Proc. Nat. Acad. Sci. USA
Vol. 73, No. 2, pp. 442–446, February 1976
Biochemistry

Order of transcription of genes of vesicular stomatitis virus

(coupled transcription and translation/eukaryotic cell-free system/ultraviolet irradiation/mechanism of transcription/gene order)

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Communicated by James E. Darnell, December 3, 1975

ABSTRACT **The effect of ultraviolet radiation on the expression of the genes of vesicular stomatitis virus (VSV) was studied in a cell-free system which executed coupled transcription and translation of the viral genes. In this system, which contained detergent-activated virus and a cytoplasmic extract of mouse L-cells, three of the five viral proteins (N, NS, and M) were synthesized accurately and efficiently; a putative precursor to the viral glycoprotein (G) was also made, but synthesis of the L protein was not detected. In response to irradiation of VSV, the synthesis of each protein in the coupled system displayed a characteristic single-hit inhibition. This permitted calculation of the apparent target-sizes for expression of the different viral genes relative to the entire genome (3.6 to 4.0 $\times 10^6$ daltons) as measured by loss of infectivity. These are: N, 0.55 $\times 10^6$; NS, 0.83 $\times 10^6$; M, 1.12 $\times 10^6$; G, 1.76 $\times 10^6$ daltons of RNA. Only the N protein gene has a target-size which corresponds to that predicted from the molecular weight of its messenger RNA (molecular weight: 0.55 $\times 10^6$). The target-sizes for the other three genes are two to four times larger than expected, and are not proportional to the molecular weights of their corresponding messenger RNAs (molecular weights: NS, 0.28 $\times 10^6$; M, 0.28 $\times 10^6$; G, 0.7 $\times 10^6$). This polar effect of UV irradiation is inconsistent with independent transcription of each of the genes of VSV. Rather, the target-sizes appear to be cumulative, suggesting that transcription initiates at a single point on VSV RNA and proceeds in the order 3′ - N - NS - M - G - (L) - 5′.**

Vesicular stomatitis virus (VSV) (Indiana serotype) is a rhabdovirus containing as its genome a single negative strand of RNA of molecular weight 3.6 to 4.0 $\times 10^6$ (1). This RNA contains the genes for the five known virus-specific proteins, all of which are components of the virus particle: L, the virion-associated transcriptase (RNA nucleotidyltransferase, EC 2.7.7.6); G, the virion glycoprotein, which is a component of the viral lipid envelope; N, the nucleoprotein; NS, a minor protein of unknown function which is associated with the viral core; and M, the matrix protein, another component of the viral envelope (2). Five corresponding species of messenger RNA have been extracted from infected cells, and characterized by translation in cell-free systems (3, 4). The molecular weights of these mRNAs are: L, $\geqslant 1.65 \times 10^6$; G, 0.7 $\times 10^6$; N, 0.55 $\times 10^6$; M and NS, both 0.28 $\times 10^6$ (5). The mRNAs are synthesized by the viral transcriptase and are complementary to the viral genome (6, 7).

In vitro, low concentrations of non-ionic detergents remove the envelope of VSV and activate the virion transcriptase (8). Like the polysomal RNA from infected cells, the transcription product is complementary to the viral genome and can be translated *in vitro* into authentic viral proteins (9) although the synthesis of L protein from mRNA made *in vitro* has not been reported.

However, the detailed mechanism of VSV mRNA synthesis and its relationship to replication is unclear. In particular it is not known whether the individual mRNAs result from independent initiation of transcription at five sites on the genome, or whether they arise by sequential read-through from a single initiation site, followed by processing of a large precursor molecule. Clearly, a minor adaptation of the latter mechanism could provide a pathway to an intact positive copy of the genome RNA, a necessary intermediate in replication. In this paper we describe experiments designed to show whether VSV has a single or multiple sites for the initiation of transcription.

A major effect of low doses of ultraviolet light on nucleic acids is to produce covalent thymine or uracil dimers in regions containing T-T or U-U sequences (10, 11). Transcription beyond such dimers is blocked (12). We therefore investigated the effect of UV irradiation of VSV on the expression of the viral genes. Expression of the genes for N, NS, M, and G proteins was assayed by measuring synthesis of the viral proteins *in vitro* in a coupled transcription–translation system that makes these four proteins under the direction of detergent-activated VSV. If the genes are transcribed independently, then the sensitivity of each to UV irradiation of the virus should be proportional to the size of the gene; i.e., the target-size for each gene will be directly related to its physical size. On the other hand, if transcription initiates at only one point on the genome, and the mRNAs are synthesized by read-through and processing, then the sensitivity of a gene will depend strongly on its position in the genome, since its target-size will include all proximal genes. In the latter situation, the gene order can be determined by reference to the relative sensitivities of the individual genes. The same approach has been used to map the ribosomal RNA genes in *Escherichia coli* (13) and mouse L-cells (14).

MATERIALS AND METHODS

Cells and Virus. The heat-resistant strain of VSV (Indiana serotype; obtained originally from Dr. A. F. Holloway) was grown in African green monkey kidney (Vero) cells and purified by sucrose velocity gradient centrifugation and glycerol–potassium tartrate equilibrium density gradient centrifugation (15). The purified virus was stored at −70° in 10 mM Tris·HCl, pH 7.6, containing 100 mM NaCl and 1 mM EDTA. Standard preparations contained about 10^{10} plaque-forming units/ml [assayed on monolayers of Vero cells (16)], and 0.2 mg of protein per ml. For the growth of radioactively labeled virus, L-[^{14}C]arginine and L-[^{14}C]lysine, each at 1.1 Ci/mol and 0.16 mM, were present from 2.5 to 20 hr after infection.

UV Irradiation. Standard preparations of VSV were exposed to UV radiation essentially as described by Marcus and Sekellick (17), but without prior dilution. The dose rate was 6.5 ergs mm^{-2} sec^{-1} (1 erg = 10^{-7} J). Under these conditions, infectivity was lost at an exponential rate with a 37% ($1/e$) survival dose of 120 ergs mm^{-2}. We attribute this

Abbreviation: VSV, vesicular stomatitis virus.

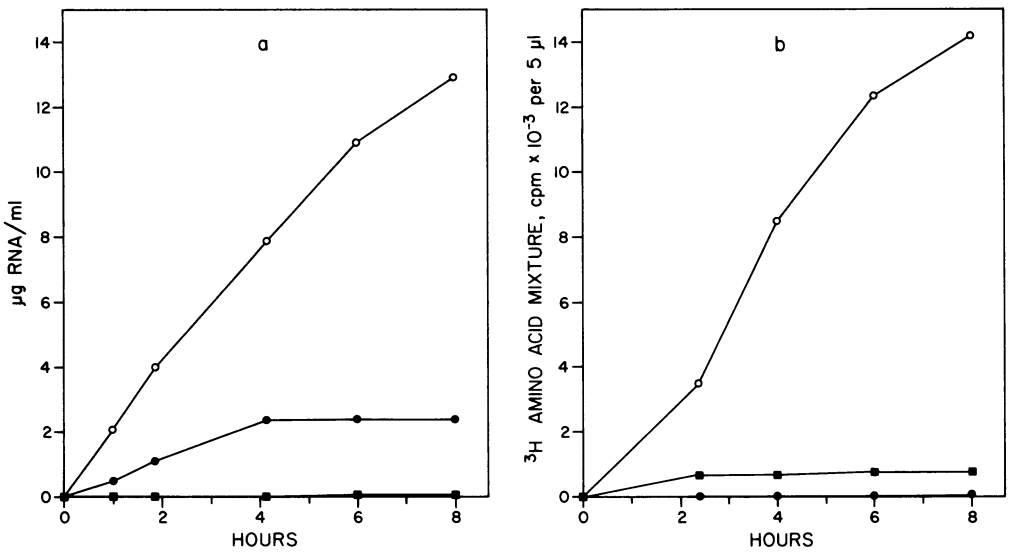

FIG. 1. Kinetics of incorporation of label from (a) [^{3}H]UTP and (b) a mixture of ^{3}H-labeled amino acids in the coupled transcription-translation cell-free system. Aliquots (5 μl) were taken at the times shown and assayed for radioactivity insoluble in trichloroacetic acid at 0° (panel a) or trichloroacetic acid at 85° (panel b). ■, Cell extract without VSV; ●, VSV (50 μg/ml) without the cell extract; ○, cell extract plus VSV (50 μg/ml).

anomalously high value to the high concentration of virus during irradiation (cf. 17).

Cell-Free RNA and Protein Synthesis. Cell-free protein synthesizing systems were prepared as described previously (18) from mouse L-929 cells grown in suspension culture. Reaction mixtures for coupled transcription and translation of VSV RNA contained: 30 mM Tris·HCl, pH 7.6; 33 mM NH_4Cl; 7 mM KCl; 4.5 mM Mg-acetate; 1 mM dithiothreitol; 0.2 mM spermidine; 1 mM ATP; 0.5 mM GTP, CTP, and UTP; 10 mM creatine phosphate; 80 μg of creatine kinase per ml; 50 μM appropriate unlabeled amino acids; 0.05% Triton-N101; 50 μg of purified VSV per ml; and 30% cell-free extracts (to give a final concentration of about 1.5 mg of protein per ml). Cell-free protein synthesis was unaffected by 0.05% Triton-N101. To follow RNA synthesis, we added 100 μCi/ml of [^{3}H]UTP (Amersham/Searle Corp., specific activity 49 Ci/mmol). To follow protein synthesis we added 100 μCi/ml of a reconstituted protein hydrolysate containing 15 ^{3}H-labeled L amino acids, but lacking asparagine, cysteine, glutamine, methionine, and tryptophan (Schwarz/Mann), or 100 μCi/ml of a mixture of L-[4,5-^{3}H]lysine (Amersham/Searle Corp., 18 Ci/mmol) and L-[5-^{3}H]arginine (Amersham/Searle Corp., 22 Ci/mmol). Incubation was at 30°. Aliquots were spotted on Whatman GF/A paper discs and washed either with cold 5% trichloroacetic acid (0°) containing 40 mM sodium pyrophosphate (to follow RNA synthesis) or with hot trichloroacetic acid (85°, to follow protein synthesis). The dried filters were analyzed in a liquid scintillation counter.

Polyacrylamide Gel Electrophoresis and Fluorography. In preparation for polyacrylamide gel electrophoresis, the protein products of the cell-free system (37 μl) were digested for 15 min at 30° with ribonuclease A (50 μg/ml) in the presence of 10 mM EDTA and 100 mg of casein hydrolysate per ml. Sodium dodecyl sulfate and 2-mercaptoethanol were added to give final concentrations of 1% and 2%, respectively, and the samples were heated at 100° for 2 min. After cooling, the samples were made 2 M in urea, and 0.01 M in sodium phosphate, pH 7.6. They were then subjected to electrophoresis on 10% polyacrylamide-dodecyl sulfate slab gels (17 cm long × 0.3 cm thick) according to the method of Weber and Osborn (19), except that the gels contained 6 M urea and were prerun for about 2 hr with a solution of 1% 3-mercaptopropionic acid and 6 M urea in electrophoresis buffer. Electrophoresis was at 60 mA for 60 hr, after which the gels were stained with Coomassie Brilliant Blue, destained, and either subjected to fluorography by the method of Bonner and Laskey (20) (for ^{3}H-labeled proteins; Fig. 3) or each channel was sliced transversely into 2 mm fractions (for double-labeled proteins; Fig. 2). The gel slices were dissolved in 0.5 ml of 30% H_2O_2 at 60° for 5 hr, mixed with 7.5 ml of Aquasol (New England Nuclear), and analyzed in a liquid scintillation counter under double label counting conditions which gave negligible spillover of ^{3}H counts into the ^{14}C channel, and 16.2% spillover in the other direction. For accurate measurement of the amount of radioactivity in ^{3}H-labeled proteins, appropriate bands were cut out of the dried gel slab using the fluorograph as a guide. The dried gel fragments were dissolved in 1 ml of 30% H_2O_2 at 60° for 24 hr, mixed with 12 ml of Aquasol, and analyzed in a liquid scintillation counter.

RESULTS

Coupled Transcription and Translation *In Vitro*. The RNA synthesis induced by 50 μg/ml of purified VSV in the presence of 0.05% Triton-N101 and a cell-free system derived from mouse L-cells is shown in Fig. 1a. VSV transcription was stimulated and prolonged by the addition of the cell extract, and a linear rate of RNA synthesis for up to 12 hr has been observed. A strong stimulation of amino acid incorporation occurred in response to VSV transcription (Fig. 1b), and a linear rate of protein synthesis for up to 10 hr (after a short lag) has been observed under these conditions. We attribute this unprecedented endurance of cell-free protein synthesis to the continuous production of new mRNA. The total amount of VSV mRNA synthesized was relatively low (about 12 μg/ml at 8 hr) compared with the amounts which normally saturate cell-free systems, and accordingly, protein synthesis was proportional to RNA synthesis under the conditions of the experiments.

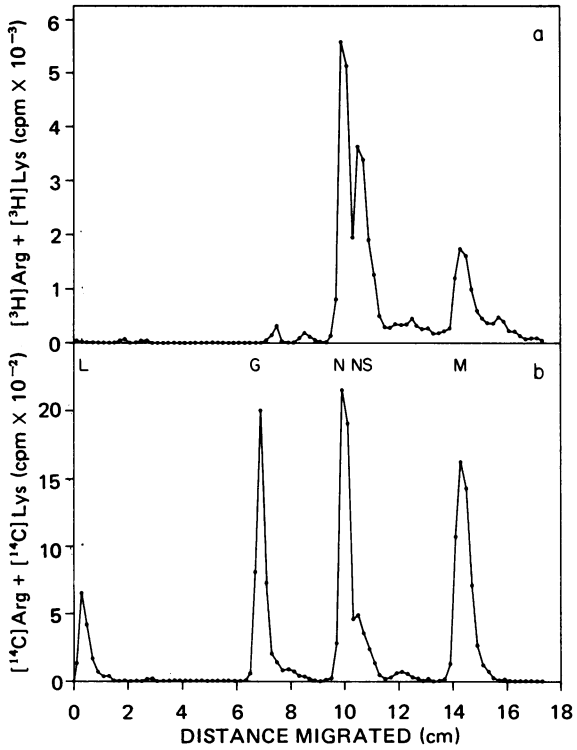

FIG. 2. Polyacrylamide gel electrophoresis of the protein products of the coupled system directed by VSV (panel a; [^{3}H]arginine- and [^{3}H]lysine-labeled), and of purified VSV (panel b; [^{14}C]arginine- and [^{14}C]lysine-labeled). An aliquot of the cell-free system (40,000 ^{3}H cpm) was taken after 8 hr incubation and prepared for electrophoresis as described in the *text*. The sample was mixed with purified VSV (20,000 ^{14}C cpm) and subjected to electrophoresis on a 10% polyacrylamide gel (19). Transverse slices (2 mm) of the gel were dissolved and subjected to double label analysis in a liquid scintillation counter. The ^{3}H cpm in panel a have been corrected for 16.2% spillover from ^{14}C and for the radioactivity contributed by the endogenous products of the coupled system (minus VSV). Only three endogenous products were detected: a peak of 1150 cpm at 4.3 cm, a peak of 350 cpm at 5.6 cm, and a peak of 300 cpm at 8.1 cm. Electrophoresis is from left to right.

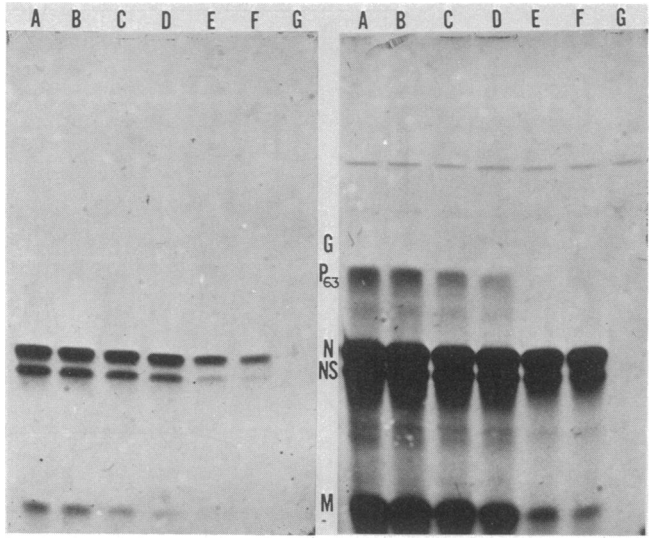

FIG. 3. Polyacrylamide gel electrophoresis of the protein products made in the coupled system directed by VSV which had been exposed to UV radiation. Samples of purified VSV (0.2 mg of protein per ml) were exposed to UV radiation for various times and then used to direct coupled transcription and translation for 8 hr in an L-cell extract as described in the *text*. The protein products (labeled with a mixture of 15 ^{3}H-labeled L amino acids) were subjected to electrophoresis on a 10% polyacrylamide slab gel in the presence of 0.1% sodium dodecyl sulfate. The positions of the radioactive proteins were determined by fluorography (20) of the stained, dried gel. The positions of the authentic viral proteins were determined from the stained gel. Products directed by: (A) VSV, no irradiation; (B) VSV, 10 sec irradiation; (C) VSV, 20 sec irradiation; (D) VSV, 40 sec irradiation; (E) VSV, 100 sec irradiation; (F) VSV, 125 sec irradiation; (G) no VSV, no irradiation. Two fluorographs of the same gel slab are shown; the one on the right has been overexposed to reveal the P_{63} protein.

The products of cell-free protein synthesis (labeled with [^{3}H]arginine and [^{3}H]lysine) were mixed with authentic VSV proteins (from purified virus labeled with [^{14}C]arginine and [^{14}C]lysine) and analyzed by electrophoresis on sodium dodecyl sulfate–polyacrylamide gels (Fig. 2). The three major products of the cell-free system corresponded exactly in electrophoretic mobility with the viral proteins N, NS, and M. A minor product at 7.5 cm had an apparent molecular weight of 63,000 and probably corresponded to a nonglycosylated polypeptide precursor (P_{63}) of the viral glycoprotein (G), which has been described by other workers (4, 9, 21). Preliminary analysis of the tryptic peptides of these four proteins confirms their identification as the products of translation of the N, NS, M, and G protein genes of VSV (data not shown). No product corresponding in size to the viral L protein was detected under these conditions.

UV Irradiation of VSV. The effect of UV irradiation of VSV was determined by using irradiated virus to direct protein synthesis in the coupled system. The products were subjected to electrophoresis on 10% polyacrylamide gels containing sodium dodecyl sulfate, and the radioactive proteins were detected by fluorography of the dried gel (Fig. 3). Levels of irradiation which introduced up to 8.5 UV hits per genome on average (as determined by loss of viral infectivity, Fig. 4a) did not result in the appearance of new protein bands nor any evidence of increased early quitting. On the other hand, the synthesis of G, N, NS, and M proteins decreased with increasing time of irradiation, which was in accordance with the decreased rates of RNA and protein synthesis induced by irradiated virus (Fig. 4b). In order to measure the exact amount of each protein synthesized, the appropriate bands were cut out of the gel, dissolved, and assayed by liquid scintillation counting. The results are shown in Fig. 4c–f. Each protein showed an inhibition of synthesis which followed single-hit kinetics, although their individual sensitivities differed. The UV doses corresponding to 37% survival were: N protein, 825 ergs mm^{-2}; NS protein, 553 ergs mm^{-2}; M protein, 410 ergs mm^{-2}; G protein, 260 ergs mm^{-2}. The dose corresponding to 37% survival of viral infectivity was 120 ergs mm^{-2}.

DISCUSSION

These results show that the transcription executed by detergent-activated VSV can be coupled with cell-free protein synthesis to produce viral proteins. Similar results have been obtained coupling the transcription of Newcastle disease virus with translation by cytoplasmic extracts of L-cells or primary chick embryo cells (L.A.B., C.N.W., and L. E. Hightower, unpublished results). The efficiency and endurance of protein synthesis in the coupled system far exceed those of conventional cell-free systems, and under optimum conditions 1 μg of mRNA directs the synthesis of about 0.1 μg of protein. After this work was completed, Breindl and

Table 1. Comparison of the target-sizes and mRNA sizes for N, NS, M, and G proteins

Viral function*	D° (ergs mm^{-2})†	Target-size‡ (daltons of RNA $\times 10^{-6}$)	RNA size§
Infectivity	120	3.82	3.82
N protein	825	0.55	0.55
NS protein	553	0.83	0.28
M protein	410	1.12	0.28
G protein	260	1.76	0.7

* Viral infectivity was measured by plaque assay (14); synthesis of N, NS, M, and G proteins was measured in the coupled cell-free system (see legend to Fig. 4).
† D° is the UV dose which corresponds to 37% survival of infectivity or protein synthesis.
‡ The target-size for loss of infectivity was assumed to correspond to the molecular weight of VSV genome RNA. The other target-sizes were calculated as being inversely proportional to the corresponding D°.
§ VSV genome RNA, ref. 32; VSV mRNAs, ref. 5.

Holland (21) reported the development of a coupled system using VSV ribonucleoprotein cores and extracts of various eukaryotic cells, but the translation in their system was much shorter-lived and only about 5% as efficient as that described in this paper.

The major protein products are the viral proteins N, NS, and M. A small amount of P_{63}, a putative nonglycosylated precursor to the viral glycoprotein, is also made, but, in agreement with the results of other workers (9, 21), no product corresponding in size to the viral L protein (molecular weight 190,000) has been detected (Figs. 2 and 3). Since the *in vitro* products of VSV transcription contain sequences complementary to almost the entire genome (22), the lack of L protein synthesis is probably due to a post-transcriptional deficiency of the cell-free system.

The use of UV irradiation to measure the target-sizes for expression of the different genes of VSV depends on the fact that transcription beyond a UV hit is blocked (12, 14). Thus the nucleotide sequences in a transcriptional unit which are distal to the site of a UV hit will not appear in the transcription product and will not be translated into viral proteins. This permits calculation of the target-size of the transcriptional unit. In principle, any inhibitor of RNA chain elongation could be used (cf. 23), but UV has the advantage that the target-size of each gene, measured *in vitro*, can be calculated as a fraction of the target-size of the intact RNA measured from viral infectivity. Assuming that the UV target for infectivity is the complete viral RNA, and that it contains a random distribution of potential sites for UV hits, the target-size for each gene which directs the synthesis of a protein in the coupled system can be calculated in terms of RNA molecular weight.

In Table 1, the target-sizes calculated from the data in Fig. 4c-f are compared with the molecular weights of the mRNAs for the different viral proteins. Clearly, there is no direct relationship between target-size and mRNA size. The two values are in close agreement for the N protein, but for the other proteins, encoded in both larger (G) and smaller (NS and M) messengers, the target-sizes exceed the mRNA size by two to four times. This is also shown in Fig. 4c–f where the broken lines indicate the UV sensitivities predicted from the molecular weights of the mRNAs for the four proteins, assuming that each is transcribed from an independent initiation site. This suggests that the targets for NS, M, and G include regions which are not present in the corresponding mRNAs. However, the viral RNA is not sufficient-

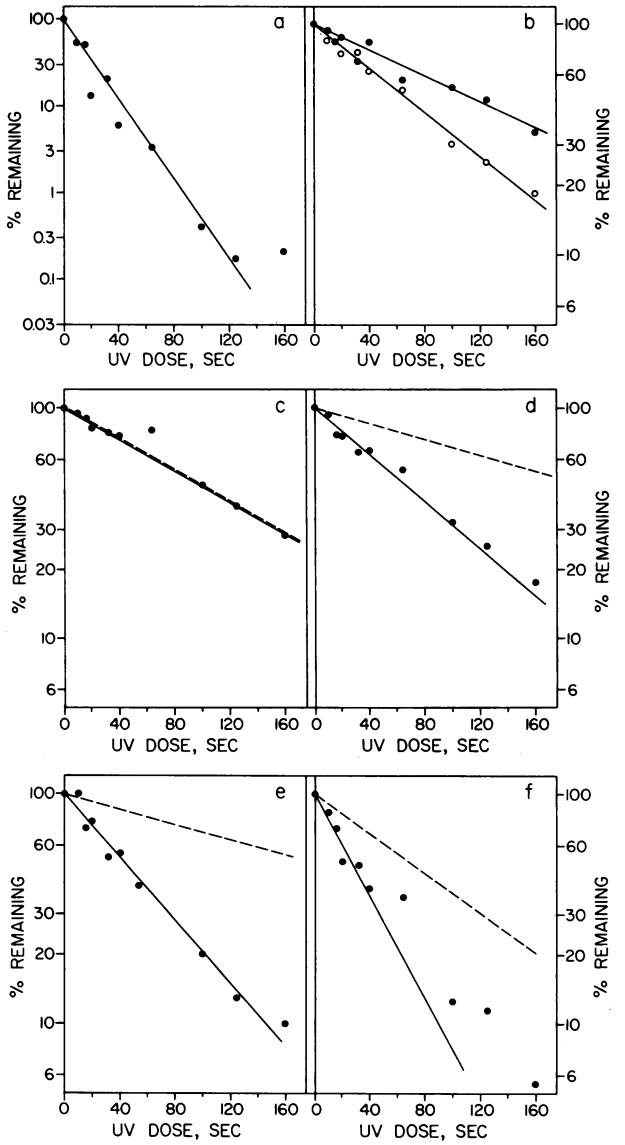

FIG. 4. The effect of UV irradiation of VSV on: (a) its infectivity, measured by plaque assay on Vero cells; (b) the rates of transcription (●) and translation (○) directed in the coupled cell-free system, and measured between 2 and 4 hr of incubation; (c) synthesis of N protein, measured from polyacrylamide gel patterns of the protein products directed in the coupled cell-free system after 8 hr incubation (see *Materials and Methods* and Fig. 3); (d) synthesis of NS protein, measured as for (c); (e) synthesis of M protein, measured as for (c); (f) synthesis of P_{63} protein (G), measured as for (c). In each case, the amount remaining has been plotted (on a semi-log scale) as a percentage of the activity (infectivity, etc.) of unirradiated VSV. The UV dose-rate was 6.5 ergs mm^{-2} sec^{-1}. In panels c–f, the broken lines indicate the UV sensitivities which are predicted from the molecular weights of the corresponding mRNAs, assuming that each is transcribed from an independent initiation site. The solid lines in panels c–f indicate the UV sensitivities which are predicted from the molecular weights of the corresponding mRNAs, assuming that they are transcribed from a single initiation site and in the order N, NS, M, G. The deviation from linearity in the response of P_{63} to increasing doses of UV probably reflects the difficulties involved in measuring such small amounts of minor product.

ly large for these regions to be untranslated "lead-in" sequences which are lost by post-transcriptional mRNA processing. The sum of the target-sizes for N, NS, M, and G (4.26×10^6 daltons) exceeds the molecular weight of the VSV genome, even without the L protein gene, which alone occupies about half the viral RNA (24). Hence, the UV targets for the expression of the N, NS, M, and G protein genes in the coupled system must be overlapping, as would be the case if they were transcribed from a single independent initiation site. In this situation, the UV sensitivity of each gene would depend on its position in the transcriptional unit, and its target.would include all proximal genes. Thus, the results indicate that the transcriptional order of the VSV genes is N, NS, M, G. The target-sizes which are predicted on the basis of this scheme and the known molecular weights of the messenger RNAs are: N, 0.55×10^6; NS, 0.83×10^6; M, 1.11×10^6; and G, 1.81×10^6 daltons of RNA, which are in close agreement with the measured target-sizes for these four genes. The segment of the genome remaining has a molecular weight of 2×10^6, which is an appropriate size to encode the L protein.

In functional terms, the results show that in the gene sequence N, NS, M, G transcription of any gene is dependent on the prior transcription of all the preceding genes. The simplest explanation of this obligatory order of transcription is that it reflects the physical order of the genes of the RNA. The transcriptase initiates at a single site at or shortly before the start of the N gene, and proceeds to read through the genes in the sequence 3′ - N - NS - M - G — 5′ with no possibility of initiating at any other site. An alternative model which we cannot rule out is that the order of transcription results from separate initiation events occurring in an obligatory sequence. The results of ribonucleoside [γ-^{32}P]triphosphate labeling experiments show more than one labeled sequence (25) but the origin of these sequences is unclear in the light of the discovery of capping of VSV mRNA (26).

The L protein gene must lie either before N (and be transcribed independently) or after G (in which case it could either be transcribed independently, or, as seems more likely, by continued read-through). Partial mapping of the genome by hybridization with RNA from various defective viral particles has located the L protein gene at one (unidentified) end (27) or near (28) the 5′ end of the genome, so it seems probable that the gene order of VSV is 3′ - N - NS - M - G - L -5′, and that the five genes are transcribed from a single initiation point.

We presume that the individual mRNAs are generated from the transcribing complex by cleavage of the nascent positive RNA strand, although other mechanisms can be imagined. In the absence of cleavage, the product of the reaction would be an intact positive copy of the VSV genome which is, of course, a necessary intermediate in replication, so transcription and replication may share a common process of positive strand synthesis. The mechanism of transcription proposed here would, in the absence of modulation, produce equimolar amounts of the five mRNAs; the available data from the studies of VSV-infected cells support this prediction (28).

Finally, the results offer an explanation for the origin of the phosphate group in the β position of the blocked 5′-terminal structure of VSV mRNA (26). For VSV, this phosphate group is derived from the capping GTP, whereas for the other viruses studied [reovirus (29), cytoplasmic polyhedrosis virus (30), and vaccinia virus (31)] it derives from the terminal nucleotide of the uncapped mRNA. We suggest that this difference arises because the substrate for capping in the latter cases is the biosynthetic 5′ terminus, where the potential exists for a 5′-diphosphate residue; however, for VSV the substrate for capping is a 5′ terminus generated by cleavage, where no such potential exists. If this is the case, the origin of the β phosphate in the capping group of eukaryotic mRNA may serve to identify messengers which arise by cleavage of precursors.

We thank Margaret Sekellick for doing the plaque assays; Drs. Philip Marcus, Bob Warrington, and Ian Kerr for helpful discussion; and the members of the molecular virology journal club where these experiments were conceived. This work was supported by Grant no. CA 14733 from the National Cancer Institute, and benefited greatly from use of the Cell Culture Facility which was also supported by this grant.

1. Wagner, R. R. (1975) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum Press, New York), Vol. 4, pp. 1–93.
2. Wagner, R. R., Prevec, L., Brown, F., Summers, D. F., Sokol, F. & McLeod, R. (1972) *J. Virol.* **10**, 1228–1230.
3. Morrison, T., Stampfer, M., Baltimore, D. & Lodish, H. F. (1974) *J. Virol.* **13**, 62–72.
4. Knipe, D., Rose, J. K. & Lodish, H. F. (1975) *J. Virol.* **15**, 1004–1011.
5. Rose, J. K. & Knipe, D. (1975) *J. Virol.* **15**, 994–1003.
6. Huang, A. S., Baltimore, D. & Stampfer, M. (1970) *Virology* **42**, 946–957.
7. Mudd, J. A. & Summers, D. F. (1970) *Virology* **42**, 958–968.
8. Baltimore, D., Huang, A. S. & Stampfer, M. (1970) *Proc. Nat. Acad. Sci. USA* **66**, 572–576.
9. Both, G. W., Moyer, S. A. & Banerjee, A. K. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 274–278.
10. Wacker, A. (1963) *Prog. Nucleic Acid. Res. Mol. Biol.* **1**, 369–399.
11. Miller, R. L. & Plagemann, P. G. W. (1974) *J. Virol.* **13**, 729–739.
12. Michalke, H. & Bremer, H. (1969) *J. Mol. Biol.* **41**, 1–23.
13. Hackett, P. B. & Sauerbier, W. (1974) *Nature* **251**, 639–641.
14. Hackett, P. B. & Sauerbier, W. (1975) *J. Mol. Biol.* **91**, 235–256.
15. Obijeski, J. F., Marchenko, A. T., Bishop, D. H. L., Cann, B. W. & Murphy, F. A. (1974) *J. Gen. Virol.* **22**, 21–33.
16. Marcus, P. I. & Sekellick, M. J. (1974) *Virology* **57**, 321–328.
17. Marcus, P. I. & Sekellick, M. J. (1975) *Virology* **63**, 163–190.
18. Friedman, R. M., Metz, D. H., Esteban, R. M., Tovell, D. R., Ball, L. A. & Kerr, I. M. (1972) *J. Virol.* **10**, 1184–1198.
19. Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412.
20. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46**, 83–88.
21. Breindl, M. & Holland, J. J. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 2545–2549.
22. Bishop, D. H. L. (1971) *J. Virol.* **7**, 486–490.
23. Perry, R. P. & Kelley, D. E. (1970) *J. Cell. Physiol.* **76**, 127–139.
24. Moyer, S. A. & Banerjee, A. K. (1975) *Cell* **4**, 37–43.
25. Roy, P. & Bishop, D. H. L. (1973) *J. Virol.* **11**, 487–501.
26. Abraham, G., Rhodes, D. P. & Banerjee, A. K. (1975) *Cell* **5**, 51–58.
27. Leamnson, R. N. & Reichmann, M. E. (1974) *J. Mol. Biol.* **85**, 551–568.
28. Stamminger, G. & Lazzarini, R. A. (1974) *Cell* **3**, 85–93.
29. Furuichi, Y., Morgan, M., Muthukrishnan, S. & Shatkin, A. J. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 362–366.
30. Furuichi, Y. & Miura, K. (1975) *Nature* **253**, 374–375.
31. Ensinger, M. J., Martin, S. A., Paoletti, E. & Moss, B. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 2525–2529.
32. Repik, P. & Bishop, D. H. L. (1973) *J. Virol.* **12**, 969–983.