Comparison of Dot Molecular Hybridization and Enzyme-Linked Immunosorbent Assay for Detecting Tobacco Mosaic Virus in Plant Tissues and Protoplasts

Ilan Sela, Michal Reichman, and Arthur Weissbach

Professor and graduate student, respectively, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100 Israel; and associate director, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.
This work was done while the senior author was on sabbatical leave at the Roche Institute of Molecular Biology.
Accepted for publication 31 August 1983.

ABSTRACT

Sela, I., Reichman, M., and Weissbach, A. 1984. Comparison of dot molecular hybridization and enzyme-linked immunosorbent assay for detecting tobacco mosaic virus in plant tissues and protoplasts. Phytopathology 74:385-389.

A randomly ^{32}P-labeled DNA probe was prepared by reverse-transcribing tobacco mosaic virus (TMV) RNA to produce single-stranded complementary DNA (cDNA). A dot hybridization technique using a (^{32}P)cDNA probe was adapted for detecting TMV-RNA in crude leaf sap and from protoplasts derived from cultured cells. Tissue homogenates or lysed protoplasts were briefly denatured and applied directly to a nitrocellulose membrane without further clarification. As little as 2.5 pg of purified TMV-RNA can be detected. The rate of synthesis of TMV-RNA as measured by the dot hybridization technique was similar to that obtained with the enzyme-linked immunosorbent assay (ELISA) for the appearance of the TMV capsid protein. However, the dot hybridization method is about twice as sensitive and detects the appearance of new TMV-RNA earlier in infection than the ELISA method which only detects new capsid protein.

Detection of sequence homologies in viral genomes has served as a basis for classification of viral diseases in plants (5,6,18). These studies involved the extraction and purification of the viral nucleic acid and its hybridization, in solution, with a complementary DNA (cDNA) probe made on a template of the known viral nucleic acid.

Recently, a dot hybridization technique in which DNA probes are used for detecting prolactin mRNA in crude lysates of rat pituitary tumor cells was reported (17) and a similar assay was developed by Owens and Diener (12) for detecting viroids. In addition, Garger et al (4) used end-labeled fragments of double-stranded RNA (dsRNA) to detect plant RNA viruses by dot blot hybridization.

During a study of the replication of nucleic acids in plant cells, we utilized a simple dot hybridization test for detecting tobacco mosaic virus (TMV)-RNA in plant leaf cells and cultured plant cells. We compared this method with enzyme-linked immunosorbent assay (ELISA) to test its utility as a diagnostic procedure in plant virology and as a general method for detecting picogram quantities of nucleic acid in plant cells.

MATERIALS AND METHODS

RNA preparations. TMV-RNA free from any other contaminating nucleic acid was obtained by extensively purifying TMV by the procedure of Devash et al (2) prior to the phenol extraction of its RNA. Cesium chloride gradient centrifugation of the virus was performed twice and the final TMV preparation was adjusted to 10 mg/ml in H_2O and filter sterilized. The phenol-extracted TMV-RNA was kept sterile and stored at −80 C.

Nucleic acids from tobacco leaves were extracted by homogenizing 20 g of fresh tissue in 100 ml of 0.05 M glycine buffer, pH 9.3, containing 0.1 M NaCl, 0.01 M EDTA, 25% phenol, 25% chloroform, and 0.1% sodium dodecyl sulfate (SDS). Following centrifugation, the aqueous phase was re-extracted with water-saturated phenol until the interphase appeared to be clear. The nucleic acids were precipitated with ethanol and kept sterile thereafter.

Gel electrophoresis. Nucleic acid was electrophoresed in 1% agarose slab gels under denaturing conditions (14). RNA was dissolved in 0.018 M Na_2HPO_4, 0.002 M NaH_2PO_4, 50% formamide, 6% formaldehyde (pH 8.0), and heated to 65 C for 5 min. After the solution cooled, 20% glycerol and 0.1% bromophenol blue were prepared in 50% formamide and an equal volume was added to the RNA before the mixture was applied to the gel. The gels were prepared in 6% formaldehyde in the above buffer and the electrode solution was the same buffer containing 3% formaldehyde. Electrophoresis was performed at 30 V until the tracking dye migrated about 0.75% of the gel length.

Under these denaturing conditions, the chloroplast ribosomal RNA species were not resolved from cytoplasmic ribosomal RNA (see text); however, when the same RNA preparations were electrophoresed on polyacrylamide-agarose composite gels (1), sharp bands and better resolution were obtained.

Reverse transcription of TMV-RNA. Reverse transcription was performed, using calf thymus DNA fragments as primer, essentially as described by Taylor et al (15). The reaction mixture (30 μl) composition was as follows: 100 mM tris-HCl pH 8.3, 40 mM NaCl, 10 mM $MgCl_2$, 7 mM dithiothreitol, 2 mM sodium-pyrophosphate (to prevent the formation of double-stranded DNA), 50 μg calf thymus DNA primer fragments, 50 μg TMV-RNA, 100 μM of each of dATP, dGTP, dTTP, 100 μCi of α-^{32}P-dCTP (Amersham, >3,000 Ci/mmole) and 30 units of avian myeloblastosis virus reverse transcriptase (Life Sciences Inc., St. Petersburg, FL 33710). Incubation was for 30 min at 37 C and the reaction was terminated by adding EDTA to a final concentration of 25 mM. The volume was then brought to 200 μl with 10 mM tris-HCl pH 7.0, 0.1 M NaCl, 0.1% SDS, and 20 μl of denatured salmon-sperm DNA (2 mg/ml) was added as a carrier. The mixture was passed through a column (0.6 × 25 cm) of Sephadex G-50 equilibrated with the above buffer and the excluded radioactive peak was collected, brought to 0.3 N with NaOH, and incubated at 37 C for 30 min to hydrolyze the RNA and denature the DNA. Sodium acetate was then added to 0.3 M and the mixture was neutralized with HCl. This was followed by the precipitation of the ^{32}P cDNA with ethanol. The total amount of radioactivity in the final cDNA preparation approached 10^8 counts per minute, indicating that the dCTP was almost totally incorporated into DNA. The cDNA transcripts were 70–200 bases long and the hybridization efficiency to an excess of TMV-RNA was 50–60%.

S1-nuclease assay. The single-stranded specific S1 nuclease was employed according to Dodgson and Wells (3) with some modification. The reaction mixture (1 ml) consisted of 50 mM tris-acetate, pH 4.7, which contained 1.8 mM $ZnSO_4$, 0.3 M NaCl,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

50 μg of undenatured salmon-sperm DNA, 15 μg of denatured salmon-sperm DNA, 750 units of S1 nuclease (Sigma Chemical Co., St. Louis, MO 63178) per milliliter, and the radioactive DNA, as specified. The reaction was at 37 C for 1 hr.

Northern transfers. RNA was transferred from agarose gels to nitrocellulose membrane with 20 × SSC (1 × SSC consists of 0.15 M NaCl; 0.015 M Na-citrate) as described by Thomas (16). The RNA was fixed on the membrane by baking in vacuo at 80 C for 2 hr and was hybridized to the ^{32}P-cDNA probe as follows: The membrane was prehybridized for 1 hr at 42 C in a sealed plastic bag in 5 × SSC, 50 mM Na-phosphate pH 6.5, 50% formamide, 100 μg/ml of denatured salmon-sperm DNA, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone 360, 0.02% bovine serum albumin, and 0.1% SDS. The solution was then replaced with a similar one containing 10% dextran sulfate and the ^{32}P-cDNA probe (1–2 × 10^6 cpm). Hybridization was carried out at 42 C for 12 hr, after which the membranes were washed twice (30 min each time) with 0.1 × SSC, 0.1% SDS at 37 C dried and autoradiographed.

Dot hybridization. The hybridization of the cDNA to TMV-RNA, which had been spotted and fixed on nitrocellulose membranes directly was examined as follows: One volume of TMV-RNA (5 mg/ml) was incubated with 0.6 volume of 20 × SSC and 0.4 volume of 36% formaldehyde at 65 C for 15 min and then serially diluted with 15 × SSC (17). A nitrocellulose membrane was wetted with 2 × SSC and mounted over a filter paper (Whatman 3 MM) in a suction manifold. In later experiments, the S & S minifold apparatus (Schleicher and Schuell, Keene, NH 03431) was used. Aliquots, 100 μl each, of the various dilutions of TMV-RNA were applied to the nitrocellulose with suction. The nitrocellulose membrane was then baked in vacuo at 80 C for 2 hr and hybridized with the DNA probe (2 × 10^6 cpm) as described above.

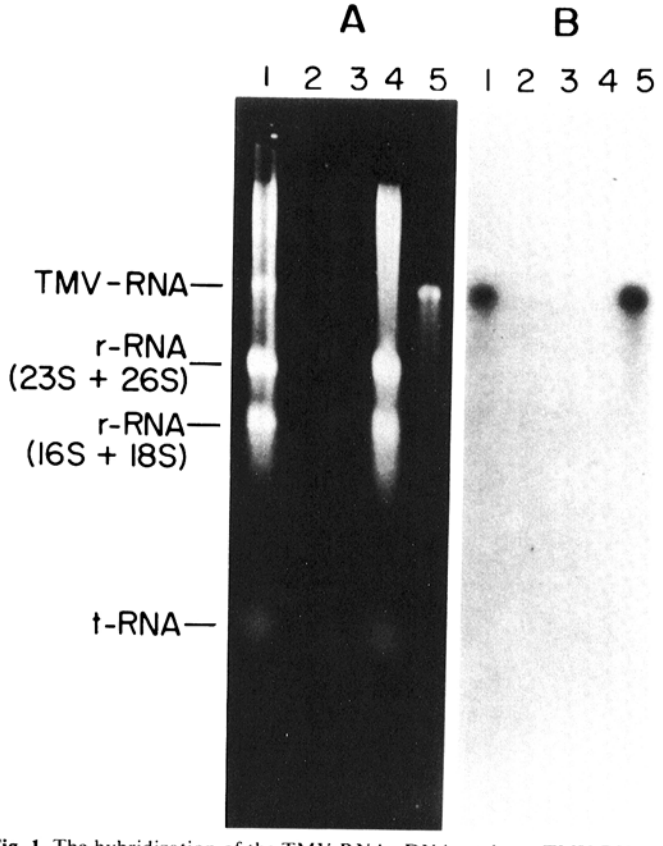


Fig. 1. The hybridization of the TMV-RNA cDNA probe to TMV-RNA. TMV or tobacco cell RNA was **A,** electrophoresed through an agarose gel as described in the text and made fluorescent with ethidium bromide. **B,** "Northern" transfers of the same gel were hybridized with the ^{32}P-cDNA probe. The RNA preparation in the various channels were: Tobacco RNA (5 μg) mixed with TMV-RNA (1 μg) (channel 1), empty channels (channels 2 and 3) tobacco RNA only (5 μg) (channel 4) and TMV-RNA only (1 μg) (channel 5).

Autoradiograms were scanned and their peaks were integrated with a Cliniscan densitometer (Helena Laboratories, Beaumont, TX 77704). After autoradiography, the nitrocellulose dots were removed and their radioactivity was determined in a liquid scintillation spectrometer.

Preparation, inoculation, and maintenance of protoplasts. Cultured tobacco cells, *Nicotiana tabacum* L. 'Wisconsin-38' were grown in suspension in salt solution, as described by Murashige and Skoog (10). The culture medium (MS-S) contained 800 mg casein hydrolyzate, 10 mg thiamine HCl, 0.5 mg folic acid, 0.5 mg biotin, 0.5 mg pyridoxine, 0.5 mg nicotinic acid, 2 mg glycine, 0.2 mg benzyladenine purine, 0.6 mg naphthalene acetic acid, 100 mg myo-inositol, and 30 g sucrose per liter at pH 5.7. The cultures were shaken in the dark at 28 C and transferred every 3–4 days.

Protoplasts were prepared from the above cell suspensions under sterile conditions. The cells were pelleted by centrifugation at 300 *g* for 5 min and washed twice by centrifugation in a solution (VIM [8]) containing 13.5% mannitol, 0.2 mM KH_2PO_4, 1 mM KNO_3, 0.1 mM $MgSO_4$, 10 mM $CaCl_2$, 1 mM KI, and 0.01 mM $CuSO_4$, pH 5.8. The cell pellet was then resuspended in VIM containing 1.5% cellulase, 0.1% macerozyme, 0.01% pectolyase (all filter-sterilized) at a ratio of three volumes per volume of packed cells and incubated for 3 hr at 37 C. The cells and the resulting protoplasts were then centrifuged, washed twice with 13.5% mannitol, layered on a 23% sucrose solution, and centrifuged at 600 *g* for 15 min. The top layer, enriched with protoplasts, was diluted with the above mannitol solution, pelleted down, and resuspended in 13.5% mannitol. The protoplast suspension was adjusted to 10^5 protoplasts per milliliter and mixed with an equal volume of a TMV solution (2 μg/ml) in the presence of poly-L-ornithine (both filter sterilized) as described by Loebenstein and Gera (9).

The metabolizing protoplasts were resuspended in MS-S media supplemented with 12% mannitol following TMV-inoculation. The protoplast concentration was adjusted to 10^6/ml distributed in droplets of 25 μl each in petri dishes, and kept in a humid chamber at 28 C under constant illumination. This procedure ensured a proper energy source and aeration, so that protoplasts maintained and supported TMV multiplication for 90–120 hr as compared to about 48 hr when kept in VIM alone. Recently, Kikkawa et al (7) have reported a very similar procedure to inoculate tobacco protoplasts derived from cell suspension.

The cellulase and macerozyme used in this procedure were from Yakult Pharmaceutical Industry Co., Nishinomaya, Japan; Pectinolyase was obtained from Seishin Pharmaceutical, Tokyo.

Enzyme-linked immunosorbent assay (ELISA). TMV replication was determined by ELISA as described by Orchansky et al (11).

Application of plant material to nitrocellulose membrane. One-gram samples of leaf tissue were homogenized in 0.5 ml of 0.01 M sodium phosphate, pH 7.6, containing 0.1% SDS, to remove the TMV capsid protein. Protoplasts (at a concentration of 5 × 10^5–1 × 10^6 per milliliter) were also lysed with SDS. The lysates were treated with SSC and formaldehyde as described for TMV-RNA and applied to nitrocellulose membrane.

RESULTS

Analysis of TMV-cDNA. The ^{32}P-labeled cDNA complementary to TMV-RNA was digested with S1 nuclease to determine its degree of single-strandedness. The S1 assay was carried out in the presence of increasing amounts of ^{32}P-labeled cDNA (final concentration, 1.2 × 10^4–1.2 × 10^5 cpm/ml). The acid-precipitable radioactivity in control reactions from which the enzyme had been omitted ranged between 9,959 and 111,150 cpm. In the presence of S1 nuclease, however, the acid-precipitable counts ranged from 283 to 639 cpm, respectively, indicating that 97–99.5% of the cDNA was hydrolyzed by S1 nuclease.

To study the specificity of the hybridization of the cDNA probe to its RNA template, TMV-RNA and tobacco-leaf RNA were denatured with formamide and formaldehyde and electrophoresed on formaldehyde-agarose slab gels. The RNA was then transferred to a nitrocellulose membrane and hybridized with the ^{32}P-cDNA

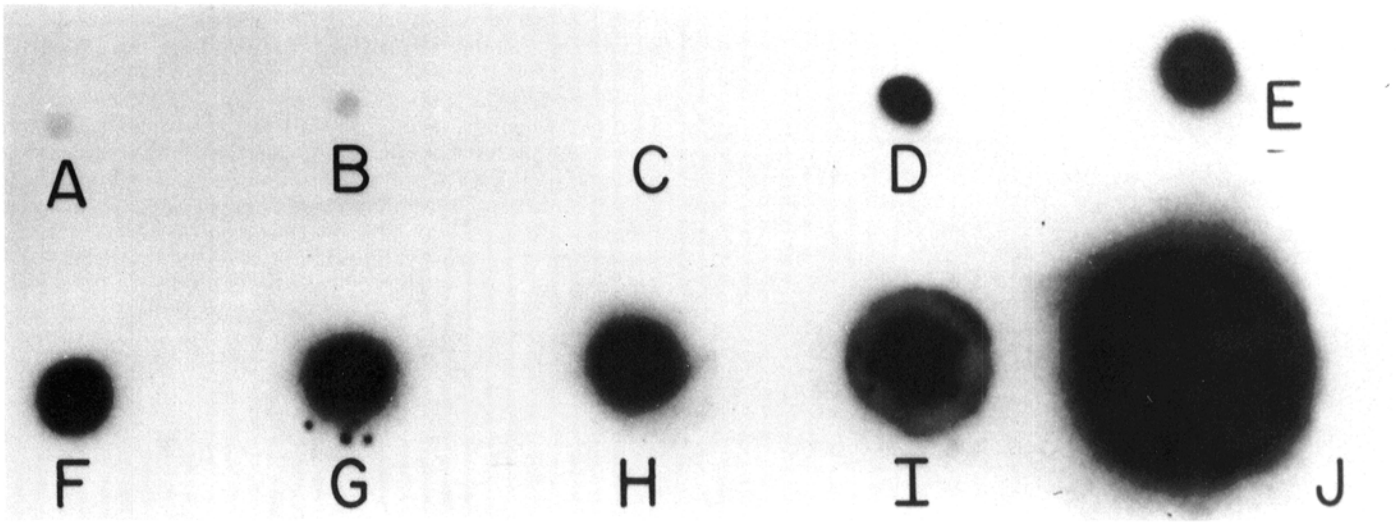


Fig. 2. Dot hybridization of the probe DNA ($\sim 10^8$ cpm/μg) to TMV-RNA. Tenfold dilutions of TMV-RNA were treated with formaldehyde and applied to a nitrocellulose membrane and hybridized. The size of each drop was 100 μl and the spots contained the following amounts of RNA: 2.5 pg (A), 25 pg (B), 250 pg (D), 2.5 ng (E), 25 ng (F), 250 ng (G), 2.5 μg (H), 25 μg (I), 250 μg (J), or no RNA (C).

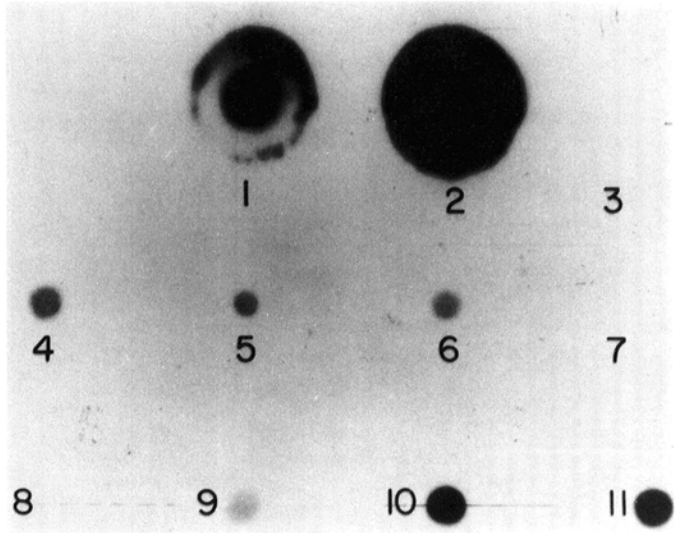


Fig. 3. Dot hybridization of crude extracts from TMV-infeoted leaves. Extracts were treated, applied to the nitrocellulose membrane and hybridized as described in the text. Various RNA preparations were also dotted as controls. Each dot corresponds to either an extract from 0.1 g of fresh tissue or to 100 μg of RNA. Upper row: RNA extracted from TMV-infected tobacco leaves (dot 1), from purified TMV (dot 2) and from uninfected tobacco leaves (dot 3). The middle row represents crude extracts from leaves of TMV-infected *Nicotiana glutinosa* (dot 4), TMV-infected tomato (dot 5), TMV-infected *Gomphrena globosa* (dot 6), and uninfected tobacco leaves (dot 7). Dots 8, 9, 10, and 11 were made from crude extracts of TMV-infected tobacco leaves at 12, 24, 48, and 72 hr postinoculation, respectively.

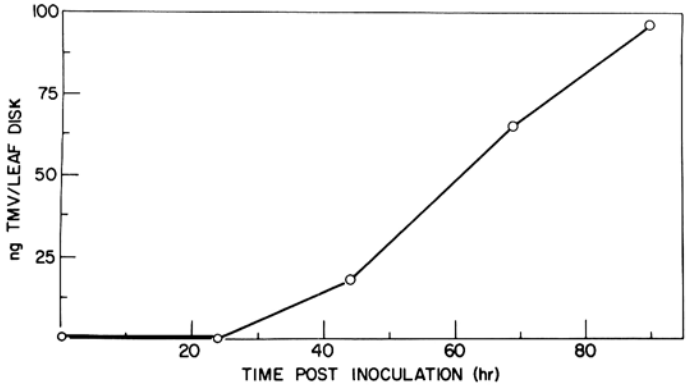


Fig. 4. Growth curve of TMV in tobacco leaf tissue as measured by the enzyme-linked immunosorbent assay (ELISA) method. Samples were taken from the same leaves as for dots 9–11 shown in Fig. 3 and additional samples were measured at 0 time and at 90 hr.

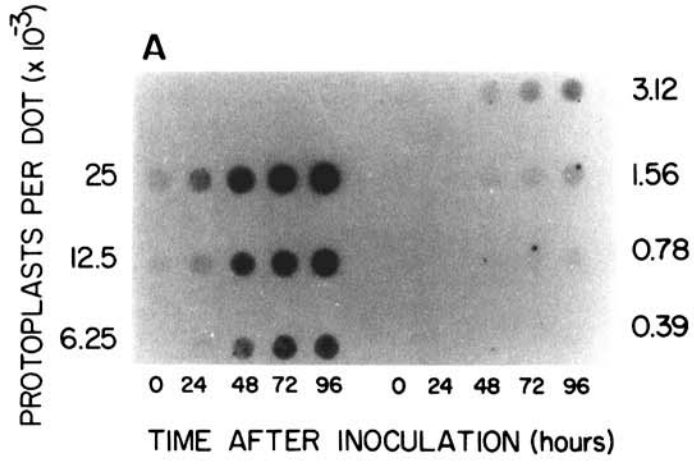
probe. The cDNA probe hybridized specifically to TMV-RNA (Fig. 1B, Lanes 1 and 5) and not to any other RNA found in uninfected tobacco leaves (Fig. 1B, Lane 4).

The hybridization of the cDNA to TMV-RNA was also investigated by the dot hybridization method. Purified TMV-RNA can be detected, even at the picogram level, in this simple manner and the radioautographic intensity of the dots increases as the TMV-RNA content increases (Fig. 2). A linear relationship was not obtained from a cpm count of the radioactive spots and precise quantification of TMV-RNA by this method is not yet possible. When a ^{32}P cDNA of $\sim 10^8$ cpm/mg was used as the probe, the radioactivity (cpm) in the nitrocellulose membrane dots containing increasing amounts of TMV-RNA was as follows: 2.5 pg = 256; 25 pg = 753; 250 pg = 1,485; 2.5 ng = 3,195; 25 ng = 4,444; 250 ng = 8,654; 2.5 μg = 8,277; 25 μg = 6,885; and 250 μg = 141,148. However, the relationship to radioactivity is close to log-linear at the lower range of RNA concentrations.

Applicability of the dot hybridization method for the detection of TMV-RNA in crude leaf extracts. Samples of leaf homogenates, treated as described above, were applied to nitrocellulose membranes and hybridized with the TMV-cDNA probe. In addition, disks were punched from TMV-infected leaves and their TMV capsid protein content was determined by ELISA.

TMV-RNA in the infected leaves was easily detected and the hybridization was specific for TMV-infected tissue and to the RNA extracted from it (Fig. 3). Furthermore, the dot intensities increased with time following inoculation with TMV. A parallel increase in the TMV capsid protein content in the same tissue, as determined by ELISA, also occurs (Fig. 4).

Measuring TMV infection in protoplasts. Protoplast infection by TMV was also examined by the dot hybridization technique and the ELISA method in tobacco cell protoplasts obtained from a suspension culture of tobacco cells. At various intervals after TMV inoculation of the protoplasts, they were collected, lysed, applied to nitrocellulose membranes and hybridized as described above. An increase in TMV-RNA content occurs in the protoplasts with time, indicating that replication of the viral genome is taking place (Fig. 5). To confirm this, in another experiment, two sets of protoplasts were put aside at various time intervals. One set was treated and dot hybridized with ^{32}P cDNA as above, except that each lysate was applied to a separate circle (25 mm) of nitrocellulose membrane,



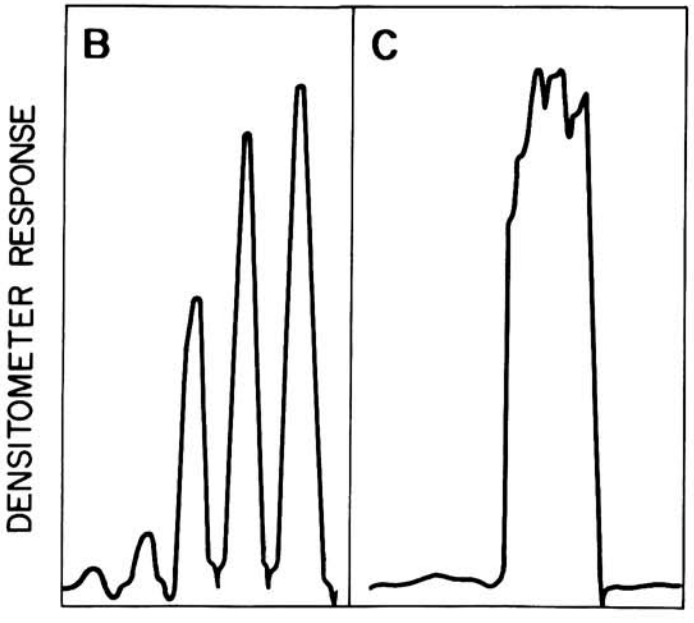


Fig. 5. A, Autoradiogram of dot blot hybridization of lysed TMV-inoculated tobacco protoplasts. After lysis and RNA denaturation, serial twofold dilutions were made starting from 25,000 cells per 100 μl applied to a nitrocellulose membrane dot. **B,** Densitometer scan of the dots of the highest cell density (25,000 protoplasts per dot). **C,** Scan of the dots at the lowest cell density (about 400 protoplasts per dot). The densitometer was set to a higher sensitivity in **C** than in **B.**

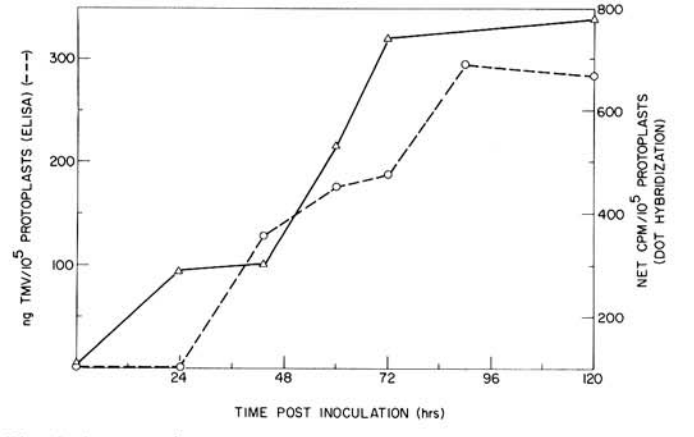


Fig. 6. A comparison between the growth curves of TMV in tobacco protoplasts as determined by measuring capsid protein by enzyme-linked immunosorbent assay (ELISA) and by dot hybridization to TMV-RNA ^{32}P cDNA.

and all further steps took place in 20-ml glass scintillation vials. In aliquots of the other set, taken at the same time, the protoplasts were lysed, without SDS, by diluting them (1:4) in the ELISA sample buffer, and TMV coat protein synthesis was followed by ELISA. The comparison of the growth curves obtained by both methods indicates that synthesis of new TMV takes place in the protoplasts (Fig. 6). The dot hybridization assay detected TMV-RNA replication earlier than the ELISA detection of the coat protein. The sensitivity of the detection of TMV in tobacco protoplasts by dot blot hybridization is also demonstrated in Fig. 5. The lysed protoplasts were diluted with 15 × SSC before being applied to the nitrocellulose membrane. Under these conditions, TMV-RNA could be detected in as few as 400 protoplasts 96 hr postinoculation. Although this is difficult to detect visually, it is

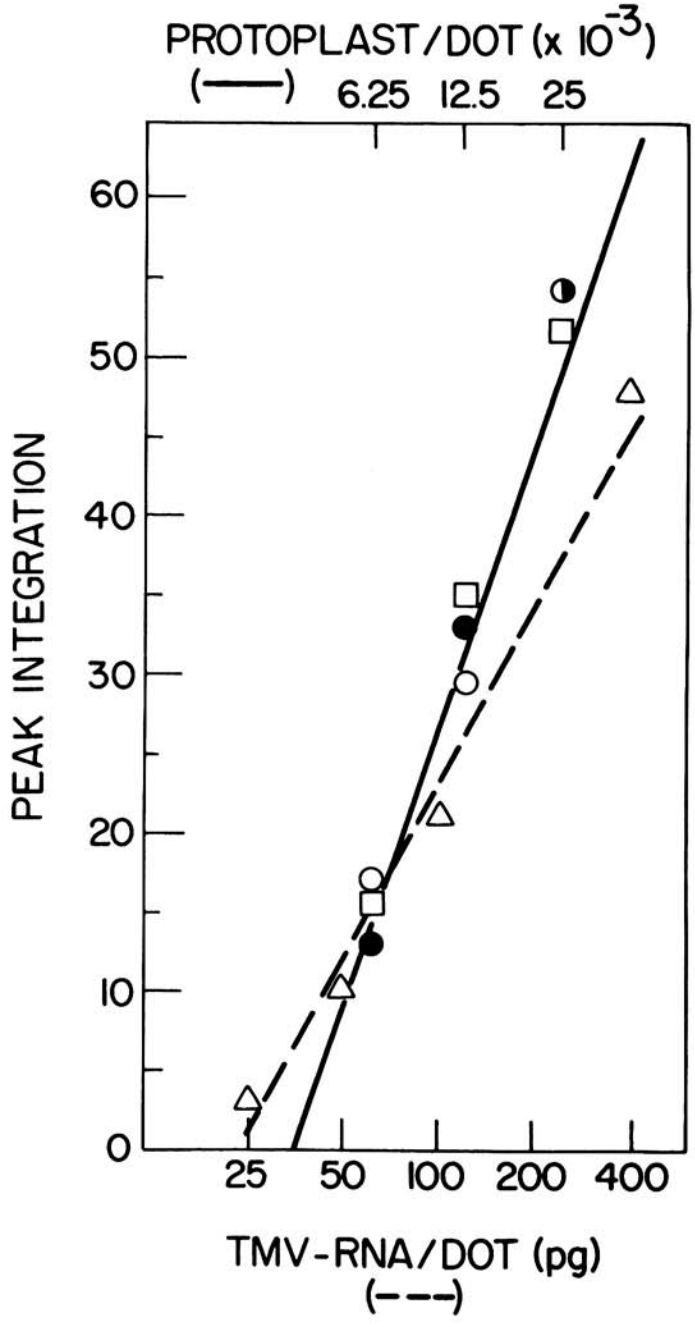


Fig. 7. Relationship between the TMV-RNA concentration and the integrated peak areas of the densitometer scans of the autoradiograms. The same film as in Fig. 5 was scanned along its ordinate, so that the cell densities were correlated to the peaks. ×, •, and □ represent dots at 48, 72, and 96 hr postinoculation, respectively. Data from serial dilutions of TMV-RNA (25–400 pg per dot) on another film were also scanned and plotted (△).

easily detected by using a densitometer scan.

When the film shown in Fig. 5 was scanned along its ordinate by measuring cell dilutions rather than time, it was possible to demonstrate a log-linear relationship between cell densities and the integrated peak area (Fig. 7). This was also true with similar dilutions of TMV-RNA as discussed below.

DISCUSSION

The present paper describes a dot hybridization method which can be used to detect TMV-RNA replication in the crude saps of leaf tissue and in protoplasts derived from cultured cells. The major usefulness of this method is that it permits the direct detection of the viral genome in cells from whole plants or from tissue culture. Thus, early events of viral infection can be detected before any measurable amount of virus protein is produced. Similarly, unexpressed or latent infections should be detectable in this manner.

The dot hybridization assay is very specific, easy to perform, and does not require the isolation of viral RNA. In comparison to ELISA, which is one of the most sensitive diagnostic methods available, dot hybridization is both simpler and more sensitive and the radiolabeled cDNA probe can be rapidly prepared from TMV-RNA by reverse transcription with a commercially available enzyme.

The dot hybridization assay detects 2.5 pg of TMV-RNA (Fig. 2). Assuming 5% RNA in the virion, the theoretical level of detection for TMV should be about 50 pg. The end point of TMV detection by ELISA in our experiments is 5–10 ng per assay. The sensitivity of the dot hybridization method is about twice as great as that of ELISA for detecting purified TMV. This is close to the values actually obtained. For instance, from the data in Fig. 6, it can be calculated that at 90 hr after TMV inoculation, the detection of virus by the ELISA test would require a minimum of 25,000–30,000 protoplasts, whereas with the dot hybridization method, 96 hr postinoculation, a sample containing 400 cells is sufficient (Fig. 5). At this level, the densitometry of the autoradiographs is more convenient than radioactive counting, since a dot containing 20 cpm is easily visualized. Because the scanner integrates the peaks by comparing a particular peak's area to that of the total area of all peaks, one cannot plot more than three or four dilution points on a curve such as that in Fig. 7. Hence, at present, quantification is possible only within a limited range.

In many experiments in plant virology, viral nucleic acids are radiolabeled by introducing radioactive precursors into leaf strips, sometimes even by vacuum infiltration (13). In this procedure, biological events may take place under suboptimal conditions. This paper reports a method which provides advantages for investigating nucleic acid metabolism in intact tissue under normal growth conditions. Furthermore, the dot hybridization method is obviously useful, not only for the detection of viral genomes, but also for detecting the metabolic fate of other nucleic acids in a tissue.

LITERATURE CITED

1. Dahlberg, A. E., Dingman, C. W., and Peacock, A. C. 1969. Electrophoretic characterization of bacterial polyribosomes in agarose-acrylamide composite gels. J. Mol. Biol. 41:139-147.
2. Devash, Y., Hauschner, A., Sela, I., and Chakraburtty, K. 1981. The antiviral factor (AVF) from virus-infected plants induces discharge of histidinyl-TMV-RNA. Virology 111:103-112.
3. Dodgson, J. B., and Wells, R. D. 1977. Action of single-stranded specific nuclease on model DNA heteroduplexes of defined size and sequences. Biochemistry 16:2374-2379.
4. Garger, S. J., Turpen, T., Carrington, J. C., Morris, T. J., Jordan, R. L., Dodds, J. A., and Grill, L. K. 1983. Rapid detection of plant RNA viruses by dot blot hybridization. Plant Mol. Biol. Rep. 1:21-25.
5. Gonda, T. J., and Symons, R. H. 1978. The use of hybridization analysis with complementary DNA to determine the RNA sequence homology between stains of plant viruses: Its application to several strains of cucumo-viruses. Virology 88:361-370.
6. Gould, A. R., and Symons, R. H. 1977. Determination of sequence homology between the four RNA species of cucumber mosaic virus by hybridization analysis with complementary DNA. Nucleic Acid Res. 11:3787-3802.
7. Kikkawa, H., Nagata, T., Matsui, C., and Takebe, I. 1982. Infection of protoplasts from tobacco suspension cultures by tobacco mosaic virus. J. Gen. Virol. 63:451-456.
8. Loebenstein, G., Cohen, J., Shabtai, S., Coutts, R. H. A., and Wood, K. R. 1977. Distribution of cucumber mosaic virus in systemically infected tobacco leaves. Virology 81:117-125.
9. Loebenstein, G., and Gera, A. 1981. Inhibition of virus replication released from tobacco mosaic virus-infected protoplasts of local lesion responding tobacco cultivar. Virology 114:132-139.
10. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15:473-497.
11. Ortchansky, P., Rubenstein, M., and Sela, I. 1982. Human interferon protects plants from virus infection. Proc. Nat. Acad. Sci. USA. 79:2278-2280.
12. Owens, R. A., and Diener, T. O. 1981. Sensitive and repaired diagnosis of potato spindle tuber viroid disease by nucleic acid hybridization. Science 213:670-671.
13. Piazzolla, P., Toussignant, M. E., and Kaper, T. M. 1982. Cucumber mosaic virus RNA synthesis by CARNA 5 in tobacco. Virology 122:147-157.
14. Rave, N., Crkvenjakov, R., and Bvedtker, H. 1979. Identification of procollagen mRNA transferred to diazobenzyloxymethyl paper from formaldehyde agarose gels. Nucleic Acid Res. 6:3559-3567.
15. Taylor, J. M., Illmenser, R., and Summers, J. 1978. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochem. Biophys. Acta 442:324-330.
16. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Nat. Acad. Sci. USA 77:5201-5205.
17. White, B. A., and Bancroft, F. C. 1982. Cytoplasmic dot hybridization. J. Biol. Chem. 257:8569-8572.
18. Zaitlin, M., Beachy, R. N., and Bruening, G. 1977. Lack of molecular hybridization between RNAs of two strains of TMV: A reconsideration of the criteria from strain relationships. Virology 82:237-241.