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Identifying the interaction between AtSRFR1 with AtTPL and AtTLR3 in planta

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Plant disease caused by pathogens results in large economic losses in crop yield annually. Our previous study found a negative regulator of effector triggered immunity in Arabidopsis thaliana. Mutations in SRFR1 (Suppressor of rps4-RLD) enhance resistance to the pathogenic bacterium Pseudomonas syringae pv. tomato strain DC3000 expressing avrRps4. We hypothesize that SRFR1 functions similar to Ssn6, a possible ortholog of SRFR1. Ssn6 - Tup1 is a well- known conserved system of transcriptional repression in eukaryotes. We are investigating whether SRFR1 interacts with Tup1-orthologs of Arabidopsis. We chose TOPLESS (TPL), which functions as a transcriptional repressor regulating shoot development, and TOPLESS RELATED (TLR). We first amplified cDNAs of TPL and TLR from Arabidopsis and cloned them into the entry vector of the Gateway compatible system. We then subcloned TPL and TLR into various vectors using Gateway system. These vectors introduced sequences of various tags such as Myc or HA at the 5' end. Using Agrobacterium -mediated transient expression in Nicotiana benthamiana, we will test for interactions between SRFR1 with TPL and TLR. Protein samples will be extracted for co-immunoprecipitation assay to reveal the presence or absence of interactions between these proteins. We will perform western blots to look for these interactions. Cloning TPL and TLR transcripts into plasmid vectors for Agrobacterium transformation required the majority of time and effort for this project. If the two proteins interact, it will be evidence of the importance for SRFR1 in transcriptional repression.