Sendai Virus-Induced Transcriptase from Infected Cells: Polypeptides in the Transcriptive Complex

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A Sendai virus-induced transcriptase-template complex was isolated from the cytoplasm of infected cells by combined sedimentation and isopycnic centrifugation. This transcriptive complex banded at 1.27 g/cm³ in D₂O-sucrose gradients. It contained two polypeptides, the viral nucleocapsid structure unit (molecular weight, 60,000) and the largest virion polypeptide (molecular weight, 75,000). The buoyant density, chemical composition, and electron microscopic appearance of the transcriptive complex indicate a structure like that of viral nucleocapsids.

We and others have described ribonucleic acid (RNA)-dependent RNA polymerases (transcriptases) in paramyxoviruses (7, 8, 17, 19) and in cells infected by paramyxoviruses (11, 18, 19). There is evidence that the virion transcriptase of one paramyxovirus, Sendai virus, resides in nucleocapsids (17), but the disposition of the transcriptase found in Sendai virus-infected cells late in infection has not been clarified. The template for the intracellular enzyme is probably 50S viral genomic RNA (19); this might well be encapsidated, by analogy with the virion transcriptase template. Furthermore, nucleocapsids have physical properties which would place them in the "microsomal" fractions heretofore used as sources of cellular Sendai virus-induced transcriptase (11, 19). In this paper, we describe isolation of the Sendai virus-induced transcriptase-template complex from infected cells, and we show that this "transscriptive complex" resembles viral nucleocapsids.

MATERIALS AND METHODS

Virus. A Sendai virus clone free from incomplete virions was grown in chicken embryo lung (CEL) cell cultures at 30 C as described earlier (19).

Infection. CEL cell monolayer cultures in 150-mm plastic dishes were infected at an input multiplicity of about 5 plaque-forming units (PFU) per cell and incubated at 30 C for 86 to 96 hr (19). By this time, the average culture had produced 10⁶ PFU (2 × 10³ PFU per cell).

Preparation of cytoplasmic extract. Cells were suspended in phosphate-buffered saline (PBS) by scraping. They were collected by centrifugation at 500 × g for 5 min, and were resuspended in ice-cold RSB (15) at a concentration of 10⁶ cells/ml. After 30 min at 4 C, cells were disrupted by 10 strokes of a tight-fitting Dounce homogenizer (Kontes Glass Co.). Nuclei and other rapidly sedimenting structures were removed by centrifugation at 12,000 × g for 10 min. The supernatant fluid was designated cytoplasmic extract.

Isolation of transcriptive complex. The enzymatically active complex was concentrated by sedimentation and banded isopycnically in a single step. We layered 27 ml of cytoplasmic extract on an 8-ml linear D₂O-sucrose gradient (1.15 to 1.33 g/cm³) which had been formed above a 3-ml cushion of 1.36 g/cm³ D₂O-sucrose. All solutions contained 0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 0.03 M NaCl (pH 8.0) in 99 mole % D₂O (BioRad Laboratories). The preparation was centrifuged at 26,000 rev/min for 3.5 hr at 10 C in a Spinco SW27 swinging-bucket rotor. Fractions were collected from the top with an ISCO (Instrumentation Specialties Co.) model D fractionator. Densities were determined by weighing 0.100-cm³ portions in a micropipette.

Radioisotopic labeling. Proteins were labeled with 5 μCi of ¹³C-amino acid mixture (reconstituted protein hydrolysate 3122-08, Schwarz BioResearch) per ml of Eagle's minimal essential medium MEM (5) containing one-tenth the usual amino acid concentrations. Labeled precursors were added 48 hr after infection and incubation for an additional 48 hr was at 30 C.

Virion proteins were labeled the same way, except that 25 μCi of ³H-amino acid mixture (3130-08, Schwarz BioResearch) per ml was used. Labeled virus was collected from culture medium by centrifugation, and 1,000S virions were isolated by rate zonal centrifugation in sucrose gradients (19).

Acrylamide-gel electrophoresis. Before electrophoresis, proteins were boiled for 1 min in 0.01 M sodium phosphate, 1% sodium dodecyl sulfate (SDS), and 1% β-mercaptoethanol (pH 7.2). Gels were formed from 10% acrylamide (recrystallized from CHCl₃), 0.27% N,N'-bis-methylene acrylamide, 0.05% TEMED (N,N',N"-tetramethylethylenediamine), 0.1% ammonium persulfate, 0.1 M sodium phosphate (pH 7.2), and 0.1% SDS. The electrophor-
esis buffer was 0.1 M sodium phosphate-0.1% SDS (pH 7.2). All gels were prerun for 1 hr to remove persulfate. Glycerol was added to each sample to a final concentration of 12% (v/v). Samples of 0.05 to 0.4 ml were loaded on the gels, and electrophoresis was performed at 45 v for 16 hr. After electrophoresis, gels were frozen and divided into 1-mm slices with stacked razor blades.

**RNA polymerase assay.** Sendai virus-induced RNA transcriptase was assayed as described before (19). We added 0.025 ml of each D2O-sucrose gradient fraction to each reaction mixture to make a final volume of 0.1 ml.

**Radioactivity measurements.** All gradient fractions and enzyme reactions were processed as described previously (9, 19). Gel slices were incubated overnight at 37 C in counting vials with 10 ml of toluene-based scintillant containing 3% (v/v) Protosol (New England Nuclear Corp.). They were then counted in a liquid scintillation counter.

**RESULTS**

Isolation of an enzyme-containing structure from infected cells. Our attempts to fractionate the Sendai virus-induced transcriptase activity in microsomal pellets of infected cells were unsuccessful. We could not disaggregate microsomal pellets with nonionic detergents like Nonidet P-40 or Triton N-101, or by prolonged homogenization. We therefore devised a method to concentrate the enzyme without pelleting. As described in Materials and Methods, we centrifuged a large volume of cytoplasmic extract on a small D2O-sucrose gradient, which banded the enzyme-containing structure isopycnically.

Most of the transcriptase activity was found in a single component at a density of 1.27 g/cm³ (Fig. 1). The enzyme made RNA complementary to Sendai virion 50S RNA, since 90% of the product

![Fig. 1. Isolation of transcriptase-containing structure. Infected cell cytoplasm was centrifuged into a D2O-sucrose gradient and fractionated as described in Materials and Methods. Radioactivity represents 3H-guanosine monophosphate incorporated in a 5-hr transcriptase assay (19). Protein was determined by the Lowry (10) method, with bovine serum albumin as standard. Fractions 1 through 29 (arrow) represent 1 ml and fractions 30 through 60 represent 0.25 ml. Ordinate values are quantities per fraction, not concentrations.](http://jvi.asm.org/)

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became ribonuclease-resistant on annealing with 50S RNA, whereas self-annealing was only 5% (Table 1). In contrast with the distribution of enzyme activity, most of the cell protein remained above the gradient (Fig. 1). However, a large part of the cytoplasmic protein labeled with 14C-amino acids in the previous 48 hr banded with the 1.27 g/cm³ structure (Fig. 2). This was entirely virus-specific protein (see below).

Enzyme activity was stable for at least 24 hr in D₂O-sucrose. Thus, it was possible to determine that 1.27 g/cm³ was truly the isopycnic density of the enzyme-containing structure, there being no shift in its position after 24 hr of centrifugation.

**Virus-specific polypeptides in the enzyme-containing structure.** In the following, we number Sendai virion polypeptides according to Mountcastle et al. (13); we did not identify polypeptide 4 of these authors, so it is not marked in our figures. Many of the polypeptides labeled from 48 to 96 hr after infection appeared to be virus-specific, as they migrated like virion polypeptides in polyacrylamide gels (Fig. 3). This was least obvious in the nuclear-large particulate fraction (Fig. 3A), but this fraction contained peaks corresponding to the smallest virion polypeptide (peak 6), smaller glycopolypeptide (peak 5), nucleocapsid structure unit (peak 3), larger glycopolypeptide (peak 2), and largest virion polypeptide (peak 1). Virus-specific glycopolypeptides and the smallest polypeptide, thought to be a viral envelope protein (13), would be expected in this cell fraction, because cell surface membranes sediment here (14), and paramyxovirion envelope proteins are incorporated into cell surface membranes (2).

Although equal amounts of radioactivity were present in nuclear and cytoplasmic fractions, cytoplasmic extracts contained fewer polypeptides and these appeared to be mainly viral. Cytoplasm was markedly enriched in the largest virion polypeptide and nucleocapsid structure unit, and contained less material qualifying as the larger glycopolypeptide or smallest polypeptide (Fig. 3B). There was also some labeled protein migrating between the smaller glycopolypeptide and the smallest polypeptide.

The 1.27 g/cm³ structure contained only the nucleocapsid structure unit (molecular weight, 60,000) and the largest virion polypeptide (molecular weight, 75,000; Fig. 4).

**Relationship between enzyme-containing structures and viral nucleocapsids.** Electron microscopy revealed viral nucleocapsids in the enzyme-rich 1.27 g/cm³ fraction (Fig. 5). This density is, in fact, where Sendai virus nucleocapsids were shown to band by Blair and Robinson (1). In addition, there was viral 50S RNA in this fraction (Fig. 6). In the presence of actinomycin D (Fig. 6B), 18S RNA, which may represent viral transcripts (16, 19), was also found. We will call this complex of 50S template and transcriptase (and perhaps nascent product) a "transcriptive complex." The finding of nucleocapsid protein and morphologically identifiable nucleocapsids in the same fraction indicates that the transcriptive complexes are structures identical to or very similar to nucleocapsids.

**DISCUSSION**

We have presented evidence in this paper indicating that the Sendai virus-induced transcriptase-

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### Table 1. Hybridization of the product made by the 1.27 g/cm³ structure

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ribonuclease-resistant counts/ min</th>
<th>Percent ribonuclease resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-annealed</td>
<td>32</td>
<td>4.8</td>
</tr>
<tr>
<td>Annealed with virion RNA</td>
<td>666</td>
<td>91</td>
</tr>
</tbody>
</table>

*Annealing was at 80 C for 1 hr in 0.1 ml of 0.3 M NaCl, 0.03 M sodium citrate (pH 7.0). Sendai virion 50S RNA, when present, was at 20 µg/ml. Half of each sample was treated with 10 µg of pancreatic ribonuclease A per ml for 30 min at 24 C before acid precipitation and counting.

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**Fig. 2. Distribution of labeled polypeptides in cytoplasmic extract.** Cells were labeled with 14C-amino acids and fractionated as described in Materials and Methods. Centrifugation of cytoplasmic extract was as in Fig. 1. Fractions through 25 (arrow) represent 1 ml; later fractions represent 0.25 ml. Ordinate values are amounts per fraction.
template complex (transcriptive complex) in cell cytoplasm is a structure with physical and morphological properties of viral nucleocapsids. In addition, it contains 50S viral RNA and nucleocapsid structural polypeptide. The accumulation of Sendai virus nucleocapsids in infected cells during the extended virus replication cycle (1, 4) correlates with our ability to measure relatively abundant transcriptase activity in these cells, compared to the Newcastle disease virus transcriptase (18).

Our transcriptive complexes may not be identical to the nucleocapsids found in virions, since the complexes have large amounts of the largest virion polypeptide associated with them. This polypeptide has not previously been found in paramyxovirus nucleocapsids isolated from virions or from infected cells (3, 12). However, isolation procedures employed in these other studies included treatments with deoxycholate or high salt (cesium chloride) concentrations, or both, and these agents may remove the largest virion polypeptide. We have found that transcriptase activity is lost after deoxycholate or cesium chlo-
Fig. 4. Polyacrylamide-gel electrophoresis of \(^{14}\)C-polypeptides from the 1.27 g/cm\(^3\) structure isolated from a cytoplasmic extract of infected cells. \(^{3}\)H-polypeptides from Sendai virions were added before electrophoresis. In this experiment, which gave good resolution of the larger virion polypeptides, the smallest virion polypeptide was partially run off the gel. In other experiments, where this polypeptide was completely included, there was no trace of \(^{14}\)C-polypeptide from infected cells at the same position.

Fig. 5. Electron micrograph of Sendai virus-induced transcriptase-containing fraction, negatively stained with phosphotungstate. \(\times 65,000\).
ride interacts with enzyme-active fractions from infected cells (H. O. Stone and D. W. Kingsbury, unpublished data).

Thus, the largest virion polypeptide is implicated in transcriptase function, either as an enzyme component or in a regulatory role. But the evidence is circumstantial, and it appears to be contradicted by the abundance of this polypeptide in virions and in association with the cellular transcriptive complex (Fig. 4). Assuming 2,400 to 3,000 structure units per nucleocapsid (6), and molecular weights of 60,000 for the structure unit and 75,000 for the largest polypeptide, one can calculate from data in Fig. 4 that there are about 400 copies of the largest virion polypeptide relative to each nucleocapsid in virions, and about twice as many in the transcriptive complex from infected cells. This would seem to be a superabundance of transcriptase, if that is the largest polypeptide’s function.

By comparison, if it is assumed that each nucleocapsid contains only one enzyme molecule of molecular weight about 100,000, this would represent only about 0.1% of the aggregate mass of nucleocapsid structure unit polypeptides in virions. This amount cannot be demonstrated in the polyacrylamide gel patterns obtained thus far with paramyxovirus polypeptides.

It should be noted that the transcriptase-containing subviral structure obtained when Sendai virions are disrupted with nonionic detergent (H. O. Stone and D. W. Kingsbury, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 219, 1972) has more polypeptides associated with it than the cellular transcriptive complex described in this paper (H. O. Stone and D. W. Kingsbury, unpublished data). It seems reasonable to designate each structure a “transcriptive complex,” since each contains a transcribing enzyme and template, but they must be distinguished as virion and cellular transcriptive complexes, because of their different origins. The apparent compositional difference may not be significant, because both transcriptive complexes have similar buoyant densities (H. O. Stone and D. W. Kingsbury, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 219, 1972), and it seems unlikely that all of the polypeptides which band with the virion complex are involved in transcription.

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