injury, play in interpreting injury signals and correctly activating jun-1 and fos-1 to enable regeneration.

Clear research/project statement occurs in 1st paragraph

Justifies model organism

The freshwater planarian Schmidtea mediterranea is widely used as a model organism for investigating the genetic basis of regeneration due to their ability to regenerate almost any part of their anatomy; including the ability to regenerate an independent body from fragments as small as 10,000 cells (5). The robust regeneration response in planaria depends on a population of pluripotent stem cells called neoblasts maintained in adult planaria. Neoblasts are responsible for replacing differentiated cells throughout the body during normal, homeostatic growth as well as providing replacement cells during the regeneration response (3).

Identifies gap in knowledge

Research in planarian regeneration has identified several physiological responses to injury that are necessary for successful regeneration. These include an increase in neoblast proliferation, migration of neoblasts to the injury site, and an apoptotic response (4). In addition, transcriptional responses to injury have been identified. While all planarian injuries induce a common transcriptional wound response immediately post injury, wounds which require regeneration are followed by a second response that develops 3-24 hours post injury and varies with wound severity and location (2). Identifying how planarians temporally calibrate different transcriptional responses to different injuries, and how expression of these wound-induced genes controls the physiological injury responses, is a major focus of planarian research.

Justifies why gap in knowledge should be filled

Two genes that may link the transcriptional and physiological injury responses are jun-1 and fos-1 (4). Jun-1 and fos-1 are expressed within one hour of wounding, and their expression scales with injury severity (unpublished data). Inhibition of jun-1 and fos-1 has been correlated with defects in injury-induced neoblast proliferation and injury-induced apoptosis as well as other regeneration defects (unpublished data, 4). This implicates jun-1 and fos-1 as important regulators of the injury response and regeneration. Jun-1 and fos-1 are of special interest as they code for two proteins that combine to form activating protein 1, a transcription factor associated with cell proliferation, apoptosis, differentiation, and wound healing in several animal species (6, 7). However, little is known about how jun-1 and fos-1 are activated in response to injury. Activation of jun-1 and fos-1 expression is likely controlled by factors from the family of immediate early genes, genes that exhibit an extremely rapid increase in expression in response to stimuli (8). Not only are immediate early genes active with kinetics consistent with regulation of jun-1 and fos-1, several immediate early genes have been implicated in regulating homologs of these factors in other systems (8). This project seeks to determine the role of several candidate immediate early action genes in activating the expression of jun-1 and fos-1 to further our understanding of how this important transcription factor promotes regeneration.

Identifies gap in knowledge

States specific research aims of the project, narrowed from project statement in intro

Justifies why gap in knowledge should be filled

Explains criteria for study subject selection

To investigate the effects of immediate early action genes on jun-1 and fos-1 expression, before the summer I will choose ten genes of interest from a list of twenty-four previously identified immediate early genes based on their role in jun and fos expression in other animals. I will then perform RNA interference (RNAi) experiments for each of the ten genes. RNAi uses double stranded RNA (dsRNA) administered during feedings to repress expression of specific

Mentions and explains controls

genes. In the RNAi worms, I will use in situ hybridization, a technique that uses colored or fluorescent probes to bind and tag target mRNA transcripts, to measure intensity and localization of gene expression of jun-1 and fos-1 at several timepoints after a variety of injuries of different severities. Deviations in jun-1 and fos-1 expression between control and experimental RNAi conditions will indicate the role of each target gene in the activation of jun-1 and fos-1 after injury. In parallel, worms from each RNAi condition will be amputated and allowed to regenerate, with in situ hybridizations against the neuronal gene cintillo, which marks regenerated brain cells and thus acts as an indicator of the extent of regeneration (9). To complete this portion of the project, for the first 1.5 weeks, I will synthesize the dsRNA required to perform RNAi experiments for each of the ten genes. This process involves the synthesis of sense and antisense RNA from plasmids, followed by annealing to form the target dsRNA. For the next 2 weeks, I will administer the dsRNA to planarians to begin the RNAi process. Then, for the next 1-1.5 weeks, I will perform the necessary amputations on the worms, fix the samples and perform in situ hybridizations to tag transcripts within each of the worms. For each of the 10 genes, RNAi will be performed on 30 worms. 10 of the worms will be decapitated and tagged for cintillo, 10 will be injured and tagged for jun-1, and 10 will be injured and tagged for fos-1.

Pattern of describing a step of methodology then explaining why the step is taken and connecting back to research aim

A timeline helps show how the project is feasible in 8 weeks

Following this data collection process, I will focus on data analysis. First, I will spend 3 days imaging the worms using single color fluorescence microscopy, maintaining consistent imaging conditions. The final 1 to 1.5 weeks of the project will then be spent using the images to quantify and analyze the effects of inhibition of each target immediate early response gene on jun-1 and fos-1 expression and overall regeneration ability. The effect of suppressing each gene on the intensity and distribution of the jun-1 and fos-1 signals will be quantified using the image analysis software CellProfiler. CellProfiler will also be used to count the number of cintillo positive brain cells regenerated following head amputation of RNAi animals. After normalization to the size of the worm, this count provides a widely accepted quantification of the extent of regeneration. Additionally, any significant morphological defects during regeneration will be noted for each of the ten RNAi conditions of interest. For genes that are required to activate jun-1 and fos-1, I will expect to see reduction in the localization and/or intensity of jun-1 and fos-1 expression after injury compared to worms treated with control dsRNA with no planarian target. In addition, inhibition of these genes should disrupt overall regeneration to a similar degree as inhibition of jun-1 and fos-1. Genes that do not affect jun-1 and fos-1 expression should demonstrate similar localization of jun-1 and fos-1 to the RNAi controls at various timepoints after injury. The families of genes that affect jun-1 and fos-1 expression will inform further research on how an organismal injury state can be translated into molecular signals which correspond to and direct the injury response at the cellular level.

Expected results demonstrate that the research is capable of interpreting the results of the analysis process in terms of how they help to answer the research question

Not clear how the reduction will be seen / what qualifies as a reduction

I am well prepared to carry out this project as I have been working in the Petersen Laboratory since November 2017. For the first few months, I shadowed a graduate student studying the wound response, and in February I began to work on my own independent project. I will continue to work on a several smaller projects related to jun-1 and fos-1 throughout the rest of the school year. While completing these projects, I will perform all the major protocols required for this project, including RNAi synthesis and feeding, in situ hybridization, imaging, and image analysis, so I will be well prepared to handle the experimental techniques required for the project. Additionally, through the completion of my current projects, I will gain a sense of significance of this project and thus an additional appreciation for and connection to the work.

My work thus far in the Petersen lab has been instructive as I intend to attend graduate school in biology and I am considering a career in research. However, time constraints during the academic year limit the scope of the projects I can complete. Spending a summer immersed in a larger scale independent project will allow me to continue to investigate my interest in research and develop skills and experience to make me a competitive candidate for graduate school. Completion of this project will provide a basis for additional research conducted in the Petersen lab next year and may contribute to an authorship on research published by the lab.

References

1. Vriz, S. Reiter, S. Galliot, B. (2014) Cell death: a program to regenerate. Current topics in developmental biology. 108, 121-151
2. Wurtzel, O. Reddien, P. et al. (2015) A generic and cell-type-specific wound response precedes regeneration in planarians. Developmental Cell. 35, 632-645
3. Reddien, P. Alvarado, A. (2004) Fundamentals of planarian regeneration. Annual Review Cell Developmental Biology. 20, 725-757
4. Wenemoser, D. Reddien, P. (2010) Planarian regeneration involves distinct stem cell responses to wounds and tissue absence. Developmental Biology. 344, 979-991
5. Salo, E. (2006) The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes). BioEssays. 28, 546-559
6. Hess, J. Angel, P. Schorpp-Kistner, M. (2004) Ap-1 subunits: quarrel and harmony among siblings. Journal of Cell Science. 117(25), 5965-5973
7. Shaulian, E. (2010) AP-1 — The Jun proteins: Oncogenes or tumor suppressors in disguise? Cellular Signaling. 22(6), 894-899
8. O'Donnell, A. Odrowaz, Z. Sharrocks, AD. (2012) Immediate-early gene activation by the MAPK pathways: what do and don't we know? Biochemical Society Transactions. 40(1), 58-66
9. Hill, E. Petersen, C. (2015) Wnt/Notum spatial feedback inhibition controls neoblast differentiation to regulate reversible growth of the planarian brain. Development. 142, 4217-4229