J. gen. Virol. (1971), **13**, 361–364
Printed in Great Britain

Defective Strains and Phenotypic Mixing

(*Accepted* 17 *August* 1971)

This communication discusses recent work on phenotypic mixing with various viruses and describes relevant results with PM_2 strain of tobacco mosaic virus (TMV) and another defective strain which we have recently isolated. The term 'phenotypic mixing' is used here to mean the coating of the nucleic acid of one virus or virus strain with the protein of another.

The defective TURIN strain of TMV was isolated from a pepper plant growing in an experimental plot at the University of Turin and we shall call it the 'TURIN' strain. The pepper plant was also infected with cucumber mosaic virus and potato virus Y; the TURIN strain was freed from these by taking inoculum from single lesions in *Nicotiana glutinosa* and *Datura stramonium*. In tobacco, cv. 'White Burley' or 'Samsun', the TURIN strain produced either necrotic rings, some of which were incomplete, or solid necrotic local lesions; the virus did not invade the plant systemically. The necrotic rings or lesions, 3 to 6 mm. in diameter, tended to spread along the veins and to form oak-leaf patterns of irregular areas surrounding the midrib and secondary veins. The symptoms closely resemble those caused by potato mop-top virus (Harrison & Jones, 1970). In *N. glutinosa* and tobacco cv 'Xanthinc' the TURIN strain produced solid necrotic lesions, indistinguishable from those caused by type TMV and differed from the necrotic rings caused by potato mop-top virus.

Dip preparations of sap examined in the electron microscope did not show any virus particles, in contrast to the many rods usual in similar preparations of sap with type TMV. A few particles of varying length but 17 nm. wide were found after the TURIN strain was concentrated from a large volume of sap; the particles appeared to be similar to those of TMV. Sap inoculated to 'Samsun' leaves dusted with carborundum produced very few lesions; more lesions were produced when the leaves were inoculated with sap extracted in 0·01 M-thioglycollic acid and 0·01 M-DIECA. Such extracts produced on average 17 lesions per half leaf whereas extracts in 0·02 M-phosphate buffer pH 7, or in 0·2 % (w/v) sodium sulphite in water produced only 7. Phenol extracts of leaves were twice as infective as extracts in thioglycollic acid and DIECA, suggesting that much of the infective virus in the plant occurs as free RNA. Phenol extracts were obtained by triturating 0·2 g. of infected leaves in 2 ml. of 0·02 M-phosphate buffer pH 7, 2 ml. of water saturated phenol, and 0·4 ml. of 3·8 % (w/v) bentonite in water.

As with PM_2 strain of TMV and potato mop-top virus the infectivity of the TURIN strain seems to be associated with a large cellular component that sediments during centrifugation for 15 min. at 8000 *g*. The supernatant fluid from such a centrifugation when inoculated to 'Samsun' tobacco produced no lesions and the resuspended pellet 27 lesions per half leaf. Sap extracted in different ways lost its infectivity after 24 hr at 20°, even with 0·02 % (w/v) azide added as a preservative. It also lost its infectivity when heated for 10 min. at 55° or diluted 1/100 in water. The properties described for the TURIN strain place it in the group of defective strains of TMV (Kassanis & Woods, 1969).

The Nigerian cowpea virus (CV), which is distantly related serologically to TMV, formed phenotypic mixtures with PM_2 when both multiplied together (Kassanis & Bastow, 1971*a*). Similarly, it formed phenotypic mixtures with the TURIN strain when both were inoculated to 'Samsun' tobacco, kept at 20°, conditions in which both viruses multiply readily. This was shown when, 1 week later, the sap was extracted from the inoculated leaves, aged for

3 days, a treatment which normally inactivates the TURIN strain, and inoculated at different dilutions to 'White Burley' tobacco plants kept at 20°, a condition in which the TURIN strain but not CV multiplies well. Necrotic rings characteristic of infection by the TURIN strain were produced by the most dilute inocula. That the TURIN strain RNA was contained in the coat protein of CV was indicated by the fact that sap remained infective and able to cause the characteristic symptoms when kept for many months at 20°. With less-dilute inocula there was some interference by CV because the lesions formed were necrotic rings with a bright yellow centre. Inoculum from such lesions, aged for 3 days and diluted, gave the characteristic necrotic rings of the TURIN strain when inoculated to 'White Burley' tobacco.

Table 1. *Pairs of viruses that failed to form phenotypic mixtures*

Potato mop-top virus	Nigerian cowpea virus
PM_2	Tobacco ringspot virus Potato virus X Potato virus Y Potato aucuba mosaic virus Lucerne mosaic virus Cucumber mosaic virus, green strain Potato mop-top virus
Tobacco necrosis virus, unstable variant	Nigerian cowpea virus

Table 2. *Pairs of viruses that form phenotypic mixtures*

Barley yellow dwarf virus	
Two serologically unrelated isolates (Rochow, 1970)	
Tobacco mosaic virus	
Ni-118	Type strain (Atabekov *et al.* 1970)
Ni-118	Dolichos enation mosaic virus (Atabekov *et al.* 1970)
Ni-118	Nigerian cowpea virus (Kassanis & Bastow, 1971*b*)
PM_2	Nigerian cowpea virus (Kassanis & Bastow, 1971*a*)
Turin strain	Nigerian cowpea virus (the present paper)

The instability in sap of such strains as the TURIN strain, PM_2 (Kassanis & Bastow, 1971*a*) and the unstable variant to tobacco necrosis virus (Kassanis & Welkie, 1963) makes them suitable for demonstrating phenotypic mixing. We tested the last two viruses in mixed infection with various serologically unrelated viruses to find whether they produce phenotypic mixtures. Table 1 shows that none of the unrelated viruses produced phenotypic mixtures, while Table 2 shows that with one exception phenotypic mixing was reported between related viruses. Another common feature is that phenotypic mixing occurred only with mixtures in which one of the viruses had defective protein. Isolates of barley yellow dwarf virus seem exceptions to the generalizations, because they are not known to have defective protein or to be serologically related, yet the RNA of one can be coated by the protein of the other in plants infected with both (Rochow, 1970). However, some remote relationship may be found, as all the other pairs known to produce phenotypic mixtures are distantly related, except for the pair Ni-118 and type TMV.

Seemingly, defective viruses (defectiveness concerning the protein) either produce no functional protein or not enough to coat the RNA; with Ni-118 this happens when the plants are kept over 30°.

When reconstituting particles from RNA and protein of different strains of TMV, the

yield of infective particles and their stability is greater the closer the serological relationship between the strains (Fraenkel-Conrat & Singer, 1957). This suggests a contradiction between reconstitution *in vitro* and phenotypic mixing in plants, because attempts to demonstrate phenotypic mixing between PM_2 and type TMV, which are closely related, failed (Kassanis & Woods, 1969), and although phenotypic mixing between Ni-118 and type TMV, which are closely related, is claimed, the results are in some doubt (Kassanis & Bastow, 1971*b*). Experimental difficulties, in demonstrating phenotypic mixing between closely related strains, may explain the seeming contradiction. The viruses have the same host range, similar other properties, and their serological similarity prevents specific tests of the neutralization of infectivity. However, it is possible that infection by one strain may exclude a closely related strain from multiplying in the close proximity required for phenotypic mixing to occur.

Phenotypic mixing may occur between unrelated viruses because Rochow (1970) showed it with two isolates of barley yellow dwarf virus that seem serologically unrelated, but this is not substantiated by the results *in vitro*. Matthews (1966) found that TMV protein can form rods with turnip yellow mosaic virus RNA; the yield of rods was less than with TMV RNA, and coating with TMV protein did not enhance the infectivity or stability of turnip yellow mosaic RNA. The reconstituted product was susceptible to ribonuclease, as also found with other virus combinations (Breck & Gordon, 1970). Although it might seem that, once the RNA and protein subunits are made, additional information is not required for the assembly of the complete virus particles, some results from the reconstitution experiments suggest that the RNA plays a more positive role in the structural stability of the virus, and in making the protein subunits form a shell in a particular configuration around the RNA. Okada *et al.* (1970) found that the RNA of type TMV was reconstituted into infective particles equally well using either its own protein or that of the cucumber strain of TMV (the two differ considerably serologically), but reconstitution was poor when both proteins were present in the reaction mixture. However, they claim that the reconstitution in a mixture of the two proteins proceeded well when the RNA was previously partially reconstituted with one of the proteins, and seemingly recognized that protein. If this claim is correct phenotypic mixing could not be expected, or at least not often, with related viruses. Table 1 shows that this is probably so. Except between the two isolates of barley yellow dwarf virus, the examples of phenotypic mixing demonstrated are between a strain with, and one without, defective protein. In these circumstances, a choice of protein is not presented to the RNA. On the contrary, one protein has a choice of two RNAs, and it is possible that this fact allows newly formed defective mutants to survive by being coated with the protein produced by the parent virus.

Rothamsted Experimental Station B. KASSANIS
Harpenden, Hertfordshire M. CONTI*

REFERENCES

ATABEKOV, J. G., SCHASKOLSKAYA, N. D., ATABEKOVA, T. I. & SACHAROVSKAYA, G. A. (1970). Reproduction of temperature-sensitive strains of TMV under restrictive conditions in the presence of temperature-resistant helper strain. *Virology* **41**, 397.

BRECK, L. O. & GORDON, M. P. (1970). Formation, characterization, and some photochemical properties of a hybrid plant virus. *Virology* **40**, 397.

FRAENKEL-CONRAT, H. & SINGER, B. (1957). Virus reconstitution. II. Combination of protein and nucleic acid from different strains. *Biochimica et biophysica acta* **24**, 540.

* Supported by a grant of the NATO Science Fellowship Programme; now at the Laboratorio di Fitovirologia Applicata, Torino, Italy.

HARRISON, B. D. & JONES, R. A. C. (1970). Host range and some properties of potato mop-top virus. *Annals of Applied Biology* **65**, 393.

KASSANIS, B. & WOODS, R. D. (1969). Properties of some defective strains of tobacco mosaic virus and their behaviour as affected by inhibitors during storage in sap. *Annals of Applied Biology* **64**, 213.

KASSANIS, B. & BASTOW, C. (1971*a*). *In vivo* phenotypic mixing between two strains of tobacco mosaic virus. *Journal of General Virology* **10**, 95.

KASSANIS, B. & BASTOW, C. (1971*b*). Phenotypic mixing between strains of tobacco mosaic virus. *Journal of General Virology* **11**, 171.

KASSANIS, B. & WELKIE, G. W. (1963). The nature and behaviour of unstable variants of tobacco necrosis virus. *Virology* **21**, 540.

MATTHEWS, R. E. F. (1966). Reconstitution of turnip yellow mosaic virus RNA with TMV protein subunits. *Virology* **30**, 82.

OKADA, Y., OHASHI, Y., OHNO, T. & NOZU, Y. (1970). Sequential reconstitution of tobacco mosaic virus. *Virology* **42**, 243.

ROCHOW, W. F. (1970). Barley yellow dwarf virus: phenotypic mixing and vector specificity. *Science, New York* **167**, 875.

(*Received* 12 *July* 1971)