The mode of cauliflower mosaic virus propagation in the plant allows rapid amplification of viable mutant strains

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We inoculated the leaves of turnip plants (Brassica campestris spp. rapa cv. Just Right) with two cauliflower mosaic viruses (CaMVs) with different small mutations in a dispensable region of the viral genome, and followed the spread of the virus infection through the plant. Surprisingly, analysis of viral DNA in single primary chlorotic lesions revealed the presence of both mutants. In contrast, the secondary chlorotic lesions and systemically infected leaves contained virus molecules of either one or the other type only. Infection of plants with different ratios of the two reporter viruses showed that this ratio is not conserved during systemic virus spread. Infection with CaMV DNA in the form of heteroduplexes containing a single mismatched base pair, in which each strand carried a distinct diagnostic marker, provided us with evidence that the mismatch was subjected to a repair process in the host plant.

Introduction

Cauliflower mosaic virus (CaMV) is one of the best studied dsDNA plant viruses. Its life cycle and potential use as a vector in genetic engineering of plants have been investigated (Gronenborn et al., 1981; Hohn et al., 1982, 1987; Brisson et al., 1984; Gronenborn, 1987). Following infection, the nick-overlap triple helices remaining in the viral DNA as vestiges of the previous replication cycle are first repaired in the host cell nucleus to produce a closed double-stranded circular genome (Howell & Hull, 1978; Ansa et al., 1982; Hull & Covey, 1983a; Maule, 1985). At this stage, host enzymes might be involved in the correction of mismatched bases. The viral DNA is then transcribed to yield a polycistronic mRNA of genome length, the 35S mRNA, which later serves as the template for synthesis of viral DNA by reverse transcription (Toh et al., 1983; Hull & Covey 1983b; Pfeiffer & Hohn, 1983; Hohn et al., 1985; Takatsuji et al., 1986).

In nature, plants are infected by virus particles that are injected into the leaf by aphids. In the laboratory, infection can be accomplished by rubbing native or cloned viral DNA on to leaves using an abrasive powder (Shepherd et al., 1970; Lebeurier et al., 1980). The infected leaf displays primary chlorotic lesions in the form of chlorotic spots approximately 3 mm in diameter within 9 to 14 days. Some days later, secondary chlorotic lesions, similar in appearance to the primary lesions, appear on uninoculated leaves. Systemic infection follows, which is characterized, in the case of turnip plants infected by virus strain CM4-184 (Howarth et al., 1981; Gardner et al., 1981; the predecessor of all constructions used in this study), by general vein clearing in all new leaves.

Our current knowledge of the progress of the spread of the infection within a plant is limited. Primary chlorotic lesions have been reported to contain viral DNA detected by a leaf blotting technique (Melcher et al., 1981), and the amount of viral DNA per unit area of leaf has been estimated (Melcher et al., 1986). However, it is difficult to quantify the amount of DNA present in single lesions by these techniques. Recombination between viral DNAs during systemic infection has been shown to occur (Lebeurier et al., 1982; Walden & Howell, 1982, 1983; Choe et al., 1985; Dixon et al., 1986; Grimsley et al., 1986), but it has not been shown directly whether primary chlorotic lesions can contain more than one replicating type of virus. Similarly, it is not known whether secondary chlorotic lesions contain one or both kinds of virus particle after infection with a mixture of strains.

As the fidelity of RNA polymerases and reverse transcriptases is generally low, the replication cycle of CaMV leaves ample scope for the generation of new virus strains. The establishment of such novel mutant strains depends on their ability to spread through the plant at least as well as the parent. What criteria govern this process?
We addressed all of the above questions in this study by infecting plants with two distinguishable CaMV mutants (which we will refer to subsequently as 'types') and analysing the progeny viruses in single chlorotic lesions. Thereby we gained information about the systemic spread of the virus, a process that may influence the genetic variation present within a population of viruses.

In addition, we investigated whether viral DNA present in the nucleus of infected cells is accessible to the enzymatic pathways involved in mismatch correction by inoculating host plants with virus genomes in the form of DNA heteroduplexes that contain a single mismatch in a predefined position. The fate of the mismatch in vivo was followed by performing a restriction digestion of the progeny viral DNA. If, after inoculation, reverse transcription occurs before repair, we would expect to see only one type of virus corresponding to the strand transcribed; if repair occurs first, then the kind of virus observed would depend on the direction of the repair.

Methods

Viruses. To facilitate discussion we arbitrarily define mutant types carrying existing and new mutations (produced by in vitro manipulation of the DNA sequence in the dispensable gene II region) using the letters A, B, C and D. All are derivatives of strain CM4-184 (Howarth et al., 1981; Gardner et al., 1981). Types A and B have already been described [Bakkeren et al., 1989, in which they are named Ca355 and Ca355THBI, respectively; Ca355 (Brisson et al., 1984) has a 12 bp intergenic region between open reading frames I and III that contains a XhoI site]. Type B carries an oligonucleotide (not present in type A) that can be cut with EcoRV (Fig. 1). Types C and D differ by a single nucleotide that generates an extra BstNI site in type D (Fig. 1), and are described in more detail below.

Constructions. We took advantage of the mismatch repair-deficient Escherichia coli strain mutS (Radman & Wagner, 1986) to reduce the number of oligonucleotides we required for construction of CaMV types C and D. We introduced an oligonucleotide pair containing a G/T mismatch into the CaMV Ca355 genome at the XhoI site (Fig. 2a). The ligation of fragments or oligonucleotides and transformation of E. coli were handled as previously described (Maniatis et al., 1982). As the mutS strain is deficient in mismatch repair, the G/T heteroduplex gave rise, after transformation into bacteria, to two mutants that differed by a single base pair (Fig. 2b). These clones provided the means for the generation of CaMV DNA with a single defined mispair by annealing of the appropriate plus and minus strands produced from the pTZ clones (Fig. 2c). Single-stranded DNA synthesis of pTZ19U or R clones was done after slight modification of the protocol described previously (Mead et al., 1986). First, the helper phage M13K07, which provides the necessary proteins for ssDNA synthesis, was grown with an increased concentration of kanamycin (50 mg/ml). This prevented the growth of helper phage with a spontaneously deleted kanamycin resistance gene. Second, after the addition of M13K07, the culture was allowed to stand for 5 min to allow adsorption and import of the helper phage into cells. The synthesized ssDNA was isolated following standard protocols for M13 (Maniatis et al., 1982). The G residue-containing plus strand was synthesized in pTZ19 U and the T residue-containing plus strand by using pTZ19 R (Fig. 2c).

Fig. 1. Restriction maps of mutant CaMV genomes. CaMV genomes are represented by thick horizontal bars, with map coordinates shown underneath. All figures relate to DNA lengths in kb. Those above the bars show sizes of restriction fragments larger than 1 kb for EcoRV (types A and B) or BstNI (types C and D). Asterisks mark restriction enzyme sites showing differences between CaMV types.

Fig. 2. Construction of mismatch-containing CaMV. The indicated oligonucleotide containing a G/T mispair was introduced into the XhoI site of the deleted gene II (step a). Transformation in E. coli mutS yielded both type C (A/T, ScaI intact) and type D (G/C, BstNI intact) (step b). From these constructs, the plus and minus strands were synthesized using the pTZ vector (step c). Annealing of the appropriate strands gave CaMV dsDNA containing well defined mismatches (step d). Upon digestion with SalI (step e), linear CaMV containing a defined mispair was released and used for inoculation of plants.

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\begin{align*}
\text{EcoRV} & : 1.6 \ 3.1 \ 2.9 \\
\text{BstNI} & : 1.6 \ 2.1 \ 1.0 \ 2.9
\end{align*}
\]
two strands resulted in a G/T heteroduplex-containing CaMV (Fig. 2d). Appropriate amounts of ssDNA (10 to 50 µg of each strand) were annealed in annealing buffer (10 mM-NaCl, 1 mM-Tris-HCl, pH 7.5) in a volume giving a dsDNA concentration of 5 µg per 20 µl. After 5 min heating at 80°C and slow cooling to room temperature, the dsDNA was digested with restriction endonucleases to verify the annealing efficiency. Upon digestion with Sall, a linear full-length CaMV genome was produced (Fig. 2e) which proved to be fully functional.

Plant infection. CaMV-carrying plasmids or freshly annealed dsDNA was digested with Sall to give linear CaMV DNA without bacterial vector DNA. The largest leaf of a 2-week-old turnip plant was covered with a very fine layer of celite powder. Linear CaMV DNA (5 to 10 µg) in 20 µl of water was placed onto the leaf and spread very gently over the surface of the leaf with the broad end of a Pasteur pipette (three to six strokes). This treatment had to be gentle enough to prevent too many cuts to the cuticle, which would cause the leaf to dry out. A few minutes after the infection was performed, the inoculated leaves were sprayed with water to provide enough moisture for the maintenance of turgor.

CaMV isolation from whole leaves. Whole leaf extractions were done by homogenizing whole leaves without the main veins and isolating CaMV DNA as described previously (Gardner & Shepherd, 1980), 20 to 30 days after inoculation.

CaMV isolation from single chlorotic lesions. Single chlorotic lesions with a diameter of 1 to 3 mm were cut out 9 to 14 days after inoculation as 5 mm diameter discs. Each disc was put into an Eppendorf tube with small holes in the cap and was dried in a lyophilizer at 6666 to 13 332 Pa for 48 h. The dried disc was crushed with a glass rod against the wall of the Eppendorf tube until a fine green powder was spread over the walls of the tube. The powder was mixed in 50 µl of UTESP (1.5 M-urea, 50 mM-Tris-HCl pH 7.5, 50 mM-EDTA pH 7.5, 350 mM-NaCl, 2% Sarkosyl, 100 µg/ml proteinase K) and incubated for 15 min at 70°C. Phenol/chloroform (1 : 1, 50 µl) was added as soon as the solution cooled down to room temperature. After brief vortexing, the tube was allowed to stand for 10 min at room temperature and was then centrifuged for 5 min in an Eppendorf centrifuge (12000 g). The supernatant was transferred into a new tube. The phenol/chloroform with the interphase was mixed with 30 µl TE (10 mM-Tris-HCl, 1 mM-EDTA pH 7.5) and, after a second centrifugation, the second supernatant was combined with the first. The phenol/chloroform extraction was repeated until no interphase appeared (two or three times). The solution was cooled on ice and a one-fifth volume of 10-5 M-ammonium acetate was pipetted into the tube, followed by two volumes of ethanol. The mixture was kept on solid CO2 for 1 h and then centrifuged for 15 min at 4°C at 20000 g. The pellet was briefly dried and then resuspended in 20 µl of TE buffer. Aliquots (4 µl) were usually used for restriction analysis.

Restriction analysis. Restriction enzyme digestions were performed following procedures recommended by the enzyme manufacturers. After electrophoresis in a 0.8% agarose gel containing 10 µg/ml ethidium bromide using 1 x TAE running buffer (Maniatis et al., 1982) the quantity of digested CaMV DNA from a single chlorotic lesion extract was not visible under u.v. light. Therefore, Southern blots were performed, using a Zeta-probe nylon membrane (Bio-Rad) following the protocol supplied by the manufacturer. The DNA probe was labelled with 32P/ATP (Amersham) by the random primer DNA labelling method described previously (Feinberg & Vogelstein, 1983). The intensity of single bands visible on the autoradiograph (Kodak X- Omat AR Film) was scanned using a Shimadzu DF-2 densitometer.

Results

Extraction of viral DNA from a single chlorotic lesion

Routine, 5 to 10 ng of CaMV DNA was extracted from 5 mm discs containing a CaMV lesion 1 to 3 mm in diameter (see Fig. 2 and Methods).

Since the inoculum of DNA was spread over most of the inoculated leaf, it was necessary to prove that CaMV DNA was present only in the chlorotic lesions. For this reason, green pieces from an inoculated leaf were also analysed. We analysed the total DNA extracted from leaf pieces that were 10-fold greater in size than the test leaf discs and contained no visible chlorotic lesions, as a control for the presence of inoculated DNA. Southern blot analysis produced no detectable signal on the autoradiograph (Fig. 3, lane 5), demonstrating that the concentration of CaMV DNA in the green areas is at least 1000-fold lower than that in chlorotic lesions.

Fig. 3. Quantification of CaMV DNA isolated from chlorotic lesions. Lanes 1 to 4 are controls representing a dilution series of CaMV DNA isolated from a plasmid and digested with EcoRV (the largest band contains vector sequences in addition to CaMV sequences). Lane 1, 10 ng CaMV DNA; lane 2, 1 ng; lane 3, 0.1 ng; lane 4, 0.01 ng. In lane 5, DNA isolated from a green leaf disc of size 10-fold that of the chlorotic lesion; discs (see Methods) was loaded. EcoRV digests of one-quarter of the CaMV DNA isolated from primary chlorotic lesions from leaves inoculated with CaMV types A and B at a ratio of 1:1 are shown in lanes 6 to 11. Arrows A or B indicate bands characteristic for these CaMV types (3-1 kb or 2-1 kb, respectively), whereas A + B bands are found in both types of CaMV. Thus, the intensity of band B is decreased in lesions containing mainly type A viruses (lane 11), whereas that of the uppermost 3.1 kb band (which lies close to the 2.9 kb band found in both types) is decreased in type B lesions (lane 9). Lanes 6, 7, 8 and 10 show that both virus types are present in most primary chlorotic lesions. Lanes 9 and 11 show chlorotic lesions of mainly one type. Comparison of the signal intensity with that of the dilution series shows that 5 to 10 ng CaMV DNA is present in a single chlorotic lesion.
Fig. 4. Analysis of the viral DNA content of primary chlorotic lesions.
Southern blot of BstNI-digested CaMV DNA isolated from primary chlorotic lesions from plants infected with CaMV types C and D at 1:1 in lanes 1 to 8, 3:1 in lanes 9 to 11 and 1:3 in lanes 12 to 14. Arrows C and D emphasize the characteristic DNA fragments for each type of CaMV (2.1 kb and 1.7 kb, respectively). Lanes 2, 4, 6 and 8 contain DNA from colonies of type C, lane 7 that from type D, and lanes 1, 3 and 5 that from mixed chlorotic lesions (from infection at 1:1). Lane 9 contains DNA from mixed lesions, whereas lanes 10 and 11 contain type D DNA (1:3 inoculation). Lanes 12 to 14 contain DNA from type C lesions (infected 3:1).

However, we cannot rule out the possibility that a limited number of microscopic lesions (invisible to the naked eye) was present in these regions.

Analysis of primary chlorotic lesions

To establish whether the primary chlorotic lesions arose as the result of a single infection event (similar to viral plaques in bacterial systems), we infected plants with CaMV types A and B at a 1:1 ratio and then analysed the chlorotic lesions, which appeared 9 to 14 days after infection. These yellowish spots were cut out and the viral DNA was isolated. Southern blot analysis of the viral DNA (Fig. 3) revealed that four of the six primary chlorotic lesions analysed contained both types of CaMV (Fig. 3, lanes 6, 7, 8 and 10), type B being predominant in all of the mixed spots. Two spots displayed either phenotype A (lane 11) or B (lane 9). We speculated that the bias favouring type B might possibly be brought about by the presence of the oligonucleotide insert in the gene II region. Therefore we constructed CaMV types C and D, which differ by only a single base pair and thus are most unlikely to differ in growth rate. Analysis of eight spots resulting from inoculations with a 1:1 mixture of C and D gave six mixed and two pure primary chlorotic lesions (Fig. 4), and confirmed that a single chlorotic lesion can contain more than one type of CaMV. Infection of leaves with different ratios of the two types demonstrated that this ratio did affect the relative yields of the progeny virus (Fig. 4, lanes 9 to 11, C:D 1:3; lanes 12 to 14, C:D 3:1). In addition, although both virus types were approximately equally represented in the spots containing only a single type, the mixed spots did not contain equal proportions of the two viruses.

Analysis of secondary chlorotic lesions

After 18 to 22 days the same plants inoculated with a mixture of CaMV types A and B (1:1) developed secondary chlorotic lesions on young uninoculated leaves. DNA isolation from single secondary chlorotic lesions followed by restriction digestion and Southern analysis showed that each single secondary chlorotic lesion contained a pure population of one virus type only (Fig. 5). Comparison of the two virus types in one leaf revealed that virus type B was predominant. This bias reflected that observed in the primary lesions.

To confirm the observation that one virus type tends to predominate in the secondary chlorotic lesions, whole leaves showing secondary chlorotic lesions but no vein clearing were harvested and CaMV DNA was isolated as described previously. As expected, Southern blot analysis revealed the existence of a strong bias in favour of virus type B in the three plants infected with a mixture of CaMV types A and B (data not shown). Analysis of whole infected leaves obtained following inoculation with mixtures of virus types C and D is shown in Fig. 6.
Table 1. Analysis of systemically infected leaves of plants infected with CaMV types C and D

<table>
<thead>
<tr>
<th>Plant no.</th>
<th>Ratio of infection (type C:D)</th>
<th>Ratio in progeny viruses (C:D)</th>
<th>Type D (%)*</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1:1</td>
<td>1:8</td>
<td>86</td>
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<tr>
<td>2</td>
<td>1:1</td>
<td>1:1</td>
<td>45</td>
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<tr>
<td>3</td>
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<td>2:1</td>
<td>30</td>
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<tr>
<td>4</td>
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<td>6</td>
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<td>2:1</td>
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<tr>
<td>7</td>
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<td>1:1</td>
<td>50</td>
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<tr>
<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>1:3</td>
<td>1:1</td>
<td>43</td>
</tr>
</tbody>
</table>

* Determined using a densitometer.

and Table 1. Despite the fact that the infections were performed with viral DNA at a ratio of 1:1 (and using the same plants used for analysis of the primary lesions), the relative amounts of the two progeny virus populations found in single leaves harvested from six plants ranged between 9:1 and 1:8 (Table 1). Thus the ratio of infection with the two CaMV DNAs is not conserved during propagation, even when there is no bias in growth rate favouring one mutant type.

Mismatch repair

Complete virus genomes carrying either a G/T or an A/C mispair in the disposable gene II region were used for inoculation. The advantage of our strategy (described in Methods; see Fig. 2), for the creation in vitro of single base mismatches or larger mispaired regions was that relatively large amounts of DNA could be made with no other changes in the genome. As in the case of virus mixtures, plants infected with CaMV containing a G/T or an A/C mispair showed systemic infection symptoms 18 to 22 days later. Single leaves were harvested and CaMV DNA was isolated. The results of the analysis are presented in Table 2. As would have been expected from the data described above, the two virus forms are not propagated in a predictable manner and thus analysis of the isolated progeny viral DNA cannot provide quantitative results. However, as CaMV propagates, in the first instance, by transcription of the minus strand, each deviation in the progeny virus population from 100% G/C (in the case of the G/T mispair) or 100% A/T (in the case of the A/C mispair) pays witness to the fact that the heteroduplex DNA was accessible to the host metabolic pathways prior to the transcription step. As shown in Table 2, both types of mispair gave rise to the two different virus forms. Of 10 plants inoculated with the G/T heteroduplex, six yielded 100% G/C-containing progeny. Of the three plants treated with the A/C construct, all appeared to have undergone changes brought about by host enzymes. Thus it appeared that there were differences in the efficiency and/or direction of repair of the two mispairs. Two different interpretations of these results are possible. (i) Mismatch repair in plants is an efficient process that corrects A/C mispairs with a random bias, converting G/T mispairs preferentially to G/C. (ii) Mixed lesions are the result of one or more rounds of DNA replication using host enzymes prior to a transcription/reverse transcription step giving rise to two types of virus. In view of the observed bias, explanation (i) seems to be the most likely, but we cannot rule out the occurrence of (ii).

Discussion

CaMV is known to tolerate small insertions in the gene II region, in which they do not affect the viability of the virus (Gronenborn et al., 1981; Brisson et al., 1984; Bakkeren et al., 1989). We took advantage of this and
introduced short oligonucleotides into this region. Since these inserts contained new restriction endonuclease sites, we could follow the fate of the inoculated DNA in the plants by carrying out restriction analysis of the isolated progeny viral DNA.

During the course of these experiments, we have developed a novel technique for the introduction of virus mutants into the plant, using annealed ssDNAs produced in vivo from viral DNA cloned in E. coli. This technique is reliable, and we have used it to study various aspects of virus multiplication and spread. Since the ssDNAs can be produced independently from bacterial strains, it is also possible to vary the chemical properties of the complementary strands. Thus it will be possible in the future to study, for example, the effects of methylation by choice of the E. coli strain used for cloning.

A chlorotic lesion was typically found to yield 5 to 10 ng of CaMV DNA, confirming and extending previous observations (Melcher et al., 1981, 1986). In contrast, no CaMV signal was detectable in the green area of a leaf. This finding indicates that virus replication is largely confined to chlorotic lesions.

Many of the primary chlorotic lesions contained both types of virus. This suggests that they arose as a result of infections involving several CaMV molecules. This observation suggests that there are only a limited number of 'competent' infection sites, but that at these sites numerous different molecules can initiate an infection.

It was interesting to observe that in the mixed primary lesions resulting from inoculation of leaves with virus types C and D (which differ by a single base pair only), the two viral DNAs also were not found in equal proportions. There appeared to be a random bias for one type. This may be due to temporal differences in the infection process, i.e. one virus type entering an infected cell later than the other. The deviation from 1:1 in the composition of the progeny virus mixture would be determined by the difference in time between the initiation of replication of the two types. Alternatively, our observations may reflect stochastic variations due to a low initial m.o.i. However, it should be noted that in nature the virus is injected by an aphid, and little is known about the size and position of the inoculum (see below).

All secondary chlorotic lesions proved to contain only one type of virus. This result provided us with a new insight into the process of virus propagation within the plant. It demonstrates that secondary chlorotic lesions are most likely caused by the growth of a population from a single virus particle. Furthermore, the two virus types spread through the plant in an unpredictable manner, one type spreading in one plant, the other type in another. We can only speculate that this phenomenon could be related to which type of virus first reaches a vascular bundle; replication of this type within the vascular tissue would lead to colonization of distant tissues by this type, especially if colonized tissue cannot support invasion by another type of the virus, as reported previously (Tomlinson & Shepherd, 1978; Zhang & Melcher, 1989). As the primary lesions often contain unequal amounts of the two viruses, the plant will be infected by the type that predominates in the primary lesion situated nearest a vein. This effect would be further enhanced if the primary chlorotic lesions already contain regions of different virus types, a question which we did not address in this study due to technical limitations. Observations on plants inoculated with mixtures of various CaMV strains (Zhang & Melcher, 1989), or on virus progeny recombined from the genomic DNA of a plant carrying transgenic copies of CaMV DNA (Gal et al., 1991), also support the notion that an infection usually becomes fixed for one type of virus molecule. These observations, together with the data presented here, suggest that the recombination events observed by other authors (Lebeurier et al., 1982; Walden & Howell, 1982, 1983; Choe et al., 1983) most likely occur in the primary chlorotic lesions, rather than at later stages of systemic spread.

Although our laboratory technique for infection is quite different from natural infection, nevertheless we have gained valuable information about the systemic spread of the virus. In nature, an aphid injects its style into plant tissues, attempting to locate vascular tissue. In the initial stages of this process, virus particles will be delivered to various cell types in the leaf tissue. In the laboratory, abrasion of leaves is perhaps more haphazard but, in contrast to natural inoculation, we can inoculate mixtures of virus types in defined ratios.

Strong evidence that viruses move from cell to cell through plasmodesmata has come to light (for reviews see Hull, 1989; Melcher, 1990). Although we have no direct evidence, our observations support the notion that this cell-to-cell movement is a limiting factor in virus spread; sorting of virus strains within a plant would occur rapidly if the virus particle entering a cell has a good chance of beginning a replication cycle before other particles enter. We speculate that this kind of propagation leads to an increase in the genetic diversity of virus strains within a population, and that it might also be important in the production and maintenance of genetic variation in other vascular plant pathogens.

We wanted to exploit CaMV as a vector for the analysis of the efficiency and direction of mismatch correction in plants. The system was based on that used previously in the investigation of mismatch repair in mammalian cells (Brown & Jiricny, 1987, 1988). However, the success of this approach depended on the possibility of using chlorotic lesions in a way analogous...
to that used for the virus plaques obtained with simian virus 40 or bacteriophages (Radman & Wagner, 1986; Brown & Jiricny, 1987). Unfortunately, primary chlorotic lesions were found to contain a mixture of viruses, and the ratio of progeny viruses obtained from the secondary chlorotic lesions did not reliably reflect that of the inoculum. These observations preclude the use of this system for quantitative studies of mismatch repair. Nonetheless, our data, albeit severely limited, suggest the existence of an active mismatch repair system in turnip and thus, presumably, in dicotyledonous plants.

Thus, mismatch correction in plants warrants a detailed study, because there appear to be differences in the efficiency and/or direction of repair of the two mispairs. This observation suggests that mismatch repair, rather than 'replication' repair, occurs. Our previous studies have revealed that in mammalian cells the G/T mispair is corrected with a very high efficiency and mostly to a G/C. In contrast, the A/C mismatch is corrected less efficiently and with no significant bias (Brown & Jiricny, 1987, 1988). We interpreted these data as evidence for the existence of a specific mismatch repair system which can counteract the potential mutagenic effects of the spontaneous hydrolytic deamination of 5-methylcytosine. In addition, this system would protect the cell from changes in the methylation pattern of the DNA, which is of importance in maintaining the differentiated phenotype of the cell by playing an important role in the regulation of gene expression (Hare & Taylor, 1985). Indeed, later studies demonstrated the existence of a G/T mismatch-specific thymine DNA glycosylase, which initiates the repair process by excising the mispaired thymine residue to generate an apurinic site (Wiebauer & Jiricny, 1990). As plant DNA is known to be significantly more methylated than that of vertebrates, the dangers of 5-methylcytosine deamination in this system must be even more pronounced (Gruenbaum et al., 1981). It would seem reasonable to expect that a G/T-specific mismatch correction system exists in these organisms. Our limited results obtained in planta suggest this to be the case.

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References


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