Expression of Wound Tumor Virus Gene Products In Vivo and In Vitro

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Infection of *Agallia constricta* vector cell monolayers with wound tumor virus results in the synthesis of 12 virus-specific polypeptides. Confirmation that these polypeptides are virus encoded rather than virus induced was obtained by cell-free translation of in vitro synthesized viral mRNA. In addition, transcription by purified wound tumor virus particles was coupled with translation of the resulting transcripts in a wheat embryo cell-free extract. Six previously described structural polypeptides, one presumptive structural polypeptide, and five previously unidentified nonstructural polypeptides were synthesized in infected vector cell monolayers, in cell-free extracts directed by in vitro synthesized viral mRNA, and in the homologous plant cell-free system, in which viral transcription was coupled with translation. Pulse-chase experiments revealed no evidence of precursor-product relationships for the wound tumor virus-specific polypeptides.

Wound tumor virus (WTV) replicates in a wide range of plant hosts and in several species of leafhoppers which act as vectors (2). This virus contains 12 double-stranded RNA segments as its genome complement (17), a virion-associated RNA polymerase (1) that catalyzes the synthesis of single-stranded RNAs complementary to all 12 gene segments (21), and guanyl and methyl transferases that are involved in modifying the 5'-terminal ends of the single-stranded transcripts (22). Although seven structural polypeptides of WTV have been identified (20), it has not yet been shown rigorously that these polypeptides are primary gene products. WTV nonstructural gene products have not been identified previously.

There are several mutant populations of WTV that are able to replicate and induce tumors in plant hosts but have lost the ability to replicate in, and thus be transmitted by, the insect vector (18, 19). This loss of transmissibility is accompanied by the deletion or modification of any of four specific gene segments and by a concomitant loss in infectivity of vector cell monolayers that normally support the replication of standard WTV (18). As part of our attempt to understand the molecular basis of tumor induction, loss of transmissibility, and the WTV-vector-host relationship, we identified the gene products expressed in WTV-infected vector cell monolayers and in several cell-free extracts directed by WTV mRNA or by purified viral particles.

MATERIALS AND METHODS

Cells and virus. *Agallia constricta* line AC20 cells, originally established by Chiu and Black (6), were

obtained from H. T. Hsu, American Type Culture Collection. Cell monolayers were maintained at 28°C in growth medium prepared as described by Liu and Black (11). WTV KB inoculum (9) and WTV antisera originally deposited by L. M. Black with the American Type Culture Collection were also obtained from H. T. Hsu. Standard WTV was purified by the method of Reddy and Black (16) from frozen tumors of WTV-infected sweet clover plants, which were generously supplied by L. M. Black. Sucrose gradient fractions containing purified WTV particles were pooled and stored at -80°C.

Preparation of virus inoculum and infection of vector cell monolayers. AC20 cells were infected with KB inoculum and passaged 10 times, after which more than 90% of the cells in a monolayer stained positive with fluorescein-conjugated WTV antibody. Inoculum was prepared from the infected monolayers between passages 10 and 20 as follows. Infected cell monolayers were harvested by trypsinization and washed with a buffer containing 0.1 M histidine and 0.01 M MgCl₂, pH 6.2 (His-MgCl₂ buffer). Cells were allowed to swell in 2 packed cell volumes of reticulocyte standard buffer (10 mM NaCl, 10 mM Tris, pH 7.4, 1.5 mM magnesium acetate) and lysed by Dounce homogenization. One volume of 2× His-MgCl₂ buffer was added to the cell lysate, and cell debris was removed by centrifugation at 2,000 rpm for 10 min. The supernatant was diluted 2.5-fold with His-MgCl₂ buffer and used immediately for infecting vector cell monolayers.

AC20 cells were plated onto 8-cm² culture dishes at a cell density chosen to give a confluent monolayer on the day of inoculation. After removal of the growth medium and two washes with His-MgCl₂ buffer, each monolayer received 0.5 ml of virus inoculum or His-MgCl₂ buffer. Adsorption was at 28°C for 2 h with periodic shaking. After the adsorption period, the inoculum was removed, and the monolayers were washed with growth medium and then incubated at 28°C in 1 ml of growth medium.

For each infection a number of AC20 cell monolayers grown on glass cover slips were also infected, and the progression and extent of infection of these cells were then monitored by using the fluorescent antibody assay (9, 15).

In vivo [^{35}S]methionine incorporation studies. WTV-infected and mock-infected AC20 monolayers in 8-cm² dishes were washed twice with growth medium lacking both methionine and serum and then incubated in 0.5 ml of this medium at 28°C for 20 min. A 50- μCi amount of [^{35}S]methionine (New England Nuclear Corp.) in 400 μl of growth medium was added to each monolayer after removal of the preincubation medium, and incubation was continued for 15 min. The labeling medium was then removed, and the monolayers were washed twice with ice-cold growth medium containing unlabeled methionine at a concentration which was 10,000-fold in excess of the normal complement of methionine in the growth medium. Monolayers were incubated for 15 min at 28°C with warm growth medium plus excess methionine, washed twice with phosphate-buffered saline, drained, and lysed in 100 μl of electrophoresis buffer (0.1 M dithiothreitol, 2% sodium dodecyl sulfate [SDS], 0.08 M Tris, pH 6.8, 10% glycerol, 0.4% bromophenol blue). [^{35}S]methionine incorporation into protein was determined as described by Mans and Novelli (12).

In vitro synthesis and purification of WTV RNA. Purified WTV particles were concentrated by centrifugation of virus from sucrose gradient fractions at 40,000 rpm in a Spinco type 50 rotor for 2 h. The resulting pellet was suspended in the transcription reaction mixture at a final concentration of 1 mg/ml. The reaction mixture contained 100 mM Tris (pH 8.0), 7.5 mM magnesium acetate, 100 μM S-adenosylmethionine, 2 mM ATP, 2 mM GTP, 2 mM CTP, 0.2 mM [^3H]UTP (125 $\mu\text{Ci}/\mu\text{mol}$; New England Nuclear Corp.), 20 U of creatine phosphokinase (Sigma Chemical Co.) per ml, 10 mM creatine phosphate (Sigma), and 3 mg of bentonite per ml. Incubation was at 28°C for 4 to 6 h, and incorporation of [^3H]UTP into RNA was determined as described previously (13) for both in vitro transcription and RNA synthesis in the coupled transcription-translation system.

Bentonite was removed at the end of the reaction, and the supernatant was adjusted to 20 mM EDTA and 0.5% SDS. The reaction mixture was extracted twice with water-saturated phenol and twice with ether, and the RNA was then precipitated with ethanol. The ethanol precipitate was collected by centrifugation, and the resulting pellet was dissolved in 0.5 ml of a solution containing 0.01 M magnesium acetate, 0.05 M NaCl, and 0.5% SDS. This solution was passed through a Sephadex G-50 column (45 by 1 cm) equilibrated with the same buffer, and the excluded column fractions containing RNA were collected and precipitated with ethanol. Two additional ethanol precipitations of the RNA were performed with 0.1 M sodium acetate, pH 6.0. The final pellet was dissolved in a small volume of water and stored at -20°C. A portion of the purified in vitro synthesized RNA was analyzed in a 15 to 30% sucrose gradient as described previously (21).

Cell-free protein synthesis. A cell-free protein-synthesizing system was prepared from wheat embryos

by the method of Roberts and Paterson (23), excluding the preincubation step. Conditions for translation and determination of the level of [^{35}S]methionine incorporation into protein were as described previously (4). For efficient synthesis of high-molecular-weight polypeptides, the wheat embryo lysate was supplemented with 1 μg of mouse L-cell S-200 extract for each 50 μl of reaction mixture. The preparation of the L-cell S-200 extract was performed as follows. L cells grown in a suspension culture were collected by centrifugation and washed twice with phosphate-buffered saline. The cells were allowed to swell in 2 packed cell volumes of a solution containing 10 mM Tris (pH 7.5), 10 mM KCl, 1.5 mM magnesium acetate, and 6 mM mercaptoethanol for 10 min at 4°C and were lysed by Dounce homogenization. The cell lysate was centrifuged at 10,000 rpm for 10 min, and the resulting supernatant was again centrifuged at 45,000 rpm in a Spinco type 50 rotor for 2.5 h. The resulting S-200 supernatant was frozen in 50- μl samples and stored in liquid nitrogen.

A messenger-dependent reticulocyte lysate was purchased from New England Nuclear Corp. Conditions for translation in this cell-free lysate were similar to those originally described by Pelham and Jackson (14), except that lysates contained 50 μg of rat liver tRNA (GIBCO Laboratories) per ml and 20 μg of polyinosinic acid-polycytidylic acid (PL Biochemicals) per ml.

Conditions used for the syntheses of RNA and protein in the coupled transcription-translation system were similar to those described previously for the coupled system directed by vaccinia virus in the wheat embryo cell-free lysate (3) and are discussed in detail in the text. For each reaction, purified WTV particles were collected from the stored sucrose gradient fraction by centrifugation and suspended directly in the reaction mixture, usually at a concentration of 1 U of optical density at 260 nm per 100- μl reaction volume.

Polyacrylamide gel electrophoresis. Samples from translation reaction mixtures and vector cell lysates were prepared and analyzed by electrophoresis on 12.5% SDS-polyacrylamide gels followed by autoradiography, as described previously (3).

RESULTS

Expression of WTV gene products in vivo. Infection of AC20 cells by WTV results in a persistent infection with no evidence of cytopathic effects (7). Furthermore, persistently infected vector cells can be maintained in continuous culture and yield highly infectious virus after as many as 32 passages (8). The implication of these results is that little reduction of host protein synthesis can be expected after WTV infection. It was therefore reasoned that in order to detect virus-specified or virus-induced polypeptides against a high background of host protein synthesis, it would be necessary to establish conditions that would result in the infection of more than 70% of the cells in a monolayer in a fairly synchronous manner.

Exposure of vector cell monolayers to fresh

concentrated lysates prepared from persistently infected cells by Dounce homogenization was found to be the most effective method of infection. The progression and extent of infection were followed by staining infected cell monolayers with fluorescein-conjugated WTV antibody. As Fig. 1 shows, the majority of cells in the infected monolayers showed points of fluorescent stain throughout their cytoplasm between 6 and 12 h postinfection. Between 12 and 24 h postinfection the points of stain increased to granule size. Fluorescent granules filled the entire cytoplasm of the majority of the cells stained between 24 and 48 h postinfection.

Once conditions for a large-scale synchronous infection had been determined, infected cell monolayers were pulse-labeled with [^{35}S]methionine, and the labeled cell extracts were analyzed by SDS-polyacrylamide gel electrophoresis. Gel autoradiographs of the [^{35}S]methionine-labeled extracts of mock-infected and WTV-infected cells prepared at 8, 12, 16, and 22 h postinfection revealed the appearance of 11 bands, which increased in intensity with time after infection (Fig. 2). The approximate molecular weights of these polypeptides were deter-

mined by taking the averages of values obtained in six separate gel runs at two different acrylamide concentrations and were 155×10^3 , 130×10^3 , 108×10^3 , 76×10^3 , 72×10^3 , 57×10^3 , 52×10^3 , 42×10^3 , 39×10^3 , 35×10^3 , and 19×10^3 . A comparison of the electrophoretic pattern of the purified WTV particles with the pattern of lysates from pulse-labeled cells revealed that five previously identified structural polypeptides (10, 20) comigrated with the *in vivo* labeled polypeptides having molecular weights of 155×10^3 , 108×10^3 , 57×10^3 , and 42×10^3 . It has been reported previously (20) and is shown below that the fastest migrating structural component of WTV particles (Fig. 2, 42,000 daltons) is composed of two polypeptides. Reddy and MacLeod (20) also reported that WTV contains two additional structural polypeptides, one of 130,000 daltons, which corresponds to the 130,000-dalton polypeptide in Fig. 2, and one of 96,000 daltons, which presumably corresponds to either the 76,000- or 72,000-dalton polypeptide in Fig. 2. These two additional structural polypeptides, which are thought to comprise the outer amorphous shell of WTV particles (20), were not observed in WTV particle preparations obtained

FIG. 1. Staining of AC20 cells with fluorescent anti-WTV serum at varying times after infection with WTV. Cells were fixed and stained as described previously (15) and photographed under a fluorescence microscope at a magnification of $\times 400$. (A) Background staining of mock-infected AC20 cells. Increase in intensity of staining after infection with WTV at 12 h (B), 24 h (C), and 48 h (D).

FIG. 2. Autoradiograph of an SDS-polyacrylamide gel analyzing [^{35}S]methionine-labeled extracts of mock- and WTV-infected vector cells. The arrows on the left indicate the migration positions of molecular weight markers (Bio-Rad), and the arrows on the right indicate the positions of the viral polypeptides synthesized after WTV infection. Also shown at the extreme right are the positions of the WTV structural polypeptides designated according to the nomenclature presented in Table 1. The numbers at the top signify the times postinfection (in hours) at which pulse-labeling was performed; the channel marked M contained the cytoplasmic extract from mock-infected cells.

during the course of this study. Lewandowski and Traynor (10) also failed to observe these structural components in their virus preparations. The remaining polypeptides in Fig. 2, which had apparent molecular weights of 52×10^3 , 39×10^3 , 35×10^3 , and 19×10^3 , and either the 76,000-dalton polypeptide or the 72,000-dalton polypeptide represent previously unidentified WTV-specified nonstructural polypeptides.

Expression of WTV gene products in vitro. To confirm that the polypeptides which appeared in vector cells after WTV infection are virus encoded rather than virus induced, RNA was synthesized in vitro by purified WTV particles and then translated in cell-free extracts prepared from wheat embryos and rabbit reticulocytes. Sucrose gradient analysis of in vitro synthesized WTV RNA revealed four peaks of radioactivity, which were in the following sedimentation coefficient (corrected to water at 20°C) size classes: 26S, 25S, 19S, and 15S (Fig. 3). A shoulder of radioactivity sedimented at 6S. This profile agrees with that previously reported by Reddy et. al. (21) and suggests that full-length transcripts were generated under the conditions used.

Translation of in vitro synthesized WTV RNA in a wheat embryo cell-free system resulted in the synthesis of polypeptides which comigrated with all of the WTV polypeptides expressed in vivo, with the exception of the 72,000-dalton

FIG. 3. SDS-sucrose gradient analysis of in vitro synthesized [^3H]UTP-labeled WTV RNA. A portion of the in vitro synthesized RNA was centrifuged at 26,000 rpm for 17.5 h at 25°C in a Beckman SW41 rotor through a linear 15 to 30% sucrose gradient in 0.1 M NaCl-0.01 M Tris (pH 7.5)-0.001 M EDTA-0.5% SDS. Fractions (400 μl) were collected from the bottom of the tube, and 10- μl samples were counted in Aquasol. The positions of 28S, 18S, and 4S RNA markers are indicated by arrows. The direction of sedimentation was from left to right.

polypeptide (Fig. 4A). However, a major *in vitro* product of 74,000 daltons migrated just slightly slower than the *in vivo* 72,000-dalton polypeptide.

An additional experiment was performed to determine whether the slower migrating *in vitro* product represents a primary gene product corresponding to the *in vivo* 72,000-dalton polypeptide which may have undergone some post-translational modification. WTV-infected and mock-infected AC20 cells at 24 h postinfection

were pulse-labeled with [35 S]methionine for 10 min, and the label was chased for 30, 60, and 120 min. There was no evidence for precursor-product relationships between, or gross post-translational modifications of, the WTV-specified polypeptides during the 2-h chase period (Fig. 5). Additional experiments will be required to show rigorously that the 74,000-dalton *in vitro* product corresponds to the 72,000-dalton polypeptide observed in the extract of WTV-infected vector cells and to determine the nature of the

FIG. 4. Autoradiogram of SDS-polyacrylamide gels analyzing 35 S-labeled translation products synthesized in WTV-infected vector cells and in a wheat embryo cell-free system programmed by *in vitro* synthesized WTV RNA (30 μ g/ml) or purified WTV particles. The arrows on the left sides of the gels indicate the migration positions of the molecular weight marker proteins. The arrows on the right of (A) indicate the position of WTV structural polypeptides. The numbers on the right sides of the gels correspond to the apparent molecular weights of the WTV gene products. (A) 35 S-labeled extracts from mock-infected and WTV-infected AC20 cells were run in lanes 1 and 2, respectively; lane 3 contained the wheat embryo cell-free translation reaction mixture programmed by 30 μ g of *in vitro* synthesized WTV RNA per ml. The polypeptides synthesized in the coupled system are shown in lane 4. (B) Analysis of the products of a separate translation reaction shows that the *in vitro* product corresponding to the fastest migrating WTV structural component is composed of two polypeptides (lane 1). Lane 2 demonstrates the *in vitro* synthesis of the 155,000-dalton structural polypeptide.

FIG. 5. Autoradiogram of an SDS-polyacrylamide gel analyzing ^{35}S -labeled whole cell extracts of mock- and WTV-infected AC20 cells obtained in a pulse-chase experiment. Four mock-infected and four WTV-infected vector cell monolayers at 24 h postinfection were each pulse-labeled with 200 μCi of [^{35}S]methionine for 10 min. One of each set of monolayers was immediately harvested and prepared for gel analysis as described in the text. The other three monolayers of each set were chased at 28°C for 30, 60, or 120 min in the presence of a large excess of unlabeled methionine and then harvested as described above. The arrows on the left indicate the migration positions of molecular weight marker proteins, and the arrows on the right indicate the positions of the WTV-specified polypeptides. The numbers at the top indicate the times (in minutes) of the chase period. Lanes 1 through 4, Whole cell extracts of mock-infected cells; lanes 5 through 8, extracts of WTV-infected cells. Each lane received 400,000 hot acid-precipitable counts per minute, and exposure was for 2.5 days.

difference in the apparent molecular weights of these two polypeptides.

There are several additional points with respect to the *in vitro* translation products that

deserve comment. It was found early in this study that the efficiency with which high-molecular-weight WTV polypeptides were synthesized could be improved considerably by adding small

quantities of a 200,000- $\times$ -g supernatant of mouse L-cell lysates to the wheat embryo translation system. The mechanism responsible for this phenomenon is unknown. However, even under optimum conditions the synthesis of the 155,000-dalton polypeptide varied depending on the preparation of WTV RNA. This polypeptide is absent in Fig. 4A, but its *in vitro* synthesis was clearly demonstrated among the products of a separate translation reaction (Fig. 4B, lane 2). Furthermore, the 155,000-dalton polypeptide synthesized either *in vivo* or *in vitro* comigrated with the largest of the WTV structural polypeptides (data not shown).

Reddy and MacLeod (20) reported that the lowest-molecular-weight structural WTV polypeptides comigrate under normal conditions of gel electrophoresis but could be resolved into two components with molecular weights of 35,000 and 36,000. These structural polypeptides correspond to the 42,000-dalton band in Fig. 4A, which also comigrated with the smallest structural component of purified WTV particles. As Fig. 4B shows, the *in vitro* synthesized 42,000-dalton product could also be resolved into two components. Since the wheat embryo cell-free system is thought to be free of post-translational modifying activities (24), it appears that these two structural polypeptides represent individual gene products and are not derived one from the other by some post-translational modification.

A gel pattern similar to that shown in Fig. 4A was obtained when polypeptides synthesized in a rabbit reticulocyte lysate programmed by WTV RNA were analyzed, except that syntheses of polypeptides of more than 100,000 daltons were inefficient with this system under the conditions used. Since no additional information was obtained from gel profiles of these translation products, the data are not presented.

Also visible among the polypeptides synthesized in the wheat embryo system in response to WTV RNA was a prominent band migrating between the 52,000- and 42,000-dalton bands (Fig. 4A). This band does not correspond to any polypeptide synthesized in WTV-infected vector cells, nor was it observed in reticulocyte lysates programmed by WTV RNA. The identity of this polypeptide must await partial peptide analysis of the *in vitro* translation products.

Since different values for the apparent molecular weights of the WTV structural polypeptides were obtained in this and each of two previous reports (10, 20), the following nomenclature for the WTV-specified polypeptides is suggested. The prefix P is used to indicate a structural polypeptide, whereas the prefix Pns denotes a nonstructural polypeptide. Each polypeptide is assigned a number denoting the order of electro-

phoretic migration, with the slowest migrating polypeptide receiving the number 1. Thus, the 155,000-dalton polypeptide is designated P1, the 19,000-dalton polypeptide is designated Pns 12, etc. Further studies will be required to determine which prefix is appropriate for the 76,000- and 72,000-dalton polypeptides. For the present, these polypeptides are designated P or Pns 4 and P or Pns 5, respectively. Table 1 summarizes the apparent molecular weights of the WTV products synthesized *in vivo* and *in vitro*, as well as the proposed designations.

Coupling WTV transcription with translation. Given the difficulties involved in studying mechanisms by which WTV transcription and translation are regulated at the molecular level in a plant host, an attempt was made to couple transcription by purified WTV particles with translation in a plant cell-free extract. The approach taken was to first determine the level of RNA synthesis catalyzed by purified WTV particles under conditions that were optimal for mRNA-directed protein synthesis in the wheat embryo lysate. The major differences between the standard *in vitro* transcription conditions and those used in the translation system in-

TABLE 1. *Apparent molecular weights and nomenclature of WTV gene products synthesized in vivo and in vitro*^a

Apparent mol wt of WTV polypeptide synthesized in infected AC20 cells	Apparent mol wt of WTV polypeptide synthesized <i>in vitro</i>	Designation
155,000	155,000	P1
130,000	130,000	P2
108,000	108,000	P3
76,000	76,000	P or Pns 4
72,000	74,000 ^b	P or Pns 5
57,000	57,000	P6
52,000	52,000	Pns 7
42,000	42,000	P8
41,500	41,500	P9
39,000	39,000	Pns 10
35,000	35,000	Pns 11
19,000	19,000	Pns 12

^a The apparent molecular weights of the *in vivo* and *in vitro* synthesized WTV polypeptides were derived by averaging values obtained from six separate polyacrylamide gel electropherograms. With respect to the proposed nomenclature, the prefix P signifies that the polypeptide is a viral structural polypeptide, whereas the prefix Pns signifies that the polypeptide is a nonstructural virus-encoded protein. The structural and nonstructural polypeptides are assigned numbers which increase with decreasing molecular weight.

^b The *in vitro* synthesized 74,000-dalton polypeptide was tentatively designated as the cell-free product corresponding to the 72,000-dalton polypeptide observed in lysates of WTV-infected vector cells.

volved a reduction in the Mg^{2+} concentration and the absence of the RNase inhibitor bentonite. Unfortunately, RNase is found as a contaminant in commercial preparations of creatine phosphokinase, and attempts to remove this contamination while retaining kinase activity have met with only limited success. Nevertheless, RNA synthesis by WTV particles was linear for at least 4 h in the wheat embryo lysate at a rate 25% of that obtained under standard conditions. Incorporation of nearly 30 pmol of [3H]UTP into RNA was catalyzed by 200 μg of purified virus particles in 2 h (Fig. 6A). Little [3H]UTP incorporation into acid-precipitable material was observed in the absence of virus.

Next, it was determined that addition of virus particles to the wheat embryo lysate in the absence of RNA synthesis resulted in no reduction in the level of exogenous mRNA-directed pro-

tein synthesis (data not shown). Addition of virus particles to the translation system under conditions which support both RNA and protein syntheses resulted in the incorporation of [^{35}S]methionine into protein for approximately 2 h; this incorporation was linear for the first 60 min (Fig. 6B). Only endogenous levels of amino acid incorporation were observed in the absence of added virus.

[^{35}S]methionine incorporation in the virus-directed coupled system was optimized with respect to each reaction ingredient (Table 2). Protein synthesis was dependent on the presence of the methyl donor *S*-adenosylmethionine during RNA synthesis. The optimal concentration for Mg^{2+} was 1 mM more than the concentration of total nucleoside triphosphates in the system, whereas the optimum concentration of monovalent cation was found to be 120 mM K^+ when the acetate salt was used. Addition of amino acids or nucleoside triphosphates in excess of the concentrations shown in Table 2 resulted in no increase in protein synthesis. A distinct optimum was observed for the amount of wheat embryo extract added, but no clear optimum was determined for the virus concentration.

An analysis of the [^{35}S]methionine-labeled products synthesized in the coupled system revealed that the same translation products were made in the wheat embryo extract when synthesis was directed either by purified WTV particles or by exogenous *in vitro* synthesized WTV RNA (Fig. 4A). Again, the efficiency with which high-molecular-weight polypeptides were synthesized in the coupled system was increased by the addition of a small quantity of mouse L-cell S-200 extract to the wheat embryo cell-free translation system.

These results represent the first report of a homologous *in vitro* system in which the transcription by a plant virus was coupled to translation in a plant cell-free lysate and should prove useful in studies aimed at elucidating the molecular mechanisms involved in regulating WTV transcription and translation in plant hosts. This system can be adapted easily for use in studies on the regulation of macromolecular synthesis by other plant viruses which contain virion-associated transcriptases.

DISCUSSION

An understanding of the mechanisms by which viruses are transmitted from host to host is often of critical importance in the control of viral diseases. Many of the arthropod-borne viruses of animals and plants are transmitted to their animal or plant hosts only after productive multiplication in the insect vector. In most in-

FIG. 6. RNA and protein syntheses in the coupled transcription-translation system. (A) RNA synthesis. The reaction mixture contained 20% wheat embryo extract, 15 mM creatine phosphate, 11 U of creatine phosphokinase per ml, 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.5), 2.6 mM dithiothreitol, 120 mM potassium acetate, 0.3 mM spermidine, 0.1 mM *S*-adenosyl-*L*-methionine, 4.5 mM magnesium acetate, amino acids at 0.05 mM each (except methionine), 1.7 mM ATP, 0.7 mM GTP, 0.5 mM CTP, 200 mCi of [3H]UTP per μ mol (final concentration, 0.1 mM), and 200 μg of purified WTV particles per ml. Symbols: $\circ$, [3H]UTP incorporation in the absence of virus; $\bullet$, [3H]UTP incorporation in the presence of added virus particles. (B) Protein synthesis. [^{35}S]methionine incorporation into protein was determined as described in the text. Reaction conditions were similar to those described for (A), except that unlabeled UTP was present at a concentration of 0.1 mM and the reaction mixture contained 50 μ Ci of [^{35}S]methionine. Symbols: $\circ$, [^{35}S]methionine incorporation in the absence of virus particles; $\bullet$, [^{35}S]methionine incorporation in the presence of added WTV particles.

stances, infection of the host by these viruses results in severe cytopathic effects, yet these same viruses readily establish persistent infections in their vectors with no expression of cy-

TABLE 2. *Optimal conditions for protein synthesis in the wheat germ cell-free system coupling transcription by WTV particles with translation^a*

Reaction mixture constituent(s)	Optimum concn
Wheat embryo extract ^b	20.0 OD ₂₆₀ units/ml ^c
K ⁺ (acetate salt) ^b	120.0 mM
Mg ²⁺ (acetate salt) ^b	4.5 mM
Amino acids ^d	50.0 μ M each
ATP ^d	1.7 mM
GTP ^d	0.7 mM
CTP ^d	0.5 mM
UTP ^d	0.5 mM
S-adenosylmethionine ^b	0.1 mM
Spermidine ^b	0.3 mM
Dithiothreitol ^d	2.6 mM
Creatine phosphokinase ^d	11.0 U/ml
Creatine phosphate ^d	15.0 mM
HEPES-KOH (pH 7.6) ^{d,e}	20.0 mM
L-cell S-200 ^d	2.0–20.0 μ g/ml
WTV particles	ND ^f

^a The effects of varying the concentrations of individual reaction constituents on the level of [³⁵S]methionine incorporation in the coupled system programmed by WTV particles were determined.

^b Reagent showed a clear concentration optimum.

^c OD₂₆₀, Optical density at 260 nm.

^d No clear optimum was observed for this reagent, but the concentration shown was saturating for protein synthesis.

^e HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

^f ND, Not determined.

topathology. WTV provides a very suitable system for studying certain aspects of this type of virus-vector-host relationship because there are mutant populations of this virus which have lost the ability to replicate in the vector cells while retaining the ability to replicate and induce tumor formations in the plant host (19). As an initial step in elucidating details of the virus-vector-host relationship at the molecular level, we identified and compared WTV gene products synthesized in infected vector cells and in cell-free translation systems directed by WTV mRNA.

The apparent molecular weights of the six structural, one presumptive structural, and five nonstructural WTV polypeptides identified in this study (Table 1) correspond well with the estimated molecular weights of the WTV gene products previously published by Reddy and Black (19) (Table 3). In this respect all but one (the 72,000-dalton polypeptide) of these WTV-specified polypeptides meet the three criteria outlined by Both et al. to define the primary gene products of reovirus (5). (i) They are present in infected cells after pulse-labeling. (ii) They co-electrophorese with cell-free translation products synthesized in response to purified viral mRNA. And (iii) they exhibit molecular weights consistent with the coding capacities of the gene segments. Furthermore, pulse-chase experiments revealed no precursor-product relationships for the virus-specified polypeptides.

Final determinations of whether the 76,000- or the 72,000-dalton polypeptide corresponds to the seventh structural polypeptide reported by Reddy and MacLeod (20) and whether the

TABLE 3. *Comparison of estimated and apparent molecular weights of WTV genome segments and gene products*

Genome segment	Mol wt of RNA ($\times 10^6$) ^a	Mol wt of gene products		
		Estimated ^a	In vivo ^b	In vitro ^b
1	2.9	161,000	155,000	155,000
2	2.40–2.48	133,000–137,000	130,000	130,000
3	2.20–2.25	122,000–124,000	108,000	108,000
4			76,000	76,000
5	1.78–1.80	99,000–100,000	72,000	74,000
6	1.10–1.15	62,000–67,000	57,000	57,000
7	1.05–1.15	58,000–64,000	52,000	52,000
8	0.83–0.90	46,000–50,000	42,000	42,000
9	0.57–0.63	32,000–35,000	41,500	41,500
10	0.55–0.61	31,000–34,000	39,000	39,000
11	0.54–0.60	30,000–33,000	35,000	35,000
12	0.32–0.35	18,000–19,000	19,000	19,000

^a Estimated molecular weight values for genome segments and gene products were taken from Reddy and Black (19).

^b Values for the apparent molecular weights of in vivo and in vitro synthesized WTV gene products were derived from Table 1. The assignment of gene segments to the corresponding gene products for which they code has not yet been established.

74,000-dalton in vitro product corresponds to the 72,000-dalton polypeptide observed in infected vector cells must await further studies. These studies include the purification of highly radioactive virus particles from infected vector monolayers, a variety of in vivo labeling studies aimed at detecting subtle post-translational modifications, partial peptide analysis of in vivo and in vitro synthesized gene products, immune precipitation studies, and the assignment of gene products to the corresponding gene segments. A study of the assignment of gene products is now in progress and is a necessary step to the eventual identification of the functions of the gene products coded for by the four gene segments involved in the loss of transmissibility (18, 19).

The development of a homologous plant cell-free system which supports the simultaneous transcription and translation of WTV mRNA provides an additional tool for elucidating certain aspects of the virus-vector-host relationship. Further refinement of this and similar cell-free systems may lead to a clearer understanding of the strategies used by viruses that depend on replication in their insect vectors for efficient transmission to their plant or animal hosts.

ACKNOWLEDGMENTS

We thank Hei Ti Hsu for furnishing AC20 cells and instructions for their culture. We are most indebted to L. M. Black for graciously providing materials, instruction, and insight. His assistance and pioneering work made this study possible.

This work was supported in part by Public Health Service grant 1 R01 AI 16075-01 from the National Institute of Allergy and Infectious Diseases.

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