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RNA Polymerase in Mumps Virion

JACQUELINE P. BERNARD[1] AND ROBERT L. NORTHROP[2]

Department of Microbiology, Rush Presbyterian St. Luke's Medical Center and Graduate College, University of Illinois at the Medical Center, Chicago, Illinois 60612

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Mumps virions of the Enders' strain were examined for polymerase activity in vitro. An RNA-dependent RNA polymerase was found to be associated with the virion. The general properties of the reaction appear to be similar to those described for other paramyxoviruses.

RNA transcriptase has been demonstrated in several RNA viruses whose naked genome lacks infectivity. This includes vesicular stomatitis virus (VSV) of the rhabdovirus group (1-4) and Newcastle disease (6) and Sendai viruses (8-10) of the paramyxovirus group. Because both the template and the polymerase are virion associated it has been possible to study the product of these reactions in vitro. In this paper, data are reported indicating that another paramyxovirus, mumps virus (MV), also has an RNA-directed RNA polymerase.

The Enders' strain of MV, propagated in embryonated hen eggs, was used to infect chicken embryo lung (CEL) cells prepared according to the method of Darlington et al. (5). Approximately 5 days after inoculation all cells became hemadsorption positive (HAD +) by using chicken erythrocytes, and the fluids were harvested and centrifuged, and the concentrated virus was purified by sucrose gradient centrifugation for use in the polymerase assay. 50*S* viral RNA was prepared from the purified virus. The hybridization method used here has been described elsewhere (7).

When different concentrations of viral proteins were assayed for 120 min at 28 C in the reaction mixture described in Fig. 1, the amount of [^{3}H]GMP incorporated into an acid-insoluble product was proportional to the protein content of the sample. The average incorporation was 13 and 18 pmol per mg of protein during the first and second hour, respectively, and it increased during a 19-h incubation period. The reaction was not sensitive to either DNase or actinomycin D. Polymerase activity was not demonstrated in the pellet of centrifuged culture fluids before all the cells became HAD +. The reaction was absolutely dependent on the presence of viral protein (Table 1) and the omission of ATP, CTP, and UTP inhibited the activity 25 to 50%, which may be explained if traces of these nucleotides contaminated the preparation. Removal of DTT had no effect on the reaction and the omission of Triton N101 reduced the activity of the complete mixture 24%. A temperature of 20 C, a pH of 8, and a magnesium concentration of 0.4 μM (Fig. 2) were optimal for the reaction which were similar to those described for Sendai virus (9), but different from those described for VSV (2), as might be expected if the cofactor requirements of the polymerase were specific for the virus group.

The product of both a 2- and 5-h reaction was repeatedly found to be 4 to 5*S* (Fig. 3A and B, respectively) compared to the sedimentation characteristics of CEL cell ribosomal RNA. Over 80% of this product was sensitive to RNase. In annealing experiments using the entire product of a 4-h reaction and 2,500 μg of 50*S* viral RNA per ml resulted in about 69% of the product becoming RNase resistant (Table 2). However, this was only 22 to 25% greater than that present in the nonannealed and self-annealed samples. The high percentage of RNase resistant material in the nonannealed and self-annealed samples may have been due to the presence of complexes formed by the product and endogenous viral RNA template in the reaction mixture. No attempt was made to examine this possibility by purifying the 4 to 5*S* component by density gradient centrifugation for annealing experiments because the amount of product made in 4 h was too small.

After incubating the assay for 19 h the 4 to 5*S* product was again demonstrated and, in addition, a heavier 10 to 18*S* RNA was also apparent (Fig. 3C). Both the 4 to 5*S* and 10 to 18*S* products were partially RNase resistant. Four

[1] Present address: Laboratoire de Genetique des Virus C. N. R. S., 91190-Gif sur Yvette, France.

[2] Present address: School of Public Health, University of Illinois at the Medical Center, Chicago, Ill. 60680.

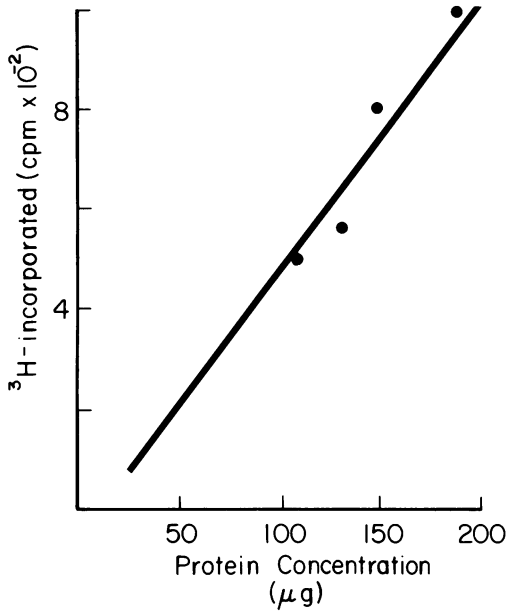

FIG. 1. *Relation between protein concent and polymerase activity. The polymerase reaction mixture consisted of 0.05 M Tris-hydrochloride, pH 8.0; 0.01 mM [^{3}H]GTP; 0.7 mM each ATP, CTP, UTP; 0.1 M NaCl; 4 mM magnesium acetate; 3 mM DTT; and 0.08% (wt/vol) Triton N101. To 50 μliters of this mixture was added 50 μliters of virus suspension diluted to contain the indicated amounts of protein, and divided into two 50-μliter portions. One portion was kept at 0 C and the other was incubated at 28 C for 2 h. The reaction was terminated by adding 0.3 ml of yeast RNA (2 mg/ml) and 5 ml of 5% cold trichloroacetic acid. After 10 min the samples were centrifuged at 1,000 rpm for 10 min at 4 C. The pellets were washed with 5 ml of fresh acid and finally dissolved in 0.5 ml of water. A 10-ml amount of Aquasol was added to each sample, and radioactivity was measured in a Tri-Carb scintillation counter. The value obtained for the aliquot incubated at 4 C (average 70 counts/min) was subtracted from corresponding value of the portion incubated at 28 C. Each value plotted is the average of two similar portions.*

TABLE 1. *Requirements of mumps virus polymerase activity*

Reaction mixture[a]	Counts/min × 10^{-3} per mg of protein	Inhibition (%)
Complete	6.9	0
Minus virions	0.02	100
Minus ATP	3.17	54
Minus CTP	5.35	23
Minus UTP	5.18	25
Minus DTT	7.39	0
Minus Triton N101	5.29	24

[a] Complete reaction mixture consisted of the same components as described in Fig. 1 containing 180 μg of viral protein.

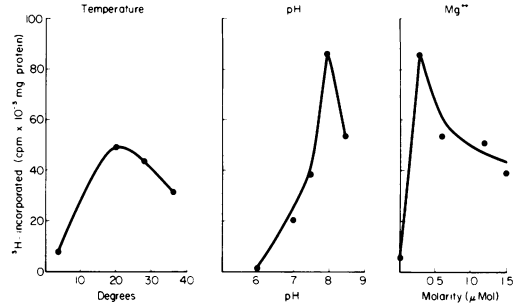

FIG. 2. *Effect of different temperatures, pH, and magnesium levels on polymerase reaction. Mumps virus (14 μg of protein) was incubated in polymerase assays as described in Fig. 1 for 16 h: (A) at different temperatures, (B) at various pH, and (C) at different concentrations of magnesium acetate.*

phenol-cresol extractions, instead of the usual two, did not alter this degree of RNase resistance. Boiling and quick cooling the entire product resulted in only minor changes in the sedimentation characteristics of the 4 to 5*S* component, whereas the 10 to 18*S* material was no longer evident, suggesting that it contained double-stranded RNA. After purifying the 4 to 5*S* component 8 to 16% of it specifically annealed with 50*S* viral RNA and 29 to 38% of the 10 to 18*S* component annealed with viral template (Table 2).

The RNA of a 17-h reaction was next analyzed by gradient centrifugation under conditions that the 50*S* viral RNA was retained in the lower ⅓ of the gradient as described in Fig. 4. The majority of radioactivity was as expected in the 4 to 5*S* and 10 to 18*S* components, but there was little evidence of radioactivity associated with the virion RNA.

These data suggest that mumps virions contain an RNA transcriptase whose characteristics are similar to those of other paramyxoviruses. Less that one-sixth of the 4 to 5*S* RNA product was complementary to the viral template RNA, and none of it appeared to be double-stranded RNA. Whether the remaining portion was the product of nonviral template-polymerase activity, of nuclease attack on newly made virus-specific strands, or artifact is not known.

With regard to the 10 to 18*S* product it appears to represent replicative intermediates on the basis of its relatively high resistance to RNase before and after self-annealing, its increased RNase resistance after hybridization with added 50*S* viral RNA, and because of its sensitivity to boiling and rapid cooling. Finding little product associated with the 50*S* virion RNA but detecting it predominantly as 10 to

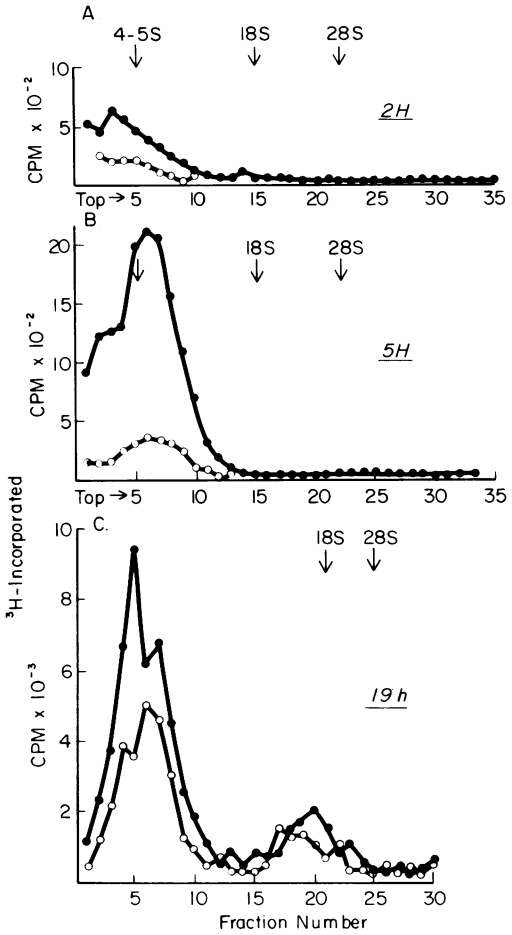

FIG. 3. *Gradient centrifugation of mumps virus polymerase product. Reaction mixtures were incubated at 28 C for 2, 5, and 19 h (A, B and C, respectively). RNA was extracted with phenol-cresol and precipitated with ethanol. The precipitates were dissolved in TS buffer and layered on separate 10 to 30% sucrose gradients in TS buffer containing 0.5% SDS. Gradients A and B were centrifuged for 16 h at 20,000 rpm in an SW.27 rotor at room temperature; gradient C was centrifuged for 18 h at 20,000 rpm in an SW 25.1 rotor at room temperature. Fractions (1 ml) were collected from the top of the gradients; 0.4-ml portions of each fraction were treated with 10 μliters of RNase (5 mg/ml) at 37 C. Both portions of each fraction were precipitated with trichloroacetic acid and radioactivity was measured. Symbols: ●, control; O, RNase treated.*

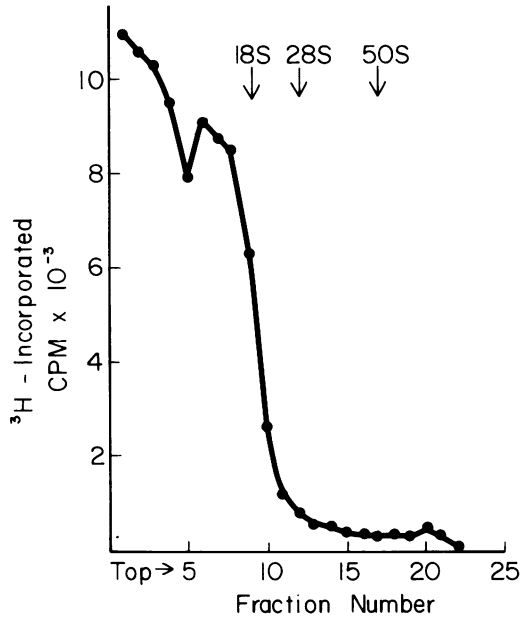

FIG. 4. *Characterization of the product of a 17-h reaction. A 1.5-ml amount of reaction mixture was incubated for 17 h and the phenol-cresol-extracted RNA was layered on a 10 to 30% glycerol gradient in TS buffer containing 0.5% SDS and centrifuged for 3 h at 36,000 rpm in an SW 41 rotor at room temperature. ^{14}C-labeled RNA extracted from uninfected chicken embryo fibroblasts was centrifuged at the same time on a separate gradient for sedimentation reference.*

TABLE 2. *Hybridization of polymerase products from 4- and 17-h reactions*

Labeled product	Treatment[a]	Counts/min per sample		RNase resistant (%)	Specific annealing (%)
		Control	RNase		
Nonpurified 4 h product[b]	Not annealed	1,346	635	47	
	Self-annealed	2,217	977	44	
	50*S*-annealed[c]	1,615	1,125	69	22-25
Purified 17 h product[d]					
4-5*S*	Not annealed	684	247	36	
	Self-annealed	494	213	44	
	50*S*-annealed	490	256	52	8-16
10-18*S*	Not annealed	632	237	37	
	Self-annealed	465	217	46	
	50*S*-annealed	445	336	75	29-38

[a] The method of hybridization was performed as described in reference 6.

[b] The polymerase product from a 4-h incubation was phenol extracted and alcohol precipitated with no further purification.

[c] A 2,500-μg amount of 50*S* viral RNA per ml was used in the annealing.

[d] A 1.5-ml reaction mixture was incubated for 17 h and phenol-cresol-extracted RNA was layered on a 10 to 30% glycerol gradient in TS buffer containing 0.5% SDS and was centrifuged for 3 h at 36,000 rpm in an SW 41 rotor at room temperature. Fractions (0.5 ml) were collected from the top of the gradient; 0.1 ml of each fraction was precipitated with cold trichloroacetic acid and radioactivity was measured. The remaining portions of fractions 1 to 4 were pooled for the 4 to 5*S* component, and fractions 6 to 10 were pooled for the 10 to 18*S* component and alcohol precipitated for the hybridization experiments. ^{14}C-labeled RNA, extracted from uninfected chicken embryo fibroblasts, was centrifuged at the same time on a separate gradient for sedimentation reference.

18*S* components could be explained by the findings of Huang et al. (6). They showed that as the incubation period of the polymerase reaction increased more and more complexes of template and complementary strands formed and these sediment more slowly and more heterogenously than does the virion RNA alone.

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