Programmed cell death (PCD) is a highly regulated process that is required in all multicellular organisms for roles in development and responses to both abiotic stresses and biotic insults. In plants, while clear evidence of PCD has been characterized, the underlying mechanisms regulating its activity are largely unknown. Investigation into these mechanisms has been focused on comparison to animal apoptosis, a well understood form of PCD, which is characterized by the release of cytochrome C from the mitochondria and subsequent activation of cell-executing caspases. While plant PCD shares similar morphologies to animal apoptosis, orthologs of key apoptotic regulators are absent in plant genomes, complicating research (1).

Despite this lack of conservation, it has been shown that apoptotic regulators from animals can suppress classical PCD characteristics in plants when expressed ectopically. The Inhibitor of Apoptosis protein from Spodoptera frugiperda (SfIAP) confers salt and heat stress tolerance in tobacco, and resistance to necrotrophic pathogens in tomato, both responses demonstrating a cell protective effect (2). Protein-protein interaction studies of the function of SfIAP revealed interactions with members of the plant-specific SQUAMOSA promoter-binding protein (SBP or SPL, depending on species) transcription factor family (3). Of the SBP proteins identified via protein interaction with SfIAP, the two members SlySBP8b and SlySBP12a induced cell death when transiently expressed in Nicotiana benthamiana, indicating their role in PCD (3). Subcellular localization analysis of the two SBPs revealed that while SBP8b is entirely nuclear localized, SlySBP12a expression is split between the nucleus and the ER membrane (3). Sequence analysis of the two proteins revealed a transmembrane domain (TMD) in SlySBP12a that controls localization, with truncated SlySBP12a lacking the TMD being entirely nuclear localized. Comparing the cell death effects of truncated SlySBP12a to wild type, the resulting cell death is attenuated, indicating that localization from the ER membrane is a level of regulation for this gene. The ER membrane has been widely studied as a critical sensor and regulatory hub for stress responses, with many membrane-tethered transcription factors functioning to regulate biotic and abiotic responses and determine cell fate (4). With the localization of SlySBP12a and the importance of membrane-tethered transcription factors highlighted in stress, it has been hypothesized that SlySBP12a is proteolytically cleaved from the ER membrane in response to stress factors then translocates to the nucleus to activate genes involved in cell death.

The overall goal of my research progress is to characterize role that SBP transcription factors, particularly SlySBP8b and SlySBP12a, have on cell death pathways within plants. SfIAP works through an unknown mechanism to inhibit cell death, and interacts with these transcription factors, so it stands to reason that regulation of SlySBP8b and SlySBP12a is important for pro-survival signaling in planta, and that these transcription factors must have some manner of interaction with PCD regulatory mechanism. Identification of the associating proteins and the genes activated by these transcription factors are of key interest for identifying PCD-associated genes and characterizing their function.

SBP family members (or SPL as they are referred to in other plant species) have been shown to play a diverse role in plant development including juvenile to adult phase transition, trichome development, apical dominance, and pollen sac development (29). Silencing of the SBP-box gene Colorless non-ripening (Cnr) in tomato results in fruit with delayed ripening, a phenotype that is seen in tomatoes overexpressing SfIAP (25, 30). Although much is known about the role of SBP family members in plant development, only a few studies have linked these transcription factors to stress responses. For example, the N immune receptor of *Nicotiana benthamiana* has been shown to associate with NbSPL6 and is required for the activation of HR cell death (31). In response to copper deficiency, Arabidopsis SPL7 activates the transcription of microRNAs and other genes involved in copper uptake (32). Another Arabidopsis SBP family member, AtSPL14, has been implicated in cell death caused by the mycotoxin FB1. The Arabidopsis atspl14 mutant was shown to be insensitive to FB1 (33), another phenotype that we have observed in SfIAP-overexpressing tomato plants (25).

While we have performed a majority of our analyses in N. benthamiana due to its ease of transformation as a model plant, we are interested in expanding this proposed mechanism of cell death regulation we have uncovered to other more economically relevant species. SBP transcription factors are found in all land plants, but the family has undergone extensive duplication and functional divergence, requiring experimental validation to expand hypotheses beyond a single species (46,47). While all SBPs are named for their characteristic DNA binding domain, the sizes and functions of these transcription factors varies between family members and species. For example, Arabidopsis thaliana contains 16 SBP genes, 19 members in tomato, 19 in rice, and 27 in apple.

My analysis will focus on using phylogenetic inference as a method to investigate the SBP gene family of *Nicotiana benthamiana*, which remains unannotated. While closely related species such as *Nicotiana tabacum* and *Solanum lycopersicum* have partial annotation of the SBP family genes, more distant model species such as *Arabidopsis thaliana* have complete annotations with partial experimental validation. To identify closely related SBP family members in *N. benthamiana* and predict function, I collected coding sequences (CDS) of all predicted SBP sequences from *N. benthamiana*, *A. thaliana*, and *S. lycopersicum* and generated a tree to guide investigation of homologous function.

**Methods**

Data

Sequences were obtained from the Plant Transcription Factor Database v5.0, an online database from the developers of PlantRegMap. The PlantTFDB has lists of TFs with detailed annotation based on literature with links to external databases for further study. PlantTFDB also contains predicted TFs from released draft genomes, but lack annotation. Querying the SBP family on PlantTFDB, I downloaded all CDS from *N. benthamiana, S. lycopersicum,* and *A. thaliana* and merged them into a single FASTA file. As an outgroup, I chose the copper responsive transcription factor CrCRR1 from the green algae *Chlamydomonas reinhardtii,* as was shown to have homology to the *A. thaliana* SBP gene AtSPL7. CDS of CrCRR1 was obtained from the Uniprot database

Multiple Sequence Alignment

Maximum Likelihood

Bayesian Inference