Isaac Dripps, Biology and Chinese

University: Grinnell College
Year in School: Senior
Hometown: Glen Carbon, Illinois
Faculty Mentor: Dr. Patrick Shiu, Biological Sciences
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Investigating the relationship between repeated DNA and gene silencing in the model organism Neurospora crassa

Isaac Dripps, Thomas Hammond, and Patrick Shiu

In order to maintain genomic integrity, organisms must possess the capability to combat unwanted elements such as retroviruses and transposons. In the filamentous fungus Neurospora crassa, the expression from repeated genetic elements is turned off, or silenced, in a post-transcriptional manner. It is thus possible to induce gene silencing in N. crassa by inserting tandem copies of a transgene into its genome. However, the number and arrangement of transgenes required to activate this process, known as quelling, is unclear. Additionally, silenced strains tend to revert to an unsilenced phenotype after several generations. With the goal of creating a genetically-defined and stably quelled strain, we are examining the effect of various tandem transgene repeats on N. crassa. We have designed plasmid vectors containing 1 to 11 ~1kb fragments of the N. crassa caratenoid pigment producing gene, albino-1 (or al-1). Depending on the number of these constructs in a transformant, the al-1 gene could remain unsilenced (orange conidia), or be partially to completely silenced (light orange to white conidia). Transformants are currently being assayed for their spore color as well as the presence or absence of the transgene repeats. Although this work is still in progress, we have identified a transformant with a putative 5-repeat al-1 transgene that displays a slight quelling phenotype (yellow-light orange conidia). These data suggest that quelling requires the introduction of at least 5 copies of a transgene.