Saint Louis Encephalitis Viral Ribonucleic Acid Replication Complex

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Pulse-labeled Saint Louis encephalitis viral ribonucleic acid (RNA) is found in the cytoplasm of infected cells associated with a membranous structure which sediments with an average value of 250S. The integrity of the complex is destroyed by detergents and ribonuclease; however, it is stable in ethylenediaminetetraacetic acid (EDTA) which differentiates this structure from cellular polyribosomes. With cultures in which cellular RNA was highly labeled prior to infection, ribosomal RNA could not be demonstrated in the complex isolated from EDTA-sucrose gradients. Single-stranded 43S and the 26S and 20S forms of viral RNA were found in the complex. Viral RNA polymerase activity in sucrose-gradient fractions sedimented in the same region as the fractions which contained the pulse-labeled viral RNA. The polymerase incorporated ³H-guanosine triphosphate into acid-precipitable material in the absence of added template. It was also found that the replication complex contains viral-specific proteins.

Synthesis of group A arboviral ribonucleic acid (RNA) and envelopment of the nucleocapsid are associated with membranous structures (7, 8, 10, 17, 18). Intracellular, complete, group B arbovirus particles are observed inside membrane-bound cytoplasmic vacuoles although the role of these structures in viral macromolecular synthesis and virion envelopment is not clear (6, 15, 16). Caliguri and Tamm (3, 4) have reported that translation and transcription of polioviral RNA are associated with distinct types of membranes which can be separated by isopycnic sucrose centrifugation. In picornavirus-infected cells, RNA synthesis is initiated in 130S structures found in the smooth membrane fraction which mature to form the late replication complex that has a sedimentation coefficient of approximately 250 (3, 4). This mature structure contains viral RNA polymerase, single-stranded RNA, the replicative 20S form, and the replicative intermediate 26S form of viral RNA (4, 9). This structure is defined as the viral-RNA replication complex (9). Cytoplasmic membrane-associated (MRC) structures similar to the picornavirus replication complex are also the site of viral RNA synthesis in cells infected with the group A arboviruses (7, 10).

This communication describes the isolation and preliminary description of a replication complex from BHK-21/13 cells infected with Saint Louis encephalitis (SLE) virus. This structure has an average sedimentation coefficient of 250, contains

viral RNA polymerase, single-stranded viral RNA, and the replicative forms of SLE viral RNA. Viral-specific proteins appear to be an integral part of the SLE viral complex.

MATERIALS AND METHODS

Virus and cell culture. Pools of SLE virus and pig kidney cell monolayer cultures were prepared according to procedures described previously (11, 20).

Baby hamster kidney cells (13) obtained from the American Type Culture Collection were grown in a lactalbumin medium (20).

Infection procedure. Confluent monolayer cultures of BHK cells were infected with SLE virus at a multiplicity of 10 plaque-forming units per cell. After an absorption period of 1 hr, warm Eagle's medium (5) with actinomycin D (0.5 µg/ml) and 5% calf serum was added, and cultures were incubated at 37 C.

Cell fractionation. Cultures were pulse-labeled for 15 min with ³H-uridine (50 µCi/ml) in single-label experiments. In dual-label experiments, 50 µCi of ³H-uridine/ml and 10 µCi of ¹⁴C-leucine/ml were used. After the radioactive pulse, cells were scraped off the glass and collected by centrifugation, and cytoplasmic extracts were prepared (7). In some experiments, cytoplasmic extracts were solubilized with Brij-58 or sodium deoxycholate (DOC; 0.5% for 10 min at 0 C) prior to sucrose gradient analysis. Extracts were layered over a 28-ml, 5 to 40% (w/v) sucrose gradient prepared in either reticulocyte standard buffer (RSB; reference 22) or ethylenediaminetetraacetic acid (EDTA) buffer (9) and centrifuged at 25,000 rev/min for 120 min at 4 C in a Beckman SW-27 rotor. Frac-

tions collected from the gradients were analyzed for optical density, trichloroacetic acid-precipitable radioactivity (20), or both.

Enzyme assay. Viral RNA polymerase activity in cell extracts and gradient fractions was determined by the microtechnique of Levin and Friedman (12). Fifty lambda of the enzyme preparation was incubated at 37 C for 60 min in a total volume of 0.1 ml which contained 0.05 M tris(hydroxymethyl)aminomethane, pH 8.0; 0.01 M $MgCl_2$; 0.001 M dithiothreitol, 0.025 μ g of actinomycin D, 0.01 M phosphoenol pyruvate; 4 μ g of pyruvate kinase, and 10 nM each of adenosine triphosphate, uridine triphosphate, cytidine triphosphate, and 3H -guanosine triphosphate (GTP; 2.9×10^6 counts per min).

Analysis of RNA associated with cytoplasmic structures. RNA was extracted from pooled, sucrose gradient fractions with sodium dodecyl sulphate-phenol at 37 C and analyzed by sucrose rate zonal centrifugation (20). One-half of each fraction was hydrolyzed with ribonuclease (20 μ g/ml), and each sample was precipitated with 10% trichloroacetic acid, collected on glass fiber filters, and washed several times with 5% trichloroacetic acid, and dried, and the radioactivity was determined (20).

Immunoprecipitation of cytoplasmic fractions. Sucrose gradient fractions (200S to 300S) of cytoplasmic extracts of 3H -leucine-labeled SLE viral-infected or noninfected cells were pooled and dialyzed overnight at 4 C against 0.02 M phosphate buffer, pH 7.4. An amount (25 μ liters) of the dialyzed sample was layered over 25 μ liters of SLE virus antibody-containing mouse ascitic fluid (2) and incubated at room temperature overnight. A positive test was indicated by a zone of precipitate which formed at the interphase of the solutions containing homologous antigen and antibody. Immunological precipitation of 3H -leucine-labeled protein in pooled gradient fractions was quantitated by the radioimmune precipitation technique (19).

Chemicals. Ribonuclease was purchased from Worthington Biochemical Corporation, Freehold, N.J., and ribonuclease-free sucrose was from Mann Research Laboratories, N.Y. Actinomycin D was a gift of Merck-Sharp and Dohme Research Laboratories, Rahway, N.J. 3H -uridine and 3H -guanosine triphosphate were obtained from Schwarz/Mann, Orangeburg, N.Y.; ^{14}C - and 3H -leucine were purchased from New England Nuclear Corporation, Boston, Mass.

RESULTS

Cytoplasmic fractions associated with viral RNA synthesis. Cytoplasmic extracts prepared from noninfected cells were divided, and a portion was incubated with either DOC or Brij-58 before centrifugation on RSB-sucrose density gradients (Fig. 1). The A_{260} gradient profile of cytoplasmic extracts prepared without detergent treatment shows materials which sediment with a size distribution of 300S to 236S, 74S ribosomes, and lighter material. Brij-58 treatment prior to gra-

dient analysis increased the size distribution of material in the major A_{260} peak from 340S to 200S. Sucrose gradients of extracts after DOC treatment contained relatively less material in the 200S to 300S region of the gradient.

Cells infected for 18 hr were labeled with 3H -uridine for 15 min; cytoplasmic extracts were prepared and treated with either DOC or Brij-58 and centrifuged through preformed RSB-sucrose gradients (Fig. 2). The gradient profile of non-detergent-treated cytoplasmic extracts had a 3H -uridine peak that contained approximately 40% of the total radioactivity and sedimented with an average value of 300S (Fig. 2A). Three smaller peaks of radioactivity were also observed at 250S, 180S, and 140S. After incubation of the extract with ribonuclease, less than 10% of the radioactivity observed in the fast-sedimenting material of the untreated extract sedimented at 200S to 300S. Incubation of the extracts with either Brij (Fig. 2B) or DOC (Fig. 2C) prior to

FIG. 1. Effect of sodium deoxycholate and Brij-58 on cytoplasmic extracts from noninfected BHK cells. Cytoplasmic extract was prepared, divided, treated with either DOC or Brij-58, and centrifuged through preformed reticulocyte standard buffer-sucrose gradients. Centrifugation was performed with a SW-40 rotor at 25,000 rev/min for 120 min at 4 C. Fractions (0.5 ml) were collected and analyzed for optical density at 260 nm. Symbols: (○) untreated cytoplasmic extract; (▲) Brij-58-treated cytoplasmic extract; (●) deoxycholate-treated extract.

FIG. 2. Effect of sodium deoxycholate, Brij-58, and ribonuclease on the cytoplasmic extracts from Saint Louis encephalitis virus-infected cells. At 18 hr postinfection cultures were pulse-labeled with ^3H -uridine ($50 \mu\text{Ci/ml}$) for 15 min. Cytoplasmic extracts were prepared, divided, incubated with deoxycholate or Brij-58, and centrifuged through preformed reticulocyte standard buffer-sucrose gradients. Fractions of 1.0 ml were collected, and one-half of each fraction was incubated with $10 \mu\text{g}$ of ribonuclease at 37°C for 10 min. Ribonuclease-treated and nontreated fractions were analyzed for acid-precipitable radioactivity. Total ^3H -uridine radioactivity ($\bullet$); ribonuclease-resistant ^3H -uridine radioactivity ($\circ$). A, nondetergent treated; B, Brij-58 treated; C, deoxycholate treated.

centrifugation dissociated most of the ^3H -uridine radioactivity from structures which sediment at 200S to 300S. When the 250S ^3H -uridine-labeled peak from infected cells was isolated from RSB-sucrose gradients, treated with Brij or DOC, and recentrifuged, the gradient profiles were similar to those presented in Fig. 2A and 2B, respectively. Unlike polyribosomes from noninfected cells, which were stable to recentrifugation after Brij-58 treatment, the faster sedimenting ^3H -uridine-labeled structures from virus-infected cells are dissociated by the nonionic detergent. These results indicate that SLE viral RNA is associated with detergent and ribonuclease-sensitive structures which are distinct from polyribosomes observed in uninfected cells.

Characterization of the replication complex. Since the rapidly sedimenting ^3H -uridine-labeled structure from SLE virus-infected cells sedimented in sucrose gradients in a manner similar to polyribosomes, it was thought it might contain viral messenger RNA (mRNA) and ribosomes or it could be a replication complex. Since EDTA is known to cause dissociation of mRNA and ribosomes (9), its effect on the sedimentation profile of the complex was investigated. Cytoplasmic extracts were prepared from cultures labeled with ^3H -uridine and ^{14}C -leucine for 30 min at 16 hr postinfection. Half of the extract was adjusted to 0.02 M EDTA and analyzed by sedimentation on

an EDTA-sucrose gradient (Fig. 3A). The control extract was analyzed by sedimentation on a RSB-sucrose gradient (Fig. 3B). The A_{280} profile of the control (Fig. 3B) shows two peaks at approximately 250S and 180S, the 74S single ribosomes, and lighter materials. In the RSB-sucrose gradient, most of the ^3H -uridine radioactivity remained near the top of the gradient with a small amount of labeled material sedimenting in a peak at 250S. The A_{280} profile of EDTA-treated extracts (Fig. 3A) did not have a major peak at 180S (polyribosomes) but does show a broad peak sedimenting in the 200S to 300S region of the gradient. Radioactive RNA and protein cosedimented in the EDTA-sucrose gradient in the peak at 250S. These results differentiate between structures which contain pulse-labeled viral RNA and are stable in EDTA (Fig. 3A) and the polyribosomes which sediment at approximately 180S and are dissociated in the presence of EDTA.

To investigate whether cellular ribosomal RNA is an integral part of the EDTA-resistant structure, uninfected BHK cells were incubated in ^3H -uridine-containing medium for 24 hr to label cellular ribosomal RNA. After an additional 12 hr in medium without label, the cells were infected for 16 hr in the presence of actinomycin ($0.5 \mu\text{g/ml}$) and pulse-labeled with ^{14}C -leucine for 15 min. Cytoplasmic extracts prepared from the cells were divided and sedimented through either EDTA-

FIG. 3. Effect of ethylenediaminetetraacetic acid (EDTA) on the replication complex. ³H-Uridine (50 μ Ci/ml) and ¹⁴C-leucine (10 μ Ci/ml) were added to cultures 16 hr postinfection. Incorporation was stopped after 30 min. (A) part of the extract was adjusted to 0.02 M EDTA and sedimented in an EDTA-sucrose gradient. (B) control was sedimented in an reticulocyte standard buffer-sucrose gradient. Symbols: (●) acid-precipitable ³H-uridine radioactivity; (○) acid-precipitable ¹⁴C-leucine radioactivity; (—) A₂₈₀.

sucrose or RSB-sucrose gradients. Fig. 4A shows that upon EDTA treatment most of the ³H-uridine in RNA from prelabeled cells sediments at the top of the gradient at approximately 74S with a coincident peak of ³H-uridine and ¹⁴C-leucine radioactivity sedimenting at approximately 250S. In contrast to this, RSB-sucrose gradients of extracts from prelabeled cells (Fig. 4B) contained a large prominent peak of ³H-uridine and ¹⁴C-leucine radioactivity which sedimented at 180S to 250S.

Since a small percentage of ³H-uridine in the EDTA-sucrose gradient sedimented in the heavy region of the gradient (Fig. 4A), fractions in the 200S to 250S region of the gradient were pooled and resedimented in EDTA (Fig. 4C). Resedimentation of the 250S peak in EDTA shifted more

than 90% of the ³H-uridine counts in these pooled fractions to the top of the gradient. These data indicate that prelabeled cellular ribosomal RNA is partially dissociated to structures smaller than 250S by EDTA treatment and that some of the remaining RNA which sediments at 250S will partially dissociate to smaller structures if treated again with EDTA. The protein in the 250S peak which was labeled with ¹⁴C-leucine during the infection was not dissociated from the complex by this repeated EDTA treatment. These data suggest that most of the cellular RNA from the prelabeled cells which sediments with the 250S structure is loosely bound.

To determine the type of RNA left in the 250S region of the gradient from prelabeled cells after the second centrifugation in EDTA, fractions 6

FIG. 4. Absence of ribosomes in the replication complex. BHK cells were prelabeled with 50 μCi of ^3H -uridine per ml for 24 hr and then 12 hr in the absence of the isotope. The cells were infected for 16 hr in the presence of actinomycin and pulse-labeled with ^{14}C -leucine (10 $\mu\text{Ci}/\text{ml}$) for 15 min, and cytoplasmic extracts were prepared. (A) part of the extract was adjusted to 0.02 M ethylenediaminetetraacetic acid (EDTA) and sedimented through an EDTA-sucrose gradient. (B) control was sedimented through a reticulocyte standard buffer-sucrose gradient. (C) fractions 5 to 15 of the EDTA gradient indicated by the arrow in Fig. 4A were pooled, diluted with one-half volume of EDTA buffer, and analyzed in an EDTA-sucrose gradient. Symbols: (●) trichloroacetic acid-precipitable ^3H -uridine radioactivity; (○) trichloroacetic acid-precipitable ^{14}C -leucine radioactivity; (—) A_{280} .

to 18 of the gradient shown in Fig. 4C were pooled, and the RNA was extracted and analyzed in sucrose gradients. A heterogeneous mixture of ribonuclease-sensitive RNA species were found with about 22% ribosomal, 3% single-stranded viral 43S and the remainder small RNA species. The RNA from the prelabeled 250S peak sedimented only in RSB (Fig. 4B) was 90% ribosomal RNA.

Table 1 shows the percentage of ^3H -uridine counts remaining in the 250S region of the gradient following centrifugation in RSB and recentrifugation in EDTA. Following the first sedimentation in RSB, 82% of the RNA from the prelabeled cells was observed to sediment in the 200S to 300S region as compared to 15% in the

extract sedimented through EDTA. Recentrifugation of this 250S peak from prelabeled cells in EDTA dissociated about 90% of the remaining RNA from the complex to sediment near the top of the gradient (Fig. 4). The 250S peak from pulse-labeled cells contained approximately 15% of the total RNA in EDTA gradients which did not dissociate from the complex after isolation and recentrifugation.

To characterize the species of RNA which are contained in the structures which sediment in the 250S region of the gradient from infected pulse-labeled cells, cultures were infected and were pulsed with ^3H -uridine for 30 min at 16 hr post-infection, and the 250S peak was isolated from EDTA gradients. The RNA was extracted and

analyzed on sucrose gradients (Fig. 5). Three species of viral RNA are associated with the 250S cytoplasmic structure from the infected cells. The largest species has a sedimentation coefficient of 43, is ribonuclease sensitive, and is assumed to be newly synthesized single-stranded progeny type

TABLE 1. Absence of ribosomes in the replication complex^a

Type of gradient	Per cent RNA remaining in the 250S region of the gradient	
	Pulse-labeled infected	Prelabeled ^b infected
RSB	15	82
1st EDTA	17	17
2nd EDTA	12	0.01

^a RSB, reticulocyte standard buffer; EDTA, ethylenediaminetetraacetic acid.

^b Data taken from Fig. 4.

FIG. 5. Sucrose gradient analysis of RNA extracted from viral replication complex. Cultures infected for 16 hr in the presence of actinomycin were pulsed with ³H-uridine for 30 min; extracts were prepared and centrifuged through ethylenediaminetetraacetic acid-sucrose gradients. The RNA in the 250S peak was then extracted with sodium dodecyl sulfate-phenol and analyzed on sucrose gradients. Trichloroacetic acid-precipitable counts before (●) and after (○) ribonuclease treatment. The designation 28S indicates the optical density peak of ribosomal RNA.

RNA. The 26S partially ribonuclease-resistant and 20S ribonuclease-resistant forms of viral RNA were also present (20).

These data lead to the conclusion that viral RNA is contained in the 250S structure as a tightly bound integral part of the complex to which small amounts of ribosomal RNA species may be loosely associated.

RNA polymerase. Since the fast-sedimenting material in the cytoplasmic extract contained the replicative forms of viral RNA, experiments were done to determine if viral RNA polymerase activity was present in the 250S particulate fraction. Cytoplasmic extracts were prepared from cultures infected for 18 hr and analyzed on sucrose gradients. Polymerase activity in gradient fractions was determined by measuring the incorporation of ³H-GTP into acid-precipitable material. As shown in Fig. 6, fractions 8 to 13 contained most of the enzyme activity which sedimented in the same region of the gradient as pulse-labeled RNA (Fig. 3B). Treatment of extracts with DOC or Brij-58 either prior to or after gradient analysis destroyed all detectable enzyme activity.

SLE viral polymerase has properties similar to those reported for the group A arboviruses (14, 18) (Table 2). The enzyme required magnesium ions and all four nucleotide triphosphates and was inhibited by manganese ions and ribonuclease. Incorporation of ³H-GTP into acid-precipitable material was linear during the first hour of incu-

FIG. 6. Sedimentation pattern of RNA polymerase in cytoplasmic extract from cells infected with SLE virus. BHK cells were infected for 18 hr; cytoplasmic extract was prepared and centrifuged through an ethylenediaminetetraacetic acid-sucrose gradient. Fractions (1.0 ml) were collected and analyzed for RNA polymerase activity.

TABLE 2. *Effect of Mn^{2+} , Mg^{2+} , nucleotides, actinomycin D, ribonuclease, and adenosine triphosphate-generating system on Saint Louis encephalitis viral polymerase*

Reaction system ^a	Total radio-activity ^b
Complete assay.....	1,572
Complete assay without	
Mg^{2+}	315
ATP.....	173
CTP.....	250
UTP.....	187
ATP, CTP, UTP.....	73
Phosphoenol pyruvate and phosphoenol pyruvate kinase.....	817
Actinomycin D.....	1,436
Complete assay with	
Ribonuclease (0.5 μ g).....	506
Mn^{2+}	346
Uninfected cells (complete assay).....	184

^a CTP, cytidine triphosphate; UTP, uridine triphosphate.

^b Counts per minute of 3H -guanosine triphosphate incorporated into acid-precipitable material.

bation and then declined rapidly. Cytoplasmic extracts of uninfected BHK cells did not incorporate a significant amount of 3H -GTP into acid-precipitable material in the presence of actinomycin D.

Viral proteins in the replication complex. Immunoprecipitation tests were performed to determine if the protein associated with the replication complex was viral specific. Fractions in the 250S region of the gradient shown in Fig. 3A were pooled and reacted with SLE viral-hyperimmune ascitic fluid. A visible zone of precipitate developed at the interphase between the SLE viral antibody and fractions which contained the replication complex. No precipitate was observed after incubation of 250S gradient fractions from non-infected cells with viral antiserum or when normal ascitic fluid was used. Most (92%) of the radioactivity in those fractions which contained the replication complex was precipitated with SLE viral antibody, in comparison to less than 10% from cytoplasmic fractions of noninfected cells or when normal, mouse ascitic fluid was used. The replication complex therefore does contain viral-specific proteins. Whether these proteins are structural, nonstructural, or both, is not known (21).

Kinetics of labeling the replication complex. The experiments described above have established the existence of a cytoplasmic replication complex (CRC) in SLE virus-infected cells. To determine

the time in the growth cycle when this complex was most active in RNA synthesis, cultures infected for 12 hr and 18 hr were doubly labeled with 3H -uridine and ^{14}C -leucine for 15 min. Cytoplasmic extracts from these cells were analyzed on sucrose-EDTA gradients (Fig. 7). It is observed that fractions which contain maximal protein and RNA (trichloroacetic acid-precipitable radioactivity) are coincident in the gradient. The amount of ^{14}C -leucine counts in the fractions containing the CRC from 12-hr (Fig. 7A) and 18-hr infected cells (Fig. 7B) is similar, whereas the amount of 3H -uridine radioactivity in the 250S region from the 18-hr cultures had increased approximately twofold. The CRC in SLE viral-infected cells appears to contain either a constant amount of structural protein, enzymatic protein, or both, or at 12 hr postinfection the synthesis and release of protein in the complex has reached a constant rate. These experiments do not distinguish between these possibilities. However, since the CRC does not appear to contain polyribosomes and therefore is probably not involved in protein synthesis, we feel that these data probably reflect the CRC composition. The increased incorporation of 3H -uridine into viral RNA in the replication complex during the viral growth cycle is concomitant with the increase in total cell-associated viral RNA synthesis (20).

DISCUSSION

Pulse-labeled SLE viral RNA is found in a 250S cytoplasmic structure which contains viral RNA polymerase activity and the nascent and replicative forms of viral RNA. These results suggest that this structure is a replication complex, i.e., the site of SLE viral RNA synthesis. The replication complex is first detected 6 hr after infection (*unpublished data*) and is most active in RNA synthesis during the rapid phase of viral growth when the rate of viral RNA synthesis is maximal (20).

Input Semliki Forest viral RNA binds to membraneous structures which have many similarities to the SLE viral MRC structures (8, 17). The observation that cycloheximide inhibits the binding of Sindbis viral RNA to the MRC structures may explain our previous observation that cycloheximide treatment prior to the end of the latent period inhibits SLE virus growth (21). In contrast to the polioviral replication complex (9), the structure and functional integrity of the MRC structures from the Sindbis (17) and SLE viral CRC are destroyed by detergent treatment. This would suggest that the arboviral replication complex may be composed of subunits which are membrane associated. Dissociation of these

FIG. 7. Sucrose gradient analysis of the replication complex pulse-labeled early and late during viral RNA synthesis. BHK cells infected for 12 hr and 18 hr were simultaneously pulse-labeled with ^3H -uridine and ^{14}C -leucine for 15 min; cytoplasmic extracts were prepared and centrifuged through ethylenediaminetetraacetic acid-sucrose gradients. Symbols: (○) ^3H -uridine trichloroacetic acid-precipitable radioactivity; (●) ^{14}C -leucine trichloroacetic acid-precipitable radioactivity. A, 12 hr postinfection; B, 18 hr postinfection.

subunits during the isolation procedure may explain the failure of arboviral RNA polymerase preparations to synthesize progeny-type, single-stranded RNA (14). Grimley et al. (10) have described a distinct type of cytoplasmic vacuole in Sindbis virus infections which they believe to be the site of viral RNA synthesis. Many smaller vacuoles with similar features are found in the perinuclear region of SLE virus-infected cells, however, their role in viral RNA synthesis is not now clear (15; *unpublished data*).

In contrast to picornaviral polyribosomes which are stable to detergent treatment and can readily be isolated (9), repeated attempts to isolate viral polyribosomes from SLE viral-infected cells using many techniques have failed (*unpublished data*). The SLE viral CRC does not appear to be associated with viral protein synthesis or to contain ribosomes. The cellular site of SLE viral protein synthesis is not now known. The presence of viral antigen(s) in the CRC may be explained

by the incorporation of viral protein(s) into the membranes from which the complex develops or associates. The Sindbis viral envelope is acquired by budding of the nucleocapsid through cytoplasmic or internal membranes (10). The envelope contains a single polypeptide which gives the virion hemagglutinating, complement-fixing and neutralizing antibody-blocking activities (1). Viral antigen in the SLE viral CRC may reflect the presence of a viral-modified membrane as a structural part of this structure. Antigens in the CRC may be identical to the envelope polypeptides of the complete SLE virion (21). Further experiments are needed to characterize the membranes associated with the replication of SLE viral RNA, structural and nonstructural proteins, and the envelopment process.

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