S-RNase BINDING PROTEINS: FUNCTIONAL STUDIES OF THE 120kDa GLYCOPROTEIN AND ANALYSIS OF S-RNase OLIGOMERIZATION

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ABSTRACT

Flowering plants control fertilization through pollen-pistil interactions. Self-incompatibility (SI) is a well-studied pollen-pistil interaction that promotes cross-pollination. SI is controlled by a multi-haplotype locus called the *S*-locus. In *Nicotiana alata*, S-RNase is a product of the *S*-locus and regulates specificity in the pistil, while *S*-locus F-box protein (SLF) controls specificity in the pollen. The interaction between S-RNase and SLF determines whether the pollination is compatible or incompatible. In an incompatible cross, the ribonuclease activity of S-RNase inhibits pollen tube growth. Genetic experiments indicate that, in addition to S-RNase and SLF, non-*S*-factors are also required for SI. S-RNase binding proteins represent potential non-*S*-factors required for SI. Using affinity chromatography, we found that S-RNase self-associates and three homologous stylar glycoproteins - the 120kDa glycoprotein (120K), *N. alata* pistil extensin-like protein III (NaPELP III), and *N. alata* transmitting tract specific glycoprotein (NaTTS) - bind directly to S-RNase. I studied the oligomerization of S-RNase in detail and found that self-association is dependent on *S*-haplotype and buffer conditions. I determined that the components of the S-RNase complex account for 30% of soluble pistil protein. 120K is the most likely candidate for a non-*S*-factor because it enters the cytoplasm of growing pollen tubes and shows polymorphism when SI and self-compatible *Nicotiana* species are compared. To test its role in SI, I suppressed 120K expression using RNAi. Suppressing 120K caused a breakdown of SI, confirming that it functions in SI.