Collaboration Summary for NASA Early Career Collaboration Award

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My collaboration with Dr. Stephen Miller at the University of Maryland, Baltimore County (UMBC) significantly benefitted not only my research but also my professional development as an early career scientist. In the research plan I submitted for the NASA Astrobiology Early Career Collaboration Award I proposed conducting two related projects that explored the evolution of cellular differentiation in the volvocine green algae, a model system for the evolution of multicellularity and cellular differentiation. Both projects required the genetic transformation of *Volvox carteri*, a spherical multicellular green algae with ~2000-4000 cells and two distinct cell types. This technique required the use of specialized equipment, a Gene Transformation Gun which prior to my collaboration with Dr. Miller I had neither the means nor expertise to use. Through working with Dr. Miller and his research group I learned how to successfully genetically transform *V. carteri* using a Gene Transformation Gun and completed several preliminary experiments (Figure 1). I also benefitted from the exposure to a different laboratory environment and from the opportunity to meet other scholars at University of Maryland, Baltimore County. Below I address each project I originally proposed and what I accomplished toward that project through working with Dr. Miller as well as several unexpected benefits of the collaboration.

Project 1: Transgenic Characterization of *regA* from non-*Volvox* Species

During the first part of my dissertation work I discovered that the genetic basis for specialized somatic cells in *V. carteri*, the *regA* gene, arose early in the evolution of the volvocine algae and is present in species that lack cellular differentiation (Grochau-Wright et al., manuscript in preparation). This indicates that *regA* originally served a different function and was later co-opted to produce specialized somatic cells. The goal of project 1 was to determine if the *regA* gene was co-opted primarily through the evolution of the gene itself or evolution through the rest of the gene regulatory network that controls somatic cell development. In order to test these two hypotheses (*regA* evolution vs. network evolution) I proposed transforming *V. carteri* mutants that lacked specialized somatic cells due to having a mutated *regA* gene, with the *regA* gene of *Pandorina morum*, a ~16 celled colonial volvocine algae species that lacks cellular differentiation. The network evolution hypothesis predicts that the *regA* gene of diverse volvocine algae species should be interchangeable because the evolutionary-genetic changes necessary to the evolution cellular differentiation occur elsewhere in the somatic cell development regulatory network. Thus, the *regA* gene of *P. morum* would be expected to be able to rescue *V. carteri* soma-less mutants. The *regA* evolution hypothesis on the other hand predicts that the *regA* gene itself underwent key evolutionary changes in order for cellular differentiation to evolve and thus the *P. morum regA* gene would not be expected to rescue soma-less *V. carteri* mutants under this hypothesis. These hypotheses are not mutually exclusive however, since *regA* and the rest of the regulatory network may have co-evolved to produce somatic cells. This genetic co-evolution hypothesis would predict that *P. morum regA* will only partially be able to rescue soma-less mutants and thus would result in a phenotype somewhere between mutant and wild-type.

Pandorina morum and *V. carteri* last shared a common ancestor ~200 mya that lacked cellular differentiation (Herron et al. 2009). This substantial divergence time and low protein sequence conservation between the *regA* genes of *P. morum* and *V. carteri* (Grochau-Wright et

al., manuscript in preparation) present the risk of technical challenges for the transformation experiments proposed. Thus, as a first step toward accomplishing the work proposed we chose to transform *V. carteri* soma-less mutants with the *regA* gene of *Pleodorina californica,* a multicellular volvocine green algae made up of ~32-128 cells, a proportion of which are somatic and the rest are unspecialized (Coleman 2012). *V. carteri* and *P. californica* last shared a common ancestor ~75 mya that likely had specialized somatic cells and unspecialized cells (Herron et al. 2009) thus *P. californica* is often considered to be representative of an intermediary stage between undifferentiated colonial species like *P. morum* and large complex *Volvox* species with two distinct cell-types (Kirk 2005). These features make it more likely that transformation of *V. carteri* soma-less mutants with *P. californica regA* will produce successful rescues, thus we decided to test the techniques with the more closely related *P. californica* before moving on to the more diverged *P. morum.* I attempted numerous transformations with the *P. californica regA* gene and isolated 17 transformants that contained the selectable marker gene but none of those that I've tested thus far have the unselected *P. californica regA* gene and there are no apparent rescues. This low transformation efficiency is not unexpected however (Schiedlmeier et al. 1994) and indicates that additional transformation attempts are necessary in order to increase the chances of a successful transformation.

Project 2: Characterizing the Genetic Basis for soma in divergent *Volvox* Species

The characteristic traits of *Volvox* including cellular differentiation have evolved multiple times independently in the volvocine green algae (Herron et al. 2010). Understanding if these independent evolutions of cellular differentiation share a common or divergent genetic basis will shed light on determining if the pathway to complexity is constrained to a few routes or can take a variety of different routes within a single lineage. In order to determine if divergent *Volvox* species evolved cellular differentiation using a common or divergent genetic basis I proposed determining the causative mutation for a soma-less mutant of *Volvox powersii* and to use mutagenesis to create a soma-less mutant of *Volvox ferrisii* for which I would also determine the causative mutation. Collectively these two species (*V. ferrisii* and *V. powersii*) along with the relatively well-studied *V. carteri* would represent the three major independent lineages of *Volvox.* However, since one of the primary objectives of my collaboration with Dr. Miller was for me to learn how to genetically transform *Volvox* species we decided to take an alternate approach to mutagenesis while I was working in Dr. Miller's lab. Rather than mutagenesis I attempted transgenic rescue of *V. carteri* soma-less mutants using the *regA* gene of *V. ferrisii* and *V. gigas,* a sister species to *V. powersii*. If these different branches of *Volvox* evolved cellular differentiation using the same gene (*regA*) through similar evolutionary pathways then the *regA* gene of these different species may be interchangeable. Working with Dr. Miller I successfully transformed *V. carteri* soma-less mutants with the *regA* gene of both *V. ferrisii* and *V. gigas*. However these transformants did not result in a wild-type rescued phenotype. This may indicate that the genes of these divergent *Volvox* species are not interchangeable as hypothesized, but I've so far only confirmed one successful transformation for each gene thus the lack of rescue could be due to technical reasons as well, such as the gene integrating into an untranscribed region of DNA. Additional transformation experiments along with the mutagenesis method discussed earlier will progress this project forward.

Other Benefits of Collaboration

In addition to the research I accomplished with Dr. Miller my collaboration had several unexpected benefits to my development as an early career scientist. Working in a different lab doing related research was beneficial for seeing how different labs are set-up and approach doing research. For example, Dr. Miller using a different methodology for growing and maintaining volvocine algae cultures which may be useful information for future research. Dr. Miller also mentors numerous undergraduates and other non-graduate students whereas my home lab (with Dr. Richard Michod) is mostly graduate students. Seeing how Dr. Miller mentors a wide variety of students, including myself, helped me reflect on how I can better recruit and train undergraduate students. While working with Dr. Miller at UMBC I also had the opportunity to meet Dr. Stephen Freeland, a UMBC professor and astrobiologist. Dr. Freeland and I discussed our research and astrobiology education and outreach, all topics which we have passionate interests in. Thus working with Dr. Miller exposed me to different methodologies, approaches to research, and allowed me to network with diverse scientists.

Summary

In conclusion, my collaboration with Dr. Miller benefitted my research and development as an early career scientist. With Dr. Miller I was able to learn how to genetically transform *Volvox* with a Gene Transformation Gun, a technique that will be critical to the rest of my dissertation research. I was also exposed to different laboratory methodologies and approaches to research and was able to network with other researchers at UMBC. Looking ahead, I will use the methods and skills I learned with Dr. Miller to progress the rest of my dissertation research including do many more transformation experiments.

Literature Cited

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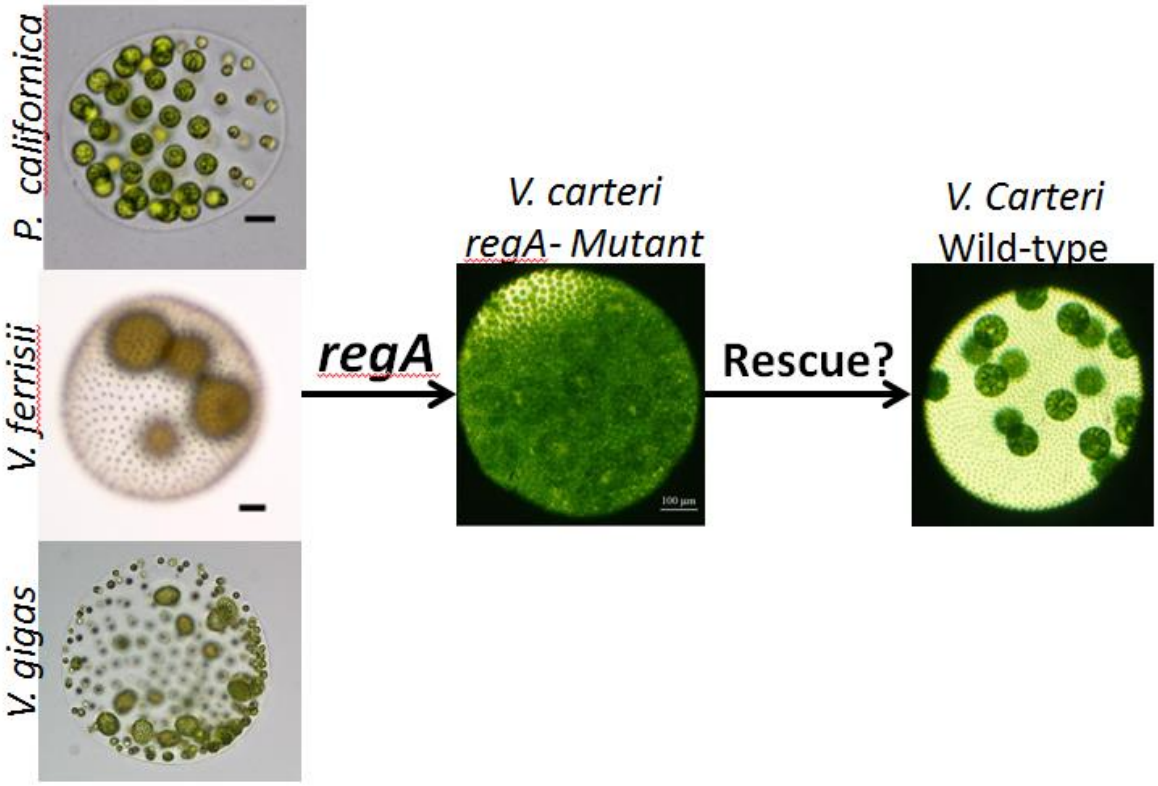

Figure 1. Summary of experiments conducted with Dr. Miller. Attempted to rescue *Volvox carteri* mutants that lacked cellular differentiation due to a mutation in the *regA* gene using the *regA* gene of diverse volvocine algae species: *Volvox gigas*, *Volvox ferrisii* and *Pleodorina californica*.