THE RELATIONSHIP BETWEEN TOBACCO MOSAIC VIRUS AND AN ACCOMPANYING ABNORMAL PROTEIN*,**

by

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The occurrence of a nucleic acid-free abnormal protein in plants infected with tobacco mosaic virus (TMV) was reported by TAKAHASHI AND ISHII (protein "X")[18], by COMMONER, NEWMARK, AND RODENBERG ("component B")[1], and by JEENER AND LEMOINE ("crystallizable antigen")[9]. Although somewhat different methods of preparation were used by these three groups of workers, the product obtained in each case is evidently the same. The protein was isolated for the present work by a procedure similar, in some respects, to that of COMMONER, NEWMARK AND RODENBERG. When this protein is mixed with protein "X" prepared by the procedure of TAKAHASHI AND ISHII, the two migrate as one electrophoretic component. Other similarities in physical and chemical properties are the subject of a separate report[15].

TAKAHASHI AND ISHII demonstrated that this protein can be caused to polymerize into rods which have the same cross section as TMV but are of varying lengths[19]. The immunological similarity which this protein bears to TMV[19,9,3] and the identity of amino acid composition [15] strongly emphasize the close structural relationship between the two. TMV has a molecular weight in the neighborhood of $5 \cdot 10^7$ whereas that of this protein in its least aggregated from appears to be less than $5 \cdot 10^4$. Its polymerization and combination with nucleic acid *in vitro* to yield infective virus particles has not been demonstrated[4,15].

The present study was undertaken to elucidate further the relationship which this protein bears to TMV insofar as its synthesis in the plant is concerned; and to determine whether it could be a degradation product of TMV resulting either from fragmentation in the extraction process or as a normal *in vivo* reversal of the synthetic mechanisms, or whether it may be a direct precursor or a by-product of the synthetic process.

MENEGHINI AND DELWICHE[14] found that ^{15}N labelled ammonium ion, when infiltrated into TMV infected leaves, was rapidly incorporated into the virus protein. From distribution of the isotope in various fractions they concluded that TMV was formed from a low molecular weight nitrogenous fraction (*e.g.*, amino acids) which was

* Reported before the thirty-eighth meeting of the American Society of Biological Chemists April, 1954[15].

** Supported by grants from the Lederle Laboratories, the Rockefeller Foundation, and the Atomic Energy Commission.

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in more rapid equilibrium with ammonium ion than were the normal plant proteins. Recent work of COMMONER, SCHEIBER AND DIETZ[2] substantiates this view although these authors conclude that "amino acid residues of TMV are not derived from the corresponding free amino acids of the host".

It appeared likely that, using a similar technique, a comparison of labelling between TMV and other protein fractions could yield information as to whether the abnormal protein accompanying TMV infection could be ruled out as either a direct precursor or a degradation product of TMV. Direct proof that such a protein is a precursor would be difficult unless the labelled protein could be introduced at the site of synthesis and the formation of labelled TMV therefrom demonstrated.

MATERIALS AND METHODS

Six weeks old *Nicotiana tabacum* plants of a Turkish variety were greenhouse-grown in four inch clay pots. Growing points and all but three or four median leaves were removed. The leaves remaining on the plants were inoculated by rubbing their top surfaces with a gauze pad soaked in a centrifugally isolated and purified solution of TMV in 0.01 M, pH 7 phosphate buffer.

At intervals after inoculation the leaves were detached and ^{15}N-labelled ammonium ion was introduced by placing their petioles in 0.02 *M* solutions of ammonium chloride containing 32 atom percent excess of the isotope and buffered at pH 7.0 with 0.01 *M* phosphate. In order to accelerate transpiration the leaves were illuminated with artificial light of about 2,000 luces intensity. When all the culture solution had been taken up, distilled water was added as needed to prevent wilting. After 24 hours, the leaves were homogenized and fractionated, and nitrogenous fractions were assayed for total nitrogen and ^{15}N content.

In one experiment after the leaves had taken up the isotopic ammonium ion they were split in half for sampling and the halves floated on large volumes of buffered solution containing unlabelled ammonium ion in order that the rate at which isotope was leached from the several fractions could be followed.

Leaf fractionation procedure

The fractionation procedure used (Fig. 1) was based on the fact that when solutions of clarified homogenate at pH 7 are subjected to high speed (80,000 × *g*.) centrifugation, the TMV can be pelleted out without significant contamination by the abnormal nucleic acid-free protein. The remaining supernatant solution when adjusted to pH 4.7 will aggregate a large bulk of proteins, characteristic of both healthy and infected plants, which can be sedimented in comparatively weak centrifugal fields (6,000 × *g*.). The abnormal protein is partially (and reversibly) polymerized at this pH and can be pelleted out at 80,000 × *g*. A further lowering of the pH to 3.8 yields another protein fraction characteristic of both healthy and infected plants. Remaining is a final supernatant solution composed principally of non-protein nitrogen.

The leaves were ground with mortar and pestle in twice their weight of 0.1 *M* pH 7.0 phosphate buffer, and further homogenized in an all glass homogenizer. Equipment and solutions were kept cold by immersion in crushed ice. Further steps in the fractionation procedure, except for adjustment of pH, were carried on in a cold room at 3–4° C., or in a refrigerated Spinco ultracentrifuge.

The homogenate was clarified at 10,000 × *g*. for 15 min, the precipitate (P_1) washed with 0.1 *M* pH 7.0 phosphate buffer, recentrifuged under the same conditions, and the supernatant solution combined with that of the first centrifugation (S_1).

P_1 is composed of cell wall debris, and the larger fragments of chloroplasts, nuclei, and other cytoplasmic particles which are insoluble in 0.1 *M* phosphate buffer. Extraction of P_1 with 2 *M* sodium chloride yielded comparable amounts of a nucleoprotein from both uninfected and infected leaves, in accord with findings of COMMONER, *et al.*[3]

Centrifugation of supernatant solution S_1 at 80,000 × *g*. for 2 hours sedimented the TMV together with some chloroplast grana and smaller cytoplasmic fragments (P_2). The P_2 pellets were resuspended in 0.01 μ, pH 7.0 cacodylate/sodium chloride (0.2/0.8) buffer, and the TMV purified by heating the buffered solution to 60° for 15 minutes or by dropping the pH to 3.8. In either case the non-TMV, protein-containing fragments were coagulated and readily sedimented by low speed centrifugation as P_3, and a nucleic acid fraction was released. Washes of precipitate P_3 with 0.01 μ pH 7.0 cacodylate buffer were combined with solution S_3. When this solution was centrifuged at 100,000 × *g*. for 1 hour, the TMV was pelleted out, leaving the nucleic acid (as determined by ultraviolet absorption spectrum) as supernatant solution S_{3a}.

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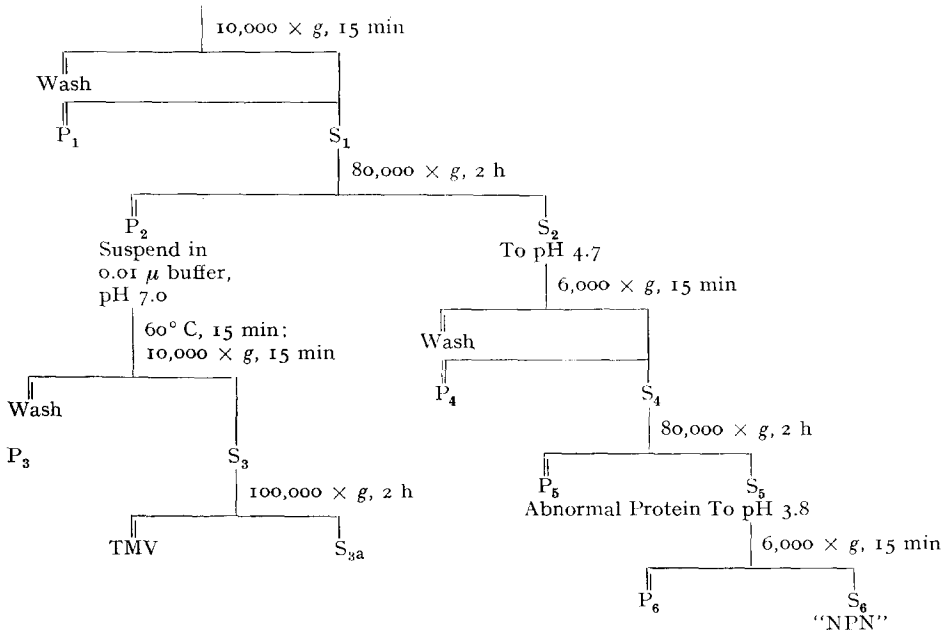
Fig. 1. Leaf Fractionation Procedure



The clear, amber-colored solution S_2, at pH about 6.5, contained the soluble non-protein and low molecular weight protein constituents. By dropping the pH to 4.7 with 1 *N* sulfuric acid the bulk of the soluble proteins were precipitated isoelectrically within an hour and removed by low speed centrifugation as P_4.

Precipitate P_4 contained the 18 S protein component of EGGMAN, SINGER AND WILDMAN[5] as well as a group of proteins which have sedimentation constants of 3 S or less. A portion of precipitate P_4 redissolves in 0.1 *M* pH 7.0 phosphate or cacodylate/sodium chloride buffers.

When the remaining solutions S_4 was centrifuged at 80,000 × *g*. for 2 hours, the abnormal protein partially polymerized at this pH was sedimented as a clear TMV-like pellet (P_5). No comparable protein was obtained from uninfected leaf tissue. Resuspension of the abnormal protein in 0.1 μ, pH 7.0 cacodylate/sodium chloride buffer, followed by clarification of the solution though low speed centrifugation and dialysis against the cacodylate buffer, yielded a solution in which over 90% of the protein migrated as a single component in the electrophoresis apparatus[15].

The solution remaining, S_5, contained those low molecular weight protein and non-protein constituents which were still soluble at pH 4.7. Most of the proteins were precipitated isoelectrically by taking the pH to 3.8 with 1 *N* H_2SO_4 and allowing the solution to stand overnight. Centrifuging at low speed yielded precipitate P_6. The nitrogen in solution S_6 was mostly non-protein (NPN), but a small amount of protein could be recovered as a denatured precipitate by heating at 60° for 15 minutes.

RESULTS

The incorporation of ^{15}N into abnormal protein

A preliminary experiment was designed to determine whether at an advanced stage of infection the abnormal protein received a label from isotopic ammonia. Four basal leaves of approximately 10 cm length, showing no external signs of mosaic, were selected from an eight week old systemically infected greenhouse-grown Turkish tobacco plant 31 days following inoculation. After administration of the isotopic ammonium ion for 24 hours as outlined above, the leaves were ground and a partial fractionation was made

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of the homogenate yielding the fractions indicated in Table I. The data of Table I show that the isotope appeared to some extent in all fractions, and the distribution was, in general, consistent with previous findings of this and other laboratories. The isotope content of the isolated tobacco mosaic virus was very low, confirming the view that isotopic nitrogen incorporated into this fraction could be explained by the formation of a small amount of new TMV without any "exchange" due to the reversible formation of the virus. Fraction P_4, constituting about 5% of the total soluble material, underwent an appreciable incorporation of the isotope. This is probably more than could be explained on the basis of new protein formation—although flooding the leaf with nitrogen, which had heretofore been limiting, undoubtedly resulted in the formation of new protein. Even more of the isotope was found in those protein fractions which were soluble at pH 4.7—fraction P_5 (the abnormal protein in infected plants) and fraction P_6. Although both of these fractions were small, they were of particular interest because of their high ^{15}N content. Only very small precipitates corresponding to P_5 were obtained with leaves from uninfected plants. These precipitates had different physical properties and appeared to be unrelated to the P_5 protein characteristic of infected plants.

The comparatively high ^{15}N content of the abnormal protein (fraction P_5), although not equalling that of the non-protein nitrogen, suggests that its synthesis was taking place, even at the advanced stage of the infection of leaves used in this experiment.

TABLE I

TOTAL NITROGEN AND ^{15}N CONTENT OF FRACTIONS FROM LEAF OF PLANT INFECTED WITH TMV FOR THIRTY-ONE DAYS, THEN EXCISED AND GIVEN $^{15}NH_4Cl$ FOR 24 HOURS. ^{15}N VALUES WERE CALCULATED ON THE BASIS OF 100 ATOM PERCENT EXCESS ^{15}N IN THE SUBSTRATE AMMONIUM ION.

Fraction	*μAtoms N per g wet weight*	*Atom % excess ^{15}N*
Total homogenate	250.0	6.8
Clarified homogenate (S_1)	13.0	11.0
TMV	39.5	0.22
pH 4.7 ppt. (P_4)	6.3	3.4
Abnormal protein (P_5)	0.9	7.8
pH 3.8 ppt. (P_6)	0.9	7.5
pH 3.8 sol., "NPN" (S_6)	35.0	26.2

Comparison of abnormal protein and TMV protein

In view of the above results, a more detailed study of the process was undertaken in Experiment II. In this case, the leaves were supplied with isotope at varying intervals after inoculation of the plant with TMV, the times being selected to cover the period of rapid formation of virus.

Twelve uniform Turkish tobacco plants, approximately six weeks old, were selected and their growing points and all leaves except the median three removed. These leaves were then inoculated with TMV. At successive intervals, one leaf was removed from each alternate plant, giving a total of six samples of six leaves each. Upon abscission from the plants, the leaves were supplied with ammonium chloride ^{15}N and allowed to stand for 24 hours. At the end of the 24 hour interval they were fractionated according to the procedure outline in Fig. 1, with the exception of one sample at the end for which the fractionation procedure was modified with ammonium sulfate precipitation of the proteins of various supernatant solutions for the purpose of electrophoretic analysis.

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Results of this experiment are presented in Table II. The general observations of Experiment I also apply to this experiment, although there was no sample for the advanced stage of infection at which Experiment I was conducted. There was a rapid incorporation of the isotope into both the abnormal protein and TMV fractions.

The abnormal protein was not present in quantities sufficient for detection and analysis until the 144 hour sample. As the infection progressed there was an increase in the ratio of abnormal protein to virus. The abnormal protein was being produced at a greater rate than the virus during the 144–240 hour period, and it was more highly labelled.

The heterogeneous fraction P_3, which includes grana and other cytoplasmic particulate material, showed a high incorporation of isotopic nitrogen, in agreement with the findings of JAGENDORF AND WILDMAN[7].

TABLE II

TOTAL NITROGEN AND ^{15}N CONTENT OF FRACTIONS FROM LEAVES WHICH WERE EXCISED AND GIVEN $^{15}NH_4Cl$ FOR 24 HOURS AT VARIOUS TIMES AFTER INOCULATION WITH TMV. ^{15}N VALUES WERE CALCULATED ON THE BASIS OF 100 ATOM PERCENT EXCESS ^{15}N IN THE SUBSTRATE AMMONIUM ION.

Fraction	*μ atoms N per g wet weight*					*Atom % excess N^{15}*				
	Hours after inoculation					*Hours after inoculation*				
	48	*98*	*144*	*195*	*240*	*48*	*98*	*144*	*195*	*240*
Clarified homog. (S_1)	236	187	129(?)	161	160	9.6	6.1	5.7	4.6	4.0
Small particulates (P_3)	12.3	19.5	10.7	21.9	8.1	3.2	3.4	3.0	1.8	2.2
TMV	0.1	3.2	9.8	16.9	33.3	—	14.7	11.1	6.0	3.5
protein	—	—	—	—	—	—	—	11.6	6.0	3.6
nucleic acid	—	—	—	—	—	—	—	4.4	3.3	2.2
pH 4.7 ppt (P_4)	55.9	35.0	62.5(?)	34.8	25.3	2.6	2.4	1.7	1.1	1.1
Abnormal protein (P_5)	—	—	0.1	1.2	4.0	—	—	—	10.0	9.2
pH 3.8 ppt (P_6)	1.7	1.1	1.9	1.9	2.8	6.4	2.5	2.2	1.9	2.1
pH 3.8 sol. "NPN" (S_6)	—	61.3	51.2	64	46.4	23.4	13.6	11.1	8.3	8.4

Metabolic stability of TMV and abnormal protein

In order to compare the metabolic stability of the living leaf of fractions already considered, as well as to gain information concerning the relationship of nucleic acids to other fractions, an experiment of a somewhat different nature was conducted. In this case (Experiment III) the median leaves of ten uniform plants were inoculated with TMV. After 72 hours all the leaves were removed from the plants and provided with isotopic ammonia for the next 47 hours. The midribs were then removed and the half leaves were divided into five similar samples. These were floated on large volumes of solutions of unlabelled ammonium chloride and phosphate buffer, and the solutions were changed daily. Leaf samples were removed at successive intervals and fractionated as before. In addition fractions of nucleic acid were obtained from TMV by the procedure of KNIGHT[10] and from the particulate fraction (P_3) by extraction with hot 5% trichloroacetic acid.

Results of this experiment are shown in Fig. 2 and 3. As was anticipated there was a rapid leaching of the isotope from the NPN fraction upon exposure of the leaf halves to the unlabelled solution. There was also a large increase in toal NPN. The total amount of isotopic nitrogen in TMV and the abnormal protein increased; the drop in $^{15}N/^{14}N$ ratio of these fractions with time being readily explained by the dilution resulting from the synthesis of new protein having a lower isotope content.

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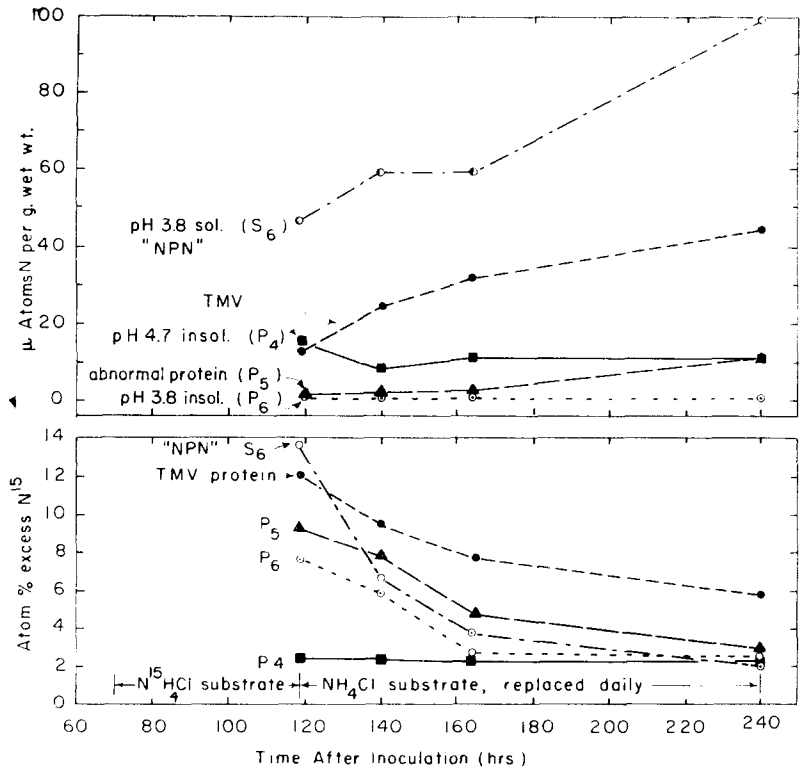

Fig. 2. Total nitrogen and ^{15}N content of leaf fractions which are soluble in 0.1 *M*, pH 7 phosphate buffer. Leaves were excised 74 hours after inoculation with TMV, floated on $^{15}NH_4Cl$ for 47 hours, and then floated on unlabelled NH_4Cl for varying times. ^{15}N values were calculated on the basis of 100 atom percent excess ^{15}N in the initial substrate ammonium ion.

These results are consistent with the results of Experiment II and with the earlier findings of MENEGHINI AND DELWICHE[13]. All point to the metabolic inertness of the bulk of the TMV synthesized in the leaf. Although, the data on the abnormal protein are not conclusive, they suggest that the protein is relatively stable.

The protein precipitable at pH 4.7 (P_4), characteristic of both infected and uninfected plants, remained more or less constant in both ^{15}N content and total nitrogen for the duration of the experiment whereas that fraction precipitated at pH 3.8 (P_6) showed a rapid loss of the isotope. It appears that the metabolic activity of this smaller fraction (P_6) was considerably greater than that of the pH 4.7 insoluble fraction (P_4).

The particulate fractions P_1 and P_3 both retained the label with little loss during the 120 hours that unlabelled ammonium ion was provided and indeed did not reach their maximal label until 24 hours after transfer to the unlabelled solution.

Incorporation of isotope into nucleic acid

A time lag was observed in the appearance of isotope in the nucleic acid fractions (Fig. 3). The extent to which the TMV nucleic acid differs from the protein portion of the virus in this regard emphasizes the non-parallel synthetic pathways of these two moieties. The time lag in the case of the nucleic acid could represent a large nitrogenous pool between the purine and pyrimidine precursors and the finished nucleic acid. Work

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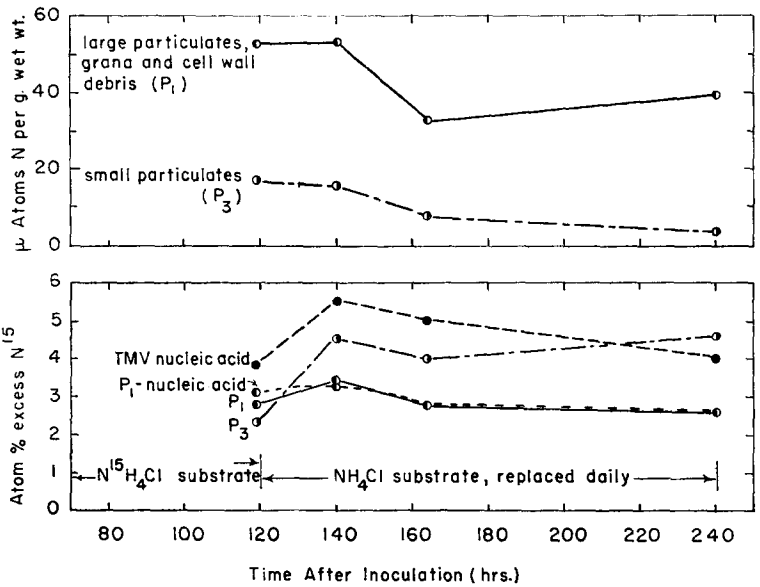

Fig. 3. Total nitrogen and ^{15}N content of leaf fractions which are insoluble in 0.1 *M*, pH 7 phosphate buffer. Values for TMV nucleic acid included for purposes of comparison. Leaves were excised 72 hours after inoculation with TMV, floated on $^{15}NH_4Cl$ for 47 hours, and then floated on unlabelled NH_4Cl for varying times. ^{15}N values were calculated on the basis of 100 atom percent excess ^{15}N in the initial substrate ammonium ion.

on the alga *Nostoc muscorum* by MAGEE AND BURRIS[12] suggests that the labelling of purines and pyrimidines probably takes place rapidly, so that the time lag in incorporation of isotope into the nucleic acid may lie between the bases and the polynucleotide.

The nucleoproteins of cell particulates

The possible implication of particulates in these synthetic processes was supported by another experiment (Experiment IV) in which uninfected and infected tobacco leaves, 19 days after inoculation with TMV, were given $^{15}NH_4Cl$ for 24 hours and then fractionated as in the earlier experiments. In addition, fraction P_1 was extracted with 2 *M* sodium chloride to yield nucleoprotein. Fraction P_3 and the residue of fraction P_1 after extraction with sodium chloride were extracted with cold and hot 5% trichloroacetic acid (TCA) to yield nucleotides and nucleic acids. There was no essential difference in the magnitude of these additional fractions between infected and noninfected plants. However, the nucleoprotein fraction was more highly labelled in both infected and uninfected plants than were the nucleotides, nucleic acids or any of the other soluble protein fractions obtained by the initial 0.1 *M* phosphate buffer extraction.

In view of the high label found earlier in the S_5 solution and succeeding fractions, a more detailed fractionation of this solution was resorted to in this experiment, using pH and temperature denaturation to obtain a number of small protein precipitates. In none of these was there found any precipitate characteristic only of the infected plant.

DISCUSSION

The nucleic acid-free abnormal protein found in TMV infected plants undergoes an incorporation of isotopic nitrogen from the non-protein nitrogen of the plant at a

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rate comparable with that of the TMV. Administration of the isotope for an interval early in the infection, when the virus is accumulating more rapidly than the abnormal protein, results in the virus that is isolated being more highly labelled. However, if the interval is chosen late in the infection, when the abnormal protein is being formed more rapidly, the abnormal protein that is isolated is labelled more highly than the virus. Therefore, neither protein *as isolated from the plant* can be derived from the other; the abnormal protein is not a degradation product of the virus *as a whole*, nor is the virus formed by a *general* polymerization of units of the abnormal protein.

Although the close relationship of the abnormal protein and virus insofar as their structure and synthesis are concerned is evident, the question as to whether the protein is a precursor or a degradation product of TMV *in vivo*, or is merely a second protein produced in infected plants is still unresolved. Our data do not exclude the possibility that the abnormal protein may be polymerized with nucleic acid to form TMV at the sites of virus synthesis in the plant cell. However, the isotope content of the abnormal protein which was isolated differed from that which would have been used for virus synthesis at the time at which the protein was isolated. The abnormal protein which was isolated could therefore represent an excess of virus precursor not utilized in virus synthesis because the protein was being produced at sites no longer synthesizing the intact virus, or because the protein had been lost by some other means from the sites of active virus synthesis to the protoplasm in general.

If the abnormal protein were an *in vivo* degradation product of the virus, the data in Table I would require that protein containing 7.8 atom percent excess ^{15}N be derived from TMV containing 0.22 atom percent excess ^{15}N. Only that portion of the virus synthesized in the period during which the leaf was exposed to the isotope would be labelled highly enough to serve as the source of the abnormal protein. The data in Table II would therefore require that during the 195–240 hour interval less than six virus particles be synthesized for every virus particle broken down. In Experiment III (Fig. 2), during the 140–240 hour interval, only three virus particles would have been synthesized for every particle broken down. Such degradation of the virus may occur as part of the process whereby the infection spreads from site to site within the cell or from cell to cell in the leaf. The spreading might be carried on only by a portion of the newly synthesized virus, with the bulk of the extant TMV being sequestered at sites no longer producing virus. Fragmentation of the infecting TMV particles into protein and nucleic acid moieties may occur in a manner analogous to the behavior of infecting bacteriophage particles, as discussed previously[14]. The abnormal protein may also result from a degradation of "superinfecting" TMV particles through a mechanism analogous to the degradation of "superinfecting" bacteriophage[6]. Alternatively, degradation of the final TMV particles synthesized at a site of virus production may occur as part of the mechanism regulating the amount of virus produced. In every case those protein fragments not re-utilized in the plant metabolism would represent the abnormal protein isolated.

It is highly unlikely that the abnormal protein could be an *in vitro* degradation product of TMV resulting from the isolation procedure. As shown previously[19], when purified TMV is carried through the isolation procedure for abnormal protein no abnormal protein is detected. In addition, when TMV is split into protein and nucleic acid moieties by heating[10], sodium dodecyl sulfate[16], urea[11] or alkali[16], the protein recovered no longer has the physical properties of the virus nor of the abnormal protein.

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The time lag observed for the appearance of isotope in the nucleic acid moiety of TMV as compared with the protein moiety emphasizes the differences in synthetic pathways of these moieties. The synthesis of the nucleic acid may involve large nitrogenous pools, possibly the nucleic acid associated with cell particulates, in addition to the pools of non-protein nitrogen.

If the final protein unit precursor of TMV possessed the dimensions of the abnormal protein then approximately 10^3 of these units would go into the virus particle. In view of the ease and rapidity with which the abnormal protein may be polymerized *in vitro* into TMV-like particles it is tempting to speculate that the nucleic acid may be synthesized to its final dimensions first and that the final act of virus synthesis may consist of a rapid polymerization of the units of TMV protein precursor around the nucleic acid core.

It is possible that the appearance of such abnormal proteins is a general phenomenon in plant virus infections; for in the case of turnip yellow mosaic virus infections MARKHAM AND SMITH[13] have also demonstrated the presence of a nucleic acid-free, non-infectious protein which otherwise resembles the virus closely in physical and chemical properties. JEENER[8] has reported upon experiments with turnip yellow mosaic virus infected plants similar to our experiments I and IV, but employing $^{14}CO_2$ as the tracer. His results, paralleling ours, show a greater incorporation of isotope into the abnormal protein than into the virus at a relatively advanced stage of infection. Here also the data do not exclude the RNA-free antigen as a possible precursor of the virus, but JEENER does not consider that they demonstrate unequivocally such a role.

SUMMARY

1. Experiments are reported in which the rate of assimilation of isotopic ammonium ion into various protein containing fractions of TMV-infected leaves was followed. Particular attention was given to an abnormal nucleic acid-free protein accompanying infection.

2. The protein sedimented by 80,000 × *g*. at pH 4.7, termed P_5, and corresponding to the protein "X" of TAKAHASHI AND ISHII, is the major abnormal protein other than the virus accompanying infection.

3. A close corresondence in isotope content appears to exist between the precursors of this protein and TMV.

4. Earlier observations on the metabolic stability of the bulk of TMV in the host cell are substantiated. The abnormal protein also appears to be relatively stable *in vivo*.

5. The extracted abnormal protein, P_5, cannot be a representative sample of a precursor of TMV, but the possibility of the same protein at the sites of synthesis being precursive is not excluded.

6. It is highly unlikely that the abnormal protein is a degradation product of TMV resulting from the extraction procedure, but the possibility is not excluded that the protein may result from an *in vivo* fragmentation of a specific portion of the total virus.

7. Isotopic ammonium ion is incorporated more slowly into the nucleic acid moiety of TMV than into the protein moiety.

8. The high activity of the particulate fractions in the incorporation of labelled nitrogen makes them suspect as the sites of virus synthesis.

RÉSUMÉ

1. Les auteurs ont suivi la vitesse d'assimilation de l'ion ammonium isotopique dans diverses fractions protéiques de feuilles infectées par le virus de la mosaïque du tabac (TMV). Une protéine anormale, sans acide nucléique, et dont la présence est liée à l'infection, a retenu particulièrement leur attention.

2. Cette protéine, qui sédimente sous 80,000 *g*. à pH 4,7 et qui est désignée par P_5, est identique à la protéine "X" de TAKAHASHI ET ISHII. C'est la principale protéine anormale, autre que le virus qui accompagne l'infection.

3. Il existe une correspondance étroite entre la teneur en isotope du précurseur de cette protéine et celle du précurseur du TMV.

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4. Les observations antérieures sur la stabilité métabolique de la masse du TMV dans la cellule hôte sont confirmées. La protéine anormale se montre également relativement stable *in vivo*.

5. La protéine anormale extraite, P_5, peut ne pas être un échantillon représentatif d'un précurseur du TMV, mais la possibilité que cette même protéine le soit sur les lieux de la synthèse n'est pas exclue.

6. Il est peu vraisemblable que la protéine anormale soit un produit de dégradation du TMV, résultant des méthodes d'extraction, mais il n'est pas impossible que cette protéine résulte d'une fragmentation *in vivo* d'une partie spécifique du virus entier.

7. L'ion ammonium isotopique est incorporé plus lentement dans la fraction nucléique du TMV que dans sa fraction protéique.

8. L'activité élevée des fraction particulaires au cours de l'incorporation de l'azote marquée amène à penser qu'elles peuvent être le lieu de la synthèse du virus.

ZUSAMMENFASSUNG

1. Es wird über Experimente berichtet, in denen die Geschwindigkeit der Assimilation des isotopen Ammoniumions in verschiedene Proteine, die in Fraktionen von TMV infizierten Blättern enthalten sind, verfolgt wird. Besondere Aufmerksamkeit wurde einem abnormen nukleinsäure-freien Protein, das die Infektion begleitet, gewidmet.

2. Das Protein sedimentiert bei 80,000 *g* bei pH 4.7. Es wird P_5 genannt und entspricht dem Protein "X" von TAKAHASHI UND ISHII. Es ist das bedeutendste abnorme Protein und ist verschieden von dem die Infektion begleitenden Virus.

3. Es scheint eine enge Beziehung im Isotopengehalt zwischen dem Vorläufer dieses Proteins und dem TMV zu bestehen.

4. Frühere Beobachtungen über Stoffwechselstabilität des Hauptanteiles des TMV in den Wirtszellen werden erhärtet. Das abnorme Protein scheint auch *in vivo* relativ stabil zu sein.

5. Das extrahierte abnorme Protein, P_5, kann kein repräsentativer Anteil des Vorläufers des TMV sein, aber die Möglichkeit, dass das gleiche Protein an den Orten der Synthese Vorläufer ist, wird nicht ausgeschlossen.

6. Es ist höchst unwahrscheinlich, dass das abnorme Protein ein während des Extraktionsprozesses entstehendes Abbauprodukt des TMV ist, aber es wird die Möglichkeit nicht ausgeschlossen, dass das Protein bei einer Fragmentation *in vivo* eines spezifischen Anteils des Gesamtvirus entstehen kann.

7. Das isotope Ammoniumion wird in den Nukleinsäureanteil des TMV langsamer eingebaut als in den Proteinanteil.

8. Die hohe Aktivität der besonderen Fraktionen beim Einbau von gezeichnetem Stickstoff in lässt es wahrscheinlich erscheinen, dass sie die Orte der Virussynthese sind.

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Received August 6th, 1954