Use of Domain-Swapping to Identify Candidate Amino Acids Involved in Differential Interactions between Two Allelic Variants of Type 1 S-Locus F-Box Protein and S_3-RNase in Self-Incompatibility Possessed by *Petunia inflata*

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Background Information

Self/non-self recognition is regulated by the polymorphic *S*-locus; matching of the pollen *S*-haplotype with one of the two pistil *S*-haplotypes results in inhibition of pollen tube growth. The *S*-locus houses *S-RNase* for pistil specificity, and, for both S_2- and S_3-haplotypes, 17 *S-locus F-box* (*SLF*) genes for pollen specificity. All SLFs are assembled into similar SCF complexes, containing Rbx1, pollen-specific Cullin1 and Skp1-like protein. According to the collaborative non-self recognition model, for a given *S*-haplotype, each SCF complex interacts with a subset of non-self S-RNases to mediate their ubiquitination and degradation by the 26S proteasome. Our lab has used a transgenic assay (Fig. 1) to determine interaction relationships of SLF proteins and S-RNases. Among those determined, S_2-SLF1 and S_3-SLF1 (an allelic pair of SLF1, differing in 44 amino acids) show differential interactions with several S-RNases, i.e., S_2-SLF1, but not S_3-SLF1, interacts with S_3-, S_7- and S_{13}-RNases (Table 1).

	S_2-RNase	S_3-RNase	S_5-RNase	S_{6a}-RNase	S_7-RNase	S_{11}-RNase	S_{12}-RNase	S_{13}-RNase	S_{16}-RNase	S_{22}-RNase	S_{24}-RNase
S_2-SLF1	–	+	–	–	+	–	+	+	–	–	
S_3-SLF1	–	–	–	–	–	–	+	–	–		

Table 1. S_2-SLF1 and S_3-SLF1 show differential interactions with three S-RNases.

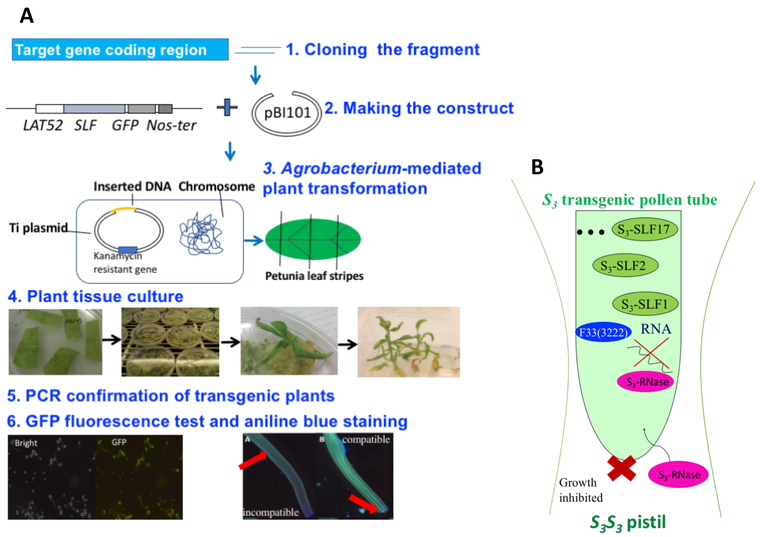

Figure 1. A. Standard procedure for generating transgenic plants expressing an *SLF* gene. B. *in vivo* functional assay

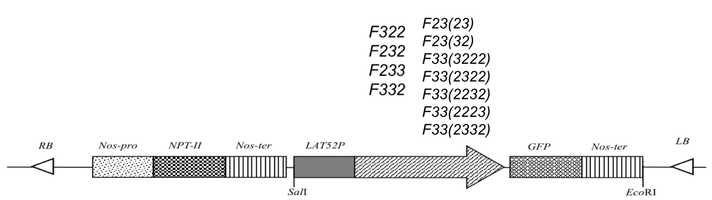

Figure 2. Transgene constructs for expressing 11 chimeric proteins of S_2-SLF1 and S_3-SLF1

Methods

To determine the biochemical basis for differential interactions of S_2-SLF1 and S_3-SLF1 with S_3-RNase, we first divided SLF1 into 3 functional domains (FD1, FD2 and FD3); generated 4 chimeric genes (*F322*, *F232*, *F233*, and *F332*) (Fig. 2); and used a transgenic assay (Fig. 1) to determine whether each encoded chimeric protein of S_2-SLF1 and S_3-SLF1 interacts with S_3-RNase. Based on the results (Fig. 3A), we further divided FD3 into 2 subdomains and then into 4 mini-domains (A, B, C, and D); generated 7 chimeric genes (Fig. 2); and similarly examined the ability of the resulting 7 chimeric proteins to interact with S_3-RNase (Fig. 3A,B). Protein structures were modeled using the I-TASSER server, and protein-protein docking analysis was performed by ClusPro (Fig. 4). FD3s of 4 SLF1s were aligned using MEGA 6 and ClustalW to identify amino acids conserved among the three that interact with S_3-RNase (Fig. 5).

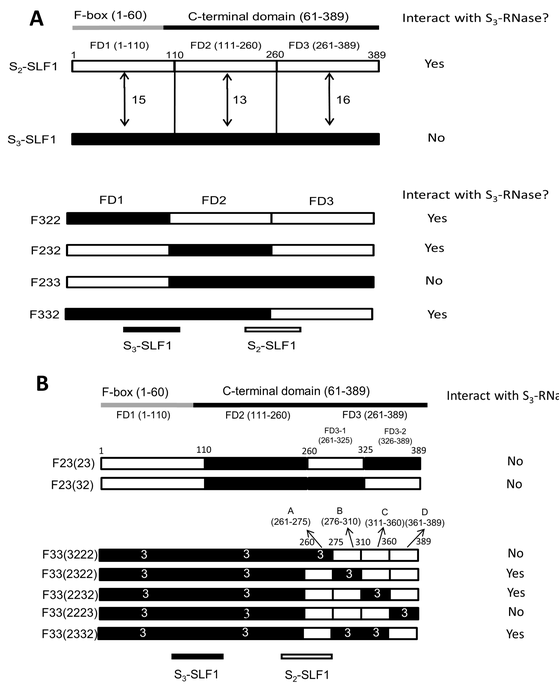

Figure 3. 11 chimeric proteins of S_2-SLF1 and S_3-SLF1. and their ability to interact with S_3-RNase.

Results

The results shown in Fig. 3 allowed us to first narrow the candidate amino acids for specific interaction of S_2-SLF1 with S_3-RNase to the 16 in FD3, and then to 4 in mini-domain A and 4 in mini-domain D. Molecular modeling of interactions between S_3-RNase and S_2-SLF1 revealed that 3 of these 8 are at the interaction surface (Fig. 4), and all 3 are conserved in S_1-SLF1 and S_{6a}-SLF1 that also interact with S_3-RNase (Fig. 5). Three of the chimeric proteins were used to determine whether FD3 alone contains the amino acids required for specific interaction of S_2-SLF1 with S_7-RNase and S_{13}-RNase. The results revealed that, unlike the case of S_2-SLF1's interaction with S_3-RNase, FD2 of S_2-SLF1 is required for interaction with S_7-RNase, and both FD1 and FD2 are required for interaction with S_{13}-RNase.

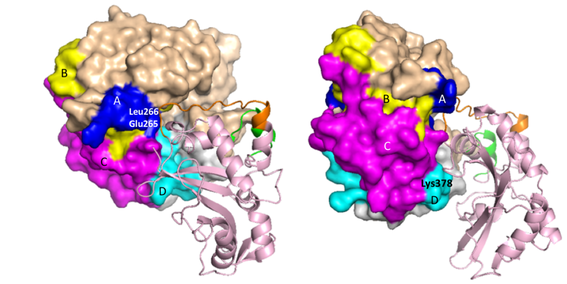

Figure 4. Computational modeling of S_2-SLF1 and molecular docking of S_3-RNase onto S_2-SLF1, as visualized in PyMOL.

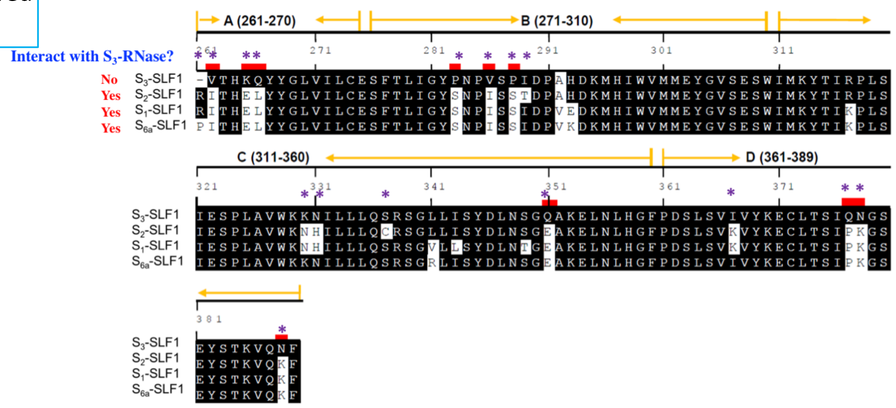

Figure 5. Alignment of deduced amino acid sequences in FD3s of three allelic variants of SLF1 that interact with S_3-RNase and of one allelic variant that does not.

Conclusion

Using domain-swapping and molecular modeling we have narrowed down the candidate amino acids for specific interaction of S_2-SLF1 with S_3-RNase from 44 that are different between S_2-SLF1 and S_3-SLF1 to 2 in mini-domain A and 1 in mini-domain D of FD3. In contrast, FD1, or both FD1 and FD2, contain(s) amino acids required for interactions with S_7-RNase or S_{13}-RNase, suggesting diversity and complexity of interactions between SLF proteins and S-RNases.

Acknowledgements

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References

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