Letter to the Editor

Viral Cross Protection: More Understanding is Needed

Milton Zaitlin

Professor, Department of Plant Pathology, Cornell University, Ithaca, New York 14853. Work supported in part by NSF Grant BMS-75-09648.

In a recent provocative Letter to the Editor of this Journal (titled "Understanding generates possibilities") deZoeten and Fulton (2) proposed an explanation for the "cross protection phenomenon" in which virus-infected plants often are protected from subsequent infection by "related" viruses, but not by "unrelated" ones. They postulated that..."the molecular basis for cross protection is the elimination of the genome (RNA) of a superinfecting related virus by its capture in the coat protein of the virus of the original infection," and further that "the formed particle is lost to subsequent infection of the host since it does not encounter an uncoating environment within the cells of the plant." I believe that their theory cannot explain cross protection, based on arguments given below. Further, I have tested their theory with coat protein-defective mutants of tobacco mosaic virus (TMV) where the defective coat protein is unable to encapsidate the viral RNA to form stable virions (11). If functional coat protein is an essential feature of cross protection as deZoeten and Fulton suggest, then these mutants should not protect against infection by conventional TMV strains. I found however, that they do offer some measure of protection, casting doubt on the role of coat protein "capture" in cross protection.

One such defective mutant (PM1) which produces insoluble defective coat protein (4) was able to protect against subsequent infection by the common (U1) strain of TMV (Table 1). The protection was not complete, probably because the defective mutant spreads slowly (11) and does not occupy all of the cells by the time the challenge virus is applied; thus, the common strain probably replicated in some previously uninfected cells. Because the defective mutant induces chlorosis on the inoculated leaf which might render the leaf tissue insensitive to any subsequent infection, I utilized a control which tested the capacity of these leaves to support the replication of the unrelated potato virus X (PVX). The replication of PVX was unaffected by the defective TMV strain, and in one case it actually was enhanced. Enhancement may not be unexpected; Rochow and Ross (9) showed that in the presence of a TMV infection, PVX antigen was increased four-fold.

My result agrees with the findings of Jockusch (6) who tested the cross protection to the common strain induced by two temperature-sensitive TMV mutants at the nonpermissive temperature of 35 C. One mutant (Ni 118) produces a nonfunctional, denatured coat protein at 35 C, is unable to encapsidate its own RNA but *does* cross protect against the common strain. Another mutant (Ni 2519) engenders a functional coat protein at 35 C, but viral assembly apparently is deficient at high temperature (1). This mutant *does not* cross protect. deZoeten and Fulton (2) cited Jockusch's 1968 study (6) as supportive of their hypothesis. They state that Ni 2519, which does not cross protect, produces no coat protein at 35 C, but this is not so; in a later study, Bosch and Jockusch (1) showed that in a small proportion of primary infected cells Ni 2519 produces a protein identical to the functional one of the strain from which it was derived. Further, their suggestion that Ni 118 allows cross protection because it

TABLE 1. Effect of infection by defective TMV mutant PM1 on yield of the common strain of tobacco mosaic virus (TMV) and of potato virus X (PVX)[a]

Expt. no.	Original inoculum		Second inoculum	TMV (mg/gm of leaf)
	(b)	none[b]	TMV	4.4
	(b)	PM1	TMV	.8
1	(c)	none	TMV	4.3
	(c)	PM1	TMV	1.6
		none	TMV	3.9
		none	TMV	5.0
				Lesions/10 leaves of *Gomphrena globosa*
	(d)	none	PVX	93
	(d)	PM1	PVX	96
2	(e)	none	PVX	119
	(e)	PM1	PVX	101
		none	PVX	71
3	(f)	none	PVX	54
	(f)	PM1	PVX	178

[a]Leaf tissue of defective TMV mutant PM1 was used to inoculate four young *Nicotiana tabacum* 'Turkish Samsun' plants 12 cm high, on the left half of each of two of the lower leaves. Four similar plants were left uninoculated for later inoculation. The plants were placed in growth chambers maintained at 25 C with a 16-hour photoperiod at about 21,000 lux. Eight days later, when the leaves showed numerous chlorotic PM1 lesions, both sides of the leaves of two plants were rubbed with TMV (0.1 mg/ml in pH 7 phosphate buffer containing Celite) and two plants with a homogenate of leaf tissue containing a mild strain of PVX (obtained from R. M. Goodman). The previously uninoculated plants were also inoculated at this time. Seven days later, leaf tissue was harvested, weighed, and frozen. On the PM1-infected leaves, tissue harvest was restricted to the symptom-bearing areas. Tobacco mosaic virus was extracted by conventional procedures using 2.5 cycles of differential centrifugation and a 60 C, 10-minute heat clarification. Virus yield was determined spectrophotometrically. Tissue containing PVX was ground in 0.1 *M* pH 7 phosphate buffer (1 ml/gm of tissue) and diluted in the same buffer for assay on *Gomphrena globosa,* using a Latin square assay design.

[b]Treatments preceded by the same letters in parentheses represent opposite halves of a given leaf.

produces coat protein ignores the fact that the coat protein is denatured at 35 C and is nonfunctional. Thus, it most probably could not serve the role they postulate for it.

I believe that it is implied in the postulate of deZoeten and Fulton that the coat protein of the superinfecting strain should be different from the coat protein of the original infecting strain, because when the superinfecting RNA is "captured" by the protein of the original strain they suggest it is, in effect, locked away. Thus, this heterologous RNA-protein interaction in some way would have to be different from the original homologous one in its capacity to disassociate. It is apparent, however, that the protein of the superinfecting strain is not always different from that of the original protecting strain, and thus would not be expected to function differently. In some cases the coat proteins of both strains are identical. For example, in the work of Rast (8) mild TMV strains are derived from the severe strain after nitrous acid treatment, and are used to protect tomato plants from more severe strains (3). Considering the small number of mutational events expected from nitrous acid treatment (12), it is probable that the coat protein of the superinfecting strain is the *same* as that of the virus which prevents its replication. Another example is the masked strain of TMV which partially protects plants against common strain (5). These two strains have coat proteins with the same amino acid composition (7) and share many other coat properties in common (10). It is hard to visualize how the RNAs would discriminate between identical coat protein molecules.

It would be appropriate if I could end this "negative" discourse with a positive declaration of the mechanism of cross protection. Unfortunately I cannot do so within the current framework of our knowledge of plant virus replication. I agree with deZoeten and Fulton that "understanding generates possibilities"; unfortunately as far as this phenomenon is concerned, we still need more understanding.

LITERATURE CITED

1. BOSCH, F. X., and H. JOCKUSCH. 1972. Temperature-sensitive mutants of TMV: Behavior of a non-coat protein mutant in isolated tobacco cells. Mol. Gen. Genet. 116:95-98.
2. DE ZOETEN, G. A., and R. W. FULTON. 1975. Understanding generates possibilities. Phytopathology 65:221-222.
3. FLETCHER, J. T., and J. M. ROWE. 1975. Observations and experiments on the use of an avirulent mutant strain of tobacco mosaic virus as a means of controlling tomato mosaic. Ann. Appl. Biol. 81:171-179.
4. HARIHARASUBRAMANIAN, V., R. C. SMITH, and M. ZAITLIN. 1973. Insoluble coat protein mutants of TMV: their origin, and characterization of the defective coat proteins. Virology 55:202-210.
5. HOLMES, F. O. 1934. A masked strain of tobacco mosaic virus. Phytopathology 24:845-873.
6. JOCKUSCH, H. 1968. Two mutants of tobacco mosaic virus temperature sensitive in two different functions. Virology 35:94-101.
7. KNIGHT, C. A. 1947. The nature of some of the chemical differences among strains of tobacco mosaic virus. J. Biol. Chem. 171:297-308.
8. RAST, A. TH. B. 1972. MII-16, an artificial symptomless mutant of tobacco mosaic virus for seedling inoculation of tomato crops. Neth. J. Plant Pathol. 78:110-112.
9. ROCHOW, W. F., and A. F. ROSS. 1954. Relative concentration of potato virus X in double and single infections. Phytopathology 44:504 (Abstr.).
10. SIEGEL, A., and S. G. WILDMAN. 1954. Some natural relationships among strains of tobacco mosaic virus. Phytopathology 44:277-282.
11. SIEGEL, A., M. ZAITLIN, and O. P. SEHGAL. 1962. The isolation of defective tobacco mosaic virus strains. Proc. Nat. Acad. Sci., USA 48:1845-1851.
12. WITTMANN, H. G., and B. WITTMANN-LIEBOLD. 1966. Protein chemical studies of two RNA viruses and their mutants. Cold Spring Harbor Symp. Quant. Biol. 31:163-172.