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Plants utilize a complex system of light responsive pathways to initiate discrete changes in the plant cell's growth and development. The Light Regulating BTB (LRB) E3 ligases are utilized in the ubiquitin-proteasome system (UPS) to target a group of photoreceptors, the phytochromes, for degradation. The UPS allows for the selective tagging and degradation of proteins in the cell. The Phytochrome B complex is stabile in far-red light but is recognized by the LRBs and broken down in red light by the LRBs. Evidence suggests that the LRBs become activated in R by forming a complete E3 ligase complex which includes the protein Cul3. We propose to investigate how the LRBs become activated and bind to Cul3 in red light in the model plant Arabidopsis thaliana. Genetic sequence alignments suggest the LRBs may be modified by the Nedd8 protein (a protein used to activate a small group of other proteins in eukaryotes) in response to red light. This project proposes to investigate whether the LRBs are modified by the Nedd8 protein by using an in vitro neddylation assay. The results of this assay will improve our understanding of how LRB E3 ligases function in modifying light responses in plants and also provide insight into neddylation and its effect on protein activity.

To test our hypothesis that the LRB proteins are neddylated, an in vitro assay using recombinantly expressed proteins will be used which eliminates the need for the genetic transformation of Arabidopsis to express the necessary tagged proteins. Neddylation is thought to be controlled by N-terminal end of the LRBs; therefore, we can test this hypothesis by using separate parts of the proteins. We will probe for neddylation using full-length LRB, as well as C terminal and N terminal portions of LRB. As a negative control the C-terminus end of LRB1 will be assayed for neddylation as that domain is not hypothesized to be involved in the neddylation process. For a positive control CUL3 will be used as CUL3 is shown to be neddylated under standard in planta conditions. RBX1 may also be included in any neddylation assay since it has been found to be a catalytic intermediate. The assay will be performed using the Abcam Neddylation assay kit that includes Nedd8 and other components necessary for neddylation with in vitro testing.

The results to date do not influence the hypothesis of whether neddylation occurs on the LRB protein. The current stage of our research is having cloned genes transfected into E. coli. We will be expressing the proteins in the hopes that we can purify them to use in the neddlyation assay. Therefore, no direct findings as to the ability or inability of neddylation on LRBs have been found. The results of the protein production and preparation are progressing, and the purification of the LRB-full length was successful, providing the first substrate for the neddylation assay. This progress is in support of the future investigation of neddylation on the LRB E3 ligase.