Comparison of viral nucleic acid intermediates at early and late stages of
cauliflower mosaic virus infection suggests a feedback regulatory
mechanism

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An important phase of the multiplication cycle of the
pararetrovirus cauliflower mosaic virus (CaMV) is
transcription of the viral minichromosome in the
nucleus. Leaves of infected turnip plants at the vein
clearing stage were found to contain a relatively low
level of minichromosome DNA, and abundant viral
transcripts and characteristic reverse transcription
products. In contrast, at the much later stage of severe
leaf chlorosis, an elevated level of minichromosome
DNA but less RNA, especially the 35S RNA reverse
transcription template, was observed. Changes in the
composition of virus nucleic acid intermediates were
also seen in roots and stems early, compared with late,
in infection. A possible feedback mechanism control-
ing the level of viral minichromosome DNA and its
importance in regulation of the CaMV multiplication
cycle are discussed in the light of these observations.

Turnip plants (Brassica rapa ssp. rapifera cv. Just
Right) were grown under glasshouse conditions with a
16 h photoperiod and inoculated as 14-day-old seedlings.
When inoculated with CaMV isolate Cabb B-JI, plants
first exhibited vein clearing symptoms typically after 12
to 14 days. By this stage, essentially all of the cells in
systemically infected leaves contain virus (Maule et al.,
1983). Vein clearing progressed towards generalized leaf
chlorosis by about 35 days post-inoculation (p.i.). From
50 days p.i. onwards, plants had severely chlorotic leaves
and were very stunted compared with non-infected
plants. We have previously found that the rate of virus
accumulation in leaves is greatest during the vein
clearing stage and tails off as the leaves progress through
leaf chlorosis (Stratford & Covey, 1988; Stratford, 1989;
R. Stratford & S. N. Covey, unpublished results). We
have studied the nuclear transcriptional and cytoplasmic
reverse transcriptional phases of the CaMV multipli-
cation cycle during the vein clearing stage at 25 days p.i.
and later during the severe leaf chlorosis stage at 50 days
p.i. A cellular total nucleic acid preparation, which
contains viral RNA together with unencapsidated viral
dNA forms, but excludes encapsidated viral DNA, was
extracted from systemically infected leaves and roots of
CaMV-infected turnip plants by a phenol-based method
Fig. 1. Two-dimensional agarose gel electrophoresis of CaMV intracellular DNAs isolated from infected turnip leaves (a and d), stems (b and e) and roots (c and f) at 25 days p.i. (a, b and c) and 50 days p.i. (d, e and f). Equal amounts of cellular nucleic acid were loaded into a single well at the top left corner of each gel, and electrophoresis in the first dimension in neutral buffer was from left to right. Electrophoresis in the second dimension, at a 90° orientation relative to the first (top to bottom in figure), was in alkaline medium. The migration in the first dimension of OC, 8 kb L and SC DNA forms is indicated. The open arrows in (a) show the presence of hairpin DNAs. The right open arrow indicates hairpin molecules without internal gaps. The left open arrow points to one arm of a chevron which actually contains two types of DNA, i.e. a thick line of heterogeneous ssDNA overlaying a fainter population of hairpin molecules with a single gap more clearly resolved in an extended form in (d). A second chevron, not so clearly resolved, containing hairpins with two gaps, is located immediately below the closed arrow indicating L DNA in (a). DNA sizes (kb) are indicated. See Turner & Covey (1988) for a detailed description of these DNA forms.

as previously described (Hull & Covey, 1983). Samples (10 µg) of total cell DNA were fractionated by two-dimensional (2D) neutral/alkaline agarose gel electrophoresis and Southern blot hybridization using 32P-labelled CaMV DNA probes as described by Covey et al. (1990b). CaMV polyadenylated RNA was isolated and analysed by Northern blot hybridization as previously described (Covey et al., 1990b).

Fig. 1(a) shows the 2D gel separation of unencapsidated CaMV DNAs isolated from turnip leaves during the vein clearing stage 25 days p.i. Randomly fragmented linear DNAs migrate during 2D gel electrophoresis to a major diagonal, bisecting each gel, since they possess the same relative mobilities in the neutral and denaturing conditions. Various molecular forms shown as lines, spots and chevron shapes have previously been shown by us to have structures consistent with DNA replication intermediates generated during reverse transcription (Covey & Turner, 1986; Turner & Covey, 1988). The SC DNA component of the CaMV mini-chromosomes migrates as a single, or sometimes double, molecular form to a characteristic position in the gel (Fig. 1a). We have also shown previously (Covey et al., 1990b) that stem and root tissue, sampled during the leaf vein clearing stage, contain less of the reverse transcription products but proportionally more SC DNA than leaves (compare Fig. 1a with b and c). The pattern of DNA forms at 50 days p.i. was significantly different to that at 25 days p.i. (compare Fig. 1a to c with d to f). This was especially obvious in leaves sampled later (Fig. 1d) which contained much more SC DNA than those sampled earlier (Fig. 1a). Minor subgenomic SC DNAs, similar to those we previously reported from turnip callus tissue (Rollo & Covey, 1985; Covey et al., 1990b), were found in leaves at both stages of infection, but were much more prominent at the later stage (Fig. 1d). The composition of open circular (OC) molecules from leaves had also changed. In particular, the 50 days p.i. sample had an abundant component of genome-length (8 kb) DNA together with various minor forms migrating
below it in the denaturing medium (Fig. 1d). The genome-length OC form which had accumulated by 50 days p.i. must comprise OC molecules with a single break in each strand since it migrated as 8 kb linear (L) molecules when denatured. It cannot therefore be a nicked form of the SC DNA, and the most likely explanation is that the 8 kb OC DNA contains two of the usual three gaps present in virion DNA. It is probably an immediate precursor to the SC DNA rather than a degradation product.

Other changes in the leaf DNA at the late stage of infection include a decline of the truncated double-stranded L DNAs of 3.8 kb and 2.4 kb, and of the two major chevron components (see Fig. 1a and d) representing both double-stranded and partially single-stranded reverse transcription products together with some hairpin forms (Turner & Covey, 1988). A population of heterogeneous hairpin molecules (which contain a single gap) extending to greater than 8 kb was found in older leaves and is probably due to aberrant plus strand priming (S. N. Covey & D. S. Turner, unpublished results). These changes are consistent with a reduction in cytoplasmic reverse transcription during the leaf chlorosis stage of infection.

Stems and roots, which already contained more SC DNA than leaves at 25 days p.i., were also found to contain enhanced levels of SC DNA at 50 days p.i., but this was less marked than in leaves. In stems at 50 days p.i., the decline in reverse transcription products was more marked than in roots. In roots at 50 days p.i., the OC DNA was clearly resolved by 2D gel electrophoresis into an upper less abundant form (closed single-stranded circle) and a lower more abundant 8 kb L form similar to that discussed for leaves above. Our interpretation of this is that the OC population contains a mixture of two-gap and one-gap virion type DNAs which are presumably immediate precursors to the SC DNA.

Steady-state polyadenylated RNA transcription products of the minichromosome SC DNA present in tissues at 25 and 50 days p.i. were analysed by Northern blotting (Fig. 2). Viral RNA from leaves at 25 days p.i. characteristically contained the two main (19S and 35S) RNA components (Fig. 2, lanes 1 and 2). Minor components included the heterogeneous background of fragmented RNAs and a minor RNA of about 500 bases. Stems from plants at 25 days p.i. contained a similar pattern of RNAs (Fig. 2, lane 3), although generally less than from leaves (Fig. 2, lane 2). Roots contained very little viral RNA (Fig. 2, lane 4), even though a significant quantity of the SC DNA transcription template was present in the tissue (see Fig. 1c).

The profile of RNA was different in tissues taken from plants at 50 days p.i. (Fig. 2, lanes 5 to 7). In leaves at 50 days p.i., although the total signal was only slightly less than that from leaves at 25 days p.i., the relative intensities of the components had altered. Most notable was the significant decline in 35S RNA relative to 19S RNA. Also, the modal size of the heterogeneous RNA background had declined from greater than 6 kb to less than 2 kb (compare Fig. 2, lanes 2 and 5). An overall decrease in the amount of viral RNA was also seen in stems at 50 days p.i. (Fig. 2, lane 6), whereas the level in roots, although very low, was not significantly changed in plants at the two sampling times (compare Fig. 2, lanes 4 and 7).

Previously, we have demonstrated that an inverse relationship exists between the relative levels of CaMV SC DNA and its RNA transcripts in different tissues of one host species (turnip) (Covey et al., 1990b) and in different host species exhibiting a variety of responses to CaMV infection (Saunders et al., 1990). The accumulation of SC DNA at a late stage of infection and the concomitant changes in RNA reported here are interesting particularly in leaves which show a differential decline of 35S RNA over 19S RNA. One possible explanation for this is that the turnover of these molecules is regulated independently, as might be expected since they serve different roles. A second explanation for the difference is more trivial and invokes random nicking of RNAs following cessation of transcription of the minichromosome. Thus, the larger RNA molecules become nicked before the smaller ones which would explain the differences observed in the two sampling times particularly as the modal size of background fragments had also decreased. This strongly suggests that RNA observed at 50 days p.i. is most probably a remnant of that synthesized much earlier and
so is likely to represent an over-estimate of the activity of the minichromosome during the chlorosis stage. Although a decline in RNA was also observed in stems, the differential effect on the level of 35S RNA was not as marked as in leaves. RNA in roots did not change significantly and this is consistent with the presence of relatively large amounts of SC DNA in roots at both of the sampling times.

The observation that SC DNA accumulates as RNA levels decline suggests that a feedback mechanism operates to maintain a low level of minichromosome DNA in the nucleus, presumably during the active replicative stage of the multiplication cycle. The feedback control might involve a viral gene product which is no longer synthesized in those situations where viral gene expression is low. Two viral gene products which could fulfil this role are the coat protein polypeptide of gene IV and the gene VI inclusion body protein. Packaging of progeny DNA into virions could prevent it being recycled to the nucleus, assuming coat protein synthesis tails off before reverse transcription. However, it is possible that the coat protein polypeptide plays an important role in reverse transcription so preferential accumulation of nascent virion DNA might not be expected to occur.

The gene VI product might be a more likely candidate to regulate movement of progeny DNA back to the nucleus since it is synthesized independently of other viral gene products via a subgenomic RNA. Although one role of the gene VI product is thought to be involvement in the trans-activation of translation (Bonneville et al., 1989; Gowda et al., 1989), the function of the gene VI inclusion body itself is not really understood. It may be an overflow vessel regulating the cellular concentration of virions. In those situations where gene expression declines (including that of gene VI), any newly synthesized virions not yet assembled into inclusion bodies could return to the nucleus to generate more SC DNA. A similar phenomenon has been observed during infections with the pararetrovirus hepatitis B (Tuttleman et al., 1986) where regulation of the synthesis of SC DNA is by the envelope protein (Summers et al., 1990). It has been proposed that the hepatitis B virus envelope protein might be analogous to the CaMV gene VI product (Hull & Covey, 1986). However, the specific role of the CaMV gene VI product, or any other virus polypeptide, in regulating the level of minichromosome DNA remains to be proven.

References


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