JOURNAL OF VIROLOGY, Mar. 1977, p. 863-871

Vol. 21, No. 3
Printed in U.S.A.

Regulation of Sendai Virus Transcription: Evidence for a Single Promoter In Vivo

KAREN GLAZIER,[1] RAJENDRA RAGHOW, AND D. W. KINGSBURY*
Laboratories of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101

Received for publication 20 September 1976

The synthesis of Sendai virus RNA species was examined after UV irradiation of cells late in infection. Compared with the inactivation of 50*S* genomic RNA synthesis, the synthesis of the group of mRNA species that sediments at 18*S* was inactivated at an average rate consistent with a process of sequential transcription from a single promoter. The rates of inactivation of the synthesis of individual mRNA's separated by polyacrylamide gel electrophoresis confirmed this and, with the aid of additional data, suggested that the order of genes in the Sendai virus genome is: 3'-NP-F_0-M-P-HN-L-5'.

Cells and DNA-containing viruses regulate polypeptide abundances largely through transcriptional control of mRNA abundances (11, 12, 29). This also seems to be true for those RNA viruses that use the negative-strand strategy of genetic information transfer, as exemplified by the rhabdoviruses and paramyxoviruses (7). In infections by the rhabdovirus, vesicular stomatitis virus (VSV), and the paramyxoviruses, Newcastle disease virus (NDV) and Sendai virus, the various virus polypeptides are made in abundances similar to their relative representation in virus particles (24, 28, 31a, 38); when the virus mRNA's are separated by polyacrylamide gel electrophoresis, their relative rates of synthesis are seen to correspond to the relative synthetic rates of the polypeptides they are thought to code for (13, 37). Furthermore, all of the mRNA's appear to be translated with approximately equal efficiencies in vivo (15).

Recent evidence suggests that the differential transcription of negative-strand virus genes is regulated by a simple mechanism. When the UV irradiation technique for mapping transcriptional units developed by Sauerbier et al. (10, 19, 20) was applied to the in vitro transcription of VSV RNA, the entire virus genome behaved as a single transcription unit, with a single strong promoter at, or close to, its 3' end (1, 5, 6). Moreover, there appears to be a polar gradient of transcription efficiencies, because the VSV gene order deduced from relative rates of mRNA or polypeptide synthesis in vivo is in excellent agreement with the gene order deduced from the UV target sizes of transcripts made in vitro (1, 5, 6, 37).

We have used the UV mapping technique to investigate the organization of the transcriptional apparatus of Sendai virus. The changes we have observed in rates of synthesis of individual mRNA species after UV irradiation of infected cells provide strong evidence that paramyxoviruses, too, transcribe their mRNA's sequentially, and the data suggest the order of genes within the virus genome.

MATERIALS AND METHODS

Virus, cells, and infection. Confluent monolayers of primary chicken embryo lung epithelial (CEL) cells were infected with wild-type Sendai virus (14) at an input multiplicity of 5 to 10 infectious units per cell and were incubated at 37°C under Eagle minimum essential medium supplemented with 3% fetal calf serum.

UV irradiation. At the time of peak virus production, 48 h after infection, the medium was removed from the infected cells and they were exposed to irradiation from a low-pressure germicidal lamp with an output predominantly at 254 nm. The dose rate was about 10 ergs/mm^2 per s, as measured by a Blak Ray ultraviolet meter, model J-225 (Ultraviolet Products, Inc., San Gabriel, Calif.).

Labeling and extraction of RNA. Immediately after UV irradiation, Eagle medium containing 50 μg of actinomycin D per ml (31) was added to the cells. After 30 min of incubation at 37°C, to assure completion of nascent transcripts, the virus-specific RNA was labeled for 3 h with [^{3}H]uridine (23 Ci/mmol, Schwarz/Mann) at a concentration of 50 μCi per ml. Cells were then dissolved in 0.01 M sodium acetate, 0.05 M NaCl, 0.001 M EDTA, 0.5% sodium dodecyl sulfate (SDS) (pH 5.0), and RNA was extracted by shaking with phenol-chloroform (1:1) at 65°C. Fresh phenol-chloroform was added to the aqueous phase, and the mixture was shaken a second time at room temperature. To the final aqueous

[1] Present address: Department of Biology, Case Western Reserve University, Cleveland, OH 44106.

layer, 2.5 volumes of ethanol was added, and this mixture was kept at −20°C overnight to precipitate the RNA.

Sucrose gradient analysis of RNA. Precipitated RNA was dissolved in 2 ml of 0.005 M Tris-hydrochloride, 0.001 M EDTA, 0.1 M NaCl, 0.5% SDS (pH 7.4; TENS buffer) and layered onto a 36-ml linear 15 to 30% (wt/wt) sucrose gradient made up in TENS. Gradients were centrifuged in a Spinco SW27 swinging-bucket rotor at 18,000 rpm at 20°C for 18 h. Radioactivity in 1-ml fractions was measured by liquid scintillation counting after acid precipitation (31).

Isolation of Poly(A)-containing mRNA. mRNA rich in polyadenylic acid [poly(A)] was selected by adsorption to and elution from oligodeoxythymidylic acid[oligo(dT)]-cellulose (4). Each sample, representing the total RNA extracted from one 100-mm dish of cells, was applied to a 1-ml column of oligo(dT)-cellulose (type T2, Collaborative Research Inc., Waltham, Mass.) in 0.01 M Tris-hydrochloride, 0.5 M NaCl, 0.001 M EDTA (pH 7.4). Poly(A)-containing RNA was eluted with 0.01 M Tris-hydrochloride, 0.001 M EDTA (pH 7.4). Fractions of the eluate containing radioactive RNA were pooled, mixed with 0.1 volume of 1 M sodium acetate and 2.5 volumes of ethanol, and placed at −20°C for 16 h or more to precipitate the RNA.

Polyacrylamide gel electrophoresis and fluorography. Poly(A)-rich RNA was collected from ethanol by centrifugation, partially dried in an air stream, dissolved in formamide containing 2 mM sodium PO_4 (pH 7.0), and applied to 4% polyacrylamide slab gels containing phosphate-buffered formamide (16). Electrophoresis was performed under a voltage gradient of 6 V per cm for 24 h with tap water cooling.

Fluorograms of the gels were made using Kodak RP Royal X-ray film hypersensitized to increase sensitivity and linearize response (9, 25).

RESULTS

UV inactivation of Sendai virus RNA synthesis. In this study, we examined Sendai virus-specific RNA synthesis at 48 h after infection, when large amounts of progeny virus genomes had accumulated within the cell and viral transcription and genome replication were proceeding at maximum rates (14, 31). After UV irradiation, total virus-specific RNA synthesis was impaired (Fig. 1). The dose-response curve was not simple first order, but was skewed towards lower inactivation rates at higher doses, an effect noted with other transcribing systems, probably deriving from the recovery of prematurely terminated RNA molecules when acid insolubility is used as the assay (19, 35). As seen in previous work, and as we show below, first-order kinetics of RNA synthesis inactivation are observed when only completed RNA molecules are measured instead (10, 19, 20). Thus, UV-induced lesions in an RNA template appear to cause premature termination of RNA synthesis in vivo, just as when DNA is the template. We also note that the UV dose range effective in the inhibition of Sendai virus RNA synthesis is comparable to that which inhibits cellular RNA synthesis (19), so the inclusion of actinomycin D during RNA labeling did not appear to have untoward effects.

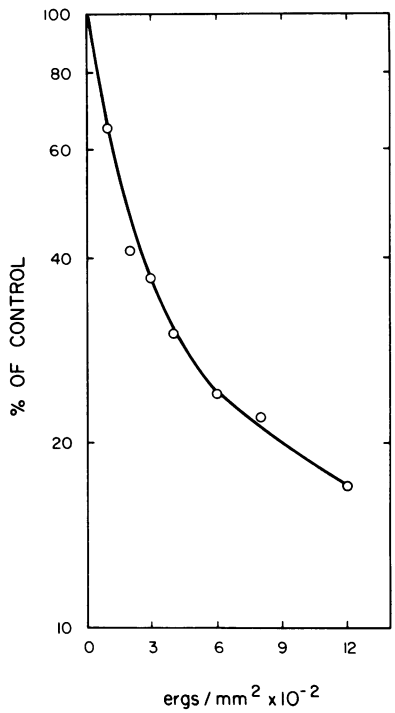

FIG. 1. *Inhibition of Sendai virus-specific RNA synthesis in CEL cells by UV irradiation. After exposure to various doses of UV light, infected cells were labeled with [³H]uridine as described in Materials and Methods and total radioactivity precipitated by 5% trichloroacetic acid was measured.*

Inactivation of transcription versus replication. Available evidence indicates that all but one of the Sendai virus mRNA's sediment together at about 18S (15a, 34), and, as we show later, they have molecular weights less than 10^6 (see also reference 13). On the other hand, the viral genome sediments at about 50S, and has a molecular weight of about 5×10^6 (23). Therefore, target considerations dictate that Sendai virus transcription will be less sensitive to UV inactivation than genome replication. More precisely, the relative sensitivities of the two functions will depend on the number of transcription units in the virus genome. We show this for the two extreme cases: (i) each gene represents a separate transcription unit

(multiple promoters), and (ii) the entire genome is a single transcription unit (a single promoter). For simplicity, we stipulate that one UV hit inactivates all distal transcription in a transcription unit, that all of the genome is devoted to the specification of transcripts, and that all transcripts are the same size and transcribed with equal efficiencies. In the first case, the relative rate of UV inactivation (R_M = transcription/replication; multiple promoters) is obviously the ratio of the target sizes of the molecules in question:

$$R_M = \frac{1}{n} \quad (1)$$

where n = the number of genes. In the second case, the target size of each transcript depends on its distance from the promoter, so the relative rate of UV inactivation (R_S = transcription/replication; single promoter) can be expressed as:

$$R_S = \frac{\sum_{i=1}^{i=n} \frac{i}{n}}{n} \quad (2)$$

where i = the gene order and n = the number of genes.

Applied to Sendai virus, if $n = 6$, we calculate that $R_M = 0.17$ and $R_S = 0.58$, a difference that should be detectable by experiment. Although additional Sendai virus genes may be discovered in the future, it is circumspect to select six as a minimal number to formulate our models, because the predicted difference between the single promoter and multiple promoter models increases as the number of genes increases. This follows from the fact that as n increases, R_M approaches zero, and R_S approaches 0.5.

As shown in Fig. 2, Sendai virus transcription, as measured by incorporation of label into 18S RNA, was, indeed, less sensitive to prior UV irradiation of the infected-cell complex than genome replication, as measured by the incorporation of precursor into 50S RNA. The surviving fraction of radioactivity appearing in each of these sedimenting classes is plotted as a function of UV dose in Fig. 3. Transcription was inactivated at 0.42 of the rate of RNA replication inactivation, a value closer to that predicted by the single promoter model. We show later that when additional data are supplied to the computation of R_S, the theoretical value approaches the experimental value more closely.

Target sizes of individual transcripts. Sup-

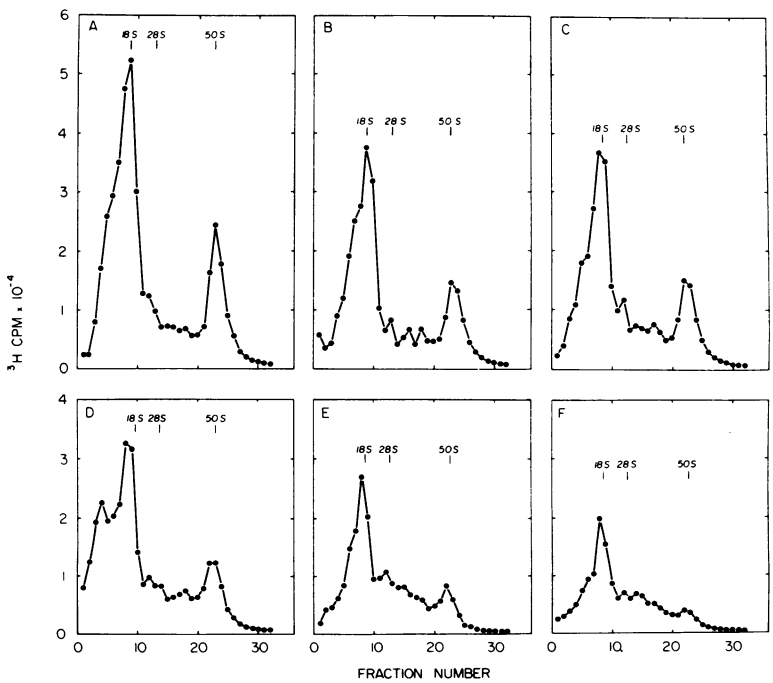

FIG. 2. *Sucrose gradient velocity centrifugation of Sendai virus RNA synthesized after UV irradiation. Doses, in ergs/mm², were: (A) 0; (B) 15; (C) 50; (D) 100; (E) 200; (F) 300. RNA was labeled, extracted, and centrifuged as described in Materials and Methods. The mobilities in the gradients of 18S and 28S CEL cell ribosomal RNAs and 50S Sendai virion RNA, as measured by absorbance at 254 nm, are indicated.*

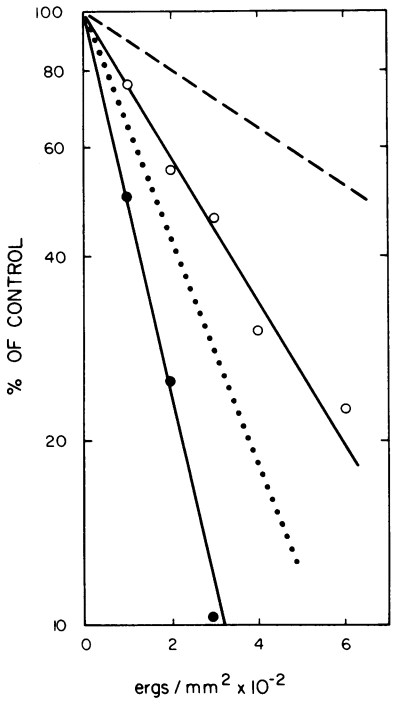

FIG. 3. *Inactivation of Sendai virus RNA replication and transcription by UV. The data points for 50S RNA synthesis (●) and for 18S RNA synthesis (○) were derived by summation of the counts per minute under the respective peaks in Fig. 2. The dashed line represents the expected inactivation rate of 18S RNA synthesis if each mRNA species were a separate transcription unit. The dotted line represents the rate of inactivation expected if all six mRNA species comprise a single transcription unit. For the derivation of these curves, see the text.*

port for the idea that the Sendai virus genome is a single transcription unit came next from examination of the UV target sizes of the mRNA's separated by polyacrylamide gel electrophoresis. To minimize overloading of the gels and to eliminate incomplete (prematurely terminated) RNA molecules, we selected RNA molecules that contained poly(A) (32) for study. As depicted in Fig. 4, five discrete species of RNA were resolved, with apparent molecular weights ranging from 0.44×10^6 to 1.4×10^6 (Table 1). When total virus-specific RNA from infected cells was examined, all of these RNA species and no others were seen in this part of the gel (K. Glazier, unpublished observation), so the selection of poly(A)-rich RNA did not appear to have introduced a serious bias. Also seen in the gels were variable amounts of heterogeneous material that migrated more slowly than the largest putative transcript (Fig. 4); this material may repre-

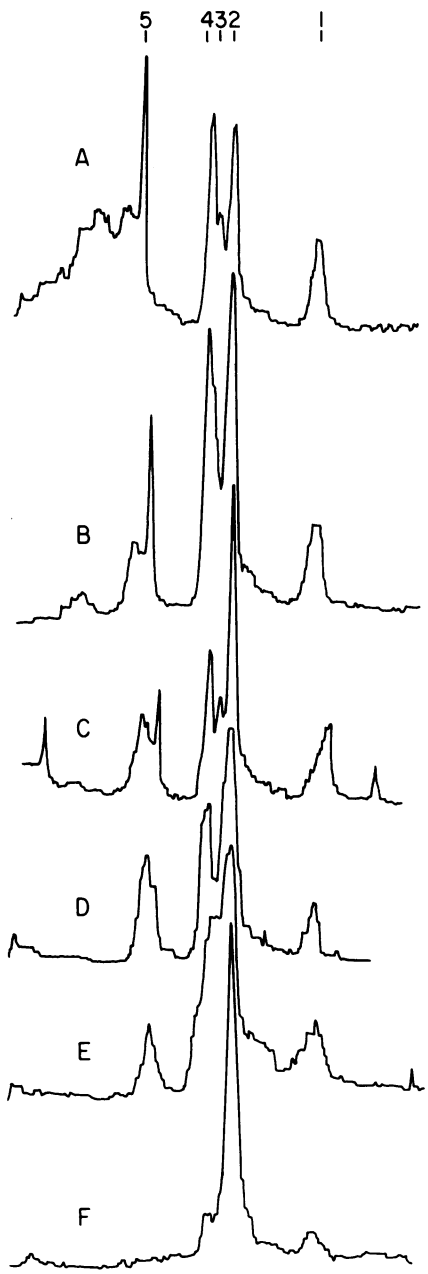

FIG. 4. *Inactivation of the synthesis of Sendai virus transcripts by UV. Doses, in ergs/mm², were: (A) 0; (B) 100; (C) 200; (D) 300; (E) 600; (F) 1,200. After irradiation, RNA was labeled and extracted, poly(A)-rich material was selected and subjected to polyacrylamide gel electrophoresis in formamide, and fluorograms were prepared as described in Materials and Methods. Shown are tracings made by an Ortec densitometer. Putative mRNA species are numbered in order of decreasing electrophoretic mobility.*

TABLE 1. *Apparent molecular weights of Sendai virus poly(A)-rich RNA species and polypeptides*

RNA species[a]	Mol wt of RNA $\times 10^{-5}$[b]	Probable gene[c]	Mol wt of polypeptide $\times 10^{-4}$[d]
5	13.7	L	16
4	8.6	F_0 and P	6.5 and 7.9
3	7.8	HN	7.2
2	7.3	NP	6.0
1	4.4	M	3.4

[a] RNA species are numbered as shown in Fig. 4.

[b] Calculated from electrophoretic mobilities in formamide-containing polyacrylamide gels, relative to the following standards (19, 37): chicken embryo cell 28*S* rRNA (molecular weight, 1.7×10^6); chicken embryo cell 18*S* rRNA (molecular weight, 0.71×10^6); *Escherichia coli* 23*S* rRNA (molecular weight, 1.07×10^6); *E. coli* 16*S* rRNA (molecular weight, 0.55×10^6).

[c] Based on the molecular weights of the polypeptides and other considerations discussed in the text.

[d] From Lamb et al. (24).

sent RNA aggregates (8) or transcriptive intermediates (31), since we did not denature our RNA preparations thermally before oligodeoxythymidylic acid fractionation.

The calculated molecular weights of the RNA species, uncorrected for poly(A) content (32, 37), are in reasonable agreement with the expected sizes of templates for the Sendai virus polypeptides (Table 1). Since there are six polypeptides and only five electrophoretic RNA species, it appears that one of the latter is comprised of two transcripts with similar molecular weights. For reasons we discuss later, we believe that the unresolved pair is in band 4, that the two messages in question are those for polypeptides F_0 and P, and that band 3 represents HN and band 2 represents NP. The assignment of band 5 to polypeptide L and band 1 to polypeptide M are more immediately obvious, since these species represent the extremes of the transcript and polypeptide molecular weight spectra and each is well separated from the remaining transcripts or polypeptides.

The lower tracings in Fig. 4 reveal that the UV sensitivities of the templates for most transcripts did not reflect the molecular weights of the transcripts. Most striking was the behavior of band 2. It was the most UV-resistant species, even though it was not the smallest. Inactivation rates for all of the RNA species are presented in Fig. 5. Band 2 was followed in order of increasing sensitivity to inactivation by bands 1, 4, 3, and 5. From these data, the UV dose required to inactivate the synthesis of each RNA species to a level of e^{-1} was determined (Table 2). Clearly, these values are not inversely proportional to the molecular weights of the RNA species, the prediction of target theory if each species represented an independent transcription unit. Instead, they fit a single promoter model with additive target sizes (1, 5, 6, 10, 19, 20). However, the target distance between RNA species 2 and 1 was disproportionately large (Table 2); our explanation is given below.

Order of genes in the genome. If all Sendai virus genes comprise a single transcription unit and if we can relate the genes to each of the RNA species that we resolved, we can establish the order of genes in the Sendai virus genome. Our argument is circumstantial, since we have not identified the gene products of these RNAs directly by their separate translation in a cell-free system. Proceeding by

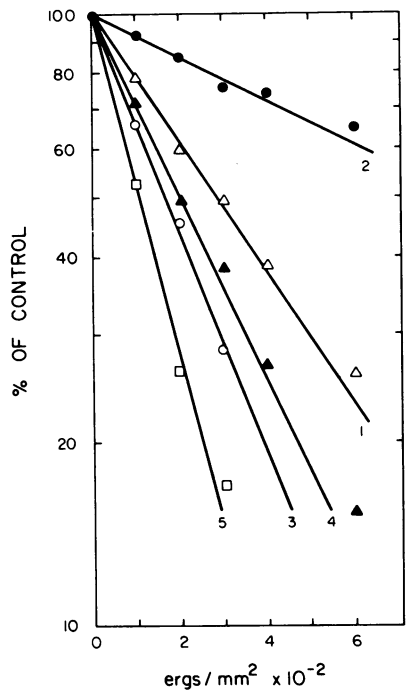

FIG. 5. *Inactivation of the transcription of Sendai virus RNA species by UV. The data points were derived from the weights of areas cut from the numbered peaks in densitometer tracings like those shown in Fig. 4, adjusted for the total radioactivity in each poly(A)-rich RNA sample as described by Portner and Kingsbury (31a). The data shown are from a single experiment, except for band 3. Since this band did not always separate clearly from adjacent bands (see Fig. 4), the data from four experiments were combined to derive the values shown. Three other experiments gave results for the remaining bands similar to those shown above.*

TABLE 2. *UV target sizes and molecular weights of Sendai virus RNA species*

RNA species[a]	Probable gene[b]	D_0 (ergs/mm²)[c]	Observed target size $\times 10^{-6}$ daltons[d]	Theoretical target size $\times 10^{-6}$ daltons[e]	
				Single transcription unit	Multiple transcription units
2	NP	1,420	0.60	0.73	0.73
1	M	434	1.96	2.03	0.44
4	F_0 and P	311	2.74	1.6 and 2.9	0.86
3	HN	241	3.54	3.7	0.78
5	L	164	5.20	5.1	1.37

[a] Numbered as in Fig. 4.
[b] As assigned in Table 1.
[c] The UV dose that reduced synthesis of the RNA species to e^{-1} of an unirradiated sample (Fig. 5).
[d] Calculated from the relationship $T = (C \times D_0)^{-1}$, where T is the target size; C is proportionality constant, based on the target size of a standard, which was chosen as the virus genome (23; Fig. 3); and D_0 has been defined.
[e] Based on the molecular weights of the RNA species presented in Table 1.

other criteria, we have already used their molecular weights to assign RNA 1 to gene M and RNA 5 to gene L (Table 1). On the same basis, RNA 2 probably represents gene NP; it is the second smallest RNA and polypeptide NP has the same rank among the primary viral translation products (24) (Table 1).

Three polypeptides, F_0, HN, and P, remain to be apportioned between two RNA species, 3 and 4. The doublet seems to be RNA 4, because it is the more abundant, and we have, in fact, occasionally seen two partially resolved components in band 4. Strict adherence to the order of polypeptide molecular weights derived from SDS-polyacrylamide gel electrophoresis (24) would assign RNA 3 to F_0 and RNA 4 to HN + P. But two of the polypeptides in question, F_0 and HN, are glycosylated, so there is some uncertainty about their molecular weights.

We think it is more likely that RNA 3 represents HN and RNA 4 represents F_0 + P, on the assumptions that the abundance of a polypeptide reflects the abundance of its message (15) (Fig. 4 and Table 3) and that the abundance of a message reflects the distance between its gene and the single transcription promoter (37) (Tables 2 and 3). The data in Table 3 are based on [^{35}S]methionine labeling, but the methionine contents of Sendai virus polypeptides are so similar that errors in the ranking seem unlikely (24).

Finally, to account for the disproportionate UV target distance between RNA 2 and RNA 1 (Table 2), we propose that one of the genes represented by the transcripts in RNA 4 intervenes between NP and M in the genetic map. F_0 is our candidate for this position, since polypeptide F_0 is synthesized more abundantly than any other polypeptide, except NP (Table 3). We explain the target size of band 4 as the average of the relatively small and relatively large target sizes of genes F_0 and P, respectively. Thus, our suggestion for the gene order of Sendai virus is: 3′-NP-F_0-M-P-HN-L-5′.

TABLE 3. *Molar rates of Sendai virus polypeptide synthesis*

Polypeptide	Relative rate of synthesis[a]	Relative molar rate of synthesis[b]
NP	27.5	4.6
F_0 + F[c]	13.4 + 8.5	2.1 + 1.7
M	10.8	3.2
P	17.0	2.2
HN	10.2	1.4

[a] Relative incorporation of [^{35}S]methionine in a 10-min pulse; from Portner and Kingsbury (31a).
[b] Calculated by dividing the relative rates of synthesis by the polypeptide molecular weights given in Table 1 and (24).
[c] F is the cleavage product of F_0 (24, 31a).

DISCUSSION

Our approach to the mapping of the transcriptional organization of the Sendai virus genome by UV irradiation has the advantage over previous studies on VSV transcriptive complexes in vitro (1, 6) of directly measuring events in the infected cell. However, Villarreal et al. (37) showed that the stoichiometry of the synthetic rates of VSV mRNA's and polypeptides corresponds to the gene order derived from UV mapping studies, and Ball (5) has more recently demonstrated that the target sizes of VSV polypeptides made after primary tran-

scription in vivo from irradiated virion templates also agree. Clearly, the evidence is mounting in support of the conclusion that the genomes of both rhabdoviruses and paramyxoviruses are organized as single transcriptional units.

In another departure from previous approaches, we did not irradiate our virus; instead, we irradiated the infected cell at a time when abundant virus-specific RNA synthesis occurs on accumulated nucleocapsid templates. We had thought that the addition of cycloheximide to the cells before irradiation might be necessary to block the production of new genomic RNA templates for transcription in cases where UV doses were insufficient themselves to abolish RNA replication (33). Significant transcription from undamaged templates would have biased the results against detection of a single-promoter genome. But despite significant RNA replication at low UV doses (Fig. 2), a multiple promoter model was clearly contraindicated (Table 2), apparently because most of the transcription that we measured derived from templates present in the cells at the time of irradiation (14).

The lack of close agreement between the simplified formulation (equation 2) of the single promoter model and the experimental measure of transcription inactivation versus replication inactivation by UV (Fig. 3) may be explained as follows. The simple model eliminated considerations like gene size differences, differential transcription rates, and recovery of all mRNA species. In particular, the transcript for gene L is not expected to be represented in the 18S population that we took as an overall measure of transcription (34). If the target sizes of Table 2 are entered into equation 2, taking the target sizes of the two components assumed to be present in RNA band 4 to be 1.6 and 2.9 (Table 2), and if the putative L gene is included, a value of 0.58 is obtained for R_S, the same as predicted by the simple model. But, if the contribution of the L gene is deleted, the value obtained for R_S is 0.39, much closer to the experimental value of 0.42.

With respect to the impact of differential rates of transcript synthesis on R_S, if promoter-proximal genes are indeed transcribed more abundantly than promoter-distal genes, this weights R_S towards lower values, independent of other considerations. Quantitation of the radioactivity in the viral RNA species from unirradiated cells gave relative molar rates of synthesis for species 2, 1, 4, and 3 that corresponded to the order of relative molar rates of viral polypeptide synthesis shown in Table 3. But species 5 had a synthetic molar abundance greater than species 3, though less than all the other RNA species (K. Glazier, unpublished data). This discrepancy challenges our gene order and the notion that gene order determines transcript abundances. However, we have found that the recovery of different RNA species varies with the conditions used for their extraction, (K. Glazier, unpublished data) and our quantitation was based solely on poly(A)-containing transcripts, so it may be premature to place undue weight on this point until more data on Sendai virus transcription rates are obtained. Different efficiencies of recovery of different transcripts would not invalidate our UV data, since we compared the changes after irradiation to the unirradiated control value for each RNA species.

The major deficiency in our data is the lack of a direct demonstration of the identity of each of the mRNA species we resolved. Until this is remedied by their individual translation in vitro, our assignment of gene order must be considered tentative. Also, it will be desirable to have independent confirmation of the map order of the mRNA's we resolved, such as by hybridization tests with RNA molecules from defective-interfering virus particles (26, 36). Assuming that we have the correct gene order, it has some interesting parallels with that proposed for VSV (3′ N-NS-M-G-L 5′) (1, 5, 6). In both cases, the most abundant polypeptide in infected cells is the nucleocapsid structure unit and its gene is promoter proximal, whereas the gene for the least abundant and largest polypeptide, the nucleocapsid-associated polypeptide, L, is placed at the opposite end of the genome. The order of intermediate genes differs, however. If gene order determines gene product abundances consistently in these systems, the differences presumably reflect the special needs of each virus for certain polypeptides in particular levels of abundance.

Regulation of gene expression by gene order has been termed "polarity" (21, 27, 29). Originally discovered in connection with nonsense mutations in prokaryotes, polarity may be a normal device for regulating the abundances of polypeptides represented by an operon or transcription unit. However, the mechanism by which polarity determines mRNA abundances is not yet clear, even in prokaryotes. Cases have been made for the primacy of a transcriptional level of control, or alternatively, for a post-transcriptional, degradative control of mRNA abundances (21, 27, 29). This is the crossroad at which our understanding of negative-strand virus transcription is poised; if tran-

scription begins only at a single promoter, how are individual mRNA species generated, and what regulates their abundances?

Previous authors (1, 5, 6) have opted for a cleavage mechanism, a theme that is currently popular in eukaryote molecular biology (11). A cogent argument in support of this idea comes from the evidence that the beta-phosphate at the 5′ end of each VSV message is derived from the inverted 7-methyl guanylate added post-transcriptionally (2), contrasting with other systems in which the beta-phosphate can be traced to the 5′ triphosphate that initiates transcription (17, 18). However, it has not been ruled out that a phosphoesterase cleaves the original terminal triphosphate to yield a free alpha-phosphate in the case of VSV. In addition, there has been no demonstration of the existence in any negative-strand virus of a nuclease with the specificity that a processing model demands. Therefore, alternative models warrant consideration. For example, the 3′ promoter might be the only "independent" promoter capable of being used in the initiation of transcription regardless of the previous location of the transcriptase that encountered it, whereas each of the distal genes might contain "dependent" promoters capable of initiating a transcript only if presented with a transcriptase that has just terminated the 3′ proximal gene. In this case, polarity could result from decreasing efficiencies of dependent promotion along the genome. This last corollary can also be applied to explain polarity if there is a cleavage mechanism, with some modification.

What impact does the discovery of a single transcription unit for negative-strand viruses have on our understanding of the relationship between transcription and the other vital form of virus-specific RNA synthesis, replication? The first step in replication involves the production of an RNA molecule with the same polarity as transcripts, but it must be the size of the genome (22). If individual mRNA's are produced by cleavage, then the mechanism for producing antigenomes must abrogate that cleavage. A virus-specific protein is clearly involved (30, 33). It could be a nuclease inhibitor or, as we have suggested earlier, it could be the structural protein of the nucleocapsid itself (22) which, in the process of encapsidating the product, would protect it from nuclease action. On the other hand, if cleavage is not involved, the nucleocapsid protein could switch on replication by acting as a suppressor of termination, analogous to the action of the product of the N gene of bacteriophage lambda (3).

For both VSV and Sendai virus, the primacy of transcriptional regulation of polypeptide abundances need not be absolute. A superimposed "fine-tuning" level of translational control has not been disproven; in fact, close inspection of our data on the relative abundance of the putative message for polypeptide L compared with available data on the abundance of polypeptide L in infected cells (24, 38) suggests that this message is inefficiently translated. A similar situation may exist for the L polypeptide of VSV (15).

ACKNOWLEDGMENTS

We thank Donna Clift for skillful technical assistance.

This work was supported by Public Health Service research grant AI-05343 from the National Institute of Allergy and Infectious Diseases, by training grant TO1-CA-05176 from the National Cancer Institute, and by ALSAC. R.R. is a Leon Journey Fellow of St. Jude Children's Research Hospital.

LITERATURE CITED

1. **Abraham, G., and A. K. Banerjee.** 1976. Sequential transcription of the genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. 73:1504–1508.
2. **Abraham, G., D. P. Rhodes, and A. K. Banerjee.** 1975. The 5′ terminal structure of the methylated mRNA synthesized *in vitro* by vesicular stomatitis virus. Cell 5:51–58.
3. **Adhya, S., M. Gottesman, and B. de Crombrugghe.** 1974. Release of polarity in *Escherichia coli* by gene *N* of phage λ: termination and antitermination of transcription. Proc. Natl. Acad. Sci. U.S.A. 71:2534–2538.
4. **Aviv, H., and P. Leder.** 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. U.S.A. 69:1408–1412.
5. **Ball, L. A.** 1977. Transcriptional mapping of vesicular stomatitis virus in vivo. J. Virol. 21:411–414.
6. **Ball, L. A., and C. N. White.** 1976. Order of transcription of genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. U.S.A. 73:442–446.
7. **Baltimore, D.** 1971. Expression of animal virus genomes. Bacteriol. Rev. 35:235–241.
8. **Bantle, J. A., I. H. Maxwell, and W. E. Hahn.** 1976. Specificity of oligo(dT)-cellulose chromatography in the isolation of polyadenylated RNA. Anal. Biochem. 72:413–427.
9. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. 46:83–88.
10. **Bräutigam, A. R., and W. Sauerbier.** 1974. Transcription unit mapping in bacteriophage T7. II. Proportionality of number of gene copies, mRNA, and gene product. J. Virol. 13:1110–1117.
11. **Brawerman, G.** 1974. Eukaryotic messenger RNA. Annu. Rev. Biochem. 43:621–642.
12. **Calendar, R.** 1970. The regulation of phage development. Annu. Rev. Microbiol. 24:241–296.
13. **Collins, B. S., and M. A. Bratt.** 1973. Separation of the messenger RNAs of Newcastle disease virus by gel electrophoresis. Proc. Natl. Acad. Sci. U.S.A. 70:2544–2548.
14. **Darlington, R. W., A. Portner, and D. W. Kingsbury.** 1970. Sendai virus replication: an ultrastructural comparison of productive and abortive infections in avian cells. J. Gen. Virol. 9:169–177.
15. **David, A. E.** 1976. Control of vesicular stomatitis virus protein synthesis. Virology 71:217–229.

15a. **Davies, J. W., A. Portner, and D. W. Kingsbury.**

1976. Synthesis of Sendai virus polypeptides by a cell-free extract from wheat germ. J. Gen. Virol. 33:117-123.
16. **Duesberg, P. H., and P. K. Vogt.** 1973. Gel electrophoresis of avian leukosis and sarcoma viral RNA in formamide. Comparison with other viral and cellular RNA species. J. Virol. 12:594-599.
17. **Ensinger, M. J., S. A. Martin, E. Paoletti, and B. Moss.** 1975. Modification of the 5'-terminus of mRNA by soluble guanylyl and methyl transferases from vaccinia virus. Proc. Natl. Acad. Sci. U.S.A. 72:2525-2529.
18. **Furuichi, Y., M. Morgan, S. Muthukrishnan, and A. J. Shatkin.** 1975. Reovirus messenger RNA contains a methylated, blocked 5'-terminal structure: $m^7G(5')ppp(5')G^mpCp^-$. Proc. Natl. Acad. Sci. U.S.A. 72:362-366.
19. **Hackett, P. B., and W. Sauerbier.** 1975. The transcriptional organization of the ribosomal RNA genes in mouse L cells. J. Mol. Biol. 91:235-256.
20. **Hercules, K., and W. Sauerbier.** 1973. Transcription units in bacteriophage T4. J. Virol. 12:872-881.
21. **Imamoto, F., and D. Schlessinger.** 1974. Bearing of some recent results on the mechanisms of polarity and messenger RNA stability. Mol. Gen. Genet. 135:29-38.
22. **Kingsbury, D. W.** 1974. The molecular biology of paramyxoviruses. Med. Microbiol. Immunol. 160:73-83.
23. **Kolakofsky, D., E. Boy de la Tour, and H. Delius.** 1974. Molecular weight determination of Sendai and Newcastle disease virus RNA. J. Virol. 13:261-268.
24. **Lamb, R. A., B. W. J. Mahy, and P. W. Choppin.** 1976. The synthesis of Sendai virus polypeptides in infected cells. Virology 69:116-131.
25. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. 56:335-341.
26. **Leamnson, R. N., and M. E. Reichmann.** 1974. The RNA of defective vesicular stomatitis virus particles in relation to viral cistrons. J. Mol. Biol. 85:551-568.
27. **Lim, L. W., and D. Kennell.** 1974. Evidence against transcription termination within the *E. coli lac* operon. Mol. Gen. Genet. 133:367-371.
28. **Lomniczi, B., A. Meager, and D. C. Burke.** 1971. Virus RNA and protein synthesis in cells infected with different strains of Newcastle disease virus. J. Gen. Virol. 13:111-120.
29. **Martin, R. G.** 1969. Control of gene expression. Annu. Rev. Genet. 3:181-216.
30. **Perlman, S. M., and A. S. Huang.** 1973. RNA synthesis of vesicular stomatitis virus. V. Interactions between transcription and replication. J. Virol. 12:1395-1400.
31. **Portner, A., and D. W. Kingsbury.** 1972. Identification of transcriptive and replicative intermediates in Sendai virus-infected cells. Virology 47:711-725.
31a. **Portner, A., and D. W. Kingsbury.** 1976. Regulatory events in the synthesis of Sendai virus polypeptides and their assembly into virions. Virology 73:79-88.
32. **Pridgen, C., and D. W. Kingsbury.** 1972. Adenylate-rich sequences in Sendai virus transcripts from infected cells. J. Virol. 10:314-317.
33. **Robinson, W. S.** 1971. Sendai virus RNA synthesis and nucleocapsid formation in the presence of cycloheximide. Virology 44:494-502.
34. **Roux, L., and D. Kolakofsky.** 1975. Isolation of RNA transcripts from the entire Sendai virus genome. J. Virol. 16:1426-1434.
35. **Sauerbier, W., R. L. Millette, and P. B. Hackett, Jr.** 1970. The effects of ultraviolet irradiation on the transcription of T4 DNA. Biochim. Biophys. Acta 209:368-386.
36. **Stamminger, G., and R. A. Lazzarini.** 1974. Analysis of the RNA of defective VSV particles. Cell 3:85-93.
37. **Villarreal, L. P., M. Breindl, and J. J. Holland.** 1976. Determination of molar ratios of vesicular stomatitis virus induced RNA species in BHK_{21} cells. Biochemistry 15:1663-1667.
38. **Zaides, V. M., L. M. Selimova, O. P. Zhirnov, and A. G. Bukrinskaya.** 1975. Protein synthesis in Sendai virus-infected cells. J. Gen. Virol. 27:319-327.