Jenna Gallegos

Major Professor: Alan Rose

Elucidating the mechanism of IME: a tool for maximizing transgene expression

Intron-mediated enhancement (IME) defines a specific process by which certain introns act by an unknown mechanism to drastically increase mRNA levels. Introns engineered for optimal enhancement in plants could yield immense increases in transgene expression for agronomic or pharmaceutical applications. It is thus important to understand the mechanism of IME.

Several observations have led us to postulate that IME acts by altering the local morphology of the promoter, rendering it more accessible to transcription machinery. First, the mechanism of IME appears to be DNA-based. Stimulating intron sequence bolsters gene expression regardless of orientation, and computational predictions of an intron's ability to boost expression improve when both strands are accounted for. Further, stimulating introns appear to effect transcript initiation as evidenced by the fact that stimulating introns can override the spatial expression of tissue specific promoters. Finally, distribution patterns of sequences associated with IME correlate with activating histone marks.

It has been shown that stimulating introns must be near the 5' end of a gene to elicit IME, but it is still unknown whether an intron can boost expression from very near or just upstream of the TSS, as we would expect if IME acts by affecting chromatin structure. When a stimulating intron was tested from various positions downstream of the first exon of the Trp1 gene in a Trp1:GUS fusion, it became virtually ineffective past 1 kb from the transcription start site (TSS). I have made constructs to test the effect of the same stimulating intron from six different positions upstream of the second exon in the Trp1:GUS construct including three upstream of the most commonly utilized TSS. Preliminary results suggest that introns within and just upstream of the 5' UTR stimulate expression. We have also observed that deleting 300 base pairs of the promoter region of the Trp1:GUS construct, including the most common TSS, does not prevent transcription in the presence of a stimulating intron. Thus introns appear to be affecting not only the level of transcript expression, but also transcription start site selection. I will further explore the relationship between introns and transcript initiation by measuring the transcript level and mapping the TSS utilized at each of the aforementioned positions. In addition to more clearly defining the relationship between introns and transcript initiation, this study will complete the first gene scale mapping of intron position versus IME.

If introns act by affecting DNA architecture, we would expect that IME signal could also simulate expression within the context of an exon. Intriguingly, An algorithm known as the IMEter, which is used to identify stimulating introns, also detects enrichment of IME signal in coding regions at the 5' ends of genes. It is possible, therefore, that IME signal can stimulate expression from anywhere near the transcription start site, but is only more commonly identified in introns due to their increased evolutionary flexibility. To determine if IME is intron-specific, I will rearrange coding sequences to incorporate IME signals that have been shown to greatly boost gene expression when located within an intron.

Further experiments to more directly identify a role for chromatin structure in IME will include computational analyses of the relationship between chromatin states and stimulating introns and DNAse hypersensitivity assays.

Despite the potential utility of IME in biotechnology, the mechanism remains elusive. This study will test the hypothesis that introns affect chromatin structure while pinpointing the ideal location for a stimulating intron. In addition to answering fundamental questions about the persistence of introns in evolution and the relationships between introns and promoters, this study has immense practical applications in providing a guide for manipulating introns in order to maximize transgene expression.

Statement of intent to pursue a career in agricultural biotechnology

Having grown up on a ranch on the eastern plains of Colorado, I am deeply invested in contributing to agricultural production and helping farmers improve yields. I have witnessed firsthand the difficulties of food production and the solutions biotechnology has to offer. I designed the proposed project specifically to have important implications in biotechnology. Stimulating introns are powerful tools for ensuring that desirable genes are expressed at appropriate levels. In addition to building skills in molecular plant biology and genomics, I am collaborating with computational biologists and engineers through the Designated Emphasis in Biotechnology program at UC Davis in order to optimally prepare myself for an industry career in agricultural biotechnology.

Awards

National Science Foundation-Graduate Research Fellowship Program $90,000 (2013-2016), Academic Excellence Scholarship from the Training Program in Molecular and Cellular Biology at UC Davis $5,000 (2012), Ronald E. Mcnair Spring and Summer program $3,800 (2011), Undergraduate Research Opportunities Program Grant $1,800 (2011), Howard Hughes Medical Initiative Grant $2,400 (2010-2011), MCDB Mentors Research Grant $500 (2010), Federal SMART Grant $6,000 (2010-2012), Ronald E. Mcnair Summer Research Program $5,000 (2010), Frank and Gina Day Scholarship $1,500 (2009-2010), Federal Academic Competitiveness Grant $2,050 (2008-2009),

Publications

In preparation: Commissioned review article for Plant Science entitled “The Enduring Mystery of Intron-Mediated Enhancement”