The Individual Roles of the Five Wall-Associated Kinases WAKs 1-5

Cody Woods, Class of 2016

Wall associated kinases (WAKs) are “wall sensor” receptors located within the plasma membrane of angiosperms that bind to pectin, a polysaccharide, present in the cell wall. WAKs contain domains (functional protein regions) in both the interior of the plasma membrane and on the exterior of the plasma membrane to interact with the cell wall. Cross-linked pectin polymers binding to WAKs have been determined through previous studies to be involved in cell expansion through activating vacuole activity within cells to increase turgor pressure. Furthermore, WAKs have also been observed to bind to fragmented pieces of pectin known as oligogalacturonides (OGs) which induces a stress response in the plant. Of these WAKs, there exists five different WAK proteins, WAKs 1-5, which in the angiosperm Arabidopsis thaliana exist in a 30kb locus on chromosome 1 and have WAK1 and WAK2 most ubiquitously expressed throughout the organism. However, the individual role of each of the five WAKs in either the cellular expansion or stress response pathway is not fully understood.

To explore the individual roles of WAKs1-5, CRISPR (clustered regularly interspaced short palindromic repeats) was used to create double stranded breaks in the DNA at a specific target site, resulting in a deletion or insertion of several bases, which allows one to selectively edit the WAK genomic locus. Using CRISPR, a WAK1 mutation in a WAK2 null background was generated. This mutation was suggested from sequencing results of a 500 base pair portion of the WAK1 gene surrounding the CRISPR target site for WAK1, in which the sequencing peaks for likelihood of nucleotide base correlation dropped on average to around 50% and began to display double-peaking patterns at approximately the location of the CRISPR target site with this pattern continuing throughout the remainder of the gene. These results are highly suggestive that we have isolated WAK1 heterozygote plants in the WAK2 null background that contain a WAK1 frameshift mutation allele, which likely produces a nonfunctional WAK1. To further examine the phenotypic impact of a WAK1 and WAK2 mutant, seeds from the heterozygous mutant plants were grown on agar plates with antibiotic selectivity. From these agar plates a suspected WAK1/2 double mutant was isolated and was seedling lethal, demonstrating the importance of WAKs for development. Repetitions of this study must be conducted to verify this result.

Development of a CRISPR complex to delete the entire WAK locus from the Arabidopsis genome by targeting CRISPR cuts at the WAK2 and WAK4 genes, the genes at the two poles of the 30kb WAK locus, was also in development this summer. The development of a successful WAK2-4 CRISPR complex transfected into Arabidopsis will allow us to further examine our current hypothesis that WAK proteins are necessary for growth and development. We also plan to use CRISPR in future studies to isolate particular WAK proteins in Arabidopsis lines to further understand the individual role of the five different WAK proteins.

Faculty Mentor: Bruce Kohorn

Funded by the NIH: INBRE

References: