The Role of EGF Domains in WAK-pectin binding in *Arabidopsis thaliana*
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The plant cell wall is made up a complex network of carbohydrates and proteins. Signaling between the extra-cellular matrix (ECM) and the interior cytoplasm of plant cells is crucial for proper cell response to the surrounding cells and environment. One subfamily of proteins that is involved in this communication is the wall-associated kinases (WAKs). WAKs are receptor-like proteins that are believed to play a significant role as sensors of cell wall structure and function (Seifert and Balukopf 2010). The five different WAKs all share a cytoplasmic serine/threonine protein kinase domain, an extracellular binding domain, and a trans-membrane domain (Kohorn et al. 2009).

It is believed that WAKs interact with the ECM and activate as well as inhibit pathways in the cytoplasm. Kohorn’s lab has shown that the de-esterified form of pectin binds to the extracellular domain of both WAK1 and WAK2 in vitro when the WAKs are expressed in the yeast *Saccharomyces cerviseae*. The role WAKs and pectin play in the activation of mitogen-activated protein kinases (MAPK) has been an important area of research. MAPKs are commonly found in eukaryotes and are involved in signaling cascades within a cell. Upon receiving developmental cues, WAK2 is believed to phosphorylate cellulose synthases, which has been viewed in vitro, leading to the activation of MAPK3. This leads to the activation of invertase and other genes, and finally results in cellular expansion. Similarly, upon receiving warnings of infection or wounding, WAK1 binds BON1, thereby stopping the inhibition of MAPK6. This activates stress genes and finally results in localized cell death to rescue the rest of the plant.

Kohorn’s lab created a new WAK2 mutant in which a tandem affinity tag (TAP) was fused to one end of the WAK2, creating a fusion protein called WAK2cTAP. Plants transformed with this mutated gene had curled leaves and shorter floral stems, indicating dwarfed cells. In a cell with the WAK2cTAP fusion protein, MAPK3 activation is lessened and cellular expansion is compromised, producing plants with a dwarfed phenotype. The WAK2cTAP fusion protein also affects WAK1, producing a response similar to that of infection or wounding, leading to necrosis. This part of the model was discerned from the fact that a MAPK6 deletion over comes the dwarfed WAK2cTAP phenotype and leads to a normal phenotype. BON1 deletion leads to pathogen response, which implies that the WAK1-BON1 complex inhibits MAPK6.

In my own research, I am looking for a suppressor at any point in one of these pathways. A suppressor is a mutation that overcomes the phenotype of the original mutation. To create a suppressor, I started with seeds with the cTAP tag on their WAK2 proteins and mutated them with ethyl methyl sulfonate (EMS) before planting them on soil. So far, I have exposed 40,000 seeds to EMS and planted them on 40 different flats. When these plants mature, I will collect seeds and plant them again. Then, I will screen for plants with the WAK2cTAP phenotype suppressed, or in other words, plants that are no longer dwarfed. I will do a western blot on these plants to makes sure the cTAP tag is still expressing. If it is still expressing, I will then do recombinant inbred mapping to find the location of the suppressor in the genome. Finally, I will be able to clone this gene. All of this will help us to understand whether the cellular expansion and death seen in these plants does in fact follow the pathway we have proposed.

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