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The Genome of Respiratory Syncytial Virus Is a Negative-Stranded RNA That Codes for at Least Seven mRNA Species

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The RNA from purified respiratory syncytial (RS) virions and the RNAs from RS virus-infected cells were isolated and characterized. The RNA from RS virions was found to be a unique species of single-stranded RNA of approximately 5×10^6 daltons. Specific annealing experiments demonstrated that at least 93% of the virion RNA was of negative (nonmessage) polarity. Eight major and three minor species of virus-specific RNA were detected in the cytoplasm of RS virus-infected HEp-2 cells. The largest intracellular RNA species comigrated with RNA from purified virions, was not polyadenylated, and was synthesized only in the presence of concomitant protein synthesis. The seven major smaller species of RNA were synthesized in the presence of an inhibitor of protein synthesis. These RNAs were all polyadenylated and were shown to be RS virus specific by their ability to anneal specifically to purified virion RNA. The sum of the sizes of the major RS virus-specific polyadenylated RNAs was sufficient to account for the coding capacity of the RS virus genome (within the limits of reliability of the methods we have used to determine size).

Respiratory syncytial (RS) virus is the most important causative agent of serious lower respiratory tract illness in children under 2 years of age. RS virus has been classified as a paramyxovirus but has been placed in the separate genus *Pneumovirus* on the basis of its morphology and the fact that, unlike other myxo- and paramyxoviruses, it does not have a detectable hemagglutinin, hemadsorption, hemolysin, or neuraminidase activity. Despite the pathogenic importance of this virus, a clear description of its molecular biology is not available. Although it is assumed that RS virion RNA is 50S, the exact size of the genomic RNA is uncertain since previous attempts to characterize the virion RNA (8, 15, 16; E. Hoffman, E. Ford, and J. Gerin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S300, p. 262) have shown 50S, 28S, 18S, and 4S RNA species. The exact polarity of the virion RNA is also uncertain as previous reports (8) demonstrated that only 47% (above background levels) of 50S RNA could be protected from RNase digestion by hybridization with polyadenylated RNA from RS virus-infected cells. Work with temperature-sensitive mutants has identified seven (or perhaps eight) genetic complementation groups (4, 10), but, beyond this, little is known about the information content of the RS virus genome. The study of virus-specific RNAs synthesized in RS virus-infected cells has been initiated (1, 5–7, 15; D. Lambert, M. Pons, and G. Mbuy, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, T120, p. 255), but is far from complete. There has been a lack of agreement concerning both the number and the size of the RNAs observed in previous studies, and evidence that the RNAs described were RS virus specific was not presented. More significant, however, is the fact that the sizes of the RNAs reported to be mRNAs were not adequate to account for even one-half of the suspected coding potential of the genome (7).

The studies described in this communication have used the techniques of high-resolution gel electrophoretic analysis and two-phase RNA–RNA hybridization to characterize the RNA from RS virions and from RS virus-infected cells. Our results show that the genome of RS virus is a single negative-stranded RNA of approximately 5×10^6 daltons and that eight major species of RNA are synthesized in RS virus-infected cells. The largest intracellular RNA is the genome-size RNA. The seven smaller RNAs are polyadenylated, RS virus-specific mRNAs which, taken together, are of sufficient size to account for the coding capacity of the genome.

MATERIALS AND METHODS

Virus and cells. The A_2 strain of RS virus, originally provided by E. Dubovi, was propagated in monolayer cultures of HEp-2 cells in Eagle minimum essential medium supplemented with 5% heat-inactivated fetal calf serum. Viral infectivity was measured by plaque assay on monolayer cultures of HEp-2 cells.

Preparation of radiolabeled RS virus intracellular RNAs. Monolayer cultures of HEp-2 cells were infected with RS virus at a multiplicity of infection of 1 PFU per cell. After 2 h of adsorption at 37°C, fresh Eagle minimal essential medium supplemented with 5% heat-inactivated fetal calf serum was added. At 14 h postinfection (p.i.), the cells were treated with 5 μg of actinomycin D per ml and, in some experiments, cycloheximide at 100 μg/ml for 2 h. The cells were then exposed to [^{3}H]uridine at 20 μCi/ml in the presence of drug from 16 to 20 h p.i. The radiolabeled infected cells were harvested, cytoplasmic extracts were prepared, and the RNA was isolated as described previously (13).

Preparation of virion RNA. Monolayer cultures of HEp-2 cells were infected with RS virus as described above. At 10 h p.i., the infected cells were treated with 0.3 μg of actinomycin D per ml, and after 1 h, fresh Eagle minimal essential medium with 20 μCi of [^{3}H]uridine per ml was added. Supernatant fluids were harvested when the cytopathic effect and formation of syncytia induced by the virus were evident (usually at 24 to 40 h p.i.). Cellular debris was removed by centrifugation at 11,000 × *g* for 20 min, and virus was concentrated by centrifugation at 65,000 × *g* for 90 min. The pellet of virus was suspended in NTE (0.01 M Tris-hydrochloride, pH 7.4, 0.1 M NaCl, 0.001 M EDTA), resuspended by sonication, and then centrifuged through a 10 to 50% linear sucrose gradient for 1 h at 150,000 × *g*. The fractions containing the visible viral band were collected, diluted with NTE, and sonicated, and the virus was rebanded by velocity sedimentation in a 20 to 60% sucrose velocity gradient at 150,000 × *g* for 2 h. The visible band was collected, virus was pelleted, and the RNA was isolated by phenol extraction and recovered by precipitation with ethanol. RNA was additionally purified by velocity sedimentation in a 15 to 30% sucrose sodium dodecyl sulfate gradient for 15 h at 19,000 rpm in an SW27 rotor. Radiolabeling of virion RNA uniformly by incorporation of $^{32}P_i$ or at the 3′ end, using RNA ligase and cytidine 3′,5′-bis[^{32}P]phosphate (^{32}pCp) was performed exactly as described previously (14). Alternatively, the virion RNA was labeled at the 5′ end as described before (11). Briefly, the 5′-terminal phosphates of the viral RNA were removed by calf intestinal phosphatase, and the enzyme was inactivated by proteinase K. The RNA was phenol extracted and ethanol precipitated. The recovered RNA was labeled by reaction with [γ-^{32}P]ATP in the presence of polynucleotide kinase from T_4-infected *Escherichia coli*. The labeled RNA was separated from residual ATP by G-50 column filtration.

RNA–RNA hybridization. RNA–RNA hybridization in solution (13), agarose gel electrophoresis, Northern blotting (14), oligodeoxythymidylate [oligo(dT)]-cellulose chromatography, and cell-free protein synthesis (3) have all been fully described before.

Chemicals. Oligo(dT)-cellulose was obtained from Bethesda Research Laboratories. Diazobenzyloxymethyl (DBM) paper was from Schleicher and Schuell, Inc. Actinomycin D was from Merck, Sharpe & Dohme. [5-^{3}H]uridine (20 to 25 Ci/mmol) was from Moravek Biochemicals. ^{32}pCp (1,000 to 3,000 Ci/mmol), [γ-^{32}P]ATP (2,000 to 6,000 Ci/mmol), and T_4 polynucleotide kinase were from New England Nuclear Corp. Carrier-free $^{32}P_i$ was from ICN Biochemicals.

RESULTS

Analysis of the RNAs synthesized in RS virus-infected HEp-2 cells. Kinetic analyses have shown that the peak rates of RS viral RNA synthesis in HEp-2 cells infected at a multiplicity of 1 PFU per cell occur between 16 and 22 h p.i. (6). Accordingly, HEp-2 cells infected with RS virus were exposed to [^{3}H]uridine in the presence of actinomycin D between 16 and 20 h p.i. The actinomycin D-resistant RNA species present in the cytoplasmic and nuclear fractions of the cell were analyzed by electrophoresis in agarose-urea gels. Eight major bands of RNA were discernable in the cytoplasm of infected cells (Fig. 1, lane A). No actinomycin D-resistant RNA species were detectable in the nuclear fraction of infected or uninfected cells (data not shown) or in the cytoplasm of mock-infected cells which had been labeled in the presence of actinomycin D (Fig. 1, lane C). The RNAs designated as 1, 2, 3, 4, 5, 7, and 8 always were evident as major species in the cytoplasm. The RNA present in band 6 was usually in much lower molar amounts than the other RNA species. Additionally, two other RNA species could be observed which migrated in front of and behind the major band designated as 5. These two species were not always evident. The identity of these minor species will be discussed later.

Effect of inhibition of protein synthesis on RNA synthesis. RS virus has been classified as a negative-stranded RNA virus. However, as mentioned above, biochemical evidence for this classification is lacking. One characteristic of negative-stranded RNA viruses is that the transcription of mRNA does not require protein synthesis, whereas replication of the genome-size RNA requires concurrent protein synthesis. Therefore, the effect of protein synthesis inhibition on RNA synthesis in RS virus-infected cells was examined to distinguish between the genomic and the mRNAs. When cycloheximide was used to inhibit protein synthesis in HEp-2 cultures infected with RS virus, it was observed (Fig. 1, lane B) that synthesis of the largest RNA species (band 8) was completely inhibited in the presence of the drug. This finding indicated that this RNA was a product of replication and that it was a candidate to be the intracellular genome-length RNA. The synthesis of all of the smaller RNA species, however, was undiminished in the presence of cycloheximide, and no qualitative or quantitative change in the pattern of these RNA species synthesized was observed. Therefore, by virtue of their ability to be synthesized in the presence of cycloheximide, this result suggested that the smaller RNAs were candidates to be mRNAs. Additionally, these data indicated that none of the smaller RNAs represented defec-

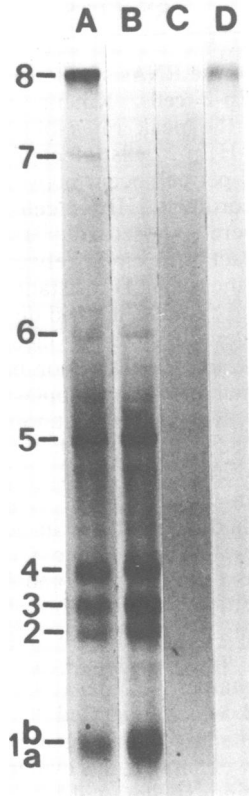

FIG. 1. Electrophoretic analysis of RNA isolated from purified RS virions and from the cytoplasm of RS virus-infected HEp-2 cells. Cytoplasmic and virion RNAs prepared as described in the text were analyzed by electrophoresis in 1.5% agarose–6 M urea gels. A fluorogram of the dried gel is shown. Lane A, [^{3}H]uridine-labeled RNA synthesized in RS virus-infected HEp-2 cells exposed to label from 16 to 20 h p.i. in the presence of actinomycin D (5 μg/ml). Lane B, [^{3}H]uridine-labeled RNA from RS virus-infected HEp-2 cells labeled 16 to 20 h p.i. in the presence of actinomycin D (5 μg/ml) and cycloheximide (100 μg/ml). Lane C, [^{3}H]uridine-labeled RNA isolated from mock-infected actinomycin D-treated cultures. Lane D, [^{3}H]uridine-labeled RNA extracted from RS virions purified through two cycles of velocity sedimentation as described in the text.

tive-interfering particle RNAs because of their resistance to inhibition by cycloheximide.

Characterization of polyadenylated RNA species. A general feature of eucaryotic mRNAs is the presence of a polyadenylate tract at the 3′ terminus of the molecule. To characterize the virus-specific RNAs present in infected cells, these RNAs were assayed for the presence of polyadenylate sequences by chromatography on oligo(dT)-cellulose. The species that bound to an oligo(dT) column and those that failed to bind to an oligo(dT) column were analyzed by electrophoresis in agarose-urea gels (Fig. 2). The seven major cycloheximide-resistant RNA species all bound to the oligo(dT) column, demonstrating that these RNAs were polyadenylated. The largest RNA, band 8, did not bind to oligo(dT), showing that it was not polyadenylated.

The presence of polyadenylate tracts on RNAs 1 to 7 suggested that these species were mRNAs; therefore, they were assayed for their ability to program protein synthesis in an mRNA-dependent, cell-free, protein synthesis system. The incorporation of [^{35}S]methionine into hot trichloroacetic acid-precipitable material was measured in micrococcal nuclease-treated rabbit reticulocyte lysates to which polyadenylate-selected RNA from RS virus-infected or uninfected cells was added to program protein synthesis. The polyadenylate-selected RNAs from actinomycin D-treated infected cells stimulated protein synthesis in the in vitro system, thereby indicating their ability to function as

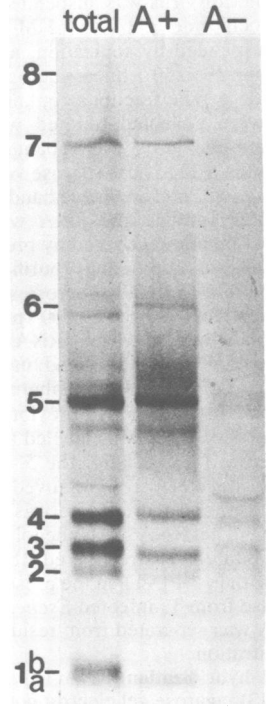

FIG. 2. Analysis of RNA species synthesized in RS virus-infected HEp-2 cells by affinity chromatography on oligo(dT)-cellulose. Cytoplasmic extracts of RS virus-infected cells labeled with [^{3}H]uridine in the presence of actinomycin D and cycloheximide were resolved by chromatography on oligo(dT)-cellulose into the bound polyadenylated (A+) and unbound nonpolyadenylated (A−) fractions. After precipitation with ethanol, these two fractions were subjected to electrophoresis on 1.5% agarose–6 M urea gels. A fluorogram of the dried gel is shown.

mRNAs. The proteins synthesized in response to these mRNAs were analyzed by electrophoresis in polyacrylamide gels and found to comigrate with RS virion proteins (manuscript in preparation).

Analysis of virion RNA. RS virions were isolated at 24 h p.i. from the supernatant fluids of RS virus-infected HEp-2 cells and purified by two cycles of velocity sedimentation. After the second velocity sedimentation, a single visible band was observed in the gradient. The visible band cosedimented with the single peak of radioactivity present in the gradient (Fig. 3A) and with the peak of virus infectivity as determined by plaque assay of the gradient fractions (Fig. 3B).

The virus present in the visible band was collected, the virion RNA was separated from protein by phenol extraction, and the recovered RNA was analyzed both by velocity sedimentation in sucrose gradients and by electrophoresis in agarose urea gels. One peak of radioactive material was observed sedimenting at approximately 50S after centrifugation (data not shown). This agrees with previous reports (8, 15, 16; Hoffman et al., Abstr. Annu. Meet. Am. Soc. Microbiol., 1978, S300, p. 262) that 50S RNA can be isolated from RS virions. Only one band of labeled material was observed after electrophoresis (Fig. 1, lane D). Therefore, contrary to previous reports (8, 15, 16; Hoffman et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, S300, p. 262), data presented here clearly demonstrate that a single species of RNA can be isolated from purified RS virions.

The RNA from purified virions comigrated with RNA 8, the largest RNA species observed in infected cytoplasmic extracts (Fig. 1, lanes A and D). This result, coupled with the observation that synthesis of RNA 8 is sensitive to cycloheximide, identified this RNA as the intracellular genome-size RNA.

Sizes of RNAs. The sizes of the RNAs synthesized in RS virus-infected cells were estimated by electrophoresis in agarose-urea gels with and without treatment with glyoxal to denature the RNA. In both cases, the molecular weights of the RS virus-specific RNAs were estimated by coelectrophoresis with RNA standards whose sizes were known from sequence analysis. The estimated molecular weights of the RNAs before and after denaturation with glyoxal are shown in Fig. 4. Assignments of glyoxal-treated and untreated RNAs are based solely on relative mobility; oligonucleotide fingerprinting is in progress to confirm our assignments.

The molecular weight of RNA 8, which we have shown to be the genomic RNA, was approximately 5×10^6 daltons under both denaturing and nondenaturing conditions. It should be noted, however, that molecular weight calculations are only approximate at this size range due to lack of accurate high-molecular-weight standards. The molecular weights of the seven major polyadenylated RNA species were determined under nondenaturing conditions as follows: RNA 7, 2.5×10^6 daltons; RNA 5, 0.74×10^6 daltons; RNAs 4, 3, and 2, 0.47×10^6, 0.40×10^6, and 0.39×10^6 daltons, respectively; RNA 1, 0.26×10^6 daltons. After denaturation with glyoxal, it was observed that RNAs 2, 3, and 4 migrated as an unresolved band with a molecular weight of 0.46×10^6 daltons. We have obtained evidence, however, that the three discrete bands separated in the absence of glyoxal represent three individual mRNA species. Each of the RNA bands separated in the absence of glyoxal encoded a single RS virus-specific polypeptide having a unique molecular weight when used to program an in vitro protein synthesis reaction (manuscript in preparation). It was also observed that after denaturation with glyoxal, the RNA which migrated most rapidly (band 1)

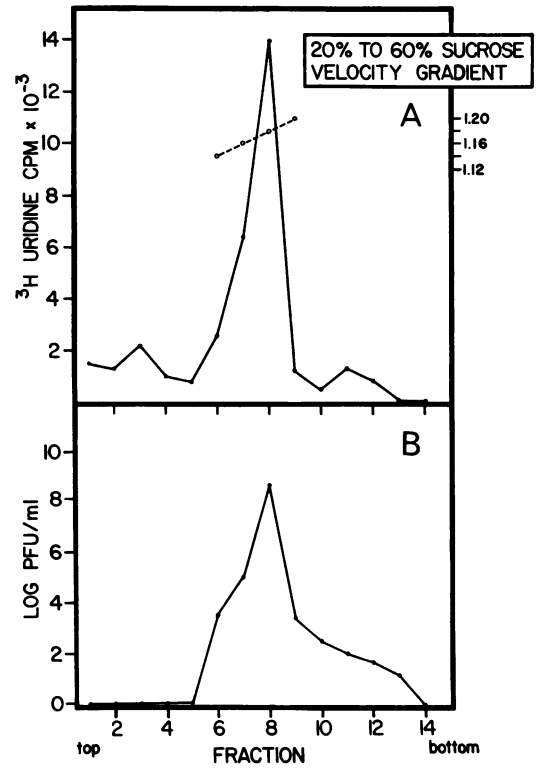

FIG. 3. Velocity sedimentation of [^{3}H]uridine-labeled RS virus. The distribution of radioactivity and infectivity after the second velocity sedimentation analysis of RS virus in a 20 to 60% sucrose gradient is shown. The gradient was fractionated, and each fraction was assayed for the presence of trichloroacetic acid-precipitable radioactivity (A) and for infectious virus by plaque assay (B).

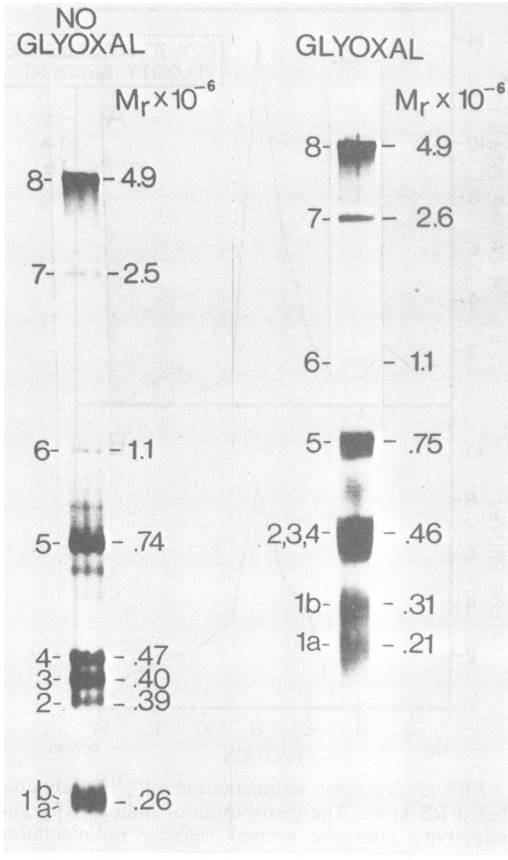

FIG. 4. Estimation of molecular weights of RNAs from RS virus-infected cells by relative electrophoretic mobility. RNAs synthesized in RS virus-infected HEp-2 cells labeled from 16 to 20 h p.i. in the presence of actinomycin D were subjected to electrophoresis in 1.2% agarose–6 M urea gels before and after treatment with 1 M glyoxal (1 h, 23°C) to denature the RNA. RNAs whose exact size is known from sequence analysis: Qβ phage, *E. coli* 16S and 23S rRNA and the four small vesicular stomatitis virus mRNAs, were subjected to electrophoresis in adjacent lanes of the same gel for use as internal reference standards. Molecular weights were calculated by plotting the square root of the relative molecular weight (M_r) versus the log mobility by the method of Lerach et al. (9).

could be resolved to two species of 0.31×10^6 and 0.21×10^6 daltons, respectively. Evidence was obtained by in vitro translation that these two bands also represented two distinct mRNA species. The nature of RNA 6 and the two minor components which migrate on either side of RNA 5 is still in question. These species of RNA, which are always present in very small amounts, disappear almost completely after denaturation with glyoxal. The identity of these bands as unique RNAs or possible polyadenylated transcription read-through products remains to be established, however.

The sum of the molecular weights of the seven major polyadenylated RNA species (RNAs 1a, 1b, 2, 3, 4, 5, and 7) is approximately 5×10^6 daltons. This indicates that these seven major polyadenylated species, if unique, could account for the coding potential of the genomic RNA.

Polarity of virion RNA. Identification of a population of polyadenylated viral mRNA species permitted the polarity of the virion RNA to be determined. [^{3}H]uridine-labeled RNA extracted from RS virions and purified as described above was annealed to increasing concentrations of oligo(dT)-selected or unselected RNA from cytoplasmic extracts of RS virus-infected HEp-2 cells. The data presented in Fig. 5 demonstrate that 93% of the virion RNA was rendered resistant to digestion with ribonuclease after annealing to polyadenylate-selected cytoplasmic RNAs from RS virus-infected cells. All of the virion RNA could be protected from digestion after annealing to total cytoplasmic RNA [unselected by oligo(dT)]. Less than 3% of the virion RNA was resistant to RNase when the virion RNA was not annealed to mRNA. These data show conclusively that RNA isolated from RS virions is single stranded and that at least 93% of the RNA is of negative-strand polarity since it anneals to polyadenylate-containing mRNA. Thus, RS virus can be characterized as a negative-stranded RNA virus.

All seven of the polyadenylated RNA species were RS virus specific. Having demonstrated that the RNA of RS virions was negative stranded and could be isolated as a single species, it was

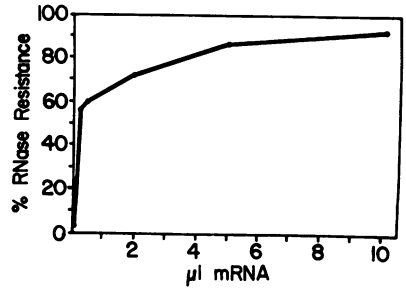

FIG. 5. Annealing of RS virion RNA and polyadenylated mRNA. [^{3}H]uridine-labeled RNA from RS virions, purified as described in the text, was annealed to increasing concentrations of unlabeled oligo(dT)-selected RNA prepared from cells infected with RS virus in the presence of actinomycin D (5 μg/ml) and cycloheximide (100 μg/ml). The percentage of trichloroacetic acid-precipitable material resistant to RNase A (25 μg/ml) was determined as a function of increasing concentrations of unlabeled polyadenylated RNA. Annealings were carried out for 8 h at 63°C. Each reaction contained 2,000 cpm.

possible to determine by hybridization whether or not the seven major RNA species synthesized in the cytoplasm of infected cells in the presence of actinomycin D were RS virus specific. To test this possibility, total cytoplasmic RNA or polyadenylate-selected RNAs from infected HEp-2 cells were separated by electrophoresis in agarose urea gels and subsequently transferred to DBM paper by the technique of Northern blotting. Virion RNA purified as described above was uniformly labeled by growing the virus in the presence of ^{32}P or was specifically labeled with ^{32}P at the 3′ or the 5′ end by using RNA ligase or nucleotide kinase, respectively. These ^{32}P-labeled virion RNAs were then used individually as probes to detect RS virus-specific sequences by two-phase RNA–RNA hybridization to the cytoplasmic RNAs immobilized on DBM paper. Data from two separate experiments are shown in Fig. 6, where total or polyadenylated cytoplasmic RNAs were probed either with uniformly labeled virion RNA or with 5′ end-labeled virion RNA. The data show that the RNA bands identified as 1, 2, 3, 4, 5, 7, and 8 all anneal specifically to RS virion RNA when 5′ end-labeled RNA is used as probe (Fig. 6). Similar results were obtained with virion RNA labeled uniformly or at the 3′ end. However, uniformly labeled or 3′ end-labeled RNA probes did not allow detection of band 7 RNA in these experiments. If the genes of RS virus are arranged as those of other negative-stranded RNA viruses, then, by analogy to these systems, this result might be expected for two reasons: (i) the large polymerase gene is located at the 5′ end of the genome (and hence would be detected most efficiently by a 5′ end-labeled probe), and (ii) it is synthesized in low amounts relative to the genes at the 3′ end (and, hence, would be relatively difficult to detect with a uniformly labeled probe). Based on the data shown in Fig. 4, which indicates that band 1 resolves to two species under completely denaturing conditions, we conclude that at least seven major polyadenylated RNA species are synthesized in HEp-2 cells after infection with RS virus and that these RNAs are all RS virus specific.

RNA in band 8 from a total cytoplasmic extract (not polyadenylate selected) also anneals to the negative-stranded virion RNA. We have shown above (Fig. 3) that this RNA is not polyadenylated. Therefore, this result shows that a genome-size, virion-complementary (positive polarity) RNA exists in RS virus-infected cells. This RNA is likely to be the template for the replication of the negative-stranded genome.

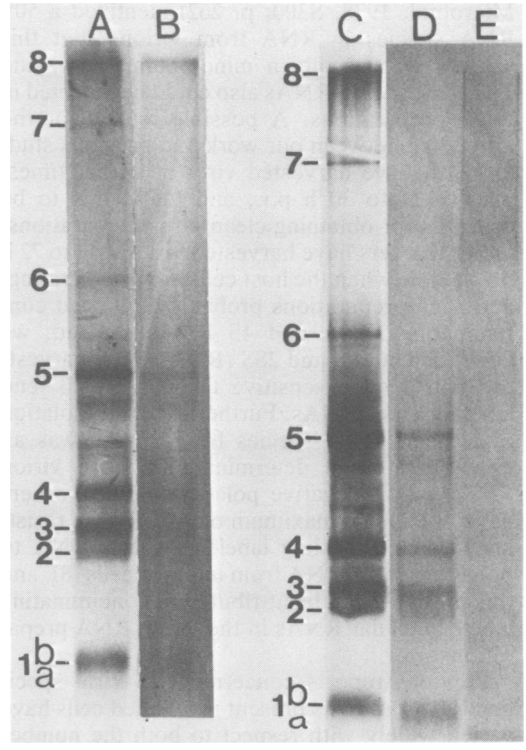

FIG. 6. Two-phase hybridization of RS virion RNA with cytoplasmic RNA from RS virus-infected cells. RNA from RS virus-infected cells labeled from 16 to 20 h p.i. with [^{3}H]uridine was separated by electrophoresis in an 1.5% agarose–6 M urea gel. The RNA in lanes B, D, and E was transferred to DBM paper, and the individual strips were annealed to purified RS virion RNA labeled only at the 5′ end with ^{32}P (lane B) or labeled uniformly with ^{32}P (lanes D and E). Lanes A and C show fluorograms of the separated total actinomycin D-resistant cytoplasmic RNAs. Lane B shows the same total cytoplasmic RNAs as those in lane A, only this sample was electrophoresed in a lane adjacent to lane A and then transferred to DBM paper by the Northern blotting procedure, after which it was annealed to purified RS virion RNA labeled at the 5′ end with ^{32}P. An autoradiogram of the washed, dried DBM strip is shown. Lanes D and E represent the polyadenylated and nonpolyadenylated fractions of the RNA separated in lane C. Lanes D and E, however, were transferred to DBM paper by Northern blotting and annealed to purified RS virion RNA labeled uniformly with ^{32}P. An autoradiogram of the washed, dried DBM strips is shown.

DISCUSSION

By using the techniques of high-resolution gel electrophoresis and two-phase RNA–RNA hybridization, we characterized both the RNA isolated from RS virions and the RNAs synthesized in RS virus-infected cells. A single species of RNA of approximately 5×10^6 daltons can be isolated from purified preparations of RS virus. More than 93% of this virion RNA is of a negative polarity. Previous reports (8, 15, 16; Hoffman et al., Abstr. Annu. Meet. Am. Soc.

Microbiol. 1978, S300, p. 262) identified a 50S RNA species in RNA from virions, but this species was usually a minor component, and 28S, 18S, and 4S RNAs also could be detected in these preparations. A possible reason for the difference between our work and previous studies is that we harvested virus at earlier times, usually 24 to 30 h p.i., and found this to be essential for obtaining clean virus preparations. Other workers have harvested virus at 48 to 72 h p.i., a time when the host cells are degenerating, and such preparations probably contained contaminating rRNA and 4S RNAs. Indeed, we could detect 18S and 28S rRNA in late-harvested virus by the sensitive technique of 3′ end labeling of the RNAs. Furthermore, the isolation of a single RNA species from virions was an essential step in determining that the virion RNA was of negative polarity. Other workers have obtained a maximum of 47% RNase resistance when annealing labeled genome RNA to polyadenylated RNA from infected cells (8), and this probably can be attributed to contaminating labeled cellular RNAs in the virion RNA preparations.

Previous reports concerning RS virus-specified RNA products present in infected cells have varied widely with respect to both the number and the size of the RNAs observed. One perplexing problem with results from previous work has been the observation that when the molecular weights of the subgenomic RNAs observed in infected cells were summed, the value calculated represented less than one-half of the possible coding potential of a 50S RNA molecule, the presumed size of the viral genome. This result could suggest several possibilities: (i) not all of the genome was expressed; (ii) the genomic RNA was not entirely of negative-strand polarity; or (iii) previous work had failed to detect all of the RNA gene products expressed. We detected eight major RNA species in cytoplasmic extracts of RS virus-infected HEp-2 cells. The largest of these RNAs, band 8, with a size of approximately 5×10^6 daltons, was shown to represent the genome-size RNA in the infected cells. Band 8 contained genome-size RNA of both negative and positive polarities, and its synthesis was sensitive to cycloheximide, indicating that it was a product of RNA replication. Therefore, the genome-size, positive-stranded RNA most likely is the template for replication of the negative-stranded genome; this, however, remains to be demonstrated. The seven RNA species smaller than the genomic RNA were all synthesized in the presence of inhibitors of protein synthesis. These RNAs were polyadenylated and could function as mRNAs in a messenger-dependent cell-free protein synthesis system. Most importantly, we showed by the technique of Northern blot analysis that all seven of these RNAs are RS virus specific. Within the limits of reliability for size determination as described here, these seven RNAs are able to account for the coding capacity of a 5×10^6-dalton genome. Therefore, it is most likely that previous reports concerning the RNAs expressed in RS virus-infected cells (7; Lambert et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, T120, p. 255) had failed to detect all of the virus-specific RNAs.

At this time, we do not wish to indicate that the seven major polyadenylated RNAs account for the entire coding capacity of the 5×10^6-dalton genome. By analogy with other negative-stranded RNA viruses, it would be reasonable to assume that small nonpolyadenylated "leader" (2) and perhaps "trailer" (12) RNA transcripts are also synthesized by the genome. An assay for these RNA species is under way. Furthermore, until a definitive correlation of the mRNA coding capacities with the viral proteins is complete and the identity of the three minor RNA species observed is clarified, the enumeration of unique gene products cannot be assumed complete.

Within the simple confines of size correlation, however, the RNAs described here do have the appropriate size to code for the RS virus structural proteins, including the large (>160,000-dalton) protein at present tentatively identified as the RNA-dependent RNA polymerase (by analogy with other negative-stranded RNA virus systems). An intracellular RNA with the appropriate size to code for the polymerase has not been detected previously by gel electrophoretic analysis. The 2.5×10^6-dalton RNA (band 7) reported here is of the appropriate size to code for this protein. Additionally, we have evidence from work in progress which shows that the five small RNAs described here, 1a, 1b, 2, 3, and 4, when used individually to program a cell-free protein synthesis system, code for proteins which comigrate with RS virus-specific proteins (manuscript in preparation).

In summary, this work demonstrates that the genome of respiratory syncytial virus is a single-stranded, negative-stranded RNA molecule of approximately 5×10^6 daltons. Upon infection of HEp-2 cells with RS virus, at least eight RNA species are synthesized: (i) genome-size RNA of both negative and positive polarities and (ii) at least seven polyadenylated, RS virus-specific mRNAs which, taken together, are sufficient to account for the coding capacity of the genome.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Baldridge, P., and L. B. Senterfit.** 1976. Persistent infection of cells in culture by respiratory syncytial virus. Proc. Soc. Exp. Biol. Med. **151**:684–688.
2. **Colonno, R. J., and A. K. Banerjee.** 1976. A unique RNA species involved in initiation of vesicular stomatitis virus RNA transcription in vitro. Cell **8**:197–209.
3. **Davis, N., and G. W. Wertz.** 1982. Synthesis of vesicular stomatitis virus negative-strand RNA: dependence on viral protein synthesis. J. Virol. **41**:821–832.
4. **Gimenez, H. B., and C. R. Pringle.** 1978. Seven complementation groups of respiratory syncytial virus temperature-sensitive mutants. J. Virol. **27**:459–464.
5. **Hodes, D. S., V. Schauf, and R. M. Chanock.** 1974. Isolation of RNA species from HeLa cells infected with respiratory syncytial virus. Proc. Soc. Exp. Biol. Med. **146**:287–290.
6. **Huang, Y., N. Davis, and G. W. Wertz.** 1981. Separation and characterization of the RNAs of human respiratory syncytial virus, p. 531–536. *In* D. Bishop and R. Compans (ed.), Replication of negative strand viruses. Elsevier/North Holland, Inc., New York.
7. **Lambert, D. M., M. W. Pons, and G. N. Mbuy.** 1981. Respiratory syncytial virus-specific RNA; p. 523–530. *In* D. Bishop and R. Compans (ed.), Replication of negative strand viruses. Elsevier/North Holland, Inc., New York.
8. **Lambert, D. M., M. W. Pons, G. N. Mbuy, and K. Dorsch-Hasler.** 1980. Nucleic acids of respiratory syncytial virus. J. Virol. **36**:837–846.
9. **Lerach, H., D. Diamond, J. Wozney, and H. Boedtker.** 1977. RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16**:4743–4751.
10. **Pringle, C. R., P. V. Shirodaria, H. B. Gimenez, and S. Levine.** 1981. Antigen and polypeptide synthesis by temperature-sensitive mutants of respiratory syncytial virus. J. Gen. Virol. **54**:173–183.
11. **Schubert, M., J. D. Keene, and R. A. Lazzarini.** 1979. A specific internal RNA polymerase recognition site of VSV RNA is involved in the generation of D.I. particles. Cell **18**:749–757.
12. **Schubert, M., and R. Lazzarini.** 1981. In vivo transcription of the 5'-terminal extracistronic region of vesicular stomatitis virus RNA. J. Virol. **38**:256–262.
13. **Wertz, G. W.** 1978. Isolation of possible replicative intermediate structures from vesicular stomatitis virus. Virology **85**:271–285.
14. **Wertz, G. W., and N. L. Davis.** 1981. Characterization and mapping of RNase III cleavage sites in vesicular stomatitis virus genome RNA. Nucleic Acids Res. **9**:6487–6503.
15. **Wunner, W. H., G. P. Faulkner, and C. R. Pringle.** 1975. Respiratory syncytial virus: some biological and biochemical properties, p. 193–201. *In* B. Mahy and R. Barry (ed.), Negative strand viruses. Academic Press, Inc., New York.
16. **Zhdanov, V. M., R. S. Dreizin, O. D. Yankevici, and A. K. Astakhova.** 1974. Biophysical characteristics of respiratory syncytial virus. Rev. Roum. Virol. **25**:277–280.