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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 S_{11} and S_{13} 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 S_2 RNase (see Figure 1). Does exchanging regions that are identical in the S_{11} and S_{13} RNases with the corresponding (divergent) regions of the S_2 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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REFERENCES

Kao, T.-h., and McCubbin, A.G. (1996). How flowering plants discriminate between self and non-self pollen to prevent inbreeding. Proc. Natl. Acad. Sci. USA **93,** 12059–12065.

Matton, D.P., Maes, O., Laublin, G., Xike, Q., Bertrand, C., Morse, D., and Cappadocia, M. (1997). Hypervariable domains of self-incompatibility RNases mediate allele-specific pollen recognition. Plant Cell **9,** 1757–1766.

Saba-El-Leil, M., Rivard, S., Morse, D., and Cappadocia, M. (1994). The S_{11} and S_{13} self-incompatibility alleles in *Solanum chacoense* Bitt. are remarkably similar. Plant Mol. Biol. **24,** 571–583.

Tsai, D.-S., Lee, H.-S., Post, L.C., Kreiling, K.M., and Kao, T.-h. (1992). Sequence of an S-protein of *Lycopersicon peruvianum* and comparison with other solanaceous S-proteins. Sex. Plant Reprod. **5,** 256–263.

Xu, B., Mu, J., Nevins, D.L., Grun, P., and Kao, T.-h. (1990). Cloning and sequencing of cDNAs encoding two self-incompatibility associated proteins in *Solanum chacoense.* Mol. Gen. Genet. **224,** 341–346.

Zurek, D.M., Mou, B., Beecher, B., and McClure, B. (1997). Exchanging domains between S-RNases from *Nicotiana alata* disrupts pollen recognition. Plant J. **11,** 797–808.

Reply

In their comments on the paper in which we show that the *S. chacoense* S_{11} RNase can be functionally converted into an S_{13} RNase when four amino acids in the HV regions are replaced with the corresponding amino acids of the S_{13} 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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main. In contrast, the part of the protein that folds to produce an active RNase probably does represent a true domain.

We further note that the structure of the fungal RNase Rh (Kurihara et al., 1992), which has the same length and identical catalytic regions as the S RNases, suggests that the amino acids corresponding to the S RNase HV regions will be located on the external surface of a solid RNase core. Thus, when we stated that in the case of the "chimeric gene products, interactions between the HV and constant regions have made the HV domains unable to display their normal allele-specific information" (Matton et al., 1997), we were not referring to the folding of the S RNase protein as a whole, as interpreted by Verica et al., but only to that part of the S RNase comprising the HV regions.

Nevertheless, we accept that the three-dimensional structure of the HV region may well be influenced by neighboring amino acid residues, and fully agree with the assessment that "the recognition function appears to be quite sensitive to disruption since relatively small alterations destroyed recognition" (Zurek et al., 1997). However, we think the lack of phenotype resulting from domain swap experiments does not constitute strong evidence for the view that residues outside the HV regions bind directly with the pollen component.

The last issue relates to the direction future experiments should take to most quickly come to terms with the S RNase recognition problem. Verica et al. suggest that to identify functionally important amino acids outside the HV regions, we could modify some of the constant amino acids in the S_{11} RNase and determine which substitutions abolish pollen recognition. In our opinion, our highly similar pair of S RNases is better suited to assessing the role of the four amino acids that differ in the HV regions.

In this regard, we have started to analyze the phenotypes provoked by other mutations in the HV regions of the S_{11} RNase; our preliminary results show that whereas all of these modified proteins retain RNase activity, the self-incompatibility phenotype of the corresponding transgenic plants varies from loss of both S_{11} and S_{13} recognition specificities to simultaneous rejection of both S_{11} and S_{13} pollen (D.P. Matton, X. Qin, G. Laublin, O. Maes, D. Morse, and M. Cappadocia, unpublished observations).

Finally, with respect to the domain swap experiments, we suggest that the possibility that the chimeric proteins have acquired a new recognition phenotype should be examined. Indeed, investigations of the *b* mating incompatibility locus in the fungus *Ustilago maydis* (Yee and Kronstad, 1993) show that some chimeric *b* alleles have a recognition specificity differing from that conferred by either parental allele. If rejection of unrelated pollen were to be observed during genetic analyses of plants carrying domain swapped S RNases, this would provide strong evidence for an effect of amino acids outside the HV regions on the recognition domains of S RNases.

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REFERENCES

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. (1994). Molecular Biology of the Cell. (New York: Garland Publishing).

Kao, T.-h., and McCubbin, A. (1997). Molecular and biochemical bases of gametophytic self-incompatibility in the Solanaceae. Plant Physiol. Biochem. **35,** 171–176.

Kurihara, H., Mitsui, Y., Ohgi, K., Irie, M., Mizuno, H., and Nakamura, K. (1992). Crystal and molecular structure of RNase Rh, a new class of microbial ribonuclease from *Rhizopus niveus.* FEBS Lett. **306,** 189–192.

Matton, D.P., Maes, O., Laublin, G., Xike, Q., Bertrand, C., Morse, D., and Cappadocia, M. (1997). Hypervariable domains of self-incompatibility RNases mediate allele-specific pollen recognition. Plant Cell **9,** 1757–1766.

Yee, A., and Kronstad, J. (1993). Construction of chimeric alleles with altered specificity at the *b* incompatibility locus of *Ustilago maydis.* Proc. Natl. Acad. Sci. USA **90,** 664–668.

Zurek, D., Mou, B., Beecher, B., and McClure, B. (1997). Exchanging sequence domains between S-RNases from *Nicotiana alata* disrupts pollen recognition. Plant J. **11,** 797–808.