Letter to the Editor

Understanding Generates Possibilities

G. A. de Zoeten and R. W. Fulton

Department of Plant Pathology, University of Wisconsin, Madison 53706.

Cross protection as a tool in plant virus control has not achieved the eminence that immunization achieved in the disease control of human and animal disease. In the absence of a well-defined immune system in plants as compared with that in animals, the basic hesitance towards cross protection and its use in control may originate in our total ignorance of its mechanism.

McKinney in 1929 (6) noticed that tobacco plants infected with a green mosaic virus (TMV) developed no yellow mosaic symptoms when subsequently inoculated with a yellow mosaic strain of the same virus. Thung in 1931 (14) was unable to isolate the second virus from doubly inoculated plants in similar experiments. The conclusion was drawn that the presence of the first virus had precluded multiplication of the second virus in the same tissues. This phenomenon: the inability to reisolate a so-called challenging virus strain from plants earlier infected with a related strain of the same virus is generally called cross protection (or premunity). Unrelated viruses do not cross protect, but, on the other hand, often enhance each other's multiplication (11) in plants that are doubly infected.

The proposed mechanisms explaining the phenomenon of cross protection have been many, and have ranged from the formation of protective substances not unlike antibodies or inhibitors, and the depletion by the first strain of essential metabolites required for multiplication of the second strain, to competition for strain-specific sites eliminating these sites for multiplication of the challenging strain.

The proposed hypotheses have not been open to experimentation to any great extent, and have only stood as hypotheses since they were proposed. Another proposed hypothesis should be considered here, however. In 1949, Kavanau (4) suggested that virus aggregates in cells already infected have specific "adsorptive" properties for related virus particles of the challenging strain. With regard to this hypothesis, Matthews (5) remarked, "It is less easy to see why cross protection is effective when the challenging strain is in the form of naked RNA. The incoming RNA might become coated with the viral subunits already present in the cell before it could begin replication."

It is this remark by Matthews that caused us to rethink the cross protection phenomenon in the light of what we know about infection and virus synthesis at the molecular level.

Wu in 1964 (15) showed that the inhibition of a TMV strain by another when both were inoculated together depends on some early events in virus replication, rather than on exclusion from the cell. This would mean then that the infectious entity gains entry to the cell, and is not eliminated before entering.

Shaw (12, 13), Kurtz-Fritsch and Hirth (8), and Merkens, et al. (7) provided circumstantial evidence that it is the virus nucleic acid that enters the cell and not the virus particles. Caspar in 1963 (1) remarked that lipid-containing structures could provide the environment affecting the stabilizing interactions within a virus particle in a way similar to nonpolar solvents and detergents, resulting in uncoating. Since the same forces involving virus assembly are involved in disassembly processes, infection sites should increase electrostatic repulsion between protein subunits and/or weaken their hydrophobic bonding. The two barriers encountered in inoculations of plant viruses satisfying the prerequisites for uncoating are the lipid-containing cuticle and plasmalemma.

Another argument for nucleic acids as the infectious entity of plant viruses entering plants may be the extremely complex regulatory mechanism that has to be postulated if the virus particle were the infectious entity entering the cell. Although the uncoating, nonpolar environment can be found in membranes within the plasmalemma of an infected cell, it is hard to understand why the incoming (infecting) particle is uncoated and those that are produced as a result of the infection are not. It seems, therefore, highly probable that viral nucleic acid is the entity entering the cell.

Cross protection works best if the challenging strain is inoculated a number of hours to a number of days after infection with the related strain has occurred. At these times, the cellular environment is conducive to assembly of virus particles of the strain which was introduced first. It is our contention 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. 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. It furthermore cannot be reisolated because of its dilution in virus particles of the virus strain of the original infection, and because of the low efficiency of plant virus inoculations (high multiplicity of infection).

Part of the proof needed for our hypothesis is provided by Jockush's (3) work in 1968 with two temperature sensitive strains of TMV. He showed that strain Ni 2519, which produced no detectable protein when kept at 35 C, would not protect the plant from subsequent infection with a vulgare strain. This was in contrast to plants infected with Ni 118, a strain which does produce protein at 35 C, but the protein does not assemble with its own nucleic acid and cross protection against the vulgare strain is observed. The prerequisite then for cross protection to work is that the challenging strain enters an infected cell at a time that the originally infecting virus is in the stage of assembly. The work of Rochow (10) and Dodds and Hamilton (2) has provided further evidence to support the idea that viral nucleic acid can be coated in vivo with either related, or even unrelated, viral coat proteins. The realization of this mechanism at work in cross protection lets us explain the baffling phenomenon of one-way cross protection in the case of elm mosaic and tomato ringspot virus. Furthermore, it explains the lack

of cross protection in the green islands in mosaic virus-infected tissues (no multiplication or assembly of virus).

Although control programs aimed at virus freedom would be preferable to a control using cross protection, we can think of practical situations where cross protection might be considered, if freedom of virus is hard to achieve or to maintain. In one such situation in The Netherlands (9), success has been claimed with cross protection as a means of control of TMV in tomato crops on a practical scale. This shows us the need of periodic reevaluation of our concepts. The rethinking process should extend itself to the epidemiological consequences, to the study of plant virus attentuation, and many cross protection-related areas of epiphytology and plant virology.

From the preceding discussion, it should also be clear that it is possible to further verify this theory experimentally.

LITERATURE CITED

1. CASPAR, D. L. D. 1963. Assembly and stability of the tobacco mosaic virus particle. Adv. Protein Chem. 18:87-121.
2. DODDS, J. A., and R. I. HAMILTON. 1974. Masking of the RNA genome of tobacco mosaic virus by the protein of barley stripe mosaic virus in doubly infected barley. Virology 59:418-427.
3. JOCKUSH, H. 1968. Two mutants of tobacco mosaic virus temperature sensitive in two different functions. Virology 35:94-101.
4. KAVANAU, J. L. 1949. On correlation of the phenomena associated with chromosomes foreign proteins and viruses, III. Virus associated phenomena, characteristics and reproduction. Am. Nat. 83:113-138.
5. MATTHEWS, R. E. F. 1970. Page 414 *in* Plant virology. Academic Press, New York. 778 p.
6. MC KINNEY, H. H. 1929. Mosaic diseases in the Canary Islands. West Africa and Gibraltar. J. Agric. Res. 39:557-578.
7. MERKENS, W. S. W., G. A. DE ZOETEN, and G. GAARD. 1972. Observations on ectodesmata and the virus infection process. J. Ultrastruct. Res. 41:397-405.
8. KURTZ-FRITSCH, C., and R. HIRTH. 1972. Uncoating of two spherical plant viruses. Virology 47:385-396.
9. RAST, A. T. B. 1972. MII-16, an artifical symptomless mutant of tobacco mosaic for seedling inoculation of tomato crops. Neth. J. Pl. Path. 78:110-112.
10. ROCHOW, W. F. 1970. Barley yellow dwarf virus: phenotypic mixing and vector specificity. Science 167:875-878.
11. ROCHOW, W. F., and A. F. ROSS. 1955. Virus multiplication in plants doubly infected by potato viruses X and Y. Virology 1:10-27.
12. SHAW, J. C. 1967. In vivo removal of protein from tobacco mosaic virus after inoculation of tobacco leaves. Virology 31:665-675.
13. SHAW, J. C. 1969. In vivo removal of protein from tobacco mosaic virus after inoculation of tobacco leaves, II. Some characteristics of the reaction. Virology 37:109-116.
14. THUNG, T. H. 1931. Smetstof en plantencel by enkele virusziekten van de tabaksplant. Pages 450-463 *in* Handel. 6 Ned.-Ind. Natuurwetensch. Congr. (Rev. Appl. Mycol. 11:750-751).
15. WU, J. H. 1964. Release of inhibited tobacco mosaic virus infection by ultraviolet irradiation as a function of time and temperature after inoculation. Virology 24:441-445.