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NOTES

Positive Identification of a Measles Virus cDNA Clone Encoding a Region of the Phosphoprotein

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A measles virus-specific cDNA clone, Cl-G, that was previously assigned as a hemagglutinin-specific clone has been reassigned as a phosphoprotein-specific clone. The nucleotide sequence of C1-G was used to deduce the amino acid sequence. A synthetic peptide was constructed from a portion of the deduced sequence, and antisera were prepared. The antibodies directed against this synthetic peptide specifically precipitated the phosphoprotein of measles virus and not the hemagglutinin.

The availability of cDNA clones specific for measles virus has greatly facilitated exploration of the molecular biology of this paramyxovirus. Rozenblatt and co-workers (5, 13) have isolated such clones and identified them as representing portions of the nucleocapsid (N), matrix (M), and hemagglutinin (HA) regions of the measles virus. These clones were prepared by oligodeoxythymidylate-primed reverse transcription of mRNA from measles virus-infected cells. These three types of clones identify three distinct classes of measles virus mRNA when used in a Northern blot analysis of total mRNA from measles virus-infected cells.

We recently completed the nucleotide sequencing of clone Cl-G, which was originally assigned as an HA clone. The complete nucleotide sequence of Cl-G, along with the deduced amino acid sequence from the single open reading frame, is shown in Fig. 1. From the nucleotide sequence, it appeared that clone Cl-G was derived from the very 3′ end of the reverse transcribed message and extended approximately 400 base pairs toward the 5′ end of the mRNA.

(A preliminary report of this work was presented at the Symposium on the Molecular Biology of Negative-Strand Viruses, Hilton Head, S.C., 1983.)

The deduced amino acid sequence for the carboxy-terminal portion of the protein raised suspicions that Cl-G may not contain a part of the HA gene. Since the HA protein is a transmembrane glycoprotein, we expected to find a hydrophobic stretch of amino acids corresponding to the transmembrane region near the carboxy terminus of the protein (assuming a conventional orientation) (6). No obvious hydrophobic domain could be identified. To positively identify the region of the measles virus genome represented in this clone, a synthetic polypeptide was synthesized that encompassed region P20 (Fig. 1). The P20 peptide was synthesized with a Beckman 990 peptide synthesizer. Subsequently, the peptide was cleaved from the resin with hydrofluoric acid and desalted on Sephadex G-10 and G-25 columns. Peptide fractions were lyophilized to dryness and purified on a preparative C18 reverse-phase column (2). The resultant peptide was then chemically cross-linked (4) to keyhold limpet hemocyanin before being used as an immunogen in rabbits. After the primary immunization, the animals were boosted with an equivalent amount of the antigen (0.5 mg). Approximately 2 weeks later, the animals were bled and antisera were obtained. The sera were ammonium sulfate precipitated and further purified by affinity chromatography with the P20 peptide coupled to Affigel 10 (Bio-Rad Laboratories, Richmond, Calif.). The crude antisera and the affinity-purified antibody were then used in immunoprecipitation studies with radiolabeled, measles virus-infected cell lysates.

Immunoprecipitation was also carried out with previously identified monoclonal antibodies to several of the measles virus proteins. Measles virus-infected CV-1 cells were radiolabeled 20 h postinfection with either [^{35}S]methionine or $^{32}P_i$ (100 μCi/ml) for 6 h. Monolayers were washed with TE buffer, and the cells were lysed by the addition of 1.5 ml of RIPA buffer (9). Cell lysates were clarified by centrifugation at 10,000 × *g* for 15 min at 4°C. Immunoprecipitation assays were performed in RIPA buffer as previously described (1, 9). Samples of the immunoprecipitated proteins were analyzed by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis (7). Gels were treated with a fluorographic enhancer (Enlightening; New England Nuclear Corp., Boston, Mass.) before being exposed to Kodak XAR-2 X-ray film and stored at −70°C. Polyacrylamide gel analysis of the immunoprecipitates from [^{35}S]methionine-labeled infected-cell lysates indicated that the affinity-purified P20 antibody precipitated a single polypeptide that comigrated with the 70,000-molecular-weight (70K) protein precipitated by the monoclonal anti-P serum (Fig. 2A). The P protein was also precipitated by the polyvalent anti-measles virus sera and was clearly distinguishable from the HA protein precipitated by this serum and by the monoclonal anti-HA serum. The 70K protein precipitated by the anti-P20 and monoclonal anti-P antisera was easily distinguished from the proteins precipitated by the anti-N protein and anti-M protein monoclonal antibodies.

The P and N proteins of measles virus are known to be phosphorylated (6). To determine whether the protein precipitated by the anti-P20 antibody was in fact a phosphoprotein, we immunoprecipitated ^{32}P-labeled infected-cell lysates (Fig. 2B). The monoclonal anti-P and the anti-P20 antisera specifically precipitated the same ^{32}P-labeled 70K polypeptide. Polyvalent anti-measles virus sera, on the other hand, precipitated both the ^{32}P-labeled P and N proteins. The 60K N protein was precipitated by the monoclonal anti-N anti-

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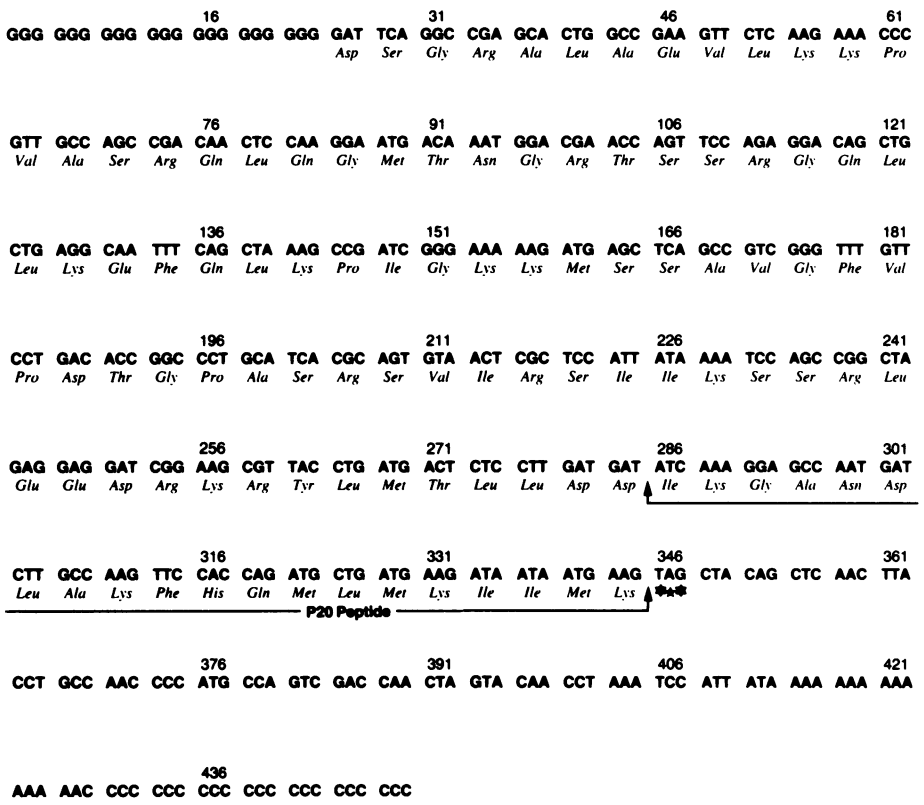

FIG. 1. Complete nucleotide sequence of clone Cl-G. Sequences were determined in both directions by the method of Maxam and Gilbert (10). The deduced amino acid sequence is presented. The bracketed region corresponds to the sequence used to construct the P20 synthetic peptide.

body and was clearly separated from the P protein. The HA protein of measles virus is not phosphorylated and thus did not appear as a ^{32}P-labeled peptide when anti-measles virus and monoclonal anti-HA sera were used to precipitate this peptide from ^{32}P-labeled lysates.

We prepared a measles library from the genomic 50S RNA of measles virus. This was done by randomly priming 1 μg of 50S measles virus RNA with salmon sperm DNA pentamers by previously described methods (12). After the tailing procedure, the cDNA was cloned into the *Pst*I site of appropriately tailed pBR322. This library, containing approximately 2,000 clones, was probed with clone Cl-G. Of six clones identified, clone pWB-3A8, containing 1,700 nucleotides of measles virus-specific information was completely sequenced. The complete and identical nucleotide sequence of clone Cl-G was found to be contained within the genomic clone pWB-3A8 (Bellini et al., unpublished data).

Both Cl-G and pWB-3A8 were used in hybrid arrest studies with oligodeoxythymidylate-cellulose-selected mRNA from measles virus-infected Vero cells. Total cytoplasmic RNA was extracted from infected CV-1 cells, and polyadenylated mRNA-enriched fractions were obtained as previously described (8). This mRNA was used in hybrid-arrest-of-translation studies under the conditions specified by Paterson et al. (11). The resultant translated peptides were immunoprecipitated with guinea pig anti-measles virus serum, and the precipitates were analyzed by sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis as described above for the P protein. Increasing concentrations of DNA from clone Cl-G or pWB-3A8, ranging from 1 to 10 μg, resulted in the total arrest of P protein synthesis compared with its synthesis in mock-arrested mRNA preparations (Fig. 3). As before, the identity of the hybrid-arrested proteins was confirmed by immunoprecipitation with both monoclonal and polyvalent antisera (data not shown).

The present report firmly establishes that clone Cl-G contains some of the coding sequences of the measles virus P gene and thus should be redesignated Cl-P. The synthetic peptide constructed was deduced from the nucleotide sequence of Cl-G. Antisera raised to this peptide clearly precipitated the 70K P protein of measles virus, in agreement with the in vitro-translated product previously observed. This same peptide is precipitated by anti-P but not anti-HA monoclonal antibody. Moreover, Cl-G and a related clone, pWB-3A8, arrested synthesis of the P protein in in vitro translation.

Fluorescence microscopy with either monoclonal anti-P or the anti-P20 antibody showed that the antigen recognized by these antibodies is internal and not on the surface of the cell, as would be expected if it were the HA protein. In double-label fluorescence experiments, the antigen recognized by anti-P20 antibodies was found to be associated with the nucleocapsids of measles virus-infected cells and with the inclusions present in late infection (R. N. Hogan, F. Rickaert, W. J. Bellini, C. Richardson, D. E. McFarlin, and M. Dubois-Dalcq, *in* D. Bishop and R. Compans, ed., The molecular biology of negative-strand viruses, in press).

The original designation of clone Cl-G as HA specific was based on several pieces of data. The clone hybrid selected an mRNA from measles virus-infected cells which, when trans-

lated in vitro resulted in the synthesis of a 70K and a small amount of a 40K polypeptide. Secondly, the 70K in vitro-translated product was precipitable by a monoclonal antibody which had been designated an anti-HA antibody by another laboratory. These results were compatible, at that time, with the idea that the in vitro-translated 70K protein was the unglycosylated form of the HA protein.

We cannot reconstruct the misdesignation of Cl-G because the monoclonal anti-HA used in the original assignment was produced by an unstable hybridoma cell line and neither the cell line nor the antibody is available to us any longer.

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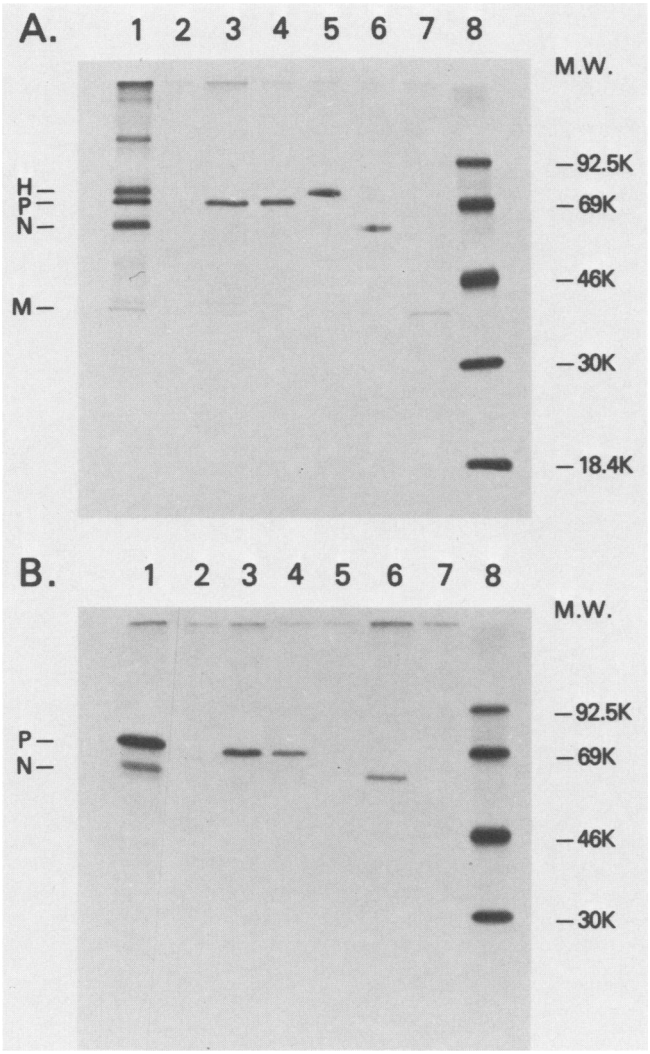

FIG. 2. Immunoprecipitation of measles virus P protein by P20 antibody. (A) [^{35}S]methionine-labeled immunoprecipitates. (B) $^{32}P_i$-labeled immunoprecipitates. Lanes: 1, guinea pig anti-measles virus serum; 2, nonimmune rabbit serum; 3, monoclonal antibody to P protein (12a); 4, affinity-purified anti-P20; 5, 6, and 7, monoclonal antibody to HA (H) (1), N (12a), and M (3) proteins, respectively; and 8, molecular weight (M.W.) standards.

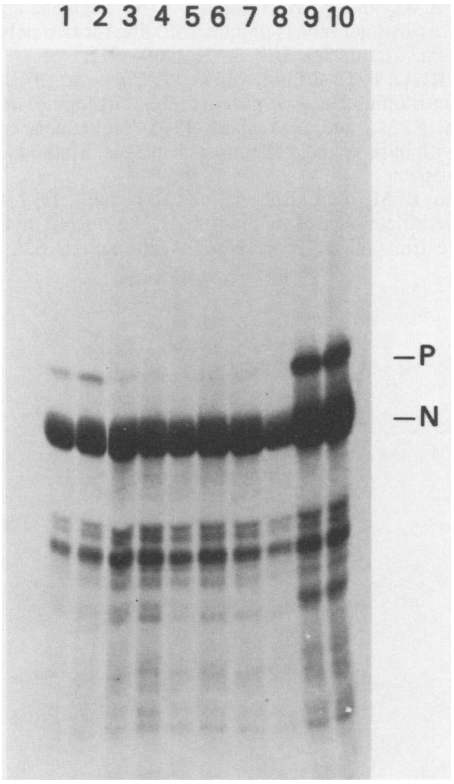

FIG. 3. Hybrid arrest of translation of measles virus P protein mRNA by clone Cl-G. Lanes: 1 and 2, mock arrest (3 μg of mRNA taken through hybrid arrest conditions with no competing DNA and subsequently translated); 3, 4, and 5, mRNA hybridized with 2, 5, and 10 μg of Cl-G DNA, respectively, and subsequently translated in vitro; 6, 7, and 8, mRNA hybridized with 1, 2, and 10 μg of pWB-3A8 DNA, respectively; 9 and 10, untreated mRNA (3 μg) translated in vitro. The positions of the P and N proteins were determined by immunoprecipitation with the respective monoclonal antibodies (data not shown).

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