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Alternative transcripts of the RPS4 resistance gene in Arabidopsis: Do they produce truncated proteins?

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Plant disease due to pathogenic infection causes large economic losses worldwide. The study of plant disease resistance will potentially lead to crop improvements in an effort to feed the growing world population. This study focuses on the *RPS4* resistance gene in *Arabidopsis thaliana*, which confers resistance to the pathogenic bacteria *Pseudomonas syringae* pv. *tomato* strain DC3000 expressing *avrRps4* in a gene-for-gene specific interaction. Like many related resistance genes, *RPS4* produces multiple alternative transcripts whose functions are not yet fully understood. The dominant *RPS4* alternative transcripts are produced via alternative splicing causing the retention of either intron 3 or introns 2 and 3. Previous research revealed the combined presence of both the full and alternative transcripts was required for proper *RPS4* function. These alternative transcripts have the potential to be translated into truncated proteins due to the presence of premature in-frame stop codons within the retained introns. The goal of this study was to detect and analyze the function of these putative truncated proteins. To detect truncated proteins, N- and C-terminal epitope tags were added to both the genomic *RPS4* sequence and cDNAs of different lengths. These constructs will be transiently expressed in tobacco leaves using *Agrobacterium*. Protein samples will be extracted for an immunoblot assay to reveal the presence or absence of truncated RPS4 proteins. Subcloning *RPS4* transcripts into plasmid vectors for *Agrobacterium* transformation required the majority of time and effort for this project. To determine the biological function of the putative truncated proteins, an *Agrobacterium*-mediated transient assay on tobacco will be performed using the same *RPS4* constructs. The plants will be observed for the presence of a ubiquitous defense response called the hypersensitive response (HR). If a certain *RPS4* construct or combination of constructs causes HR, it will lend evidence toward the functional importance of those constructs in generating defense responses. It has been hypothesized that in resting cells, R-proteins are self-inhibited by intramolecular interactions. When the plant recognizes an invading pathogen, these intramolecular interactions are disrupted, leading to defense responses. To test this hypothesis on the *RPS4* gene, another transient assay will be performed using *RPS4* constructs with N- and C-terminal serial deletions in various combinations. If a certain combination of constructs causes HR, it will provide evidence that these sequences interact intramolecularly. The serial deletion constructs are in the initial stages of development and will be used in the future to perform this experiment. The

sum of this research will improve our understanding of alternative transcripts and their function in plant resistance.