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Biochemical characterization of phosphoproteins involved in defense signaling in the plant *Arabidopsis thaliana*

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Plants use an innate immune response for protection against pathogens that can be activated by elicitor molecules, such as a peptide from the flagellar tail of bacteria or the oligosaccharide chitin. Receptors reside at the plasma membrane with the ability to bind elicitor molecules from pathogens and activate defense signaling pathways. Phosphorylation by protein kinases is a major mechanism used by cells to activate proteins and transmit intracellular signals. However, little is known about the phosphorylation-dependent signaling pathways involved in the plant cell's response to pathogens. In the Peck lab, our goal is to identify proteins that are phosphorylated in the early defense signaling pathways of the plant innate immune response, and use these proteins to decode the complex array of molecules and pathways involved. Using previous proteomic screens, the Peck lab has identified candidate proteins from the plant *Arabidopsis thaliana* that are phosphorylated in response to the perception of flagellin. Our hypothesis is that these phosphoproteins are important for defense signaling pathways. The goal of my project is to confirm that several of these proteins are indeed phosphorylated. To do this, I initially characterized peptide-specific antibodies raised against three of these proteins and used the antibodies together with isoelectric focusing (IEF) to detect phosphorylation. Each antibody was tested against plant extracts and gave a signal with immunoblot analysis at the expected molecular weight. Experiments using T-DNA knock out plants for one protein yielded results suggesting that the observed signal is indeed the protein of interest. Multiple bands were observed for each protein on IEF gels that may correlate with multiple phosphorylated forms. We are currently examining if the phosphorylation patterns of these proteins change in response to the perception of flagellin. Data obtained to date on this project will be presented.