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Transcription elongation factor of respiratory syncytial virus, a nonsegmented negative-strand RNA virus

(paramyxovirus/reverse genetics/replication/polymerase)

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ABSTRACT **RNA synthesis by the paramyxovirus respiratory syncytial virus, a ubiquitous human pathogen, was found to be more complex than previously appreciated for the nonsegmented negative-strand RNA viruses. Intracellular RNA replication of a plasmid-encoded "minigenome" analog of viral genomic RNA was directed by coexpression of the N, P, and L proteins. But, under these conditions, the greater part of mRNA synthesis terminated prematurely. This difference in processivity between the replicase and the transcriptase was unanticipated because the two enzymes ostensively shared the same protein subunits and template. Coexpression of the M2 gene at a low level of input plasmid resulted in the efficient production of full-length mRNA and, in the case of a dicistronic minigenome, sequential transcription. At a higher level, coexpression of the M2 gene inhibited transcription and RNA replication. The M2 mRNA contains two overlapping translational open reading frames (ORFs), which were segregated for further analysis. Expression of the upstream ORF1, which encoded the previously described 22-kDa M2 protein, was associated with transcription elongation. A model involving this protein in the balance between transcription and replication is proposed. ORF2, which lacks an assigned protein, was associated with inhibition of RNA synthesis. We propose that this activity renders nucleocapsids synthetically quiescent prior to incorporation into virions.**

The paramyxovirus human respiratory syncytial virus (RSV) is a nonsegmented negative-strand RNA virus (1). Information on the molecular biology of this large group of viruses is based mainly on the rhabdovirus vesicular stomatitis virus and the paramyxovirus Sendai virus (2–5). The RNA genomes of these prototype viruses are tightly encapsidated by the major nucleocapsid N or NP protein and also are associated with the nucleocapsid phosphoprotein P and the large L polymerase protein. Transcription initiates at the 3′ extragenic leader region of genomic RNA and proceeds by a sequential, polar, stop–start mechanism that produces subgenomic mRNAs. RNA replication involves a switch to readthrough synthesis to produce a genome-length positive strand replicative intermediate (antigenome), which also is tightly encapsidated and serves as the template for production of progeny genomes.

The genome of RSV is 15,222 nt long. It encodes 10 major species of mRNA and 10 major viral proteins, compared with 5–7 mRNAs for the prototype nonsegmented negative-strand RNA viruses. Several RSV proteins lack known counterparts in most or all other nonsegmented negative-strand viruses—namely, two nonstructural species, NS1 and NS2; a small hydrophobic SH membrane protein; and an internal virion protein called M2 or 22K [here designated the M2(ORF1) protein], all previously of unknown function. The M2 mRNA contains two open reading frames (ORFs) that overlap slightly in the middle of the molecule (see Fig. 4); the upstream ORF1 encodes the M2(ORF1) protein and the downstream ORF2 lacks an assigned protein (6). Other RSV proteins, such as N, P, and L, appear to have direct counterparts in the model systems (although amino acid sequence relatedness is lacking except for highly conserved polymerase motifs in L). RSV transcription is sequential and polar, at least for the 9 smaller genes. Thus, some general features of RSV molecular genetics follow those of the prototype viruses. Here, the proteins involved in RSV transcription and RNA replication were investigated by using a reconstituted minigenome system in transfected cells (7, 8).

MATERIALS AND METHODS

cDNAs. cDNA encoding the complete M2 gene [containing both ORFs, nucleotides 10–909 of the complete sequence (6)] or M2(ORF1) (nucleotides 10–594) or M2(ORF2) (nucleotides 563–835) (see Fig. 4) was cloned separately into the expression plasmid pTM1 (7). C2-LUC minigenome cDNA was prepared from C2 cDNA (7) by replacing the *Xba* I–*Pst* I chloramphenicol acetyltransferase (CAT) cDNA with a *Bam*HI–*Stu* I luciferase (LUC) cDNA (Promega). RSV-CAT-LUC cDNA will be described elsewhere (L. Kuo and P.L.C., unpublished data); briefly, it resembles the C2 cDNA but in addition contains the insertion, between the CAT gene end (GE) motif and the trailer region, of the natural 19-nt intergenic region of the NS1 and NS2 genes followed by the LUC ORF flanked by gene-start (GS) and GE motifs.

RESULTS

RSV RNA replication and transcription were studied by using short, cDNA-encoded analogs of genomic or antigenomic RNA complemented intracellularly by viral proteins expressed from cotransfected plasmids (7). Negative-sense RSV-CAT minigenome [encoded by cDNA C2 (7)] contained the CAT ORF under the control of the RSV GS and GE putative transcription signal motifs and flanked by the 3′-leader and 5′-trailer extragenic regions of the RSV genome (Fig. 1). A second cDNA, C4, encoded the positive-sense antigenome complement of C2 RNA (Fig. 1, and ref. 7). Synthesis of the C2 and C4 RNAs was driven by an adjoining promoter for phage T7 RNA polymerase, and the 3′ end of each RNA was generated by a self-cleaving ribozyme (7). Each plasmid was transfected separately into cells infected with vTF7-3, a recombinant vaccinia virus expressing T7 RNA polymerase (9), and was complemented by RSV proteins synthesized from cotransfected plasmids (7). Coexpression of the N, P, and L proteins resulted in the synthesis of the two expected products

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Abbreviations: RSV, respiratory syncytial virus; CAT, chloramphenicol acetyltransferase; LUC, luciferase; ORF, translational open reading frame; GS, gene start; GE, gene end.

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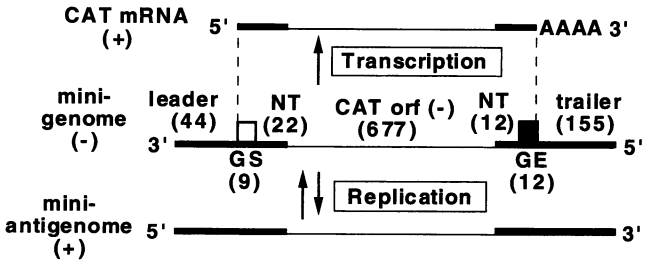

FIG. 1. The RSV-CAT minigenome (C2 RNA), miniantigenome (C4 RNA), and subgenomic polyadenylylated CAT mRNA (7). RSV-specific sequences are indicated by filled rectangles; the GS and GE transcription signals are open and filled boxes, respectively; and lengths of nucleotide segments are in parentheses. NT, nontranslated gene sequence flanking the transcription signals.

of RNA replication—namely, minigenome (Fig. 2*B*, lane 2) and miniantigenome [Fig. 2*A*, lanes 2 and 7; the miniantigenome is seen most clearly as the upper band in lane 7, the unbound (UB) fraction from oligo(dT) chromatography of the sample in lane 2]. In contrast, the mRNA synthesized by N, P, and L alone contained some complete CAT mRNA but consisted mainly of shorter, heterogeneously sized species (Fig. 2*A*, lane 2).

To examine the possibility that one or more additional RSV proteins might be required for complete transcription, genes N, P, and L were supplemented with each of the other RSV genes in turn separately and in some cases in mixtures (not shown). Only the M2 gene, which was supplied initially containing both ORFs (see Fig. 4), enhanced transcription (Fig. 2*A*, lane 3). At the optimal level of input M2 cDNA, which was 0.04 that of N cDNA on a relative molar basis, CAT mRNA was synthesized in abundance as full-length molecules (Fig. 2*A*, lanes 3 and 10). CAT assay (not shown) showed that the expression of translatable CAT mRNA was increased 32-fold between the samples represented by lanes 2 and 3. When the level of input M2 plasmid was increased progressively, a second effect on RNA synthesis was observed—namely, a progressive reduction in the synthesis of miniantigenome (Fig. 2*A*, lanes 7, 9, 11, 13, and 15), mRNA (Fig. 2*A*, lanes 10, 12, 14, and 16), and minigenome (Fig. 2*B*, lanes 2–6). It is not yet known whether mRNA synthesis was inhibited directly or as an indirect effect of reduced RNA replication.

The nature of the mRNAs produced in the absence of M2 plasmid was investigated by Northern blot hybridization with two negative-sense oligonucleotide probes specific to the 5′- or 3′-proximal region of the CAT mRNA (Fig. 3 *Upper* and *Lower*, respectively). As expected, both probes bound to

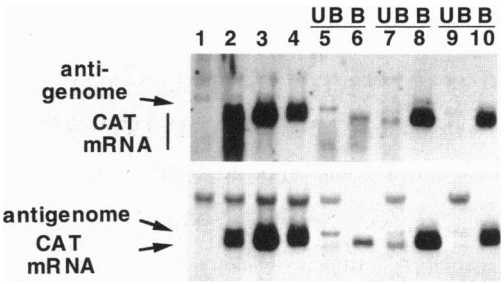

FIG. 3. Northern blot hybridization of duplicate blots (*Upper* and *Lower* panels) using 5′-end-labeled oligonucleotide probes specific to the 5′-proximal (nucleotides 105–155) (*Upper*) vs. the 3′-proximal (nucleotides 647–695) (*Lower*) region of the 732-nt CAT mRNA. Intracellular RNAs were prepared from 293 cells in which plasmid-expressed C2 minigenome was complemented by the N, P, and ΔL (nonfunctional L) plasmids (lane 1); N, P, and L (lane 2); or N, P, L, and M2 with M2 at 0.04 the relative molar amount of N (lane 3). Lane 4 contains RNA from cells that had been infected with RSV and transfected with *in vitro* synthesized C2 minigenome, a control in which all of the RSV proteins would be present and vaccinia virus was absent (7, 10). The unbound (UB) and bound (B) fractions from oligo(dT) chromatography of lanes 2, 3, and 4 are, respectively, lanes 5 and 6, 7 and 8, and 9 and 10. The 3′-specific oligonucleotide exhibited nonspecific binding to the 18S rRNA band.

antigenome (e.g., lane 5 of each panel). And both probes hybridized to complete mRNA (e.g., lane 8 of each panel). This confirmed that a small amount of complete mRNA was made in the absence of M2 plasmid (lane 6 of each panel). The 5′-specific probe hybridized in abundance to the smear of short RNAs produced in the absence of M2 plasmid (Fig. 3 *Upper*, lane 2), but the 3′-specific one did not (Fig. 3 *Lower*, lane 2). This suggested that the short transcripts were the result of premature termination of transcription rather than, for example, spurious internal initiation. The fact that some of these incomplete mRNAs bound to oligo(dT)-cellulose (Fig. 2*A*, lane 8, and Fig. 3 *Upper*, lane 6) probably can be attributed to posttranscriptional polyadenylylation mediated by the vaccinia virus poly(A) polymerase, although polyadenylylation of prematurely terminated molecules by the RSV transcriptase also is a possibility.

The incomplete mRNAs that were synthesized in the absence of M2 plasmid were detected more efficiently with the 5′-specific oligonucleotide probe (Fig. 3 *Upper*, lane 2) than with riboprobe (Fig. 2*A*, lane 2). The short end-labeled oligonucleotide probe would be the more accurate measure, since use of a uniformly labeled, partially radiolyzed riboprobe representing the entire CAT sequence together with stringent

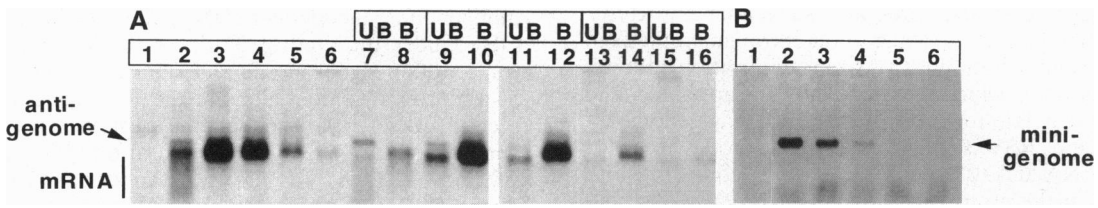

FIG. 2. Effects of M2 gene expression on RNA replication and transcription in 293 cells by plasmid-supplied C2 minigenome (*A*) or C4 miniantigenome (*B*) complemented by plasmid-supplied RSV proteins (7). Intracellular RNAs were analyzed by Northern blot hybridization with strand-specific riboprobes (7). (*A*) C2 minigenome was complemented by N, P, and nonfunctional (ΔL) L (lane 1); N, P and L (lane 2); or N, P, L, and M2 with M2 at the following molar amounts of input plasmid relative to N as 1: 0.04 (lane 3), 0.20 (lane 4), 1.0 (lane 5), or 5.0 (lane 6). The RNAs also were separated by oligo(dT) chromatography (7); the unbound (UB) and bound (B) fractions of lanes 2–6 are, respectively, in lanes 7 and 8, 9 and 10, 11 and 12, 13 and 14, and 15 and 16. The probe was the ^{32}P-labeled C2 minigenome. The dense mRNA-size band in the unbound fractions such as in lane 9 is nonpolyadenylylated mRNA; authentic RSV transcription also yields nonpolyadenylylated versions of each mRNA in comparable relative amounts. Input plasmid in each well of a six-well dish containing 1.5×10^6 cells was 0.4 μg each of N and P and 0.2 μg each of L and C2 or C4. (*B*) Cells were transfected and analyzed as described above except that the plasmid encoding the C2 minigenome was replaced with that encoding the C4 miniantigenome, and Northern blot analysis was with the C4 miniantigenome as probe. C4 miniantigenome was complemented with N, P, and ΔL (lane 1); N, P, and L (lane 2); or N, P, L, and M2 plasmid (lane 3) with M2 at the following molar amounts relative to N: 0.04 (lane 3), 0.20 (lane 4), 0.1 (lane 5), or 5.0 (lane 6).

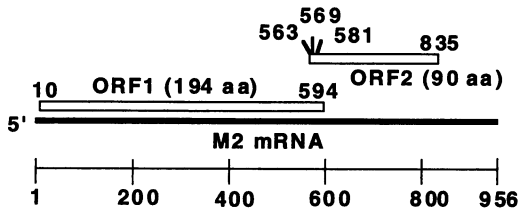

FIG. 4. Map of the M2 mRNA (black bar) showing the two ORFs (open rectangles), with potential translational start and stop sites labeled according to mRNA sequence position. The numbers in parentheses indicate the number of amino acids in the ORF products.

hybridization conditions would place a bias against detection of short species derived from one end. Importantly, phosphor imaging analysis indicated that the ratio of 5′-specific probe that hybridized to RNA produced in the absence versus the presence of M2 plasmid (Fig. 3 *Upper*, lanes 2 vs. 3) was 1.16 vs. 1.0. This quantitation was compromised somewhat because it did not exclude the miniantigenome. Nonetheless, the similarity in the amount of hybridization suggests that the number of transcription "starts" was the same in each case. Thus, the increase in synthesis of full-length mRNA effected by expression of M2 plasmid appeared to be at the level of transcriptional elongation and not at that of initiation.

The two ORFs of the RSV M2 mRNA (Fig. 4) were then placed separately into the plasmid expression vector for individual evaluation. The possibility that M2(ORF1) alone directed transcriptional elongation was assayed first with minigenome C2-LUC, in which the CAT ORF had been replaced with a longer ORF, that of LUC (Fig. 5). In the absence of M2(ORF1) plasmid, LUC mRNA was synthesized as a diffuse band of short, incomplete species (Fig. 5, lane 2) which approached in size the full-length CAT mRNA marker (Fig. 5, lane 5). The addition of M2(ORF1) plasmid resulted in a dramatic shift to the efficient synthesis of full-length LUC mRNA (Fig. 5, lane 4).

The effects of the M2 ORFs were then assayed with a dicistronic minigenome, RSV-CAT-LUC, that expressed the CAT and LUC sequences as separate mRNAs from tandem genes, each with an independent set of GS and GE motifs. In the absence of M2 plasmid, a moderate amount of full-length CAT mRNA was made, but LUC mRNA was below the level of detection (Fig. 6 *Top*, lane 2). Interestingly, the amount of full-length CAT mRNA synthesized in the absence of M2 was greater here (Fig. 6 *Top*, lane 2) than in Figs. 2*A* (lane 2) and 3 *Upper* (lane 2). This was associated with the use of different cells: the poor processivity of the transcriptase on the CAT

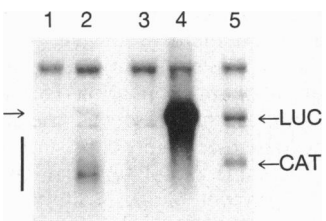

FIG. 5. Effect of M2(ORF1) on transcription of minigenome bearing the LUC gene. In HEp-2 cells, plasmid-encoded C2-LUC minigenome was complemented by plasmids expressing the N and P proteins together with ΔL (nonfunctional L) (lanes 1 and 3) or L plasmid (lanes 2 and 4) and M(ORF1) plasmid (lanes 3 and 4) supplied at 0.20, the relative molar amount of N. The arrow and vertical bar to the left indicate, respectively, the miniantigenome and prematurely terminated LUC mRNA in lane 2. Lane 5 contains CAT and LUC mRNAs expressed from the dicistronic RSV-CAT-LUC minigenome complemented with N, P, L, and M2(ORF1) as markers. Intracellular RNAs were analyzed by Northern blot hybridization with an equal mixture of negative-sense CAT and LUC riboprobes. Nonspecific binding to rRNA is visible near the top of the autoradiogram.

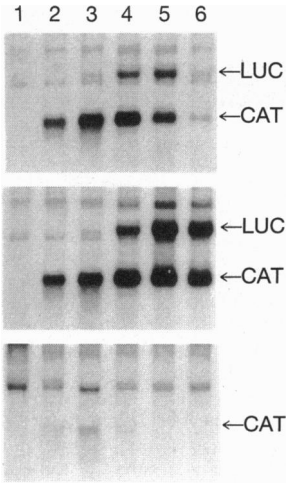

FIG. 6. Effects of M2(ORF1) and ORF2 on sequential transcription by the dicistronic RSV-CAT-LUC minigenome. In HEp-2 cells, plasmid-supplied minigenome was complemented by N, P, and ΔL (nonfunctional L) (lane 1); N, P, and L (lane 2); or N, P, and L (lanes 3–6) together with 0.008 (lane 3), 0.04 (lane 4), 0.20 (lane 5), or 1.0 (lane 6) molar equivalents relative to N plasmid of M2 (*Top*) M2(ORF1) (*Middle*), or M2(ORF2) (*Bottom*) plasmid. Total intracellular RNA was analyzed by Northern blot hybridization by using an equal mixture of negative-sense CAT and LUC riboprobes. *Top* and *Middle* are from one experiment, and *Bottom* is from a second experiment in which the overall amount of minigenome transcription was less and background hybridization was greater. The small increase rather than decrease in CAT mRNA in *Bottom* lane 3 versus lane 2 was peculiar to this one experiment. The CAT and LUC mRNAs are labeled.

gene in the absence of M2 plasmid was more pronounced in 293 (Figs. 2 and 3) than in HEp-2 (Fig. 6) cells. The inclusion of M2 plasmid (containing both ORFs) in low amounts resulted in an increase in CAT mRNA and the appearance of LUC mRNA (Fig. 6 *Top*, lanes 4 and 5). A higher level resulted in inhibition of RNA synthesis (Fig. 6 *Top*, lane 6). Expression of M2(ORF1) plasmid (Fig. 6 *Middle*) gave similar results except that the higher levels of input plasmid did not significantly inhibit RNA synthesis (Fig. 6 *Middle*, lanes 5 and 6). That the effect of transcriptional elongation required synthesis of the M2(ORF1) protein was demonstrated by the lack of activity associated with a second plasmid that contained a single nucleotide deletion at position 19, which shifted ORF1 into a second frame terminating 12 codons later (not shown). In contrast, expression of M2(ORF2) alone (linked to the strong translational start site of plasmid pTM1) was associated with inhibition of positive-sense RNA synthesis (Fig. 6 *Bottom*, lanes 3–6; note that the bottom panel is from an experiment separate from that of *Top* and *Middle* and exhibited a lower level of minigenome expression).

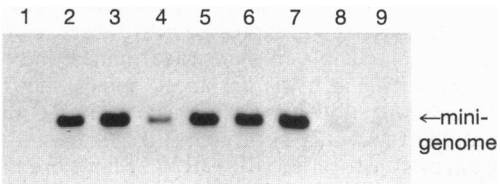

FIG. 7. Inhibition of minigenome synthesis by M2(ORF2). In HEp-2 cells, plasmid-encoded C4 miniantigenome was complemented by plasmids expressing the N and P proteins and ΔL (nonfunctional L) (lane 1) or L (lanes 2–9) together with: 0.008 (lane 2), 0.04 (lane 3), or 0.02 (lane 4) N-equivalents of M2 plasmid; or 0.04 (lane 5) or 0.20 (lane 6) N-equivalents of M2(ORF1) plasmid; or 0.008 (lane 7), 0.04 (lane 8), or 0.20 (lane 9) N-equivalents of M2(ORF2) plasmid. Total intracellular RNA was analyzed by Northern blot hybridization using the miniantigenome riboprobe.

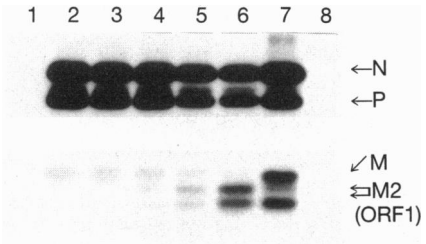

FIG. 8. RSV-specific proteins synthesized in 293 cells which were uninfected (lane 8) or infected (lane 7) with RSV or infected with vTF7-3 and transfected with the following plasmids (at the same amounts as used for complementation or as indicated): 0.5 μg of pTM1 expression plasmid lacking insert (lane 1); N, P, and L (lane 2); or N, P, and L plus the following amount of input M2 plasmid relative to N as 1: 0.008 (lane 3), 0.04 (lane 4), 0.2 (lane 5), or 1.0 (lane 6). Cells were labeled by incubation with 50 μCi of [^{35}S]methionine per ml 21–24 h after infection or transfection, and lysates were analyzed by immunoprecipitation using rabbit antisera raised against purified RSV (7), followed by electrophoresis on a 13% gel. Only the part of the gel that contains N, P, M, and M2(ORF1) is shown.

The effect of the two M2 ORFs on negative-sense RNA synthesis was evaluated with C4 miniantigenome (Fig. 7). Expression of a moderate level of complete M2 plasmid was associated with a moderate level of inhibition of minigenome synthesis (Fig. 7, lane 4); expression of the same level of M2(ORF1) plasmid lacked inhibitory activity (Fig. 7, lane 6); and expression of M2(ORF2) plasmid was strongly inhibitory (Fig. 7, lanes 8 and 9).

The level of protein expression directed by transfected N, P, and M2 plasmids (the latter containing both ORFs) vs. RSV infection was compared by metabolic labeling and immunoprecipitation with anti-RSV antibodies. Results with HEp-2 and 293 cells were similar; the latter are shown in Fig. 8. Also, since an intracellular protein corresponding to ORF2 has not yet been described, the M2(ORF1) protein was the only available marker for intracellular protein synthesis by the M2 plasmid. The labeling period (21–24 hr after infection or transfection) corresponded to the peak of viral protein synthesis late in RSV infection or to the midpoint of incubation of the plasmid-transfected cultures. The levels of N and P expressed from plasmids (Fig. 8, lanes 2–6) were similar to those in RSV-infected cells (Fig. 8, lane 7). The M2(ORF1) protein expressed by RSV migrated as two closely spaced species (Fig. 8, lane 7); multiple species have been observed previously and are due at least in part to intramolecular disulfide bonds (11). The relative abundance of the two species was somewhat different when expressed from plasmid (Fig. 8, lane 6), an effect whose basis is not yet known. The amount of input M2 plasmid that provided the greatest elongation activity (i.e., 0.04 relative to N; Fig. 2*A*, lane 3, and Fig. 6 *Top*, lane 4) was associated with levels of expressed M2(ORF1) protein (Fig. 8, lane 4), which were much lower that that observed at the peak of viral protein synthesis in RSV-infected cells (Fig. 8, lane 7). When ORF1 was expressed in the absence of ORF2, higher levels of input plasmid afforded modest increases in elongation activity (Fig. 6 *Middle*, lanes 5 and 6), showing that transcription elongation was active over a broad range of concentration of M2(ORF1) protein (Fig. 8, lanes 4–6). The inhibitory activity associated with ORF2 became predominant at a level of input M2 plasmid (1.0 relative to N; Fig. 2*A*, lane 5, and Fig. 6 *Top*, lane 6) which expressed an amount of M2(ORF1) protein (Fig. 8, lane 6) comparable to the peak in RSV-infected cells (Fig. 8, lane 7).

DISCUSSION

In a reconstituted, cDNA-expressed RSV minigenome system, the N, P, and L proteins were necessary and sufficient for RNA replication but produced mRNA consisting mostly of prematurely terminated molecules. Coexpression of both ORF1 and ORF2 of the M2 gene had two effects: at lower expression levels it conferred transcriptional processivity, whereas at higher levels RNA synthesis was strongly inhibited. These activities were independent and mapped to ORF1 and ORF2, respectively. Coexpression of M2(ORF1) with N, P, and L genes was recently shown to be required for the production of infectious RSV from a complete synthetic antigenome, confirming its importance in gene expression by infectious, replication-competent RSV genomic RNA (12).

The M2(ORF1) protein is an internal virion structural protein of low abundance that colocalized intracellularly with the N and P proteins in dense virus-specific cytoplasmic inclusion bodies (13). A protein product of ORF2 has not yet been identified intracellularly. The presence of a counterpart to ORF2 in all pneumoviruses examined to date (6, 14, 15) and the inhibitory activity associated with its expression, give strong support to its identification as an 11th RSV gene. The mechanism by which this internal ORF might be expressed during RSV infection is unknown (6).

Processive, sequential transcription in response to the expression of M2(ORF1) was maximal over a broad range of input plasmid (0.04 to 1.0 relative to N; Fig. 6 *Middle*, lanes 4–6). This was associated with levels of synthesis of intracellular M2(ORF1) protein that ranged from being below detection to being equivalent to that of RSV-infected cells at the peak of viral protein synthesis (Fig. 8, compare lanes 4–6 with lane 7). RSV infection is characterized by a slow, progressive increase in viral protein synthesis; viral proteins first become detectable by ≈8 hr after infection and increase to a maximum by 18–24 hr, which is followed by the peak of virion morphogenesis. Thus, the M2(ORF1) protein probably is active throughout the virus growth cycle. In contrast, the inhibitory effect of M2(ORF2) expressed from the complete gene occurred at a high level of input M2 plasmid (Fig. 2*A*, lanes 5 and 6; Fig. 6 *Top*, lane 6), which mimicked the level of M2 gene expression observed late in RSV infection (Fig. 8, lane 6). This suggests that, in RSV-infected cells, M2(ORF2) probably would be active late in infection coincident with virion morphogenesis and might be involved in rendering nucleocapsids quiescent prior to budding.

The M2(ORF1) protein did not appear to be required for transcription initiation, since the amount of transcription "starts" was the same in its presence or absence (Fig. 3). Also, the small amount of full-length CAT mRNA that was produced in its absence was correctly terminated and polyadenylylated, indicating that M2(ORF1) was not required for those steps (Figs. 2 and 3). Thus, its activity seemed to be restricted to mRNA elongation. Transcriptional elongation or antitermination is an important element of the regulation of gene expression in prokaryotic, eukaryotic, and viral systems (16).

The identification of an elongation factor active for the transcriptase but not the replicase was unexpected, since the two enzymes otherwise ostensively consist of the same protein species and use the same template for mRNA versus antigenome synthesis. However, analysis of intracellular Sendai virus RNAs detected significant amounts of prematurely terminated antigenome (17), indicating that the replicase of a nonsegmented negative strand virus also can be poorly processive. Perhaps the RSV replicase also has an associated elongation activity—one which is different from that of the transcriptase.

Although the balance between the synthesis of mRNA versus antigenome is thought to be a central event in the growth cycle of the nonsegmented negative-strand RNA viruses, it is poorly understood. An important distinction between transcription and replication is that the latter is obligatorily coupled with concurrent encapsidation of the nascent RNA product, although the exact nature of this coupling is not

understood (4, 5, 17). According to the simplest model, the polymerase in its default state is a transcriptase that is switched to replication by the rapid cotranscriptional binding of N protein to the leader sequence at the 5′ end of the nascent positive strand. Other models invoke changes in the conformation of the ribonucleoprotein template (18) and phosphorylation of polymerase components (19).

We suggest a model whereby the polymerase initiates, copies nonprocessively, and terminates within the promoter-proximal end of the genome unless it interacts with a transcription or replication elongation factor. Factor choice could be the switch between transcription and replication. Concurrent encapsidation of the nascent RNA product alone might constitute the replication elongation "factor," but it also is possible that a factor more directly comparable to M2(ORF1) protein is involved, such as soluble N or P protein. For example, the polymerase might contain a single binding site that can interact either with the transcription or replication elongation factor but not both. The binding of either would confer processivity, exclude the other factor, and confer a specificity such that polymerase associated with transcription elongation factor would recognize cis-acting transcription signals and execute stop-start transcription, whereas that associated with replication elongation factor would execute readthrough replication. Dissociation of a transcription elongation factor would account for the polar transcription that is characteristic of nonsegmented negative-strand RNA viruses.

A direct counterpart to M2(ORF1) has not been identified among other negative-strand RNA viruses. These viruses might indeed lack a transcription elongation factor, with RSV representing a divergent or more primitive ancestral situation. Alternatively, another protein species might serve, such as a modified form of NP or P or one of the additional products of the *P* locus such as the C, Y, X, or V proteins (4). A direct counterpart to M2(ORF2) also has not been identified, although the effect of inhibition of RNA synthesis is reminiscent of the inhibition of transcription suggested to be associated with the M protein in other nonsegmented negative-strand RNA viruses (5, 20).

These studies show that RNA synthesis by RSV is more complicated than previously appreciated for the negative-strand RNA viruses, involving at least two additional transacting viral factors. Presumably, increased complexity in gene regulation provides greater flexibility in host interaction. For example, the poorly processive mechanism proposed for the polymerase in the absence of elongation factors suggests a model for latent or persistent infection. This idling state would be reversible by the production of new M2(ORF1) protein, perhaps supplied by a low basal level of processive transcription independent of elongation factor (21) or by a surrogate transcription elongation factor.

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