

P023 Protein interactions and subcellular localization in self-incompatible *Petunia hybrida*

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In Gametophytic Self-Incompatibility, growth of self-pollen tubes is inhibited via the action of the S-RNase protein. Current hypotheses propose that in compatible pollinations, S-RNase activity is inhibited by an E3 ubiquitin ligase complex with SLF and SBP1 as critical components or alternatively, via sequestration in a vacuolar-like compartment. One aspect of both of these hypotheses is the proposal that SLF should interact preferentially with non-self S-RNases compared with self-S-RNases. To test this hypothesis, we have carried out quantitative yeast two-hybrid assays using a fluorescence β -galactosidase reporter gene. Different bait and prey constructs for S1 and S3 S-RNase were tested with SLF-S1 and SLF-S3 constructs. Initial data indicated that non-self S-RNase/SLF interactions were generally stronger than self S-RNase/SLF interactions. We are extending these experiments by using a three-hybrid approach to set up competition assays between self and non-self S-RNase and SLF proteins. In related work, we are developing methods, including HPF/FS TEM and bimolecular fluorescence complementation, to determine the subcellular location of S-RNase, SLF and SBP1 following pollination, in both compatible and incompatible pollen tubes. We will discuss our results from the above studies in the context of our long-term goal to determine the molecular basis of self-incompatibility recognition.