EVIDENCE FOR STEPS IN THE ASSEMBLY OF TURNIP YELLOW MOSAIC VIRUS NUCLEOPROTEIN

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The isolation of three nucleoprotein components (B_o, B_1 and B_2) from turnip yellow mosaic virus (TYMV) preparations by sedimentation into dense cesium chloride solutions was described previously (Matthews, 1959). Two further nucleoproteins (B_{ooo} and B_{oo}) have now been isolated. Some properties of these components are given below, together with the results of labelling experiments which suggest that the various non-infectious particles may form a series of stages in the synthesis of complete infective nucleoprotein.

Virus preparations were made from infected Chinese cabbage plants by differential ultracentrifugation, and the nucleoprotein bands were isolated as described previously (Matthews, 1959). The relative amounts of these components, listed in order of increasing density, were very approximately as follows: B_{ooo}, 1%; B_{oo}, 3%; B_o, 3%; B_1, 90%; B_2, 3%. Using inocula of equal optical density at 260 mμ the other components gave numbers of local lesions on Chinese cabbage ranging from 0.5 to 5.0% of those given by B_1. This infectivity is almost certainly due to residual contamination of these fractions with the B_1 component since their infectivity compared with B_1 decreases as purity increases following successive resedimentations into dense cesium chloride solution. The nucleoproteins are closely related serologically since they precipitated at similar rates

when mixed at equal virus protein concentrations, with a standard dilution of rabbit antiserum prepared against B_1 nucleoprotein. The percentages of ribonucleic acid (RNA) in the nucleoproteins determined from nitrogen and phosphorus analyses assuming protein and RNA contain 15% N and that RNA has 9.0% P, were as follows:- B_{oo}, 12%; B_o, 23%; B_1, 36%; B_2, 36%. So far we have had insufficient of B_{ooo} for adequate analyses.

For tests on incorporation of P^{32} or S^{35} into the nucleoproteins, single Chinese cabbage plants infected with TYMV for 16-25 days were removed from their pots and the roots carefully washed. The roots were then placed in a small beaker containing 2.0-10.0 mc. in 0.5-1.0 ml. of solution (P^{32} or S^{35} used directly as supplied by the Radiochemical Centre, Amersham, Catalogue PBS_1 and SJS_1). After plants had stood in the radioactive solution for various times the leaves were ground in a few ml. of 0.5M phosphate buffer (pH 6), together with one or two non-radioactive plants of the same batch. Virus preparations were made from the expressed sap by two cycles of differential centrifugation. The nucleoprotein components were isolated by 2-3 cycles of sedimentation into cesium chloride of density 1.38-1.42 g/ml. Fractions were then dialysed overnight against distilled water and centrifuged at 3000 r.p.m. for 10 minutes to remove any debris. In some experiments counts per minute/μg phosphorus were estimated directly on these fractions. In others, greater specificity was obtained by precipitating the isolated fractions with a B_1 antiserum; hydrolysing the RNA in the washed specific precipitate with alkali; subjecting the neutralised digest to chromatography in the isopropanol ammonia solvent (Markham and Smith, 1952), eluting the nucleotides in 0.1N HCl, and estimating counts per minute and optical density on the eluates at 260 mμ.

Representative experiments are summarised in Table 1. They

are not strictly comparable since they were carried out at various times on plants from different batches. However, they show that the nucleoproteins acquire P^{32} progressively in the order:- B_{ooo}, B_{oo}, B_o, B_2 and B_1. By 60 minutes B_o, B_2 and B_1 were uniformly labelled. When a plant was placed in excess P^{31} for 60 minutes after 30 minutes in P^{32} the order of labelling was reversed except for B_1. Presumably, if appropriate times were chosen complete reversal could be obtained.

TABLE 1 INCORPORATION OF P^{32} INTO TYMV NUCLEOPROTEINS

Time plant in P^{32}	Method of analysis	Component				
		B_{ooo}	B_{oo}	B_o	B_2	B_1
22 mins.	cpm/µg P	-[a]	-	3.8	NS[b]	0.31
38 mins.	cpm/µg P	-	-	11.5	9.1	2.3
60 mins.	cpm/µg P	-	-	17.1	16.4	15.5
30 mins.	cpm/100µg nucleotides	54	19.7	15.7	11.2	4.02
40 mins.	"	-	370	109	80	29.8
30 mins. P^{32}, then 60 mins. excess P^{31}	"	41.5	389	442	543	271

[a] - = not isolated. [b] NS = counts not significantly above background.

In tests on the incorporation of S^{35}, the fractions were isolated as for the P^{32} experiments except that the empty protein shell which constitutes about one third of the total virus protein in the plant (Markham and Smith, 1949) (here called component T) was sedimented as a band into CsCl of density 1.26 g/ml. following two cycles with CsCl of density 1.41 g/ml. Nitrogen determinations were made on aliquots of the fractions. All the virus material in the remainder of the sample was precipitated by addition of an excess of B_1 antiserum. Total counts were estimated on the washed specific precipitate and converted to c.p.m. per unit of virus protein nitrogen, assuming that all the

nitrogen estimated was virus nitrogen. Results for one such experiment, in which the plant was held in the S^{35} solution for 90 minutes, were (c.p.m./μg virus protein N): T = 45.3; B_{oo} = 2.38; B_o = 2.05; B_2 = 0.87; B_1 = 1.26. Because of error in low nitrogen values for B_{oo}, B_o and B_2 the only differences that can be regarded as significant are between T and the four nucleoproteins. The B_{ooo} component was not isolated in this experiment, but would certainly have moved clear of T in CsCl of density 1.26 g/ml.

These labelling experiments with P^{32} and S^{35} suggest either (i) that the various virus-like components are formed within the cell by separate processes which have available to them phosphorus (or sulphur) with differing specific activities, or (ii) that they form a sequence in which one is formed first and then converted to the next, or (iii) that some combination of (i) and (ii) occurs. Our tests with S^{35} support those of Jeener (1954), who, from experiments on the incorporation of C^{14} from $C^{14}O_2$, concluded that T is a precursor of complete virus. For the relation between the nucleoproteins, we favour alternative (ii) above since the order in which they become labelled with P^{32} is the same as their order of increasing content of RNA (except for B_2 and B_1 which have very similar amounts of RNA). We therefore put forward the following working hypothesis concerning the late stages in the assembly of TYMV:

1. T accumulates.
2. T acquires a small, and as yet undetermined quota of RNA to give B_{ooo}.
3. B_{ooo} receives a piece of RNA equal to about one third the total complement of the complete virus to give B_{oo}.
4. B_{oo} receives a further piece of RNA of about the same size to give B_o.

5. B_o receives another similar piece to give B_2.
6. The RNA in B_2 is "finished" in some way as yet unknown to give the complete virus B_1. There is a suggestion from some of our best analyses that while B_1 and B_2 have very similar amounts of RNA, B_2 gives a slightly greater optical density at 260 mμ per unit of phosphorus than does B_1. This difference, if real, might be due to a more random orientation of the RNA bases in B_2 than in B_1.

Accumulation of P^{32} at the tips of leaves about 12 inches from the roots may begin less than 1 minute after roots are put in the P^{32} solution, giving a rate of movement of $P^{32} > 5.0$ mm/second (for S^{35}, > 1.4 mm/second). If we neglect time taken for P^{32} to reach the leaves, it takes substantially less than 30 minutes, and probably about 15 minutes or less, for inorganic phosphate to be incorporated into the RNA of B_1.

The RNA complement of a complete TYMV particle has a weight equivalent to a molecular weight of about 1.8×10^6. The idea that most of the RNA in TYMV is assembled in pieces about one third the size of the total complement of the particle, receives support from the observation (Ralph, Lyttleton and Matthews, 1959) that at least a great part of the TYMV RNA prepared by the phenol method (Gierer and Schramm, 1956) has a sedimentation coefficient of 16-17S which would not be inconsistent with a molecular weight in the range $4\text{-}6 \times 10^5$. How such large pieces of RNA could enter the empty protein shells, if in fact these are complete shells, remains to be determined. It may be that the RNA enters as much smaller pieces, and that the B_{oo} and B_o nucleoproteins merely represent stages which exist without detectable change in density for a longer time than other intermediates. The results given here do not rule out the

possibility that additional classes of TYMV nucleoprotein particles are present in the plant in smaller amounts.

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References

A. Gierer and G. Schramm. Nature 177, 702 (1956).

R. Jeener. Biochim. et Biophys. Acta 13, 307 (1954).

R. Markham and J.D. Smith. Biochem. J. 52, 552 (1952).

R. Markham and K.M. Smith. Parasitology 39, 330 (1949).

R.E.F. Matthews. Nature, in the press (1959).

R.K. Ralph, J.W. Lyttleton and R.E.F. Matthews. Unpublished observations (1959).