The Synthesis of Tobacco Mosaic Virus RNA and Ribosomal RNA in Tobacco Leaves

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SUMMARY

Young, maturing and aged tobacco leaves were infected with tobacco mosaic virus (TMV) strain vulgar or flavum. TMV-RNA synthesis and accumulation were followed. Flavum and vulgar RNAs had different patterns of synthesis and accumulation: flavum-RNA may be unstable.

In healthy leaves, ribosomal RNA synthesis (measured by [32P] incorporation) increased to a peak before the leaf reached its maximum length then declined to 25% of the maximum as the leaf aged. TMV infection of a young leaf caused immediate and persistent inhibition of ribosomal RNA synthesis. Ribosomal RNA synthesis in older leaves showed three phases after TMV infection. (1) One day after inoculation, ribosomal RNA synthesis was higher than in healthy leaves. (2) During the main accumulation of TMV-RNA, ribosomal RNA synthesis was inhibited. (3) Following TMV-RNA accumulation, ribosomal RNA synthesis rose, often to levels higher than in healthy leaves. The half-life of ribosomal RNA in a TMV-infected leaf was found to be twice that in a healthy leaf.

These observations are discussed in relation to leaf development and virus multiplication.

INTRODUCTION

Tobacco mosaic virus (TMV) can multiply in a tobacco leaf until its RNA is 75% of the total nucleic acid present (Fraser, 1971). Certain aspects of host RNA metabolism are already known to be considerably altered after TMV infection. Chloroplast ribosomal RNA synthesis is inhibited (Fraser, 1969; Hirai & Wildman, 1969). In very young leaves, TMV infection inhibits aspects of growth, including accumulations of ribosomal RNA (r-RNA), transfer RNA and DNA. Infection of older leaves slows the loss of cytoplasmic r-RNA which occurs in the normal course of leaf senescence (Fraser, 1972).

The effects of TMV infection on cytoplasmic r-RNA synthesis have not previously been studied in detail. This paper reports an investigation of this topic. The multiplication patterns of two strains of TMV, vulgar and the more cytopathic flavum, and their effects on r-RNA synthesis were compared. These strains have different effects on other aspects of leaf RNA metabolism (Fraser, 1969, 1972). r-RNA synthesis was studied in leaves infected at different ages in the hope of understanding the effects of TMV on leaf development. An attempt was made to measure the rate of r-RNA turnover in healthy and infected leaves.

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METHODS

Plants and inoculation. Tobacco plants (Nicotiana tabacum L. var. 'Samsun') were grown in soil in clay pots. One week before inoculation, the plants were transferred to a climatic chamber and grown under continuous, mixed fluorescent and tungsten light of 7500 lx, at 25 ± 1 °C and 80 % relative humidity. Plants of similar height (20 to 25 cm) and appearance were selected. Leaves 10 or 17 cm long were primarily inoculated with TMV strains vulgare or flavum or with sterile phosphate buffer (control) and leaves 4 cm long were secondarily infected following primary inoculation of lower, expanded leaves (Nilsson-Tillgren, Kolehmainen-Sev6us & von Wettstein, 1969) as described previously (Fraser, 1972). The infected or sham-inoculated leaves were identified by light metal rings round the petiole. Equivalent leaves were used for the determination of RNA synthesis rates at various times after inoculation.

Radioactive incubation. Up to 30 days after infection, infected or sham-inoculated leaves were detached by cutting the petiole under water. The cut end was immersed in 0.5 ml water containing 0.5 mCi [32P]-orthophosphate (Radiochemical Centre, Amersham). The entire volume of radioactive solution was taken up by the leaf within 1 h. For the remainder of the incubation the leaf was floated on sterile water.

Nucleic acid extraction. Nucleic acids were extracted from 0.4 g samples of leaf tissue by a detergents–phenol procedure (Fraser, 1971), and fractionated by electrophoresis on polyacrylamide gels (Loening, 1967). The gels were scanned for u.v. extinction at 265 nm with a Joyce Loebl 'Chromoscan', frozen in solid CO₂ and sliced transversely at 0.5 mm intervals. The slices were dried on a film base and radioactivity in each slice was counted in a Packard 460 gas-flow system.

Derivation of data. The following data were derived from the u.v. extinction and radioactivity scans of gels:

1. The total incorporation of radioactivity into 25 S plus 18 S cytoplasmic r-RNA or into TMV-RNA was found by adding the radioactivities of the individual gel slices in the appropriate radioactivity peaks, and subtracting the general background of heterodisperse radioactivity on the gel, as shown in Fig. 1.

2. The weight of DNA on the gel was calculated from the area of the DNA peak in the scan of u.v. extinction. The ratio peak area/weight of DNA was determined by electrophoresis of known amounts of pure Escherichia coli DNA. DNA migrates in polyacrylamide gels as a single, sharp peak, unless very highly sheared (Loening, 1967). The DNA content of a sample of leaf material was found by this method to be $130 \pm 4 \mu g/g$ leaf (mean of eight determinations ± standard error). The DNA content was separately measured by the procedure of Schmidt & Thannhauser (1945) using the modified pre-washing procedure of Holdgate & Goodwin (1965). The value found was $144 \pm 7 \mu g/g$ leaf. This confirms that the phenol-detergent extraction and gel peak-area method provides a reliable and reproducible estimate of leaf DNA content.

3. The weight of TMV-RNA on the gel was calculated from its peak area on the u.v. extinction scan, as described by Fraser (1971). No allowance was made for double-stranded TMV-RNA, which probably has an electrophoretic mobility similar to DNA (Jackson et al. 1972). Published estimates of double-stranded TMV-RNA concentration in leaves (Shipp & Haselkorn, 1964; Babos, 1971) suggest that its concentration relative to that of single-stranded TMV-RNA is too low to introduce significant error into TMV-RNA or DNA measurements.

Method of presentation of results. [32P] incorporation into r-RNA and TMV-RNA, and
Synthesis of TMV-RNA and ribosomal RNA

Fig. 1. Polyacrylamide gel electrophoresis of [³²P]-labelled nucleic acids extracted from a 13 cm long tobacco leaf two days after inoculation with TMV strain *vulgare*. The gel had an acrylamide concentration of 2.4% and was run for 2.5 h at 5 mA, 8 V/cm gel length. The continuous curve shows extinction at 265 nm; the histogram radioactivity. The peaks are: at 1.0 cm, DNA; at 1.5 cm, TMV-RNA and at 2.8 and 4.2 cm, r-RNA. The broken line under the TMV-RNA radioactivity peak shows the delimitation of the peak for calculation of [³²P]-incorporation into TMV-RNA. The broken line under the DNA extinction peak shows how the peak area was obtained for calculation of the weight of DNA on the gel.

The amount of TMV-RNA have been expressed on a per µg DNA basis rather than on a per leaf or per µg r-RNA basis. This has several advantages. As corresponding RNA and DNA data were derived from the same gel, variation arising from differences in extraction and gel-loading was minimized. Incorporation of [³²P] into TMV-RNA and r-RNA could be directly compared. TMV infection certainly has drastic effects on leaf r-RNA contents (Fraser, 1972). If this is even partly an effect on degradation rather than synthesis, as this investigation suggests, then specific activity values for r-RNA (³²P] incorporation into r-RNA/µg r-RNA) are meaningless as a guide to r-RNA synthesis.

TMV infection affects leaf DNA content only when very small leaves are infected. Changes in DNA content of leaves during growth are given in Fraser (1972) and closely parallel changes in leaf fresh weight, i.e., curves drawn on a per µg DNA basis are essentially similar to those drawn on a per g fresh weight basis.

Reproducibility of results. Estimates of [³²P] incorporation by the gel method are likely to be free from errors arising from [³²P]-containing contaminants, as a clean, definite peak is measured. The RNA extraction procedure has been shown to give an 80% yield; reproducibility of extraction is extremely high (Fraser, 1971). A control experiment showed that measurements of [³²P] incorporation by this method are also highly reproducible. Eight identical leaves were incubated with [³²P] 5 days after inoculation with TMV. [³²P] incorporation into r-RNA was 5040 ± 356 ct/min/µg DNA (mean ± standard error) from eight samples taken from a single leaf frozen on solid CO₂ and crushed finely before sampling, and 4807 ± 678 ct/min/µg DNA from eight samples taken from eight different leaves. The standard errors show that the variation between leaves was larger than between
replicate determinations on the same leaf. The amount of variation between samples from different leaves was smaller than the differences between treatments to be reported in the results.

As a check on the reproducibility of the timecourses of r-RNA and TMV-RNA syntheses after infection or in control tissue, each of the experiments of Figs. 3, 4 and 5 was carried out three times. One example is shown; repeat experiments gave similar curves.

RESULTS

Kinetics of $^{32}$P incorporation

Incorporation of $^{32}$P into the r-RNA of detached, healthy leaves and into TMV-RNA in infected leaves continued for at least 50 h. The rate of incorporation decreased only slightly with time (Fig. 2). The rate of $^{32}$P incorporation into r-RNA in TMV-infected leaves could change suddenly, as shown in Fig. 2, depending on the time after inoculation. This was the result of changes in the instantaneous rate of r-RNA synthesis, to be demonstrated below. An incubation time of 5 h was chosen for the measurement of the 'instantaneous' rate of r-RNA or TMV-RNA synthesis. This allowed sufficient labelling for accurate measurement, and was in the early part of the incorporation curve where incorporation was near linear with time. By 5 h, the relative amount of labelling of the r-RNA precursor (Rogers, Loening & Fraser, 1970) was too small to cause significant errors in estimates of $^{32}$P incorporation into TMV-RNA, which has a similar electrophoretic mobility.

Synthesis of TMV-RNA

Young leaves were secondarily infected with TMV strain vulgar (Nilsson-Tillgren et al. 1969). The first sign of virus in the young leaves was $^{32}$P incorporation into TMV-RNA, detectable 3 days after primary inoculation of the lower leaves (Fig. 3). By this time the leaf was 4 cm long. The synthesis of TMV-RNA was at a maximum between 3 and 6 days after the first detection of virus RNA synthesis, then declined slowly until after 25 days no pulse-labelling whatsoever of TMV-RNA could be detected. The concentration of TMV-RNA in the leaf rose rapidly during virus RNA synthesis and became constant after synthesis declined, at a level of 0.5 mg TMV-RNA/g fresh weight of leaf.

The leaves primarily inoculated when 10 cm long grew to a maximum length of 17 cm. In vulgar-infected leaves, TMV-RNA synthesis was detectable by $^{32}$P incorporation 1 day after inoculation, was at a maximum rate 3 days after inoculation then gradually declined (Fig. 4). The TMV-RNA content of the leaf increased in a sigmoid fashion and became constant after 10 days.

In leaves inoculated with strain vulgar after they had reached their maximum length of 17 cm, vulgar RNA synthesis and accumulation followed timecourses similar to those observed when 10 cm leaves were inoculated, except that the initial lag in TMV-RNA synthesis was shorter and the final amount of TMV-RNA was less and was reached earlier (Fig. 5).

The multiplication of TMV strain flavum when 10 or 17 cm leaves were infected was rather different from the vulgar multiplication pattern. Flavum replicated to reach an RNA concentration only about 40% of that reached by vulgar (Figs. 4, 5). The accumulation of flavum-RNA lasted if anything only slightly longer than vulgar-RNA accumulation. $^{32}$P incorporation curves show that the labelling of flavum-RNA tended to build up more slowly than vulgar-RNA labelling, but that flavum-RNA labelling remained at a high
Fig. 2. Kinetics of $^{32}$P-orthophosphate incorporation into r-RNA and TMV-RNA in healthy and TMV strain *vulgare* infected leaves. Two days before the $^{32}$P incubation, leaves 13 cm long were inoculated with *vulgare* or sterile phosphate buffer; 4.5 h after the start of the $^{32}$P incubation, samples of control and TMV-infected leaves were cut into strips 2 x 10 mm and infiltrated under vacuum with either sterile water (A) or 50 µg/ml actinomycin D (B). The leaf strips were floated on water or actinomycin D solution in Petri dishes, under continuous illumination.

(A) ○—○, $^{32}$P incorporation into r-RNA of healthy leaf; △—△, $^{32}$P incorporation into r-RNA or TMV-infected leaf; □—□, $^{32}$P incorporation into TMV-RNA.

(B) After infiltration with actinomycin D at 4.5 h. ●—●, $^{32}$P in r-RNA of healthy leaf; ▲—▲, $^{32}$P in r-RNA of TMV-infected leaf.

level long after the rate of *vulgare*-RNA synthesis had dropped, and after a steady *flavum*-RNA level in the leaf had been reached.

*r-RNA synthesis*

In the healthy tobacco leaf, the rate of r-RNA synthesis rose to a maximum when the leaf had reached between 60 and 80% of its maximum length (Figs. 3, 4). This is slightly before
the peak in leaf r-RNA content, which occurs at about the time when maximum length is reached (Fraser, 1972). The rate of r-RNA synthesis in the healthy leaf declined as the leaf aged. The low, fairly stable rate of $[^{32}\text{P}]$ incorporation into r-RNA in aged leaves (Fig. 5) was about one quarter of the maximum rate recorded in young leaves (Fig. 4).

When a 4 cm long leaf was infected by TMV strain *vulgare* (Fig. 3), r-RNA synthesis began to decline as soon as TMV-RNA synthesis became detectable, then stayed at a constant, low rate. r-RNA synthesis did not rise after the end of TMV-RNA synthesis.
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Fig. 4. r-RNA and TMV-RNA synthesis after primary inoculation of 10 cm long leaves.
(A) $[^3P]$ incorporation during a 5 h incubation into r-RNA of healthy ($\bigcirc$--$\bigcirc$), TMV strain vulgare-infected ($\bigtriangleup$--$\bigtriangleup$) and TMV strain flavum-infected leaves ($\square$--$\square$).
(B) $[^3P]$ incorporation into TMV-RNA: $\bigtriangleup$--$\bigtriangleup$, vulgare; $\square$--$\square$, flavum.
(C) Changes in leaf content ($\mu$g/$\mu$g DNA) of TMV-RNA: $\triangle$--$\triangle$, vulgare; $\blacksquare$--$\blacksquare$, flavum.

When 10 or 17 cm long leaves were infected with virus (Figs. 4, 5), the subsequent pattern of r-RNA synthesis was very different from that in healthy leaves. In the first day after inoculation, $[^3P]$ incorporation into r-RNA in virus-infected leaves rose to a level higher than in control leaves. The post-inoculation stimulation of r-RNA synthesis in older leaves was not a result of rubbing the leaf during primary inoculation. The control leaves were sham-inoculated and showed no stimulation of r-RNA synthesis. A comparison between two healthy leaves, one sham-inoculated 1 day previously and one not, showed no significant difference in the rate of $[^3P]$ incorporation into r-RNA.

Following the initial stimulation, r-RNA synthesis in infected leaves dropped to a level below that in control leaves. This reduced rate of r-RNA synthesis coincided with the main period of accumulation of TMV-RNA. Finally, after the end of TMV-RNA accumulation,
Fig. 5. r-RNA and TMV-RNA synthesis after primary inoculation of 17 cm long tobacco leaves.

(A) [$^{32}$P] incorporation during a 5 h incubation into r-RNA of healthy (○—○), TMV strain vulgare-infected (△—△) and TMV strain flavum-infected leaves (■—■).

(B) [$^{32}$P] incorporation into TMV-RNA: △—△, vulgare; ■—■, flavum.

(C) Changes in leaf content (µg/µg DNA) of TMV-RNA: △—△, vulgare; ■—■, flavum.

the rate of r-RNA synthesis in infected leaves again rose. In leaves inoculated when 10 cm long, the rate of r-RNA synthesis after the end of TMV-RNA synthesis was higher than in control leaves for some days. This was especially true of leaves infected with TMV strain flavum. The late increase in r-RNA synthesis in leaves inoculated when 17 cm long was also more pronounced with flavum than with vulgare, but for both strains was less than the increase in leaves inoculated when 10 cm long.

Fig. 6 shows the results of an experiment which examined the effects on r-RNA synthesis of TMV multiplication in young leaves which had been artificially forced into a state
Synthesis of TMV-RNA and ribosomal RNA

Fig. 6. r-RNA and TMV-RNA labelling in tobacco plantlets. Tobacco plantlets were grown on acid-washed sand with complete mineral nutrient solution. When the longest leaf was 4 to 5 cm long, the roots were washed free from sand and placed in deionized water. Leaves more than 1 cm long were inoculated with TMV strain vulgare 2 or 6 days after transfer to water culture. Control plants were sham-inoculated with sterile phosphate buffer. For [32P] incubations, plantlets were placed with their roots in 0.5 mCi [32P]-orthophosphate in 0.3 ml water. This was taken up within 50 min. The plants were then supplied with deionized water for the remainder of the 5 h incubation. [32P] incorporation into r-RNA in healthy (○—○) and TMV-infected plants (–––), and [32P] incorporation into TMV-RNA (△—△) were determined.

similar to senescence in older leaves. The rate of r-RNA synthesis in young tobacco plantlets grown in sand with mineral-nutrient solution drops rapidly when the plantlets are transferred to deionized water culture. The content of r-RNA falls. Infection of the plantlets with TMV-vulgare 2 days after transfer, while the decline in r-RNA synthesis rate was rapid, slowed the decline. Infection with TMV 6 days after transfer, when the rate of r-RNA synthesis had stabilized at a low level, led to an r-RNA synthesis rate three times the control value.

r-RNA turnover

I tried to measure directly the effects of TMV infection on the rate of r-RNA degradation in vivo. Leaf tissue was labelled with [32P] then infiltrated with actinomycin D at a concentration of 50 μg/ml, which is sufficient to stop all further r-RNA synthesis. Changes in the radioactivity of r-RNA after administration of actinomycin D then represent a degradation timecourse. Fig. 1 shows that radioactivity was lost more slowly from r-RNA in TMV-infected leaves than in control leaves. The half-life of r-RNA in healthy leaves, under these experimental conditions, was 40 h, in TMV-infected leaves about 80 h.

DISCUSSION

TMV-RNA synthesis

The kinetics of TMV multiplication are now well documented. Commoner et al. (1950) recorded the curve of increase of TMV coat-protein. Kubo & Tomaru (1968) reported changes in the incorporation of [3H]-uridine and [14C]-valine into TMV and changes in infectivity after infection. Fraser (1972) showed the pattern of TMV-RNA accumulation and how this varies with virus strain and the age of leaf infected. The data for incorporation
of $[^{32}P]$ into TMV strain vulgare RNA and vulgare RNA accumulation shown in Figs. 3 to 5 are consistent with these earlier reports. Two features of the results, however, are new.

Twenty-five days after infection of young leaves by vulgare, no incorporation of $[^{32}P]$ into TMV-RNA could be found at all (Fig. 3B). This was not merely a result of general debilitation of leaf synthetic capacity, as the leaf was still capable of r-RNA synthesis at this time (Fig. 3A). A failure, exhaustion or inhibition of some part of the TMV-RNA synthesizing mechanism is implied.

The multiplication of TMV strain flavum RNA followed a pattern different from that of vulgare RNA. A high rate of $[^{32}P]$ incorporation into flavum RNA was maintained even after net accumulation of flavum RNA in the leaf had ceased. In contrast, the rate of vulgare RNA synthesis fell away before the maximum vulgare RNA concentration was reached (Figs. 4, 5). It is possible that flavum RNA is not completely stable, and turns over, or that flavum coat-protein synthesis later in infection is insufficient to build degradation-resistant virus particles with all the flavum-RNA synthesized. The kinetics of $[^{32}P]$ incorporation into, and accumulation of vulgare RNA are consistent with its being highly stable.

r-RNA synthesis immediately after infection

In the first 1 or 2 days after infection, r-RNA synthesis was inhibited in leaves infected when 4 cm long; vastly stimulated in leaves infected when 10 cm long and slightly stimulated in leaves infected when 17 cm long (Figs. 3 to 5). When young leaves were artificially forced into a senescent phase, a stimulation of r-RNA synthesis followed infection (Fig. 6). Kubo (1966) reported a stimulation of RNA synthesis shortly after infection with TMV. Kubo & Tomaru (1968) found no stimulation of $[^{3}H]$-uridine incorporation into 80 S ribosomes after TMV infection of young leaves, but a stimulation of incorporation in older leaves. There is therefore a fundamental difference in the response of young, and older or senescent tissues to TMV infection.

TMV infection can certainly block leaf growth, measured by increases in length, weight, DNA, r-RNA and t-RNA, if the leaf is infected while very young, e.g. about 1·5 cm long. By the time the leaf reaches a length of 5 cm, its further growth, measured by all these parameters, is substantially resistant to inhibition by TMV (Fraser, 1972). Leaves infected when 4 cm long (Fig. 3) were intermediate between these two cases. Certain parameters of growth, such as length increase (Fig. 3C) and DNA increase (not shown) were but marginally inhibited by TMV infection; the increasing rate of r-RNA synthesis which is a part of leaf growth could still be inhibited. It is remarkable that the infected leaves were able to treble their length, and to grow almost as well as healthy leaves, despite severely curtailed r-RNA synthesis.

The amount of early stimulation of r-RNA synthesis after infection of older leaves declined with the age of the leaf. This was probably a result of the inability of older, senescent leaves to support vastly increased r-RNA synthesis. The relatively small amount of TMV-RNA synthesized (Fig. 5) is another guide to the impaired synthetic capacity of very old leaves.

r-RNA synthesis during TMV-RNA accumulation

During the major TMV-RNA accumulation in the leaf, r-RNA synthesis in all ages of leaf infected was depressed to less than one half of the control leaf level (Figs. 3 to 5). At the time of maximum TMV-RNA synthesis, leaves infected with vulgare when 4 cm long showed a level of $[^{32}P]$ incorporation into TMV-RNA four times the $[^{32}P]$ incorporation into r-RNA, a convincing demonstration of how much the RNA metabolism of the young leaf is turned over to virus RNA synthesis. Corresponding values for vulgare RNA labelling: r-RNA
labelling in older leaves were 1:4:1 in leaves infected when 10 cm long and 0:6:1 when
17 cm long leaves were infected. These results are consistent with the final virus concentra-
tions reached, which are highest in young leaves (Figs. 3 to 5; Fraser, 1972). Kubo & Tomaru
(1968) reported a decrease in the rate of incorporation of [3H]-uridine into 80 S ribosomes
at this time after infection. Pring (1971) also found in barley strip mosaic virus-infected
barley leaves that more [32P] was incorporated into virus RNA than into host RNA, and
that host RNA synthesis was reduced during virus RNA synthesis.

It is tempting to try to explain the lowered rate of r-RNA synthesis during TMV-RNA
synthesis as a result of the competition by TMV-RNA synthesis for nucleotides. But
comparison of the rates of synthesis and accumulation of flavum and vulgare RNAs (Figs.
4, 5) suggests that the competitive inhibition of r-RNA synthesis from flavum RNA synthesis
should be much less than from vulgare RNA synthesis. Actually the inhibitions of r-RNA
synthesis by the two strains were very similar, suggesting that there may be some non-
competitive inhibition of r-RNA synthesis. One case is already known of an inhibition of a
specific RNA synthesis by non-competitive means after TMV infection. Just after infection,
while cytoplasmic r-RNA synthesis is unimpaired or stimulated, chloroplast r-RNA
synthesis is completely inhibited (Fraser, 1969; Hirai & Wildman, 1969).

The considerable TMV-RNA synthesis during this period makes it unlikely that the
reduction of [32P] incorporation into r-RNA was caused by some effect of infection on
nucleotide pool size or rate of incorporation of inorganic phosphate into nucleotides.

r-RNA synthesis following TMV-RNA accumulation

r-RNA synthesis after the major TMV-RNA accumulation remained inhibited in leaves
infected when 4 cm long and rose in leaves older when infected. Clearly the amount of
stimulation or inhibition of r-RNA synthesis after the end of TMV-RNA synthesis is
similar to the degree of stimulation or inhibition of r-RNA synthesis immediately after
infection in each of the three ages of leaf infected. Taking the universal depression of r-RNA
synthesis during TMV-RNA accumulation as a result of separate factors, possibly competi-
tive, there are two ways of looking at r-RNA synthesis after TMV-RNA synthesis has
declined. The effect of TMV on r-RNA synthesis seen immediately after infection may be a
long-standing one, which reappears after the separate inhibition by multiplying TMV-RNA
has lessened. The alternative explanation is based on an effect of TMV infection on leaf
development. The losses of r-RNA and t-RNA content which are features of normal leaf
senescence are retarded in TMV-infected older leaves, i.e. TMV blocks the senescent phase
of development, just as it blocks the growth phase in infected young leaves (Fraser, 1972).
The falling rate of r-RNA synthesis is also a facet of leaf senescence (Figs. 3 to 5). A blocking
of this by TMV would lead to a rate of r-RNA synthesis higher than in control leaves after
the ending of the separate inhibition of r-RNA synthesis during TMV-RNA accumulation.
This is what occurs (Figs. 4, 5). It is consistent with this theory that flavum was always
found to give a greater rise in late r-RNA synthesis than vulgare, as flavum also caused
greater retardation than vulgare of the losses of r-RNA content during ageing (Fraser, 1972).

In TMV-infected, cultured tomato root-tips (R. S. S. Fraser, U. Klöpper & D. A. Green-
berg, in preparation) we have found a similar stimulation of r-RNA synthesis after the
ending of TMV-RNA synthesis. In the healthy root, r-RNA synthesis declines with distance
from the tip. TMV-RNA synthesis is localized behind the tip. Cells further from the tip,
in which TMV-RNA synthesis has slowed, have r-RNA synthesis rates much higher than
in healthy root-tips.
r-RNA turnover

The retardation by TMV of the loss of r-RNA content during leaf ageing could be caused by a stimulation of r-RNA synthesis or an inhibition of \textit{in vivo} degradation. Considering the varied but often inhibitory effects of TMV on r-RNA synthesis (Figs. 4, 5) it seems unlikely that the maintenance of higher r-RNA content in older leaves is significantly due to increased r-RNA synthesis. The results of Fig. 1 suggest that r-RNA content is maintained because TMV infection slows the rate of \textit{in vivo} degradation of r-RNA. This conclusion assumes that actinomycin D had no effect on r-RNA degradation and that r-RNA degradation was unaffected by the complete cessation of r-RNA synthesis.

\textit{Ribosomes and TMV multiplication}

Infection by TMV forces the leaf to synthesize a vast amount of virus coat-protein, which requires ribosomes. TMV has very varied effects on r-RNA metabolism, but most of these result in situations favourable for virus multiplication. The inhibition of r-RNA synthesis during TMV-RNA accumulation reduces the competition for nucleotide substrates. Several effects combine to ensure that a high ribosome concentration is created for TMV protein synthesis: r-RNA synthesis is stimulated before and after TMV-RNA synthesis in older leaves; the normal loss of ribosomes during senescence is retarded. The permanent and severe inhibition of r-RNA synthesis when young leaves are infected can be of little significance for coat-protein synthesis, as the actual ribosome concentration in young leaves is much higher than in older leaves.

It is not known how TMV stimulates or inhibits r-RNA synthesis: whether the influence is through intermediary metabolism or if there is a direct effect on gene activity. The effects of TMV on growth and senescence of leaves open up the possibility of TMV influencing messenger RNA synthesis involved in the control of leaf development. An indication of the degree of sophistication of the control by TMV over leaf RNA metabolism is provided by the following simultaneous effects of \textit{flavum}: stimulation of cytoplasmic r-RNA synthesis (Fig. 4); retardation of \textit{in vivo} degradation of r-RNA (Fraser, 1972); inhibition of chloroplast r-RNA synthesis and acceleration of \textit{in vivo} degradation of chloroplast r-RNA (Fraser, 1969).

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