Expression of a Recombinant DNA Gene Coding for the Vesicular Stomatitis Virus Nucleocapsid Protein

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A cDNA clone containing the entire vesicular stomatitis virus nucleocapsid gene was assembled by fusing portions of two partial clones. When the cDNA clone was inserted into a new general-purpose eucaryotic expression vector and introduced into appropriate host cells, abundant N-protein synthesis ensued. The expressed protein was indistinguishable from authentic N protein produced during vesicular stomatitis virus infections. The recombinant N protein was recognized by a polyclonal antibody and two different monoclonal antibodies and could not be resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis from authentic N. Our results suggest that the recombinant N protein produced in transfected cells rapidly aggregates into high-molecular-weight complexes in the absence of vesicular stomatitis virus genomic RNA.

Recent reports suggest that the nucleocapsid protein N plays a central role in regulating the replication of vesicular stomatitis virus (VSV) (1, 18; B. Blumberg, C. Giorgi, and D. Kolakofsky, Cell, in press). In the infected cell, full-length genomic RNAs, whether negative or positive strand, are only found associated with this protein in the form of a very stable nucleocapsid structure. Viral mRNAs, which, in aggregate, contain all but 117 nucleotides of the sequences present in the genome-length positive-strand nucleocapsids, are virtually free of association with the N protein. Several reports have dealt with the speculation that RNA synthesis, once initiated, can proceed to yield either a genomelength positive strand or the five separate monocistronic mRNAs, depending upon the intracellular availability of the N protein (1, 17, 18). The common feature of these models is the speculation that during N-protein sufficiency, nascent RNA transcripts are rapidly encapsidated, a process that releases the polymerase from a site-specific attenuation and allows it to synthesize a genome-length RNA. In N-protein deficiency, as in in vitro transcription, the nascent transcripts are released; new transcripts initiated at specific sites along the templates are elongated to form the monocistronic mRNAs. Once formed, these mRNAs remain unencapsidated even during N-protein sufficiency because they do not contain the nucleation site—a sequence which is thought to be found only at the 5′ termini of the positive or negative genomic RNAs. Thus, the N protein is thought to control the balance between transcriptive and replicative RNA synthesis in the infected cell.

Direct tests of this model have not been possible because of the inability to effectively divorce genomic RNA synthesis from encapsidation, alter the putative nucleation site at will, or vary the intracellular availability of N protein. Recombinant DNA techniques and the use of chimeric cells which constitutively produce N protein may offer the degree of control over the replicative process that is needed.

In the present communication, we report the assembly of a complete DNA gene encoding the VSV N protein. We have positioned this gene in a simian virus 40 (SV40) cloning vector so that it is transcribed intracellularly as part of the major late SV40 mRNA and translated into a protein indistinguishable from its natural counterpart produced during VSV infections.

MATERIALS AND METHODS

Virus cell lines and culture conditions. The Indiana strain of VSV (Mudd-Summers strain) used throughout this work was cultured on monolayers of BHK cells and purified as described previously (19). Escherichia coli HB101, obtained from Cold Spring Harbor Laboratories, was used to replicate plasmid DNAs. Bacterial cells containing plasmids were either cultured in M9 medium with their plasmid content amplified by the addition of chloramphenicol or cultured in L broth until a stationary phase was reached. CV-1 cells transformed by an origin defective mutant of SV40, COS cells (12), were obtained from G. Khoury and were cultured in Eagle minimal essential medium
containing nonessential amino acids, 5% fetal calf serum, penicillin, and streptomycin.

**Plasmids.** pN4, a plasmid containing most of the VSV N-gene (11), was obtained from J. Rose. pBR322 and pML-2, a derivative of pBR322 which lacks “poisonous” sequences (20), were obtained from H. Boyer and M. Lusky, respectively. pMK2004 (14) was a gift from D. Helsinki.

**RNA preparations.** RNA was prepared from purified viral particles by the sodium dodecyl sulfate (SDS)-phenol-chloroform extraction method described previously (15). Deproteinized RNA was precipitated with ethanol, dissolved in a small volume of 1% SDS in 0.01 M Tris-hydrochloride (pH 7.4)-0.1 M NaCl-1 mM EDTA, and fractionated on 10 to 30% (wt/wt) sucrose gradients containing 0.5% SDS. Fractions containing the 42S RNA from standard infectious VSV were precipitated with ethanol, rinsed with 80% ethanol to remove excess salt, dried in vacuo, and dissolved in 1 mM Tris-hydrochloride (pH 7.4).

**Enzymes.** Restriction endonucleases and T4 DNA ligase were obtained from either Bethesda Research Laboratories or New England Biolabs. The Klenow fragment of the E. coli DNA polymerase I was obtained from Boehringer Mannheim. S1 nuclease was obtained from Miles Laboratories. Restriction endonuclease digestions were performed under conditions suggested by the producer or by Davis et al. (7).

**Preparation of cDNA clones of the VSV 3' terminus.** DNA complementry to the 3' end was synthesized by using VSV RNA and a primer d(ACGAAGAC) (Collaborative Research, Inc.) that is the complement of the first eight bases of VSV RNA. Reaction conditions were a modification of those previously reported (3, 25): 50 µg of RNA per ml, 3.7 µM primer, 50 mM Tris (pH 8.4), 10 mM MgCl2, 50 mM KCl, 1 mM β-mercapto-ethanol, 0.5 mM of each deoxynucleotide triphosphate, 10 µg of actinomycin D per ml, and 500 U of avian myeloblastosis virus reverse transcriptase per ml. After 90 min of incubation at 42°C, the reaction solution was adjusted to 0.3 M NaOH and heated to 50°C for 30 min to hydrolyze the RNA. The solution was neutralized with acetic acid, passed over a Sephadex G50 column, and precipitated with ethanol. The cDNA was dried in vacuo and dissolved in water. Double-stranded DNA was synthesized under the same general reaction condition, but without primer or RNA. Double-stranded cDNA was trimmed with S1 nuclease and tailed with oligodeoxyctydylid acid as previously described (9, 25).

The oligodeoxyctydylid acid-tailed cDNAs were hybridized with E. coli plasmid pBR322 which had been linearized by cleavage at the PstI site and tailed with oligodeoxyguanydilid acid, and the mixture was used to transform E. coli HB101 as described below. Ampicillin-sensitive colonies were screened by hybridization (13) with 32P-labeled leader RNA (5, 6).

**Selection of recombinant clones.** Plasmid DNA was introduced into E. coli HB101 after treatment with CaCl2 as described by Davis et al. (7). Transformed cells were plated on LB medium containing either 10 µg of tetracycline per ml, 100 µg of ampicillin per ml, or 100 µg of kanamycin per ml, depending on the plasmid. Colonies containing the desired recombinant plasmid were identified by colony hybridization by the method of Grunstein and Hogness (13).

**DNA sequence determination.** Restriction endonuclease fragments were labeled at their 3' termini with cordycepin [32P]triphosphate by the method of Tu and Cohen (24). Labeled DNAs were cut with a second restriction endonuclease, and the uniquely labeled fragments were separated by polyacrylamide gel electrophoresis as previously described (21). Labeled fragments recovered from the gel were sequenced by the chemical sequencing methods of Maxam and Gilbert (21).

**Transfection of COS cells with pJS223.** Recombinant DNA plasmids containing an intact N gene (pJS223) were introduced into COS cells by the transfection procedure of Parker and Stark (23). COS cells were grown in monolayers on either cover slips, 24-well culture plates, or 150-mm petri dishes until just confluent. After transfection, cells were cultured for 1, 2, or 3 days depending upon the experiment. Transfection efficiency, measured with supercoiled SV40 DNA and CV-1 cells, was 30 to 40%. Transfection efficiency of COS cells with pJS223 is estimated to be 5 to 20% by indirect immunofluorescence.

**Immunofluorescence.** Transfected COS cells on cover slips were washed with phosphate-buffered saline, air dried, and fixed in acetone. They were then incubated with a rabbit anti-VSV antisera (dilution, 1:20) or the supernatant from mouse hybridoma clone 4A35 (obtained from Robin Weiss) or clone V118 (Armenheier, unpublished data) secreting monoclonal antibodies to N protein, washed with phosphate buffered saline (pH 7.4), and then incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G (IgG) or fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG, respectively. Fluorescence was examined with a Zeiss fluorescence microscope. Control stainings performed with normal rabbit serum or supernatant fluids from hybridomas secreting monoclonal antibodies to unrelated proteins were negative.

**Solid-phase radioimmunoassay.** N protein from extracts of transfected COS cells was assayed in a solid-phase radioimmunoassay based on the following antibody sandwich technique. Monoclonal antibody 4A35 (subclass IgG1), partially purified by ion-exchange chromatography from mouse ascites fluid, was adsorbed to the wells of polyvinyl chloride microtiter plates in phosphate-buffered saline (pH 7.4). Nonadsorbed antibody was washed away, the plates were treated with a solution of ovalbumin (3% in phosphate-buffered saline) for 2 h, and COS cell extracts were added for 4 h (50 µl per well). The wells were then washed, incubated for 2 h with a rabbit anti-VSV serum (dilution, 1:500), washed, incubated for 2 h with 125I-labeled Staphylococcus aureus protein A (20,000 cpm per well), washed, and then counted in a Beckman LS 1600 scintillation counter. The use of radiolabeled protein A was feasible because it did not bind to the monoclonal antibody adsorbed to the plates.

**Immunoprecipitation.** 35S-labeled COS cell extracts obtained after transfection with pJS223 were incubated with rabbit anti-VSV serum for 1 h at room temperature and then mixed with fixed S. aureus cells previously incubated with an extract from unlabeled COS cells. The cells were collected by centrifugation, washed three times, and solubilized in SDS in the presence of 2-mercaptoethanol, and the soluble proteins were separated by polyacrylamide gel electrophoresis (10% acrylamide, 0.013% bisacrylamide;
0.1% SDS) on 25-cm-long slab gels. The radiolabeled proteins were visualized by fluorography.

RESULTS

Cloning the 3' terminus of VSV RNA. To produce cDNA clones in which the terminal sequences were completely represented we employed a synthetic octadexynucleotide that is the complement of the extreme 3' terminus of the VSV RNA. Using this olig deoxynucleotide as a primer, we were able to stimulate reverse transcription of the VSV RNA about 1.5-fold above that found in the absence of exogenous primer. Double-stranded cDNA transcripts were prepared, inserted into pBR322, and cloned as indicated above. Clones were screened by colony hybridization by using 32P-labeled leader RNAs, a family of short transcripts from the extreme 3' terminus of the VSV genome (5, 6). Approximately 7% of the VSV containing clones contained portions of the leader sequence that could be detected by colony hybridization. Most of these leader RNA-positive clones were incomplete and lacked one or more nucleotides of the complete 3' terminal VSV sequence. One complete clone, pJS77, was selected for further investigation.

The VSV sequences of pJS77 (approximately 360 base pairs) were excised from the plasmid and terminally labeled with cordycepin [32P]tri-phosphate. The VSV insert was cut with Bg/II, which was a unique site at position 210; the labeled fragment containing the 3' terminal sequence of VSV was purified by gel electrophoresis, and its sequence was determined by chemical sequencing (21). The results confirmed the presence of the complete VSV 3'-terminal sequence in the cloned fragment preceded by the oligodeoxycytidyl acid tail introduced by tailing with terminal transferase. We have not sequenced the entire clone since the sequence of that portion of the VSV genome has been reported (11). We have confirmed the identity of the balance of the insert by restriction endonuclease mapping. When digested with BamHI, MboII, DdeI, HinII, HincII, AccI, BglII, and AluI endonucleases, the cloned fragment yields the number and size fragments predicted from the sequence published by Gallione et al. (11).

Assembly of cDNA coding for VSV nucleocapsid protein. The clone of the 3' terminus of VSV, pJS77, described above, overlaps the portion of the N gene cloned by Gallione et al. (11). The overlapping region of the two clones contained a unique BglII site at which they could be conveniently joined to form a cDNA spanning the entire N gene. Because the N gene contains an internal PstI site, we changed the terminal sequences of each clone to XhoI sites so that the assembled N gene could be easily retrieved from the plasmid and manipulated as a single fragment. The strategy and schematic diagram of the assembly process are illustrated in Fig. 1.

The DNA segment bounded by XhoI sites

FIG. 1. Schematic representation of the procedure used to fuse two overlapping partial cDNA clones to form a complete cDNA gene of the VSV nucleocapsid protein.
contains 33 nucleotides before the ATG coding for the amino terminal methionine of the nucleocapsid protein. This sequence includes the first 13 nucleotides of the N gene which are not translated, preceded by the leader-N gene intergenic sequence, a portion of the leader gene, and six nucleotides introduced as part of the XhoI linkers. The sequence is CTCGAGGCAGGA-GAAACTTTAACAGTAATCAAAATG. The cloning method used also introduced some extraneous sequences at the distal end of the gene beyond the end of the pN4 previously characterized (11). These include a short stretch of pBR322, the remnant of the oligodeoxycytidyllic acid tail, and the XhoI site.

**pJC119, a general-purpose eucaryotic expression vector.** The construction of the SV40 expression vector pJC119 is shown in Fig. 2. pSV53, obtained from C.-J. Lai, consists of a late-region deletion mutant of SV40 cloned into the BamHI site of pBR322. The deletion extends from the HindIII site at position 1,493 (replaced by a BamHI linker) to the BamHI site at position 2,533 and removes most of the coding region of the VP1 gene, including the initiation codon. (We have employed the SV40 numbering system of Buchman et al. [3] to identify positions on the SV40 genome.) To insert a unique cloning site into this vector, pSV53 was digested with BamHI, and the termini were filled in with DNA polymerase 1 and blunt-end ligated to XhoI linkers. This SV40 fragment was then recloned into the XhoI site of pMK2004, and recombinants were identified by colony hybridization with nick-translated SV40 DNA. One such recombinant plasmid (pJC32) was passed through the dam strain GM33 to obtain an unmethylated version which could be cut with BclI. The SV40 fragment was isolated by digestion with XhoI and FnuDII (to fragment the plasmid) and purified by sucrose gradient centrifugation. After recircularization with T4 DNA ligase, the SV40 fragment was linearized by digestion with BclI and cloned into the BamHI site of the poison-negative plasmid pML2 (20) to yield pJC119. In this configuration the XhoI site in pJC119 is unique and spans the deletion in the late region, permitting the cloning of XhoI (or SalI) fragments into this site without removal of the plasmid. Moreover, the XhoI linkers reconstructed the original BamHI site six base pairs away on either side, allowing the cloning of BamHI, BglII, BclI, and SalI/A fragments into the same region. The remainder of the SV40 late

FIG. 2. Schematic representation of the construction of the general purpose expression vector, pJC119.
region is intact, retaining all of the sites required for initiation, splicing, and polyadenylation of late transcripts. Because the initiation codon of the VPI gene has been removed by the deletion, translation will initiate at the first ATG codon within a fragment cloned into the XhoI site.

Expression of VSV nucleocapsid gene in COS cells. The recombinant plasmid pJS223 contains the VSV N gene properly positioned so that it will be expressed as a late protein of SV40. This DNA and its counterpart lacking the N gene, pJC119, were introduced into separate cultures of COS cells by transfection, and the expression of the N gene was monitored by immunofluorescence. At intervals after transfections, cells were washed, fixed with acetone, and treated with monoclonal antibody directed against the VSV nucleocapsid protein. After removal of unbound antibody by serial washing, the cells were treated with fluorescein conjugate of rabbit anti-mouse IgG, washed again, and examined by fluorescence microscopy. Depending upon the experiment, between 5 to 20% of the cells showed strong fluorescence by day 2. The intensity of the fluorescence increased by day 3 and day 4. However, the cytopathic effects due to the transfection and the abortive SV40 infection also increased with time, and the number of cells adhering to the microscope slide was greatly diminished by day 4. Examples of the immunofluorescence of cells transfected with pJS223, which contains the N gene, and pJC119, which does not, are shown in Fig. 3. The fluorescence observed is notably granular—suggesting that the nucleocapsid protein may aggregate with itself or with cellular components. This suggestion is supported by the observation that the antigen found in extracts of transfected cells is heterodisperse and that approximately half of it is removed by centrifugation at 100,000 x g for 45 min.

To estimate the amount of N protein synthesized in transfected COS cells, we have employed immune precipitation with anti-VSV hyperimmune serum and S. aureus cells. COS cells were transfected with either pJC119 or pJS223
and labeled with \(^{35}\text{S}\)methionine for 24 h from the day 2 to day 3 after transfection. Cell extracts were prepared and immune precipitated with rabbit hyperimmune serum directed against VSV and fixed \(S.\ aureus\) cells, and the precipitated material was fractionated by SDS-polyacrylamide gel electrophoresis. The autoradiograms (Fig. 4) of these gels revealed a \(^{35}\text{S}\)-labeled protein in the immune precipitate of extracts of pJS223 that tracked with the virion N protein. This protein band was not apparent in polyacrylamide gel electrophoresis of extracts that were not first fractionated by immune precipitation—suggesting that the protein accounts for only a small amount of the total labeled protein. Direct estimates of the relative abundance of this labeled N protein can be made by comparing the radioactivity found in the N protein band excised from the SDS gels to the total trichloroacetic acid-precipitable label in the corresponding amount of unfractonated extract. These comparisons show that between 0.036 and 0.1% of the trichloroacetic acid-precipitable \(^{35}\text{S}\) label in the extracts are recoverable in the N protein. The N protein is synthesized only by those cells actually transfected with the plasmid pJS223, in these experiments approximately 10% of the cells. Using this number and assuming that the immune precipitation was quantitative and that the \(^{35}\text{S}\) counting efficiencies were the same for trichloroacetic acid precipitates and the excised gel slices, we calculate that 0.36 to 1% of the \(^{35}\text{S}\)methionine incorporated by transfected cells was recovered in N protein. Therefore, to our first approximation, 0.36 to 1% of the protein synthesized by pJS223 transfected cells is N protein.

**Aggregation of the nucleocapsid protein.** The immunofluorescence observed with pJS223-transfected cells was notably granular (Fig. 3). This granularity was consistently found and was not dependent upon the particular combination of immunoreagents: it was observed when either monoclonal or polyclonal anti-nucleocapsid antibodies were employed. Granular fluorescence also was observed in VSV-infected cells when they were probed with anti-nucleocapsid antiserum, suggesting that both the nucleocapsids and the N protein tend to concentrate in areas of the cytoplasm. To further explore these observations, we prepared extracts of cells expressing the cloned N gene and fractionated them by velocity sedimentation in sucrose gradients. The profile of the velocity separation (Fig. 5A) showed that the N protein is heterodisperse, with components sedimenting as fast as 200S. The expected sedimentation constant for monomeric N protein is approximately 3S. This large discrepancy suggests that the nascent N protein aggregates to form a heterogenous population of structures. Evidence for rapidly sedimenting complexes of N protein was also obtained from isopycnic centrifugation experiments. Extracts of cells transfected with either the vector containing the N gene or the vector alone were layered onto preformed linear 20 to 40% (wt/wt) CsCl gradients and centrifuged for 24 h. Fractions of the gradients were assayed for N protein by the solid-phase radioimmunoassay, for total protein by the Lowry method (data not shown), and for density. The gradient containing the extracts of cells transfected with the vector containing the N gene showed a prominent band of N protein at a buoyant density of 1.31 g/ml.
Had the association of the N protein been disrupted by the high concentration of CsCl, the monomeric N protein generated would not sediment fast enough to form a band during the centrifugation. Indeed, virtually all of the protein detected by the Lowry assay is located at the top of the CsCl gradients in fractions 15 to 23.

**DISCUSSION**

In this communication, we report the successful cDNA cloning of the VSV 3' terminus. The clone obtained, pJS77, overlaps a cDNA clone previously described (11). Using the two overlapping cDNA clones, we have assembled a complete VSV nucleocapsid gene. The assembled nucleocapsid gene is derived from two different strains of VSV (Indiana), the Mudd-Summers strain and the San Juan strain. Despite the fact that sequence differences have been noted between these two strains, the sequence of the leader-proximal portions of the N gene derived from the Mudd-Summers strain is the same as that reported for the San Juan strain (11). Consequently, the recombinant gene codes for the San Juan strain of the nucleocapsid protein.

The assembled N gene clone has noncoding or extraneous sequences flanking the coding regions. Fortunately, the 34 nucleotides upstream from the start of the coding region do not contain an ATG codon, so that protein synthesis directed by the cloned gene will initiate at the correct position, provided that an initiation codon is not supplied by the vector.

The SV40 vector employed in these studies carries a deletion that removes most of the coding region, including the initiation codon, for the major structural protein, VP1. Since the normal SV40 late mRNA promoter, splice donor, splice acceptor, termination, and polyadenylation sites are preserved in this vector, DNA inserted at the deletion site should be transcribed into a spliced, polyadenylated mRNA in which the insert sequence follows the SV40 late mRNA leader sequence. SI nuclease mapping of the transcripts from pJS223-transfected cells bears out this prediction (Condra, unpublished results).

Two additional aspects of the vector bear further comment. The vector can be replicated in both eucaryotic cells, by virtue of its SV40 origin, and procaryotic cells, by virtue of its pML2 origin. Since the pML2 portion lacks the sequences which interfere with SV40 replica-

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**FIG. 5.** Behavior of aggregated nucleocapsid protein during velocity sedimentation and isopycnic gradient centrifugation. Cytoplasmic extracts of COS cells transfected with the vector alone (O) or with the vector containing the N gene (■) were prepared and fractionated on (A) linear 10 to 30% (wt/wt) sucrose gradients (37,000 rpm, 4°C, 100 min) or on (B) linear 20 to 40% (wt/wt) CsCl gradients (33,000 rpm, 4°C, 24 h). The gradients were fractionated, and the N protein content of each fraction was determined by a solid-phase radioimmunoassay as described in the text. The density of the relevant CsCl gradient fractions was determined by weighing 100-μl samples and is shown in the insert of panel B. The positions to which 140S and 80S material sedimented was determined with nucleocapsids from VSV or the small defective particle DI 011.
tion, the vector bearing the insert can be grown in *E. coli* and used directly in appropriate animal cells without removing the plasmid sequences. However, the plasmid sequences interrupt the early region of the SV40; consequently, the T antigen necessary for replication must be supplied in trans if the recombinant is to be replicated. COS cells constitutively express T antigen (12), and SV40 vectors deficient in T-antigen expression introduced into them replicate to copy numbers as high as $2 \times 10^7$ per cell (22).

The VSV N gene, when properly positioned in the pJC119 vector and introduced into COS cells, is accurately and abundantly expressed. The product protein is recognized by polyclonal anti-VSV serum and by two different monoclonal antibodies directed against the nucleocapsid protein. Electron microscopic studies of VSV-infected cells demonstrating that nucleocapsid structures accumulate as filamentous materials in cytoplasmic inclusions (26) offer a ready explanation for the granular appearance of the fluorescence observed during viral infection. We have explored the possibility that the granular appearance of the fluorescence of the transfected cells arose from a clustering or aggregation of the N protein in the cytoplasm. The fluorescence remains granular when cells are fixed with either acetone or paraformaldehyde-glutaraldehyde. The N protein in extracts of unfixed transfected cells sediments as a heterodisperse population with some members having sedimentation constants as large as 200S. The association of the N proteins is stable in high-ionic-strength solution since they form a tight band in CsCl gradients. The small sedimentation constant expected of the monomeric N protein (3S) and the short duration of the centrifugation (24 h) indicate that the banded material must be polymeric. Thus, it appears that the N protein exists as complexes or aggregates in both fixed and unfixed cells.

At present, we cannot distinguish between the possibility that the N protein aggregates with itself to form structures composed only of N and the possibility that the N protein associates with host cellular components such as RNA or cytoskeletal elements. The buoyant density of the N protein complexes (1.31 g/ml) is the same as that of VSV nucleocapsids and very close to that expected of protein aggregates free of RNA. The behavior of purified N protein obtained from virions does not help us in deciding between the alternatives: purified N protein both self-aggregates to form complexes large enough to precipitate and can encapsidate host RNA to form nucleocapsid-like structure (Blumberg and Kolakofsky, personal communication).

Finally, the level of N protein in transfected cells is substantial and rivals that synthesized in virus-infected cells. VSV-infected cells produce about $10^7$ VSV particles each. However, several reports indicate that only 10% of the nucleocapsids are matured into progeny particles (10, 16). Assuming 1,100 N proteins per nucleocapsid (4) and $10^5$ nucleocapsids per infected cell, we estimate that the total amount of N protein synthesized by an infected cell is 8.3 pg or approximately 1% of the cellular protein. The level of N protein measured in several transfection experiments ranged from 0.36 to 1% of the newly synthesized protein. Thus, the amount of N protein synthesized in cells transfected with pJS223 approximates that found in the natural VSV infection.

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**LITERATURE CITED**

Gene Coding for VSV Nucleocapsid Protein


