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Are the Hypervariable Regions of S RNases Sufficient for Allele-Specific Recognition of Pollen?

In a recent research article published in THE PLANT CELL ("Hypervariable Domains of Self-Incompatibility RNases Mediate Allele-Specific Pollen Recognition"), Matton et al. (1997) show that the pollen recognition function of the Solanum chacoense S_{11} RNase can be converted to that of the S_{13} RNase by replacing the two hypervariable (HV) regions of S_{11} RNase with those of S_{13} RNase. From these results, Matton et al. conclude that, "one allelic form of the S RNase molecule can be converted into another by modification of the HV domains alone and that allelic
specifcity can be determined by the HV domains alone.”

Although in this case the differences in allelic specificity between these two very closely related S RNases (the S$_{11}$ and S$_{13}$ RNases differ in only 10 out of 190 amino acids, with four differences in the exchanged HV regions and six elsewhere in the molecules [Saba-El-Leil et al., 1994; Figure 1]) can be localized to the HV regions alone, it should be emphasized that this is not necessarily the case for all S RNases. Indeed, domain swap experiments performed by us (S. Huang, A.G. McCubbin, and T.-h. Kao, unpublished results cited in Kao and McCubbin, 1996; Zurek et al., 1997) suggest that regions outside the HV regions are involved in pollen recognition.

For example, when the HV regions of Petunia inflata S$_3$ RNase were replaced with those of S$_1$ RNase, the resulting chimeric protein, which had normal RNase activity, was no longer able to reject S$_3$ pollen. However, it did not gain the ability to reject S$_1$ pollen (Kao and McCubbin, 1996). In a more extensive study, nine chimeric S RNases were constructed by swapping the HV regions, as well as other regions, between the Nicotiana alata S$_{2}$ and S$_{C10}$ RNases. Again, none of the resulting chimeric S RNases was able to reject either S$_{2}$ or S$_{C10}$ pollen, despite the fact that they all had normal RNase activity (Zurek et al., 1997). These results suggest that allele-specific pollen recognition by S RNases depends on amino acids located both in the HV regions and in other parts of the molecule.

Matton et al.’s conclusion that the HV regions are required for allele-specific pollen recognition supports the results of the previous studies (Kao and McCubbin, 1996; Zurek et al., 1997). However, because the role of amino acids that are the same in the S$_{11}$ and S$_{13}$ RNases cannot be addressed in these experiments, their findings do not contradict the other conclusion drawn from domain swap experiments with more divergent S RNases—that allele-specific interactions between pollen and S RNase also requires amino acids that fall outside the HV regions.

There are some clues as to the possible locations of these amino acids. Sequence comparisons among the S RNases have revealed that there are nine scattered HV amino acids which could potentially play a role in pollen recognition (Tsai et al., 1992; Figure 1). The fact that these nine amino acids happen to be identical in S$_{11}$ and S$_{13}$ RNases does not preclude their potential importance and should not be taken to mean that the HV regions alone mediate S allele-specific pollen recognition in general. Indeed, there is no particular requirement for allelic variability at these amino acid positions; they could be identical, as they are in highly similar S RNases, or different, as they are in divergent S RNases.

To reconcile the difference between their finding and the previous results, Matton et al. suggest that one explanation for the inability of the chimeric S RNases to reject pollen in the previous studies may be that using pairs of S RNases with highly divergent sequences causes protein folding problems for the chimeric RNases (Matton et al., 1997). However, despite the overall sequence divergence between the S$_{A2}$ and S$_{C10}$ RNases, one of the chimeric S RNases constructed by Zurek et al., in which a relatively short region outside the HV regions was exchanged, is quite similar to the S$_{A2}$ RNase (only eight amino acid changes). In addition, the observation that all of the chimeric S RNases tested in the domain swap studies possessed normal RNase activity suggests that their overall conformation was unaffected (McCubbin et al., 1996; Zurek et al., 1997). These results lend further support to the hypothesis that residues outside the two HV regions are also involved in S allele specificity.

There are a number of ways in which the role of amino acids inside and outside the HV domains could be tested in the future. For example, it would be interesting to evaluate whether altering any of the conserved amino acids outside the S$_{11}$ and S$_{13}$ HV regions in positions...
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that correspond to the nine scattered HV residues (see Figure 1) results in loss of the pollen recognition function for one S RNase without affecting the other. The role of identical residues within the HV regions of the S11 and S13 RNases could be assayed in a similar fashion. Moreover, the domain swapping approach could be extended to include regions from a more divergent S. chacoense S RNase such as the S2 RNase (see Figure 1). Does exchanging regions that are identical in the S11 and S13 RNases with the corresponding (divergent) regions of the S2 RNase have an effect on pollen recognition?

In conclusion, any domain swap experiment between a pair of S RNases only demonstrates the role of those exchanged amino acids that differ between the two S RNases under study; it cannot address the role of amino acids that are conserved between the S RNases. Ultimately, determining precisely which amino acids in a specific S RNase are involved in pollen recognition will require elucidation of the crystal structure of the S RNase and identification of the pollen S allele products.

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Reply

In their comments on the paper in which we show that the S. chacoense S11 RNase can be functionally converted into an S13 RNase when four amino acids in the HV regions are replaced with the corresponding amino acids of the S13 RNase (Matton et al., 1997), Verica et al. raise three issues that we feel deserve further elaboration.

The first issue is whether recognition of an S RNase by its specific pollen counterpart only involves interactions with amino acids in the HV regions (as we have suggested) or whether amino acids all over the protein are involved (as Verica et al. and others have proposed).

Before discussing this issue, we must point out that because the structure of the S RNases and the identity of the pollen component are unknown, an extensive debate is unlikely to prove fruitful at this time. We must also recognize that differing views often arise when the same question is addressed using different experimental material. This material generally defines the experimental strategies that can be employed and, thereby, the nature of the conclusions that can be drawn.

Working with two very similar S RNases, our strategy has been to concentrate (by site-directed mutagenesis) on those amino acids that differ between the two. We concluded from our results that “one allelic form of the S RNase molecule can be converted into another by modification of the HV domains alone and that allelic specificity can be determined by the HV regions alone” (Matton et al., 1997).

In contrast, when widely divergent pairs of S RNases are examined, domain swaps replace site-directed mutagenesis as the preferred experimental strategy. The results have so far shown that swapping entire regions of one S RNase with the corresponding regions of another always seems to abolish the pollen recognition phenotype, although the RNase activity itself is conserved.

The conclusions drawn from these experiments have been that “HV regions are necessary but not sufficient for encoding S allele specificity” (Kao and McCubbin, 1997), or that “the S RNase molecule does not have a specific domain responsible for allelic recognition” (Zurek et al., 1997).

A possible resolution to these differing views involves what we perceive to be a second issue raised by Verica et al.—the nature of an HV domain and the use of RNase activity as a gauge of recognition domain integrity. To date, the terms “HV domain” and “HV regions” have been used almost interchangeably in the literature. However, strictly speaking, a domain is a “portion of a protein that has a tertiary structure of its own” (Alberts et al., 1994). Because the tertiary structures of the HV regions, either alone or in the context of an S RNase, are unknown, there is no evidence to suggest the HV regions constitute a bona fide structural do-
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