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Regulation of Expression of the Wound Tumor Virus Genome in Persistently Infected Vector Cells Is Related to Change in Translational Activity of Viral Transcripts

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The interaction between a plant virus and its insect vector was studied at the molecular level by examining wound tumor virus (WTV) gene expression in cultured cells derived from its leafhopper vector. Infection of vector cells by WTV is noncytopathic and results in an acute phase (through day 5), followed by persistence beginning with the first cell passage. Viral-specific polypeptide synthesis and viral genome RNA accumulation increased to a maximum level during the first 5 days following inoculation and then decreased as infected cells were passaged (to 5 to 20% of the level observed during the acute phase by passages 10 to 15). In contrast, viral-specific mRNAs were present at approximately the same level in the acute phase and in the early stage (passage 10) of the persistent phase of infection. Although viral transcripts isolated at different times after inoculation exhibited identical electrophoretic migration patterns, they had different functional activities in cell-free translation systems. Transcripts isolated from persistently infected cells were inefficiently translated in vitro, reflecting the situation in infected cells. These results indicate that the decline in the level of viral polypeptide synthesis associated with the persistent phase of WTV infection is related to a change in the translational activity of viral transcripts.

Insects provide the primary mode of transmission for many economically important and biologically interesting plant viruses. A significant number of insect-transmitted plant viruses replicate both in the plant host and in the vector. Cultured cell lines derived from the insect vector have proven to be suitable systems with which to investigate the complexities of such virus-vector-host interactions (for reviews, see references 4 and 5). In this regard, the cell line AC-20, derived from the leafhopper *Agallia constricta*, has been successfully used to study the transmission, multiplication, cytopathology, and primary gene products of wound tumor virus (WTV), a plant virus member of the family *Reoviridae* (2–5, 7–10, 19, 20, 22, 26–28, 30–32).

Inoculation of both the leafhopper vector and cultured vector cells with WTV results in a productive, asymptomatic, and persistent infection (4, 5, 11, 20, 22, 26). The infection that is established in cultured vector cells has the following characteristics. (i) No apparent cytopathology develops in infected cultures at any point during the infection. Persistently infected cells exhibit the same morphology, pattern of cellular protein synthesis, and growth rate as uninfected cells and do not suffer from periodic crises (4, 5, 26). (ii) Viral polypeptide synthesis increases in a linear fashion following inoculation and reaches a peak (approximately 12 to 15% of total protein synthesis) between 48 and 120 h postinoculation (p.i.; the period extending from inoculation to day 5 of infection is termed the acute phase). The rate of viral polypeptide synthesis begins to decline, relative to cellular protein synthesis, with the first cell passage (the period beginning with the first cell passage [day 5] is designated the persistent phase). By passage 10, the synthesis of viral polypeptides is reduced to a level approximately 5 to 20% of that observed during the acute phase. Synthesis of each of the 12 viral polypeptides appears to increase and decline during the two phases in a coordinated manner (22, 26). (iii) Essentially all cells in the persistently infected culture remain infected during hundreds of cell passages, as determined by the fluorescent-antibody assay (26). (iv) Infectious virus can be recovered from persistently infected cells. Inoculation of uninfected AC-20 cells with virus prepared from persistently infected cells initiates a new cycle of acute and persistent infection (26). (v) Viral polypeptide synthesis is not stimulated in persistently infected cells by superinfection with homologous virus. However, these cells are susceptible to infection by a heterologous virus (26).

To characterize further the persistent infection established in vector cells by WTV and to gain some understanding of the mechanisms governing the regulation of WTV gene expression, the kinetics of viral protein synthesis and RNA accumulation were measured in WTV-infected AC-20 cells. In addition, viral transcripts isolated from acutely and persistently infected cells were tested for functional activity in cell-free translation systems.

MATERIALS AND METHODS

Cells and virus. *A. constricta* cells, line AC-20 (7), were maintained in monolayer culture at 28°C in growth medium prepared as described by Liu and Black (17). Synchronous infections by WTV were initiated as described previously (22, 26) at a multiplicity of infection of 10 to 20 cell-infecting units per cell. Virus titers were determined, and infections were monitored by a fluorescent antibody assay (15, 27).

In vivo incorporation studies. AC-20 cell cultures, in 75-cm^2 T flasks inoculated on different days with an identical titer of WTV, were passaged and then pulse-labeled on the same day with 400 μCi of [^{35}S]methionine per ml in methionine-depleted growth medium, as described previously (22). Lysates were prepared and analyzed by electrophoresis on 12.5% sodium dodecyl sulfate (SDS)-polyacrylamide gels followed by autoradiography (22). The level of [^{35}S]meth-

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ionine incorporation was determined by the method of Mans and Novelli (18). Each gel lane received the same amount of radioactivity. The level of incorporation into individual polypeptides was determined after excised gel bands were dissolved in 15% hydrogen peroxide at 65°C overnight, followed by counting in Aquasol liquid scintillation fluid. In addition, the relative radioactivity that was incorporated was quantitated by densitometry of autoradiographs (26).

Isolation of cytoplasmic RNAs from infected cells. Infected and mock-infected AC-20 cell monolayers in 100-mm-diameter dishes initially seeded at a density of approximately 10^7 cells per dish were washed with ice-cold phosphate-buffered saline, scraped from the culture dishes, collected by centrifugation, and suspended in lysis buffer (30 mM Tris hydrochloride [pH 7.5], 5 mM KCl, 10 mM $MgSO_4$, 50 μg of heparin per ml, and 10 μg of polyvinyl sulfate per ml). Cells were lysed by the addition of Nonidet P-40 to a concentration of 0.05% and by vigorous mixing. Nuclei were removed by centrifugation at 2,000 × *g* for 5 min. The supernatant was diluted with an equal volume of 2× digestion buffer (50 mM Tris hydrochloride [pH 7.5], 0.2 M NaCl, 15 mM EDTA and 4.8% SDS) containing 20 μg of proteinase K per ml. After 20 min of incubation at 37°C, the RNA was extracted twice with water-saturated phenol and precipitated with ethanol.

To separate WTV genomic double-stranded (ds) RNA from single-stranded (ss) viral mRNA, extracted cytoplasmic RNA was incubated overnight at 4°C in 2 M LiCl. Insoluble ssRNA was collected by centrifugation at 15,000 × *g* for 15 min. The ssRNA was taken through a second round of LiCl precipitation. Genomic dsRNA was precipitated from the LiCl supernatant with ethanol after a fourfold dilution with H_2O. Genomic dsRNA was further treated with 10 μg of RNase A per ml for 30 min in 0.3 M Tris hydrochloride (pH 7.5)–0.6 M NaCl–3 mM EDTA. Following this treatment, RNA was extracted twice with H_2O-saturated phenol, precipitated with ethanol, and then suspended in an appropriate volume of 1 mM EDTA.

Dot-blot and Northern-blot analysis. The concentration of ss cytoplasmic RNA preparations was determined by measuring the absorbance at 260 nm. An equal amount of RNA from each preparation was dried; suspended in 3 M NaCl–0.3 M trisodium citrate (20× SSC); serially diluted in 20× SSC; and spotted, with the aid of a Hybri-dot manifold (Bethesda Research Laboratories, Gaithersburg, Md.), onto a nitrocellulose membrane presoaked with 20× SSC. Blots were baked under vacuum for 2 h at 80°C. Prehybridization and hybridization reactions were performed as described by Nuss and Summers (24) with 1 × 10^5 to 2 × 10^5 cpm of heat-denatured 3′-end-labeled genome RNA per ml used as a probe. Following hybridization, the blots were washed four times at room temperature with 2× SSC–0.2% SDS and twice at 72°C with 0.2× SSC–0.2% SDS–0.05% sodium PP_i, each for 30 min. Dried blots were exposed to Kodak XAR-2 film at −70°C with an intensifying screen (Lightning Plus; DuPont Co., Wilmington, Del.). Hybridization signals were quantitated with the aid of a Gelman automatic integrating densitometer (Gelman Sciences, Inc., Ann Arbor, Mich.). Absolute concentrations were determined by comparison with a standard curve generated by application of known amounts of in vitro-synthesized WTV transcripts to the nitrocellulose sheet.

For Northern blot analysis, equal amounts (25 μg) of ss cytoplasmic RNA were dried and suspended in 25 μl of 2.2 M formaldehyde in 50% formamide and denatured at 65°C for 5 to 10 min. The denatured RNA samples were electrophoresed through a 1.7% agarose gel containing 2.2 M formaldehyde and 20 mM phosphate buffer (pH 6.5) and then transferred to a nitrocellulose membrane with 20× SSC as the transfer medium (37). The Northern blots were processed as described above for the dot blots.

Genome RNA used as probe for both the dot blot and Northern blot hybridization reactions was labeled at the 3′ end with cytidine 3′,5′-[5′-^{32}P]bisphosphate ([^{32}P]pCp), as described by Bruce and Uhlenbeck (6), and passed through a Sephacryl S300 column to remove contaminants. The recovered RNA (specific activity, 5 × 10^5 cpm/μg) was analyzed for purity by electrophoresis on a 7.5% polyacrylamide gel followed by autoradiography before hybridization. ^{32}P-labeled hybridization probes were also prepared from M13 phage DNA containing inserts corresponding to WTV genome segments S11 and S7 with the universal probe primer by the procedure of Hu and Messing (12).

Quantitation of genome RNA. WTV genome RNAs isolated from infected cell cytoplasms were subjected to electrophoresis on 7.5% polyacrylamide gels and stained with silver (25, 36). The stained gels were dried on acetate sheets and quantitated by densitometry. A known amount of purified reovirus type 3 ds RNA was run in a parallel lane as a standard.

In vitro translation and immuneprecipitation. Isolated ss cytoplasmic RNA was heated for 1 min at 90°C and rapidly chilled on ice before addition at a concentration of 200 or 100 μg/ml to translation reactions containing nuclease-treated rabbit reticulocyte or wheat germ lysates (Promega Biotec, Madison, Wis.), respectively. The level of [^{35}S]methionine incorporation was determined, and the translation products were analyzed by polyacrylamide gel electrophoresis (22).

Viral polypeptide synthesis was also quantitated by immunoprecipitation. Translation reactions (50 μl) were terminated by the addition of an equal volume of 2× sample buffer (0.16M Tris hydrochloride [pH 6.8], 4% SDS, 0.2 M dithiothreitol, 20% glycerol, 0.8% bromophenol blue) and solubilized by boiling for 5 min. Equal amounts of incorporated radioactivity (400,000 cpm) were diluted with 20 volumes of immunoprecipitation buffer (50 mM Tris hydrochloride [pH 7.5], 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1.0% Triton X-100). Preimmune rabbit sera (2 μl) and protein A-Sepharose (20 μl of a 50% solution) were added, and the samples were incubated at 4°C for 1 h with shaking. The samples were clarified by centrifugation, and the supernatants were incubated in a fresh tube overnight at 4°C with 4 μl of rabbit antisera made against purified WTV and 20 μl of a 50% protein A-Sepharose solution. The antigen-antibody complex bound to protein A-Sepharose was washed with immunoprecipitation buffer containing 0.01% SDS, released by boiling in 100 μl of 1× sample buffer, and analyzed by gel electrophoresis. Radioactive polypeptides were detected by fluorography with En^{3}Hance (New England Nuclear Corp., Boston, Mass.) autoradiography enhancer.

RESULTS

Kinetics of viral polypeptide synthesis and genome RNA accumulation in AC-20 cells inoculated with WTV. We recently reported that the synthesis of viral polypeptides in WTV-infected, cultured leafhopper cells is regulated in a coordinated and selective manner (26). Synthesis of viral-specific polypeptides reached a maximum level between 48 and 120 h p.i., representing approximately 12 to 15% of the total protein synthesized. However, the rate of viral-specific polypeptide synthesis declined relative to that of host pro-

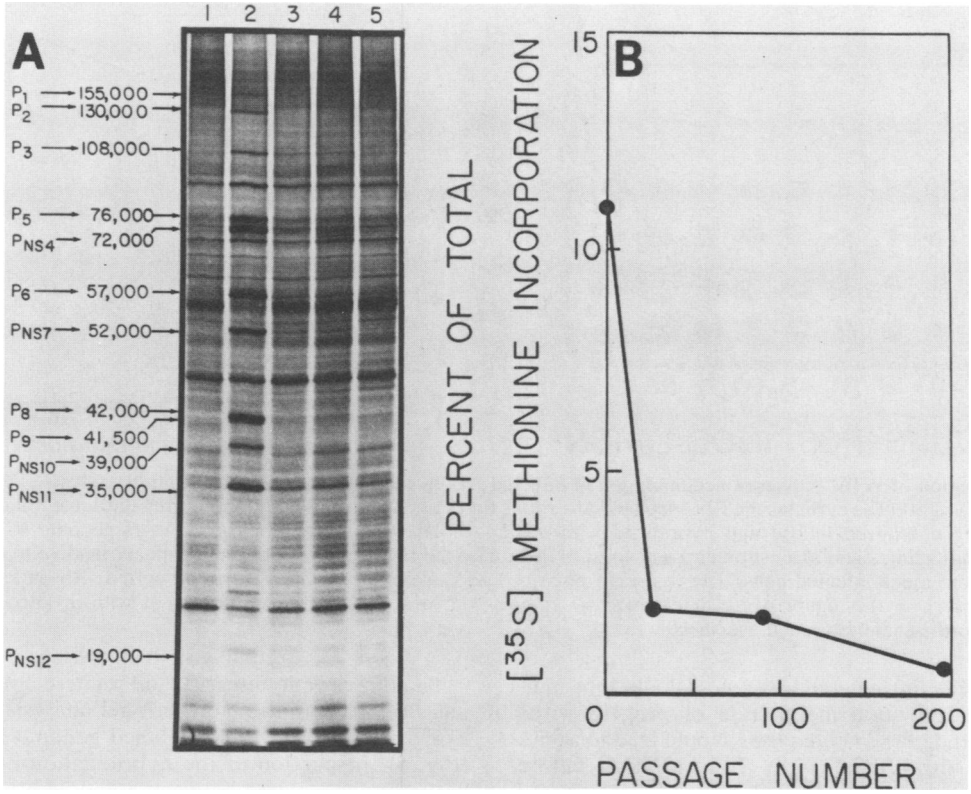

FIG. 1. Viral polypeptide synthesis in WTV-infected AC-20 cells. (A) Autoradiograph of SDS-polyacrylamide gel (12.5%) analyzing [^{35}S]methionine-labeled lysates of WTV-infected AC-20 cells. Lane 1, lysate of mock-infected cells; lane 2, lysate from infected cells pulse-labeled at 48 h p.i.; lanes 3 to 5, lysates from infected cells pulse-labeled at passages 25, 90 and 194, respectively. The location and estimated molecular weights (22) of WTV structural (P) and nonstructural (P_{NS}) polypeptides are indicated at the margins. (B) Percentage of total protein synthesis that is viral specific. The level of incorporation into viral and cellular polypeptides was determined after dissolving excised gel bands and portions of gel lanes in 15% hydrogen peroxide at 65°C overnight, followed by counting in Aquasol liquid scintillation fluid. The relative radioactivity incorporated was also quantitated by densitometry of autoradiographs (26).

tein synthesis, with the first passage of the infected culture reaching a level of approximately 5 to 20% of that occurring at 48 h p.i. by passages 10 to 15. Synthesis of viral polypeptides continued at this basal level for hundreds of subsequent passages. Gel profiles of cell lysates prepared from WTV-infected cells pulse-labeled at various times p.i. are presented in Fig. 1 to illustrate these points and to serve as a reference for subsequent experiments described in this report.

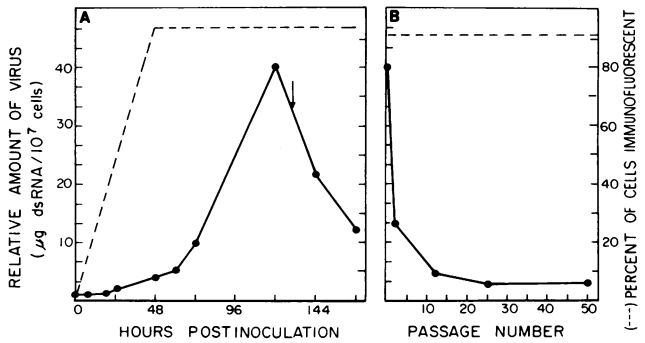

FIG. 2. Progeny virus accumulation in WTV-infected vector cells as reflected in ds genome RNA accumulation. (A) dsRNA accumulation during the acute phase of infection. Cells were passaged at 120 h p.i., as indicated by the arrow. (B) dsRNA levels in infected cells during the persistent phase of infection. In both panels, the percentage of cells in the culture that stained positive following fluorescent antibody staining is indicated (-----).

The relationship between viral-specific polypeptide synthesis and virus replication was determined by quantitating the accumulation of ds genome RNA, a function of progeny virus formation (14), with time after inoculation (Fig. 2). The intracellular concentration of genome RNA increased slightly between 36 and 72 h p.i., followed by an exponential rise to a maximum of 40 μg/10^7 cells at 120 h p.i. As in the case of viral-specific polypeptide synthesis, the concentration of genome RNA declined with the first passage of the infected culture and between passages 10 and 15 reached a basal level of approximately 5% of the value at 120 h p.i. The titer of virus recovered from acutely (48 h) and persistently (passage 50) infected AC-20 cells differed by 1 log, which was consistent with the observed decrease in the genome RNA concentration during the persistent phase (data not shown; A. J. Peterson, Ph.D. thesis, State University of New York at Albany, 1986). Thus, except for the expected initial lag in genome RNA accumulation, the synthesis of viral-specific polypeptides and the production of progeny virus exhibited similar kinetics. By analogy to human reovirus (13, 16, 29, 39), the synthesis of WTV genome RNA may be quantitatively related to viral protein synthesis.

Genome RNA was not detected in the culture fluid, indicating that most of the progeny remains cell associated. Thus, the observed reduction in intracellular virus concentration appears to be due to the dilution of virus particles

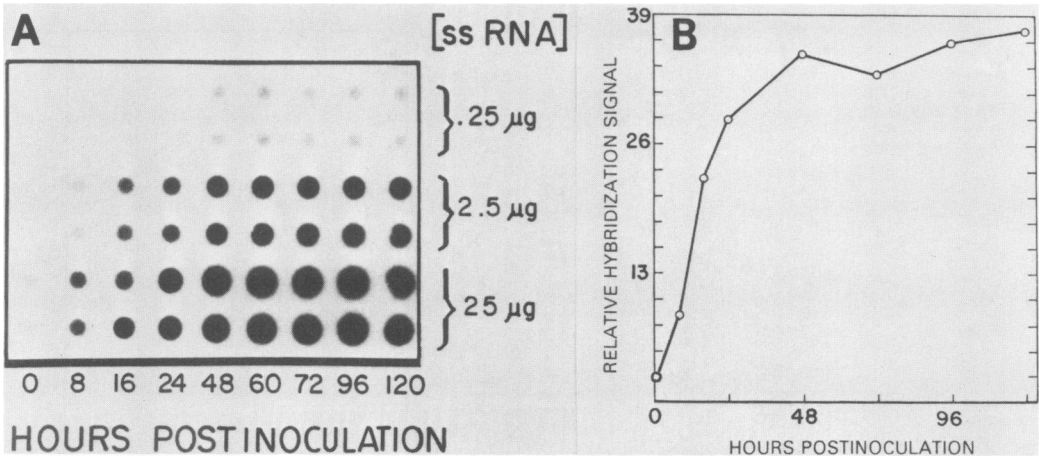

FIG. 3. Determination of WTV transcript accumulation in infected AC-20 cells by dot blot hybridization analysis. (A) Three concentrations (indicated at the right) of ss cytoplasmic RNA isolated at various times (indicated at the bottom) during the acute phase of infection were spotted in duplicate onto nitrocellulose and hybridized with melted ^{32}P-labeled, 3'-end-labeled WTV ds genome RNA in excess. (B) Quantitation of hybridization signal. Densitometry was used to quantitate the hybridization signals in panel A relative to the signal obtained for RNA isolated from mock-infected cells. The first time point (○) was taken after a 2-h adsorption period. Absolute concentrations of ssRNA (data not shown in this figure) were determined by comparison to a standard curve generated with in vitro-synthesized WTV transcripts applied to the same nitrocellulose sheet.

among daughter cells during cell division. Calculations indicated that a 95% reduction in the rate of progeny virus production at the end of the acute phase would lead to a 95% reduction in the intracellular virus concentration before passage 10. It is noteworthy that gel electrophoretic patterns of genome RNA isolated from acutely and persistently infected cells were identical with respect to relative concentrations and migration positions of individual genome segments (Peterson, Ph.D. thesis).

Accumulation of viral transcripts. For members of the family *Reoviridae*, viral transcripts function both as mRNA and as a template for negative-strand synthesis (14). Consequently, the changes in the rate of viral polypeptide synthesis and progeny virus accumulation may reflect changes in the level of viral transcripts as the infection progresses. The rate at which WTV transcripts accumulate in infected vector cells was initially determined by dot blot hybridization analysis. Equal amounts of ss cytoplasmic RNAs isolated from infected cells at different times p.i. were spotted onto nitrocellulose membranes and probed with purified, denatured WTV genome RNA labeled at the 3′ termini with [^{32}P]pCp. Hybridization signals were quantitated by densitometry. Viral transcripts were readily detectable by 8 h p.i. (Fig. 3) and increased in concentration in a linear fashion, reaching a maximum level by 48 h p.i. The concentration of viral transcripts was maintained at this level for the remainder of the acute phase.

Similar analysis of RNA isolated from persistently infected cells revealed that the concentration of viral transcripts decreased by only 5 to 20% by passage 15 and approximately 50% by passage 50 (Fig. 4). Thus, persistently infected vector cells that synthesized very low levels of viral polypeptides (passage 25; Fig. 1A, lane 3) contained only slightly lower concentrations of viral transcripts than acutely infected cells which synthesized large quantities of viral polypeptides (48 h p.i.; Fig. 1A, lane 2). It should be noted that quantitation of viral transcripts by the dot blot hybridization method is not complicated by possible contamination by ds genome RNA because genome RNA is not retained by nitrocellulose filters unless it is denatured prior to spotting (Peterson, Ph.D. thesis).

To compare the integrity and relative amounts of individual viral transcripts, Northern gel analysis was performed, using denatured [^{32}P]pCp-labeled genome RNA as a probe (Fig. 5). Inspection of the hybridization patterns for RNA preparations isolated between 8 and 24 h p.i. indicated that certain genome segments may be preferentially transcribed during the early stage of the infection. However, there were no apparent differences in the migration positions and little difference in the relative amounts of individual viral transcripts present in RNA preparations obtained from acutely (48 to 120 h p.i.) or persistently infected vector cells. Moreover, the migration positions of the transcripts isolated from infected cells were coincident with the positions of in vitro-synthesized viral transcripts, indicating that they represent intact transcripts (23).

To verify the results obtained with end-labeled genome RNA as the probe, Northern blots were hybridized with M13 phage DNA containing inserts corresponding to the negative

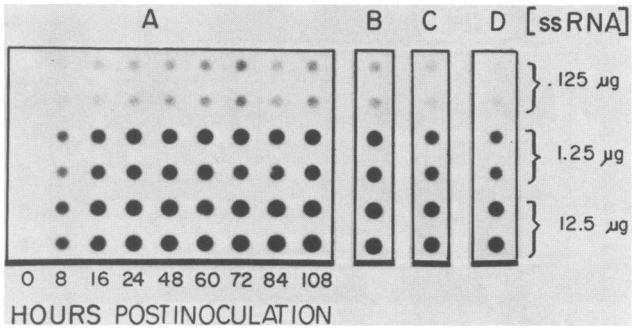

FIG. 4. Determination of WTV transcript accumulation in acutely and persistently infected AC-20 cells by dot blot hybridization analysis. To compare the level of transcripts in acutely and persistently infected cells, ss cytoplasmic RNA was isolated at various times from cells of one infected culture that was subcultured a total of 50 passages after inoculation. The RNA preparations were analyzed as described in the legend to Fig. 3. (A) RNA prepared during the acute phase. (B to D) RNA prepared from cells passaged 2, 15, and 50 times, respectively.

strands of WTV genome segments S11 and S7 (1). Transcripts derived from these two gene segments exhibited identical migration positions and were present in similar relative amounts in RNA preparations obtained from infected vector cells at 48 h p.i. and at passages 4, 8, 12, 16 and 20 (Fig. 6).

Functional activity of viral RNA isolated from infected AC-20 cells. Compared with AC-20 cells acutely infected with WTV, persistently infected cells synthesized very low levels of viral-specific polypeptides, even though viral transcripts were present at similar levels in the acute and early stages of the persistent phases of infection. To compare the functional activities of viral transcripts present in acutely and persistently infected cells, RNA samples (including the preparations that were examined by Northern hybridization analysis [Fig. 5 and 6]) were tested for the ability to direct the synthesis of viral polypeptides in cell-free translation systems. Because WTV transcripts are not selectable by oligo(dT)-cellulose chromatography (Fig. 7 of reference 1), total ss cytoplasmic RNA preparations were added to the translation systems at subsaturating levels. It must be noted that each RNA preparation contained the same concentration of viral transcripts, as determined by Northern blot analysis (Fig. 5 and 6). Each RNA preparation stimulated

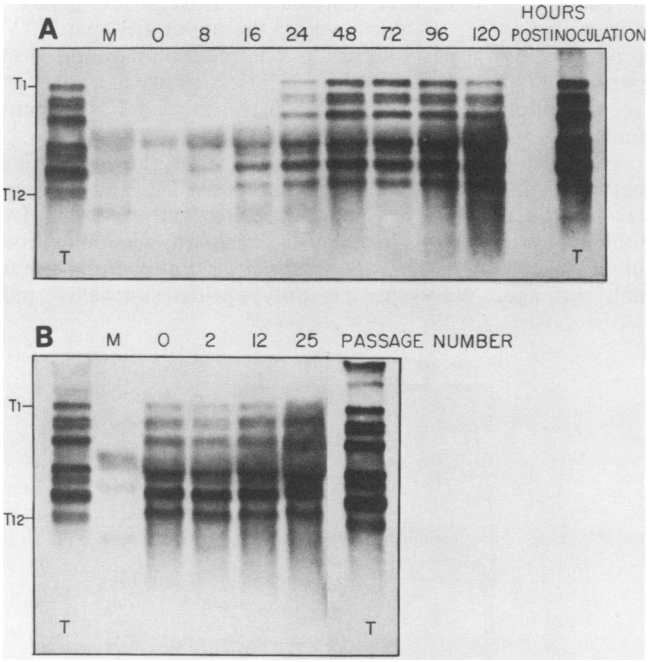

FIG. 5. Northern gel analysis of cytoplasmic RNAs isolated from acutely and persistently infected AC-20 cells. As described in the text, 25-μg quantities of ss cytoplasmic RNA isolated from WTV-infected cells were denatured, electrophoresed on a 1.75% agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized to denatured ^{32}P-labeled, 3'-end-labeled genome RNA. (A) RNAs prepared from cells during the acute phase at the times postinoculation indicated at the top of the panel. (B) RNAs prepared from subcultured infected cells at the passage number indicated at the top of the panel. RNA in the lane marked 0 was extracted at 48 h p.i. For both panels, the lanes marked M contain RNA extracted from mock-infected cells, while the lanes marked T contain in vitro-synthesized WTV transcripts. The migration positions of WTV transcripts T1 and T12 (23) are indicated at the left. Blank lanes between the lanes marked 25 and T in panel B were excised. The typical shadowing patterns resulting from the presence of abundant quantities of rRNA can be seen in all gel lanes.

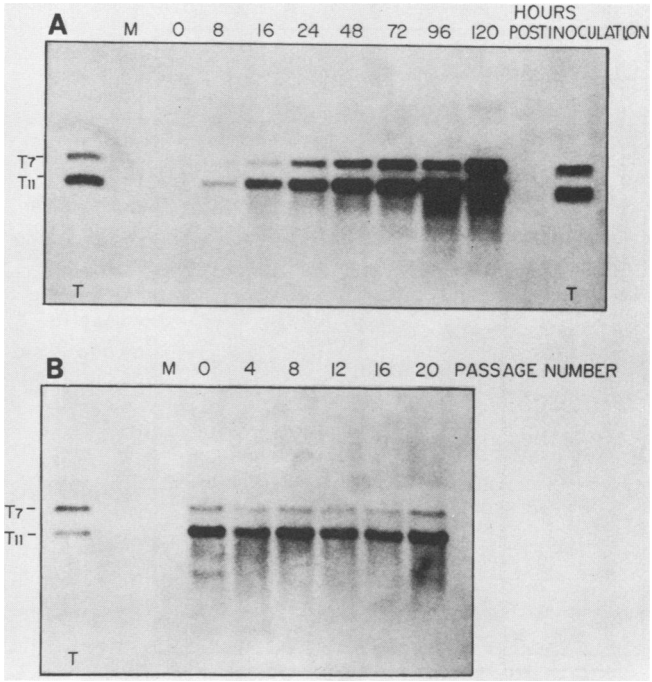

FIG. 6. Northern gel analysis of cytoplasmic RNAs prepared from infected AC-20 cells, with ^{32}P-labeled M13 clones containing inserts corresponding to the negative strand of WTV gene segments S7 and S11 used as probes. The migration positions of WTV transcripts T7 and T11 are indicated at the left. Symbols are the same as described in the legend to Fig. 5. The two labeled probes used in this analysis had different specific radioactivities.

[^{35}S]methionine incorporation to a similar extent (Peterson, Ph.D. thesis). Gel profiles of the translation products synthesized in response to the ss cytoplasmic RNA preparations were similar to the profiles of lysates prepared from pulse-labeled infected cells (Fig. 7). That is, the relative levels of viral-specific polypeptides synthesized in response to RNA prepared from acutely and persistently infected cells (compare lane 6 with lanes 7 through 9, Fig. 7) were reflective of the relative levels of viral polypeptides found in lysates prepared from these two populations of cells after pulse-labeling (lanes 2 and 3, Fig. 7). Similar results were obtained with the wheat embryo translation system (Peterson, Ph.D. thesis). Mixing experiments failed to detect any inhibitory effect by added RNAs isolated from persistently infected cells on cell-free translation of in vitro-synthesized WTV transcripts. In some experiments a slight reduction in the synthesis of polypeptides of M_r > 100,000 was observed when the translation system was programmed with RNA isolated from persistently infected cells. However, this effect occurred only with the reticulocyte lysate.

The difference in translational activities of the viral transcripts was quantitated by immunoprecipitation of labeled WTV polypeptides from in vitro translation reactions programmed with equal amounts of viral transcripts prepared from acutely or persistently infected cells. Under the conditions used, P6, a 57,000-molecular-weight component of the viral core (21), was the viral polypeptide precipitated in greatest quantity. The anti-WTV antisera precipited 10- to 15-fold more labeled WTV polypeptides from the translation reaction programmed by transcripts prepared from acutely infected cells than from reactions programmed by an equal concentration of transcripts prepared from persistently in-

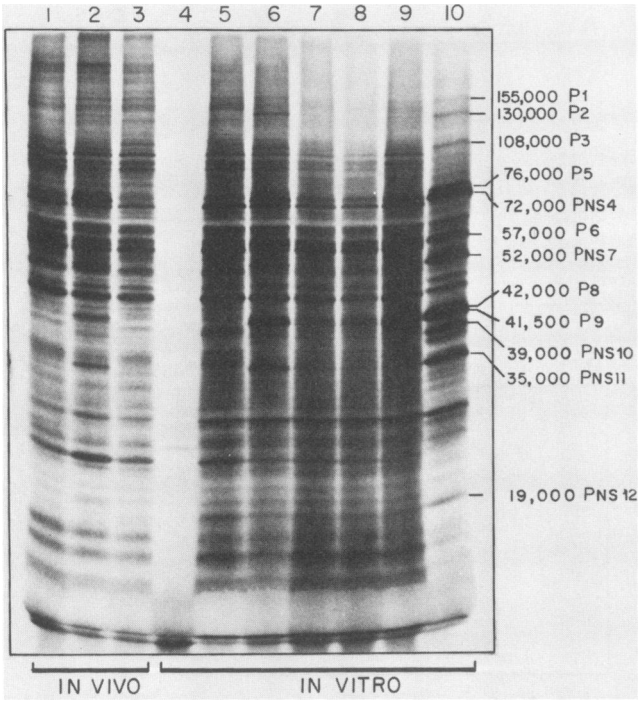

FIG. 7. In vitro translation of RNAs isolated from acutely and persistently infected AC-20 cells. Nuclease-treated reticulocyte translation reactions were programmed with cytoplasmic RNA (200 μg/ml) prepared from mock-infected or WTV-infected AC-20 cells. Reaction volumes containing equal amounts of incorporated [^{35}S]methionine were analyzed on 12.5% SDS-polyacrylamide gels. For comparison lysates were prepared from pulse-labeled mock-infected cells (lane 1), acutely infected (48 h p.i.) cells (lane 2), and persistently infected (passage 150) cells (lane 3). Lanes 4 to 9 contain in vitro translation reactions programmed with no RNA (lane 4); RNA from mock-infected cells (lane 5); RNA from acutely infected cells (48 h p.i.) (lane 6); or RNA from persistently infected cells extracted at passage 4 (lane 7), passage 8 (lane 8), or passage 12 (lane 9). Lane 10 contained in vitro translation reactions programmed with 3 μg of in vitro-synthesized WTV transcripts per ml. Bands in lane 10 not identified as viral polypeptide are the result of early termination during translation of viral mRNAs and apparent minor contamination of the WTV transcripts with host mRNAs (22). The migration positions and estimated molecular weights of WTV primary gene products are indicated at the right.

fected cells at various stages of subculturing (Fig. 8). The results suggest that the translational activity of WTV transcripts changes as the infection of AC-20 cells progresses from the acute to the persistent phase.

DISCUSSION

Information concerning the molecular biology of the reovirus multiplication cycle has been obtained primarily from studies of human reovirus replication in mouse L cells (see references 14 and 38 for recent reviews). Parental (infecting) virus particles are activated during uncoating and begin transcription of the dsRNA genome producing ssRNAs with messenger sense polarity. The viral transcripts must serve two functions: (i) as mRNA for the production of viral polypeptides by host ribosomes and (ii) as template for minus-strand synthesis, yielding progeny genome dsRNA. The latter process takes place within nascent subviral particles. Once dsRNA synthesis is completed, progeny subviral particles actively synthesize additional transcripts and are responsible for the synthesis of the majority of viral transcripts during the infectious cycle.

The kinetics of WTV macromolecular synthesis in AC-20 vector cells during the acute phase of infection (Fig. 9) were generally consistent with predictions vis-à-vis the human reovirus replication cycle model. WTV-specific protein synthesis and mRNA accumulation commenced and reached a maximum level with similar kinetics (Fig. 1, 3, and 9). Progeny genome dsRNA began to accumulate at a time (36 to 72 h p.i.) when viral protein synthesis and transcript accumulation were already proceeding at a high rate (Fig. 2). Between 72 and 120 h p.i. WTV genome dsRNA accumulation increased at a linear rate, reaching a maximum level by the end of the acute phase. Because human reovirus progeny subviral particles actively synthesize transcripts in infected cells, the rates of reovirus transcript accumulation and progeny genome dsRNA accumulation peak at approximately the same time (16, 29, 39). In contrast, the rate of WTV transcript accumulation reached a plateau before the peak of genome dsRNA (equivalent to progeny subviral particles) accumulation. The observed change in the relative concentrations of WTV transcripts and dsRNA between 72 and 120 h p.i. indicates that a significant portion of the transcript pool is used as a template for the synthesis of genome RNA during the later stage of the acute phase. Close inspection of the relative kinetics of transcript and dsRNA accumulation (Fig. 9) also suggests the possibility that WTV progeny subviral particles are less active than parental viral particles in the synthesis of transcripts; otherwise, transcript accumulation would continue to increase as dsRNA accumulated.

By the end of the acute phase of infection, the similarities between the multiplication cycle of WTV and human reovirus become fewer because infection of mouse L cells by human reovirus results in cell lysis and death, while infection of AC-20 cells by WTV is noncytopathic. Following the first cell passage, WTV-specific polypeptide synthesis and

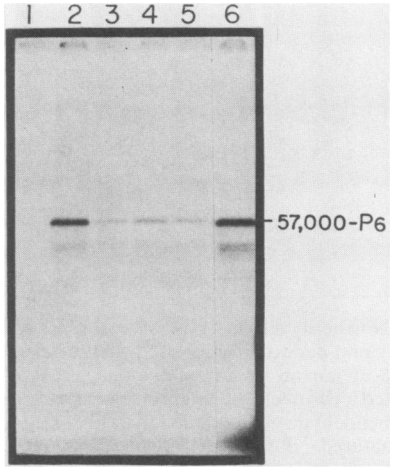

FIG. 8. Immunoprecipitation of in vitro translation reactions programmed with RNAs isolated from WTV-infected AC-20 cells. Gel lanes contain immunoprecipitates of in vitro translation reactions programmed with identical amounts (200 μg/ml) of cytoplasmic RNA isolated from mock-infected cells (lane 1); acutely infected cells (48 h p.i.) (lane 2); persistently infected cells at passage 4 (lane 3), passage 8 (lane 4), and passage 12 (lane 5); and in vitro-synthesized WTV transcripts (lane 6). The migration position and estimated molecular weight of WTV gene product P6 is indicated at the right.

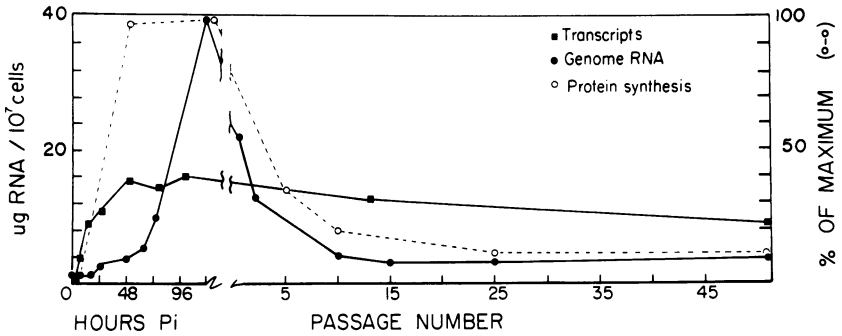

FIG. 9. Summary of the kinetics of viral macromolecular synthesis and accumulation in AC-20 cells acutely and persistently infected with WTV. Viral-specific polypeptide synthesis (○) is presented as percentage of maximum synthesis. Viral transcript (■) and genome RNA (●) accumulation is presented as micrograms per 10^7 cells.

genome RNA accumulation declined, reaching a basal level within 10 to 15 passages. A contributing factor to the decline in viral polypeptide synthesis appears to be a change in the functional activity of the pool of viral transcripts. The results of dot blot and Northern blot hybridization analysis indicate that intact viral transcripts are present in persistently infected cells (passages 10 to 25) at nearly the same concentration as in acutely infected cells (Fig. 5 and 6). However, the transcripts isolated from persistently infected cells were functionally less active in in vitro translation systems than were transcripts isolated from acutely infected cells (Fig. 7 and 8). The decline in the rate of viral polypeptide synthesis, in turn, may contribute to the decline in viral genome RNA accumulation if its synthesis is dependent on continued viral protein synthesis, as has been reported for human reovirus (13, 16, 29, 39).

Millward and co-workers have reported (34, 35, 40) that transcripts synthesized late in the infection by human reovirus progeny virions lack a 5′-terminal cap, while transcripts synthesized by infecting parental virions have a cap. Furthermore, these investigators reported that as the infection proceeds, mRNA translation shifts from a cap-dependent to cap-independent mode (33). It will be interesting to see whether the apparent reduction in functional activity of WTV transcripts associated with the persistent phase of infection involves a modification of the 5′ terminus of viral transcripts.

Information concerning virus-cell interactions and viral gene expression comes predominantly from studies of lytic viral infections. The interaction of WTV with cells of its insect vector differs significantly from the interaction between lytic viruses and their cell hosts. WTV replicates to a high titer (estimated as 1.2×10^5 virus particles per cell; from data shown in Fig. 2) without negatively affecting cellular macromolecular synthesis or integrity. It is tempting to speculate that during the evolution of this relationship it became necessary for the virus to down-regulate the expression of its genome to ensure the survival of its vector, thus increasing its chances for survival. It may be possible, once the regulatory mechanisms are understood, for this strategy to be extended to other biological systems for the purpose of manipulating gene expression.

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