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Molecular Analysis of Viral RNAs in Mice Persistently Infected with Lymphocytic Choriomeningitis Virus†

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Infection of newborn mice with lymphocytic choriomeningitis virus (LCMV) results in a lifelong persistent infection. Persistently infected animals continuously produce low levels of infectious virus and accumulate large amounts of intracellular viral nucleic acid (P. J. Southern, P. Blount, and M. B. A. Oldstone, Nature [London] 312:555–558, 1984). We have used gel electrophoresis and hybridization techniques to analyze viral RNAs that appear during the establishment and maintenance of a persistent LCMV infection in vivo to identify any role for defective and/or defective interfering RNAs. We have found a complex, heterogeneously sized population of viral RNAs in multiple independent tissues that is uniquely associated with persistent infections in vivo, but we have not yet established whether these RNAs have a causal or a consequential association with persistent infection by LCMV. Within the complex virus RNA population, full-length genomic L and S RNAs were readily detectable and represented the most abundant individual viral RNA species. RNAs apparently corresponding in size to the viral nucleoprotein and glycoprotein mRNAs could also be detected in these tissue RNA samples. The presence of glycoprotein mRNA indicates a potential mechanism of posttranscriptional regulation to account for the previously documented restriction in viral glycoprotein expression in persistently infected mice (M. B. A. Oldstone and M. J. Buchmeier, Nature (London) 300:360–362, 1982).

Newborn mice that are infected within the first 24 h of life with lymphocytic choriomeningitis virus (LCMV) develop a lifelong persistent infection and may succumb, in later life, to LCMV-associated disease (reviewed in references 3, 15, and 20). This infection is characterized by an initial phase of virus replication that lasts for 5 to 7 days and is followed by a gradual reduction in the titer of infectious virus. Low levels of infectious virus can be recovered from serum and tissue samples throughout life. Viral nucleoprotein (NP) is readily detectable within tissues of persistently infected mice, but surface expression of the viral glycoproteins (GP) is markedly reduced relative to that during an acute infection (3, 21, 37). Recently, in situ hybridization studies with whole body sections from mice have documented the appearance and maintenance of viral nucleic acid sequences in multiple major tissues during the course of persistent LCMV infection (29). Initially, when virus titers are highest, the viral nucleic acid sequences are at or below the limits of sensitivity for detection by in situ hybridization, but at later times there is a significant accumulation of viral nucleic acid in the infected animals, despite a marked reduction in the amount of infectious virus that can be recovered.

There is an extensive literature on persistent LCMV infection of cell culture systems and mice that documents the generation of a class of virus particles that are noninfectious but are able to interfere with infection by standard virus (3, 5, 12, 13, 15, 17, 23, 34–38). There has not been a complete molecular description of (defective) interfering LCMV particles (17, 32), but novel subgenomic RNAs arising during persistent infection with related arenaviruses have been described (6, 9). It is unknown whether the defective viral RNAs (i.e., deleted species) that have been described for other viral systems (2, 11, 14, 22, 24, 33) during persistent infection of cell cultures (for example, vesicular stomatitis virus, influenza virus, and Sendai virus) have any counterpart in persistent LCMV infection in animals. This paper complements and extends the previous data obtained from in situ hybridization studies by analyzing the size and polarity of viral RNAs present during persistent infection. These experiments were dependent upon the recent development of hybridization probes to monitor replication and transcription of the viral genomic L and S RNAs during infection (28, 30).

MATERIALS AND METHODS

Virus stocks and animal infections. A triple plaque purified clone (53B) of LCMV Armstrong CA1371 was used in all experiments. Virus stocks derived from the first or second passage after plaque purification were used to initiate all infections. Adult BALB/c mice (approximately 6 to 8 weeks old) were each infected by intracranial injection of 10^6 PFU; Newborn BALB/c mice (less than 24 h old) were each infected by intracranial injection of 1,000 PFU.

RNA purification from mouse tissues. Total intracellular RNA was isolated by a modification of the method of Chirgwin et al. (4). Mouse organs (brains, livers, lungs, kidneys, spleens, and blood) were removed aseptically and snap-frozen in liquid nitrogen. Single organs were placed in 2 ml of GTC (4 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% Sarkosyl [CIBA-GEIGY Corp.], 0.1M β-mercaptoethanol) and immediately homogenized with a Tekmar tissue homogenizer. Additional GTC was added, and then the thick, viscous solution was vortexed for approximately 45 to 60 s, layered over 2 ml of DEP-treated 5.7 M cesium chloride (Sigma Chemical Co.)–10 mM EDTA, and centrifuged for 16 h at 35,000 rpm at 18°C in an SW41 rotor in a Beckman ultracentrifuge. The RNA pellet was washed with 70% ethanol and then repelleted.

Nucleic acid concentration. Purified RNA pellets were suspended in sterile water, and the concentration and purity were determined by A_{260}, A_{280}, and A_{320} measurements. Additionally, the amount and integrity of rRNA were as-

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sessed by electrophoresis of total intracellular RNA on nondenaturing 1% agarose minigels.

RNA electrophoresis. RNA was denatured with 1 M glyoxal–50% dimethyl sulfoxide as previously described (16, 30). A standardized amount of total intracellular RNA (5 or 10 μg) was electrophoresed on a 1.5% agarose gel in 10 mM NaH_2PO_4–Na_2HPO_4 (pH 6.5) (18). Electrophoresis was performed at 14 mA constant current (ca. 2.5 V/cm) for 16 h with continual recirculation of the phosphate buffer.

RNA transfer and hybridization conditions. RNA was transferred from agarose gels to nitrocellulose or Nytran filters (Schleicher & Schuell, Inc.) under standard conditions (31). The filters were air dried and baked for 2 h at 80°C. Prehybridization and hybridization with nick-translated probes were performed by using 50% formamide (deionized by treatment with Bio-Rad AG 501-X8)–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–2.5× Denhardt solution–50 mM phosphate buffer (pH 6.5)–0.1% sodium dodecyl sulfate–100 μg of sonicated salmon sperm DNA per ml for 24 and 48 h, respectively. Hybridization with radiolabeled RNA probes was performed for 24 h at 55°C.

After hybridization, the filters were washed in 2× SSC–0.1% sodium dodecyl sulfate for 30 min at 37°C and in 0.1× SSC–0.1% sodium dodecyl sulfate for 30 min at 55°C. The wash in 0.1× SSC–0.1% sodium dodecyl sulfate was repeated for 30 min at a higher temperature than 55°C if indicated by excessive background.

Nitrocellulose strips with serial fivefold dilutions of LCMV cDNA sequences were included in the experimental hybridization reactions to provide a means of quantitating virus-specific sequence in the total tissue RNA samples.

Synthesis of hybridization probes. Purified LCMV cDNA sequences were labeled with [α-^{32}P]dATP and [α-^{32}P]dCTP to a specific activity of $\geq 2 \times 10^8$ cpm/μg by the procedure of Rigby et al. (25). Single-stranded RNA probes were synthesized in the presence of [α-^{32}P]UTP by using the SP6 promoter and polymerase system (19). All LCMV hybridization probes contained exclusively LCMV sequences. The SP64 vector containing a rat actin cDNA insert was generously provided by J. Nelson.

Isolation of viral ribonucleoprotein (vRNP) complexes. Viral ribonucleoprotein particles (vRNP) were isolated from brains of persistently infected BALB/c mice by a modification of the method used by Howard and Buchmeier for purification of LCMV vRNP from disrupted virions (10). Four brains from 1-month-old persistently infected BALB/c mice were placed in phosphate-buffered saline on ice and homogenized with a B Dounce in inhibitor buffer (10 mM Tris hydrochloride [pH 7.4], 20 mM NaCl, 10 mM EDTA [pH 8.0], 10 mM sodium bisulfate, 1 mM phenylmethylsulfonyl fluoride, 1 mM pepstatin A, 500 KIU of aprotinin per ml, 400 U of RNasin [Promega Biotec] per ml, 0.5% Nonidet P-40). Disruption of cells was confirmed by light microscopy. Particulate debris and nuclei were pelleted by centrifugation at 2,500 rpm in a Sorvall RT6000 centrifuge, and the supernatant (cytosol fraction) was removed. A sample of the cytosol was denatured with GTC, and the RNA was extracted (see above). The cytosol was layered over a 10 to 60% Renografin gradient (E. R. Squibb & Sons) and centrifuged at 4°C for 16 h at 35,000 rpm in an SW41 rotor. vRNP complexes were localized by centrifugation (in parallel) of intact and disrupted (vRNP) ^{35}S-radiolabeled virus on continuous Renografin gradients. Addition of cytosol fractions from tissues did not alter the gradient localization of virus or vRNP. The gradients were fractionated in 1.0-ml aliquots by dripping after bottom puncture, and RNA was recovered by two phenol extractions and one chloroform extraction of the gradient fractions. The RNA was ethanol precipitated with 30 μg of yeast tRNA added as carrier and analyzed by denaturing agarose gel electrophoresis.

RESULTS

Hybridization probes to study LCMV gene expression. cDNA cloning, hybridization experiments, and nucleotide sequencing information from several laboratories have established that the arenavirus genomic S RNA segments contain ambisense coding arrangements (1, 27, 30; summarized in Fig. 1). We have used multiple, independent cDNA clones as specific hybridization probes for either the L or S genomic RNA segments to examine LCMV gene expression

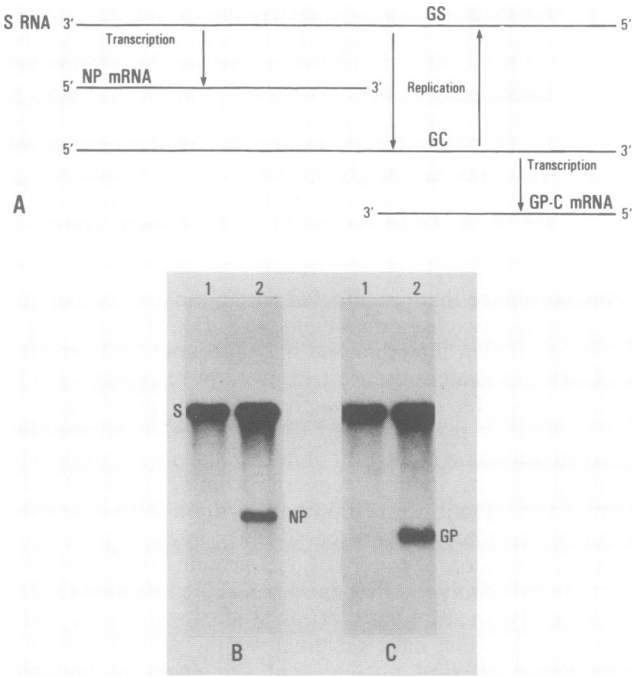

FIG. 1. Replication and transcription of LCMV during acute infection. (A) Diagrammatic representation of the proposed replication and transcription strategy of the LCMV S segment. Virion RNA (genomic sense, GS) enters the cell, and NP mRNA is transcribed from this template. Synthesis of the full-length genomic-complementary-sense RNA (GC) provides an obligatory intermediate for replication of genome RNA and a template for transcription of GP mRNA. The intergenic region, between the NP- and GP-coding regions, has a palindromic sequence which could potentially act as a transcription terminator (1, 30). (B and C) LCMV gene expression from the S segment in acutely infected BHK cells and in virions. This provides a basis for comparison with acute and persistent infection in mice. Total intracellular RNA was extracted from virions and from BHK cells acutely infected with LCMV (multiplicity of infection, 5) at 48 h postinfection and analyzed by Northern (RNA) blot. Hybridization with an NP region nick-translated probe (panel B) demonstrates a signal from the S segment in virions (lane 1) and acutely infected BHK cells (lane 2) and a single, well-defined subgenomic RNA (NP mRNA) in acutely infected BHK cells (lane 2). The hybridization signal was completely stripped, and the filter was rehybridized with a GP region nick-translated probe (panel C), which reveals signal from the S segment in virions (lane 1) and acutely infected BHK cells (lane 2) and a single, well-defined subgenomic RNA (GP mRNA) of slightly lower molecular weight than that of the NP mRNA in acutely infected BHK cells (lane 2).

during infection. Figures 1B and C show typical results in the analysis of acutely infected BHK-cell RNA samples with NP and GP coding-region-specific S probes. Hybridization with either probe reveals a well-defined pattern of viral RNAs.

Viral gene expression in persistently infected mice. The in situ hybridization experiments with whole-body sections had established that viral nucleic acid sequences rapidly accumulate during the first 2 weeks after infection of newborn mice and then are retained throughout the course of a life-long persistent infection (29). Because of the complex gene-coding arrangement within the S RNA segment (Fig. 1), it has not been possible to distinguish between replication products and transcription products by in situ hybridization. Therefore, we undertook an examination of different tissue RNA samples by using the techniques of gel electrophoresis, nucleic acid transfer, and hybridization to assess the sequence content of viral RNA species (Fig. 2). The main findings of this analysis can be summarized as follows: (i) genomic-sized S and L (data not shown) segment RNAs are the predominant individual RNA species; (ii) RNAs of similar size to the NP mRNA (not shown) and the GP mRNA are detected; (iii) there is a heterogeneous signal from (mostly) subgenomic RNA species that is first detectable 7 to 14 days postinfection; and (iv) the intensity of signal increases dramatically during the first 2 weeks postinfection. These results with kidney RNAs (Fig. 2) are representative of results with RNA from other organs (livers, spleens, lungs, and brains) that have been examined (data not shown).

Viral gene expression in acutely infected mice. Intracranial infection of adult mice with LCMV results in extensive virus replication in the infected animals and virus titers in the range of 10^6 to 10^7 PFU/g of tissue or PFU/ml of serum (3). We were interested in analyzing RNA samples taken during acute in vivo infection (7, 8) to compare with acute infections in vitro (Fig. 1) and persistent infection in vivo (Fig. 2). We found that the genomic-sized L and S RNAs and the subgenomic NP and GP mRNAs were readily detectable in total tissue RNA extracted from acutely infected mice and that these RNAs accumulated over time until the animals died 6 to 9 days postinfection (Fig. 3). Viral gene expression during acute in vivo infection therefore resembles that during the acute infection in vitro. This suggests that the complex, heterogeneously sized viral RNAs detected during persistent in vivo infection (Fig. 2) are linked to the persistent state and are not simply a consequence of any in vivo infection.

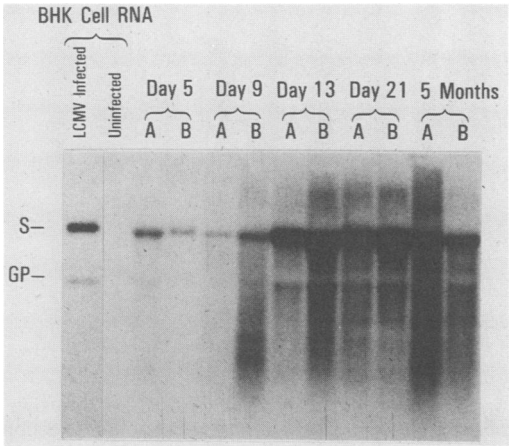

FIG. 2. LCMV gene expression in kidneys of persistently infected mice. Total intracellular RNA extracted from kidneys of persistently infected mice at 5, 9, 14, and 30 days and 5 months postinfection was size fractionated by agarose gel electrophoresis under denaturing conditions. After transfer of the RNAs to a nitrocellulose filter, hybridization was performed with a radiolabeled, nick-translated probe from the GP-coding region of the S segment. Two mice (mice A and B) were sacrificed at each time point, and uninfected and (acutely) infected BHK-cell RNA samples were included as negative and positive controls, respectively. The genomic S segment (S) and GP mRNA (GP) are indicated.

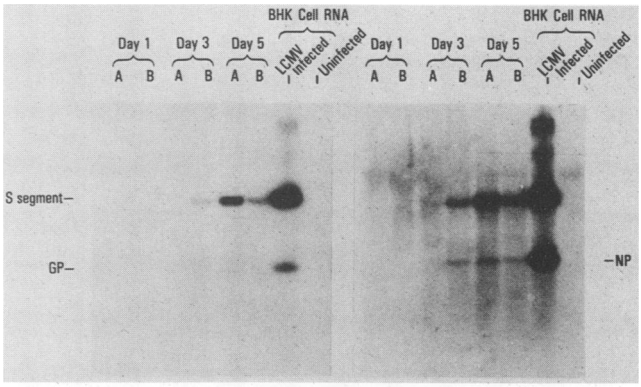

FIG. 3. LCMV gene expression in brains of acutely infected mice. RNA extracted from the brains of two acutely infected mice (mice A and B) at days 1, 3, and 5 after intracerebral infection with 10^6 PFU of LCMV was analyzed by gel electrophoresis, transfer, and hybridization (see Materials and Methods and the legend to Fig. 2 for details). The filter was hybridized with a nick-translated probe from the GP-coding region (left panel) and then stripped and rehybridized with a nick-translated probe from the NP-coding region (right panel).

Strand-specific hybridization analysis of viral RNA extracted from persistently infected tissues. As an extension to the analysis of the intracellular viral RNAs that appear during persistent infection, we have used single-stranded RNA probes to distinguish between genomic-sense and genomic-complementary-sense RNAs. To derive meaningful comparisons, we have either hybridized the same nitrocellulose filter sequentially with different probes or produced identical filters from duplicate gels run in parallel and then hybridized the filters simultaneously. The single-stranded RNA probes were prepared in vitro by using the SP6 promoter and polymerase (see Materials and Methods) and represented the same region of the viral genome labeled either in the genomic-sense or in the genomic-complementary-sense polarity. A typical result for brain RNA hybridized with probes from the NP-coding region is shown in Fig. 4. The two complementary probes detected approximately equivalent amounts (ca. 10 ng/10 μg of total intracellular RNA by reconstruction experiments) of genomic-sized S RNA. Similar results, biased slightly in favor of genomic-sense RNAs, were obtained with a pair of strand-specific S probes from the GP-coding region and with a pair of L-specific probes (not shown).

In situ hybridization with strand-specific probes. We have used the technique of in situ hybridization to whole-body sections of persistently infected mice as an alternative method of examining the polarity of the accumulated viral RNA sequences (29). The in situ results with a pair of S probes from the NP-coding region (Fig. 5) demonstrated that there was a preferential accumulation of viral genomic-sense RNAs over genomic-complementary-sense RNAs. Given the ambisense gene coding arrangement for LCMV (Fig. 1),

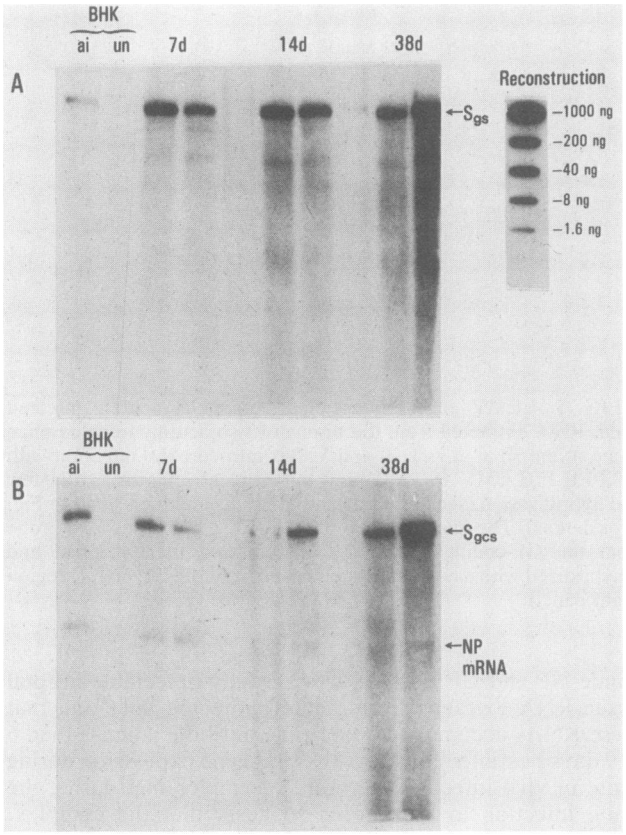

FIG. 4. Strand-specific hybridization analysis of viral RNAs extracted from the brains of persistently infected mice. Two mice were sacrificed at 7, 14, and 38 days postinfection, and RNA samples were processed as described in the text and in the legend to Fig. 2. Uninfected (un) and acutely infected (ai) BHK cell RNAs were included as negative and positive controls, respectively. The filters were hybridized with genomic-complementary-sense (panel A) and genomic-sense (panel B) single-stranded RNA probes from the NP region of the S segment. The polarities of the hybridizing genomic-sense (S_{gs}) and genomic-complementary sense (S_{gcs}) S segments and the NP mRNA are indicated. A reconstruction filter, with dilutions of LCMV cDNA sequences, was included in the hybridization reaction with the genomic-complementary-sense probe (see also Fig. 5).

in situ hybridization signals cannot necessarily be assigned to a unique viral RNA. For example, the NP genomic-sense probe will detect both NP mRNA and the full-length genomic-complementary-sense S RNA (Fig. 4B). However, any pair of strand-specific probes shows equivalent tissue distributions for the target viral RNA sequences, with the strongest signals corresponding to lacrimal gland, salivary gland, kidney, liver, and brain tissue as reported previously, and illustrates the relative accumulation of genome sense over genomic-complementary-sense RNAs.

Controls to address the problem of RNA degradation. After finding complex hybridization patterns with the tissue RNA samples from persistently infected mice (Fig. 2 and 4), we were concerned about the possibility of RNA degradation during extraction and analysis. Essentially identical hybridization results were obtained when total tissue RNA was recovered by mechanical crushing of frozen tissue followed by phenol extraction instead of tissue homogenization in guanidinium thiocyanate (see Materials and Methods). Also, denaturing gels made with either formaldehyde or methyl mercury produced results indistinguishable from those obtained with the glyoxal gels presented here. We adopted two routine controls to monitor for RNA degradation: (i) assessment of the integrity of host 28S and 18S rRNA bands in nondenaturing agarose minigels, and (ii) hybridization of the nitrocellulose filters with a probe specific for actin mRNA (data not shown). In both cases, well-defined bands were interpreted as signifying intact RNA, whereas an absence of signal or excessive low-molecular-weight streaking was consistent with RNA breakdown. On the basis of results with these controls, we consider that in vitro RNA degradation is not responsible for the heterogeneous population of viral RNAs that have been detected.

Analysis of viral RNAs within intracellular RNP. Viral RNAs do not occur as free species within infected cells, but instead are tightly associated with proteins (in particular, the LNP) to form RNP. If the complex population of subgenomic RNAs present in tissues of persistently infected mice is capable of replication, we might expect to find these RNA species copurifying with intracellular RNP. Organs from persistently infected mice were processed to dissociate intact polysomes and to provide a cytosol fraction (see Materials and Methods). The cytosol fraction was centrifuged through a 10 to 60% continuous Renografin gradient, and nucleic acid was recovered from fractions corresponding to a radiolabeled RNP marker which had been centrifuged in a parallel gradient. RNA samples were separated by denaturing agarose gel electrophoresis, transferred to a nitrocellulose filter, and hybridized with an LCMV-specific probe from the NP region. The viral RNP fraction from tissue

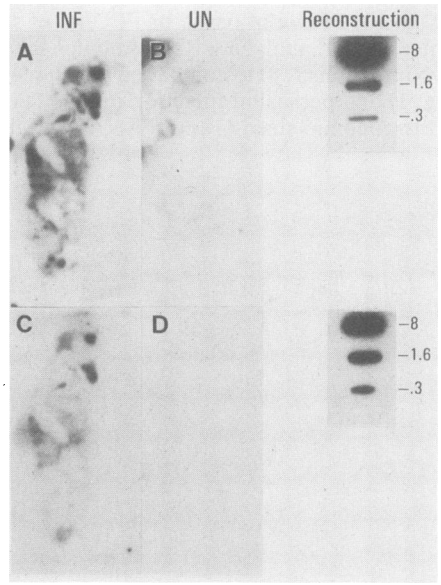

FIG. 5. Strand-specific analysis of viral RNAs by using in situ hybridization to whole-mouse sections. Whole-mouse sections from 6-week-old persistently infected and uninfected BALB/c mice were hybridized with riboprobes from the NP region of the S segment to detect genomic-sense sequences (A and B) and genomic-complementary-sense sequences (C and D). The infected and uninfected mice are designated in the figure by INF and UN, respectively. Reconstructions (serial dilutions of plasmids containing inserts with viral sequences homologous to the radiolabeled probes) are shown adjacent to the sections, with the amount of plasmid DNA indicated in nanograms. A reconstruction standard was included to determine the approximate amount of viral nucleic acid being detected and to control for any differences in the specific activity of the riboprobes.

contained subgenomic RNA species that were identical in size distribution to the RNA species present within the unfractionated cytosol preparation (Fig. 6). Subgenomic viral RNAs were not detected in other fractions of the gradient, and actin mRNA localized predominantly to less dense fractions of the gradient (unpublished observations), suggesting that the heterogeneous RNAs from tissue were present in the form of intracellular vRNP.

DISCUSSION

LCMV readily establishes a persistent infection in neonatally infected mice, adult immunosuppressed mice, and tissue culture cells (3, 15). Such persistent infections, both in vivo and in vitro, have been associated with significant reductions in the formation of infectious progeny virus and in the accumulation of viral GP at the surfaces of infected cells (21), as well as with the generation of (defective) interfering viral particles (13, 23, 37). We have performed a molecular analysis of LCMV gene expression during persistent infection to search for defective and/or defective interfering viral RNAs and have identified a complex population of heterogeneously sized viral RNAs that first appears 7 to 10 days postinfection. Viral nucleic acid sequences accumulate and are retained in most major organs during persistent infection, and heterogeneously sized RNAs are present in all tissues that have been examined (brain, liver, spleen, and kidney tissues). Apparently full-length genomic L and S RNAs have been readily detected in the total tissue RNA samples, and these genomic RNA species represent the individual most abundant L-derived and S-derived RNAs within persistently infected tissues. Viral RNAs corresponding in size to the normal NP and GP-C mRNAs (Fig. 1) have also been detected in the various tissue samples, indicating that at the level of whole organs, the phenomenon of LCMV persistence cannot simply be explained by the absence of one or more viral RNAs that are routinely found during acute infections.

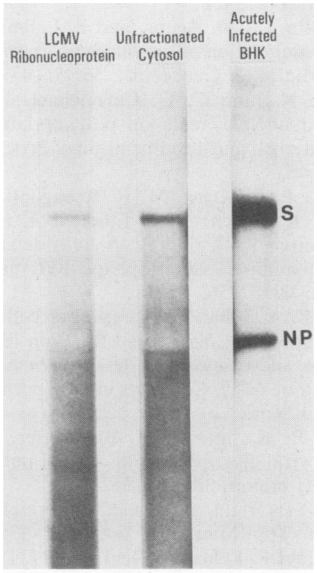

FIG. 6. Analysis of intracellular vRNP complexes. Four 1-month-old persistently infected mice were sacrificed, their brains removed, and vRNP were isolated on Renografin gradients (see Materials and Methods). RNA was extracted, electrophoresed, transferred, and hybridized with a nick-translated probe from the NP region of the S segment. RNA from acutely infected BHK cells serves as a positive control. RNA extracted from the cytosol fraction prior to centrifugation on a Renografin gradient and RNA extracted from the vRNP fraction of the Renografin gradient are shown. The S segment and NP mRNA are indicated. The heterogeneous subgenomic viral RNAs were not found in the fractions in which cellular and viral mRNAs were detected (data not shown).

To identify any unique features of LCMV gene expression during persistent infection of mice, we also used the same analytical techniques to examine LCMV gene expression during an acute infection of adult mice. There was a marked difference between the two in vivo infections, since the acute infection in mice produced a pattern of viral transcription and replication products that was indistinguishable from that produced during acute infections in tissue culture cells. This simple pattern was also observed in neonatally infected mice (animals that would become persistently infected if allowed to survive) during the first 7 days of infection. The heterogeneous population of viral RNAs has been detected only in neonatally infected mice, at least 7 to 10 days after infection at a time when the formation of infectious progeny virions has begun to diminish. Once established, the heterogeneously sized RNAs appear to be retained throughout the lifespan of persistently infected animals, and experiments are now in progress to determine the rate of turnover of the intracellular viral RNAs during persistent LCMV infection. Several independent analyses of persistently infected tissue culture cell lines have shown a limited number of well-defined subgenomic viral RNA species and no indication of the complex RNA population found in vivo (32; submitted for publication). Combining all of these observations, the heterogeneous viral RNAs appear to be specific for LCMV persistent infections in vivo, but with the present information, we cannot establish whether this is a causal or a consequential association with persistence.

Several different control experiments have demonstrated the absence of (detectable) RNA degradation during the extraction and analysis procedures. However, we have not as yet attempted to assess any process of in vivo degradation that might be contributing to the heterogeneously sized RNA population. Estimation of the intracellular half-life of vRNP complexes should indicate whether part or all of the heterogeneity is related to intracellular turnover of RNP complexes. The fact that apparently full-length genomic L and S RNAs can be recovered from tissues of persistently infected animals argues against extensive in vivo degradation. Also, the demonstration that the vRNP fraction from persistently infected tissues includes some heterogeneously sized RNAs suggests that these species may be capable of replicating. This is also consistent with the strand-specific hybridization analysis that suggested that the heterogeneous RNAs were composed of both genomic-sense and genomic-complementary-sense species, the latter being potential replication intermediates.

We have used hybridization probes that span the entire genomic S RNA segment as an initial approach to analyzing the sequence content of the heterogeneous viral RNA population. The hybridization signal was somewhat lower, but no less heterogeneous, with probes from the central region of the S segment when compared with probes from either the 5′ or 3′ terminus (data not shown). This could be explained by internal deletions that have removed various portions of the central region of the S segment but have left the termini and signals required for replication intact. We have not detected any encapsidation of the heterogeneously sized viral RNAs either by direct analysis of virion particles purified from tissue or after a single-step amplification in

vitro with BHK cell monolayers. Experiments to transfer and amplify the heterogeneously sized viral RNAs are being continued both in vivo and in vitro, using a variety of different cell types.

Some of the heterogeneity we have observed may be a reflection of multiple independent foci of infection that have developed within a given tissue. The predominant neural cell types infected (26) during acute infection (choroid plexus, ependymal epithelium, and the arachnoid cells of the meninges) differ markedly from the cells supporting persistent infection (cortical neurons, hypothalamus, and cerebellar Purkinje cells), and this may also contribute to the different patterns detected. If each focus of infection resulted in the appearance of one or a few deleted viral RNAs, as has been observed with LCMV persistence in tissue culture cells, then extraction and analysis of whole-tissue RNA would lead to superimposition of these individual deleted RNA species and creation of a highly complex population.

The detection of a distinct viral RNA of similar size to the normal GP-C mRNA in the persistently infected tissue RNA samples suggests that the regulation of GP expression observed during persistent infection cannot occur solely at the level of primary transcription. There are approximately similar amounts of this putative GP-C mRNA in acutely infected animals (either adult, fatally infected mice or neonatally infected mice within the first 7 days of infection) during the active phase of virus replication and in persistently infected animals that are producing only low levels of infectious virus. The absence of viral glycoprotein from the surface of infected cells could potentially be responsible for the failure to produce high levels of infectious virus during persistent infection and may be a critical factor in any failure of the host immune system to recognize virus-infected cells. We are currently examining whether the putative GP-C mRNA is associated with cellular polysomes in persistently infected tissues and whether the RNA extracted from tissues can be translated in vitro to learn more about the regulatory mechanisms affecting glycoprotein expression during persistent infection.

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