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Transcriptional Mapping of Vesicular Stomatitis Virus In Vivo

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Synthesis of the proteins of vesicular stomatitis virus (Indiana serotype) was studied in mouse L cells infected with virus that had been exposed to UV radiation. The UV target sizes measured during primary transcription indicated that the five genes occupy a single transcriptional unit. Thus, in infected cells, as in cell-free systems, transcription of vesicular stomatitis virus RNA initiates at a single point and proceeds in the order N, NS, M, G, and L.

Vesicular stomatitis virus (VSV) (Indiana serotype) is a rhabdovirus with a single-stranded RNA genome of negative polarity (17). During infection, the genome is transcribed into five species of mRNA (9, 13, 14, 16) that code for the five known viral proteins (5, 10, 12) and that together represent essentially all the genetic information of the virus. The enzyme that executes transcription is a component of the virion that can be activated by nonionic detergents to perform the same reaction in vitro (3). The RNA products of this reaction closely resemble authentic viral mRNA's by several criteria (13), including their translation in cell-free systems (4).

Previously, we showed that, during transcription in vitro, RNA synthesis was initiated at a single point on the genome and that the five viral genes were transcribed in an obligatory order. These conclusions were based on experiments showing that UV irradiation of VSV exerted a strong polar effect on the products of transcription. The UV target size for the expression of each gene was equal to the combined physical sizes of all the preceding genes in the order N, NS, M, G, and L (2; L. A. Ball, C. N. White, and P. L. Collins, *in* D. Baltimore, A. S. Huang and C. F. Fox [ed.], *Animal Virology*, in press). These conclusions were confirmed by Abraham and Banerjee by the same method (1).

To investigate the possibility that this mechanism of RNA synthesis was peculiar to detergent-activated virus or limited to transcription in vitro, we studied the effect of UV irradiation on the synthesis of VSV proteins in infected cells. The results reported in this paper show that the same mechanism operates in vivo as in vitro.

Mouse L cells were infected with VSV, and 1 h later the cultures were isotopically labeled for 13 min with [^{35}S]methionine. Analysis of the labeled proteins showed that four virus-specific polypeptides could be resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the infected cell extracts (Fig. 1, cf. a and g). These were identified as the viral proteins L, G, N, and M by co-electrophoresis with the proteins of purified VSV (not shown); the NS protein was obscured by the intensely labeled cellular polypeptide that migrated just in front of N.

UV irradiation of the virus before infection impaired its capacity to direct the synthesis of these four proteins (Fig. 1a through f), although each one displayed a different and characteristic sensitivity. No new proteins were detected as a result of irradiation. L protein synthesis was the most sensitive, followed in order by G, M, and N. The UV doses were sufficient to introduce up to 10 lethal hits per genome, as measured by the loss of viral infectivity. One hit corresponds to the 37% survival point and was attained by a UV dose of 70 ergs/mm^2 in these experiments. This is shown quantitatively in Fig. 2, where viral infectivity (Fig. 2a) and the synthesis of each protein (Fig. 2b-e) are displayed as functions of the UV dose received by the infecting virus. In every case, the data fitted single-hit kinetics from which the relative target sizes for transcription of the four genes could be calculated. Similar results were obtained from cells pulse-labeled 90 and 120 min after infection, but beyond that time the results were complex and not amenable to the analysis used here. We ascribe this to the onset of secondary transcription resulting from the preferential replication of undamaged genomes.

Assuming that the target for viral infectivity was the complete genome and that it contained a random distribution of potential sites for UV hits, we used the infectivity data to calibrate the target sizes for the individual genes in terms of RNA molecular weight. The results are shown in Table 1 (column 2), and are com-

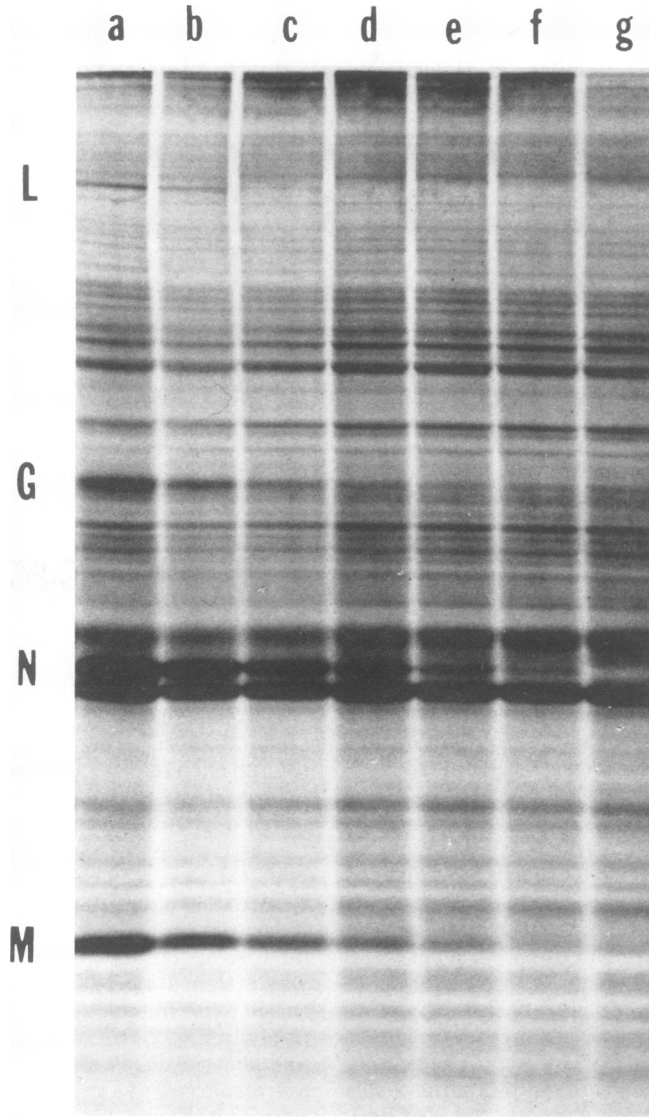

FIG. 1. *Polyacrylamide gel electrophoresis of the proteins isotopically labeled with [^{35}S]methionine in uninfected L cells and in cells infected with unirradiated and UV-irradiated VSV. An autoradiograph of the fixed, dried gel is shown. The origin is at the top, and the positions of the viral proteins L, G, N, and M are indicated. (a) Proteins from cells infected with unirradiated VSV. (b through f) Proteins from cells infected with VSV that had previously been exposed to UV irradiation: (b) 81, (c) 162, (d) 325, (e) 585, and (f) 975 ergs/mm^2. (g) Proteins from uninfected cells. All steps during the growth, infection, and isotopic labeling of the cells were done at 37°C. Mouse L cells, grown in suspension culture in modified Eagle medium containing 9% fetal bovine serum, were harvested by centrifugation and suspended in the same medium without serum at 10^7 cells per ml. Portions containing 10^6 cells were infected with unirradiated VSV (Indiana serotype, heat-resistant strain) or with VSV that had been exposed to UV radiation for various times (2). The multiplicity of infection was equivalent to 100 PFU of unirradiated virus per cell. After 30 min, the cells were diluted 20-fold with medium, stirred for an additional 1 h, and collected by gentle suction onto glass-fiber filters (7). The filters (and cells) were washed twice with methionine-free medium and then were shaken gently in 1-ml portions of medium containing 5 μCi of [^{35}S]methionine (Amersham/Searle) at a final specific activity of 36 Ci/mmol. After 13 min, the filters were washed first with their incubation medium and then with medium containing unlabeled methionine at 0°C. The cells were extracted by boiling the filters for 2 min in 0.2 ml of gel sample buffer (11), and the proteins in 50-μl portions of the extracts were subjected to electrophoresis on a discontinuous 10% polyacrylamide slab gel in the presence of sodium dodecyl sulfate (11).*

pared with the results of similar calculations based on the amounts of viral proteins (column 3) and the corresponding mRNA's (column 4)

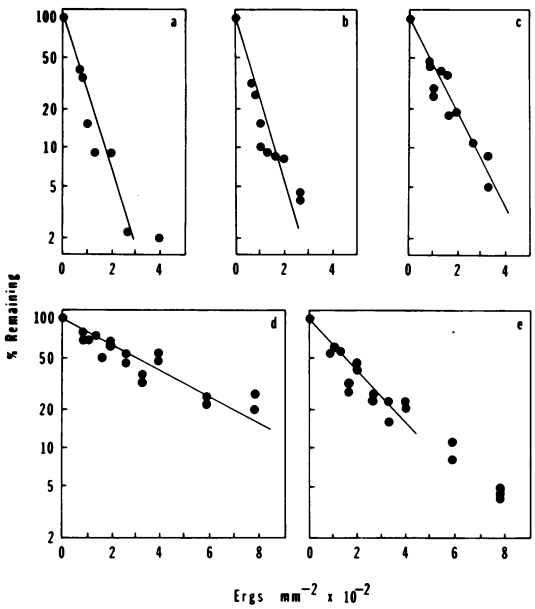

FIG. 2. *Effect of UV irradiation of VSV on: (a) infectivity, measured by plaque assay on monolayers of African green monkey kidney cells; (b through e) synthesis of the viral proteins measured 1 h after infection. (b) L protein; (c) G protein; (d) N protein; and (e) M protein. The individual proteins were quantitated by densitometry of the autoradiographs of polyacrylamide gels, under conditions where the densitometer response was proportional to the amount of radioactivity. In each case, the amount remaining is displayed on a semi-log scale as a percentage of the value for unirradiated VSV.*

synthesized in a cell-free system (2; Ball et al., in press).

It is clear that the results from the three methods are in close agreement. In vivo, as in vitro, the N protein gene is the only one with a target size that is equivalent to its physical size; for the other four genes, the target size exceeds the physical size by two to four times. Moreover, the target for the L protein gene occupies the entire genome, indicating that all the genes must lie within a single transcriptional unit. In this situation, their UV sensitivities will depend not on their physical sizes, but on their positions within the transcriptional unit, since their UV targets will include all the proximal genes (8). The target sizes that were observed in vitro and during primary transcription in vivo are in close agreement with the cumulative physical sizes summed in the order of increasing sensitivity: N, NS, M, G, and L (Table 1, column 6). This demonstrates that, during primary transcription in VSV-infected cells, RNA synthesis initiates at a single site on the genome and proceeds in an obligatory order through the genes.

The implications of this mechanism of transcription and its relationship to replication have been discussed previously (1, 2; Ball et al., in press). However, the idea that these two processes may share a common pathway of positive-strand synthesis has been strengthened by the discovery by Colonno and Banerjee of a small RNA molecule (leader RNA) that appears to represent the product of in vitro transcription of the 3' terminus of VSV RNA (6). Its presence is most consistent with the hypothesis that VSV mRNA's are generated during in vitro transcription by nucleolytic processing of a

TABLE 1. *Comparison of the target sizes for the genes of VSV measured in vivo and in vitro*[a]

Gene	Target size (daltons of RNA × 10^{-6}) determined from:			mRNA size (daltons of RNA × 10^{-6})	
	Protein in vivo	Protein in vitro[b]	mRNA in vitro[c]	Individual[d]	Cumulative
N	0.6	0.55	0.48	0.55	0.55 (N)
NS	—[e]	0.83	0.96[f]	0.28	0.83 (N + NS)
M	1.2	1.12		0.28	1.11 (N + NS + M)
G	2.1	1.76	1.72	0.7	1.81 (N + NS + M + G)
L	3.9	—[g]	3.9	2.1	3.91 (N + NS + M + G + L)

[a] The target size for the loss of viral infectivity was assumed to correspond to the molecular weight of VSV genome RNA, i.e., 3.82 × 10^6 (15). The other target sizes were calculated as being proportional to the corresponding UV sensitivity.

[b] Reference 2.

[c] Ball et al., in press.

[d] Reference 16.

[e] The NS protein was obscured by an intense cellular polypeptide with which it comigrated.

[f] The mRNA's for NS and M proteins were not separated by methods used.

[g] No L protein synthesis was detected in vitro.

(potentially) intact positive RNA strand. The results reported in this paper indicate that a similar mechanism operates during primary transcription in VSV-infected cells. Furthermore, analysis of the viral proteins made in cells infected with UV-irradiated Newcastle disease virus (L. A. Ball and L. E. Hightower, unpublished data) and analysis of the viral mRNA's made in cells infected with Sendai virus (K. Glazier, R. Raghow, and D. W. Kingsbury, personal communication) suggest that transcription initiates at a single site on the genome in paramyxoviruses also.

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