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Domain specific interaction of S-RNase binding protein with the stylar 120 kDA glycoprotein in nicotiana

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Through the process of evolution, many flowering plants have developed a biochemical mechanism to prevent self-pollination and pollination by closely related plants. Gametophytic self-incompatibility (SI) is one such system that prevents inbreeding, a well-known disadvantage for organisms. In Nicotiana, this SI mechanism is controlled by the S-locus and genes located within this locus. The S-locus encodes two highly polymorphic proteins that are directly responsible for the recognition and rejection of self-pollen, S-RNase and SLF/SFB an F-box protein. Non-S-RNase factors also play a key role in SI. The 120 kDa glycoprotein (120K) and HT-B are two style proteins known to be involved in SI. Recent research has revealed that without 120K or HT-B the SI mechanism doesn't work, thus rendering the plants self-compatible (SC). In a previous experiment, the C-terminus of 120K (120K CTD) was used as bait in a yeast-two hybrid (Y2H) screen of pollen and pistil proteins. These experiments revealed that several proteins interacted with the 120K CTD. One of these proteins was the S-RNase Binding Protein (NaSBP1). NaSPB1's interaction with 120K CTD suggests that it could be involved in SI. In vitro binding assays demonstrated that 120K CTD interacts with full length NaSBP1, but which part of the protein is responsible for this interaction? NaSPB1 consists of a 120 amino acid (aa) N-terminal domain, a 165 aa helical domain and a 47 aa RING HC domain. NaSBP1 has been cloned into pMAL-C2xMBP, an N-terminal fusion expression vector. Six different MBP::NaSBP1 fusion constructs have been designed from the three different domains of NaSBP1. Binding experiments (pull-down assays) with domain specific MBP::SBP1 clones will be performed to see what domain or domains are responsible for the interaction with 120K CTD. This will ultimately lead to a greater understanding of how the gametophytic SI mechanism works in Nicotiana.