JOURNAL OF VIROLOGY, Jan. 1995, p. 552–559
0022-538X/95/$04.00+0

Vol. 69, No. 1

Comparative Sequence Analysis of the Reovirus S4 Genes from 13 Serotype 1 and Serotype 3 Field Isolates

ROSS KEDL, STEPHEN SCHMECHEL, AND LESLIE SCHIFF*

Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455

Received 7 July 1994/Accepted 28 September 1994

The reovirus σ3 protein is a major outer capsid protein that may function to regulate translation within infected cells. To facilitate the understanding of σ3 structure and functions and the evolution of mammalian reoviruses, we sequenced cDNA copies of the S4 genes from 10 serotype 3 and 3 serotype 1 reovirus field isolates and compared these sequences with sequences of prototypic strains of the three reovirus serotypes. We found that the σ3 proteins are highly conserved: the two longest conserved regions contain motifs proposed to function in binding zinc and double-stranded RNA. We used the 16 viral isolates to investigate the hypothesis that structural interactions between σ3 and the cell attachment protein, σ1, constrain their evolution and to identify a determinant within σ3 that is in close proximity to the σ1 hemagglutination site.

The S4 gene segment of the mammalian reovirus encodes the σ3 protein (40, 44), which together with μ1/μ1C forms the bulk of the outer capsid (54). σ3 occupies the outermost position in the reovirus capsid and has an ellipsoidal, finger-like shape (14). The other reovirus proteins that constitute the outer capsid include the cell attachment protein, σ1, and the pentameric spike protein, λ2, both of which occupy positions at the fivefold icosahedral vertices (33, 61).

σ3 serves a number of distinct structural roles in the reovirus life cycle. Genetic studies indicate that polymorphisms in the S4 gene influence the stability of virus particles to inactivating agents such as heat and detergent (13). These observations led to the hypothesis that σ3 stabilizes the outer capsid in harsh extracellular environments (45). Reovirus outer capsid proteins undergo proteolysis during the course of infection (reviewed in reference 45); studies in cell culture and in vivo suggest that removal of σ3 from infecting virions is an essential step in viral entry (4, 56). The finding that viruses with temperature-sensitive lesions that map to the S4 gene (*ts*G mutants) fail to assemble outer capsids at the nonpermissive temperature suggests that σ3 plays a critical role in viral assembly (19, 42, 43).

In addition to having structural roles, σ3 may serve regulatory functions within the infected cell. Differences in the capabilities of reovirus strains to affect cellular RNA synthesis and cellular protein synthesis map to the S4 gene segment (53). σ3 inhibits in vitro activation of the interferon-induced double-stranded RNA (dsRNA)-dependent protein kinase (PKR) (26). Biochemical studies have localized σ3 to the initiation factor fraction of cells expressing a cloned S4 gene (34), and its expression stimulates translation of cotransfected reporter genes (23, 39, 52).

The structural and regulatory functions of the S4 gene product are not understood on a molecular level. Two biochemical activities have been ascribed to the σ3 protein: σ3 binds zinc (50, 51) and binds dsRNA in a sequence-independent manner (25). Zinc-binding activity localizes to an amino-terminal fragment that contains a zinc finger motif (50). A dsRNA-binding activity of σ3 localizes to a carboxy-terminal fragment (41, 50). The importance of these activities of σ3 in the reovirus life cycle remains to be determined.

Studies of the structure and functions of σ3 have taken advantage of polymorphisms among the three prototypic reovirus strains: type 1 Lang (L), type 2 Jones (J), and type 3 Dearing (D). Many additional reovirus field isolates are available for the study of polymorphic biological or biochemical properties associated with the S4 gene (24, 46–48). S4 gene sequences have been previously determined for the L, D, and J strains (3, 22, 52) and for the prototypic *ts*G mutant (*ts*G453) (8). Sequence analysis and partial peptide mapping suggest that the σ3 protein is more conserved than the minor capsid protein, σ1, which determines viral serotype and tissue tropism, but is more variable than μ1 (20, 21, 52, 60). Thus, comparative sequence analysis of the S4 gene is particularly suited to the identification of molecular determinants impor-

TABLE 1. Strains of reovirus[a]

Reovirus strain	Serotype	Species	Site of isolation	Yr isolated
D	3	Human	Ohio	1955
L	1	Human	Ohio	1953
J	2	Human	Ohio	1955
c8	3	Human	Tahiti	1960
c9	3	Murine	France	1961
c15	1	Human	Washington, D.C.	1958
c18	3	Bovine	Maryland	1961
c23	1	Bovine	Maryland	1959
c31	3	Bovine	Maryland	1959
c44	3	Bovine	Maryland	1960
c45	3	Bovine	Maryland	1960
c50	1	Bovine	Maryland	1960
c84	3	Human	Washington, D.C.	1957
c87	3	Human	Washington, D.C.	1957
c93	3	Human	Washington, D.C.	1957
c100	3	Bovine	Maryland	1960

[a] Reovirus strains Dearing (D), Lang (L), and Jones (J) are the prototypic laboratory strains for which S4 sequence information was available prior to this study. Clone 87 (c87) is the laboratory strain Abney. Field strains were isolated and initially characterized by Rosen and colleagues (24, 46–48). Serotype 3 c100 was isolated from a stock of c43; it has a electropherotype distinct from that of c43. c100 is designated serotype 3 because the sequence of its S1 gene is identical to that of c43 (9). Clone 15 was initially designated serotype 2 (46) but was recently determined to be serotype 1 (62).

* Corresponding author. Mailing address: Department of Microbiology, University of Minnesota, 420 Delaware St., S.E., Minneapolis, MN 55455. Phone: (612) 624-9933. Fax: (612) 626-0623. Electronic mail address: schiff@lenti.med.umn.edu.

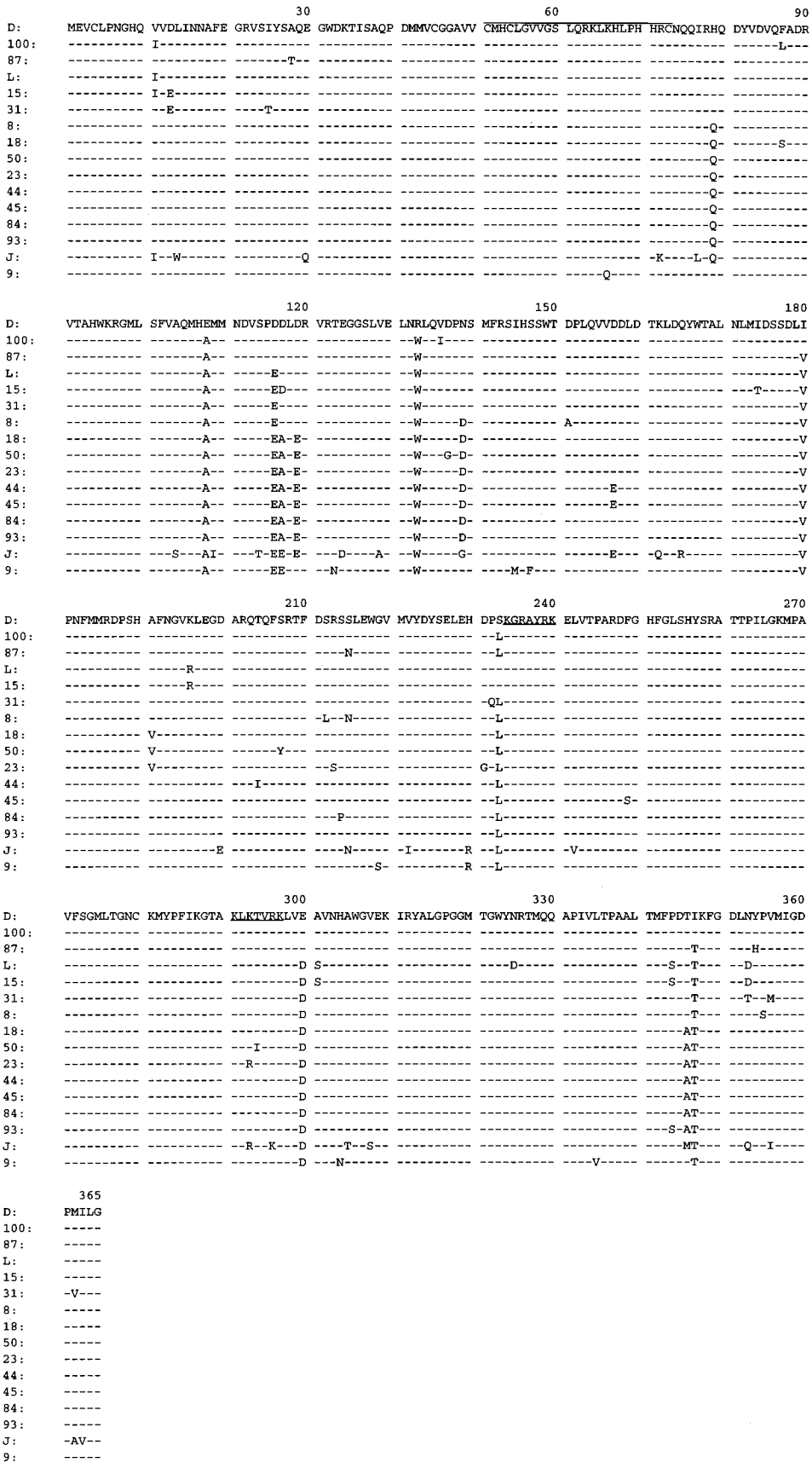

FIG. 1. Alignment of the predicted σ3 amino acid sequences of type 1 and type 3 reovirus strains. The σ3 sequences from prototypic strains included in the alignment are D (22), L (3), and J (52). The single-letter amino acid code is utilized, and any amino acid identical to that found in D is indicated with a dash. The sequence corresponding to the proposed zinc finger is overlined (38, 50). Two sequences with similarity to a characterized dsRNA-binding motif (55) are underlined.

Percent Amino Acid Homology

Percent Nucleotide Homology	D	L	J	8	9	15	18	23	31	44	45	50	84	87	93	100
D		96.7	89.6	96.4	95.6	96.4	96.2	95.6	96.4	96.2	96.2	95.6	96.4	97.8	96.4	98.1
L	93.5		90.4	96.4	95.6	99.2	96.2	95.6	96.7	96.2	96.2	95.6	96.4	96.7	97.0	97.0
J	77.4	78.2		91.2	90.1	90.1	91.5	91.5	90.7	92.0	92.0	90.9	91.8	90.7	91.8	90.4
8	90.4	90.1	79.1		95.9	96.2	97.5	97.0	96.7	97.5	97.5	97.0	97.8	97.5	97.8	96.7
9	85.6	85.7	78.8	85.0		95.3	95.9	95.3	95.9	95.9	95.9	95.3	96.2	96.2	96.2	95.9
15	93.6	99.6	78.1	90.1	85.7		95.9	95.3	97.0	95.9	95.9	95.9	96.2	96.4	96.7	96.7
18	87.8	87.3	79.2	87.5	84.2	87.4		98.9	96.4	98.9	98.9	98.9	99.2	96.7	99.2	96.7
23	87.5	87.1	79.1	87.3	83.9	87.2	99.5		95.9	98.4	98.4	98.4	98.6	96.2	98.6	95.9
31	94.6	93.1	78.1	90.2	85.6	87.3	87.1	87.0		96.4	96.4	95.1	96.7	97.0	96.7	96.7
44	88.5	88.5	84.6	87.5	84.6	88.6	96.7	96.4	87.9		99.5	98.4	99.2	96.7	99.2	96.4
45	88.6	88.6	84.5	87.6	84.5	88.7	96.8	96.5	88.0	99.7		98.4	99.2	96.7	99.2	96.4
50	88.2	87.8	79.5	88.0	84.4	87.9	98.9	98.6	87.5	96.7	96.8		98.6	96.2	98.6	95.9
84	88.5	88.5	84.7	87.9	84.7	88.5	97.2	96.8	87.9	99.4	99.5	97.2		97.0	99.5	96.7
87	98.2	93.7	78.2	90.9	85.9	93.8	88.0	87.6	87.9	88.9	88.8	88.4	88.7		97.0	98.1
93	88.5	88.6	84.7	87.9	84.7	88.7	97.2	96.8	87.9	99.4	99.5	97.2	99.8	88.7		96.7
100	98.6	93.2	77.6	90.1	85.8	93.3	87.5	87.2	94.2	88.2	88.3	88.0	88.2	97.6	88.2	

FIG. 2. Sequence comparisons done by using the homology analysis program of Lawrence and Goldman (30). Nucleotide homology was determined with the unit odds cost matrix, and amino acid homology was determined with the Dayhoff matrix.

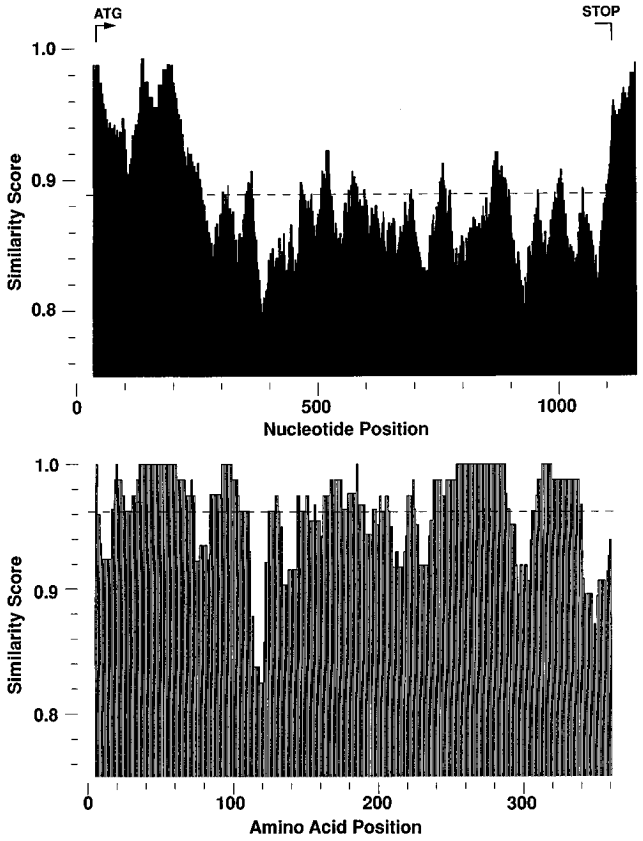

FIG. 3. The PLOTSIMILARITY program (12) was used to determine the level of identity between the 16 S4 gene sequences, using a sliding window of 30 bases (top). The analysis includes only those nucleotide positions known for all 16 strains (bases 21 through 1176). The positions of initiation and termination codons are indicated. The 16 σ3 protein sequences were analyzed by using a sliding window of 10 bases (bottom; aligned beneath the S4 coding region). In these analyses, identical positions are given a value of 1.0 and all others are given a value of 0.0. The horizontal dashed lines indicate the average level of identity of the 16 sequences.

tant for aspects of σ3 function. To facilitate the linking of biological and biochemical phenotypes of σ3 to specific sequence determinants and to better understand the natural evolution of reoviruses, we sequenced cloned PCR products representing the S4 genes from 10 serotype 3 and 3 serotype 1 natural isolates (Table 1) (24, 46–48).

Determination of S4 gene sequences from 13 reovirus strains. Genomic dsRNA was isolated from purified virions or from infected L-cell lysates (5) and used as a template for cDNA synthesis. The primers used for cDNA synthesis corresponded to the 5′-terminal nucleotides of the coding and noncoding strands of the D S4 gene (22) and contained *Eco*RI sites at their termini to facilitate cloning. The products of cDNA synthesis were amplified by PCR (49) with the same primers. PCR products were cloned into pUC18, and plasmid DNA was sequenced by using Sequenase version 2.0 (U.S. Biochemical Corp., Cleveland, Ohio). To reduce artifactual banding, dITP was used in place of dGTP in the sequencing reactions, and reaction mixtures were treated with 2.5 U of terminal deoxynucleotidyl transferase (Boeringher Mannheim Biochemical, Indianapolis, Ind.) prior to electrophoresis (16). Second clones of clone 45 (c45), c87, c93, and c100 were completely sequenced; no PCR-generated sequence changes were found. Nucleotide sequences were entered into the sequence assembly management package SAM and analyzed by using the companion sequence analysis package, EUGENE (31, 32).

We determined unambiguous sequences for the entire σ3-coding regions of the S4 genes as well as for 5′ and 3′ noncoding positions represented within the PCR products (data not shown). The S4 genes exhibited sequence variability in both 5′ and 3′ nontranslated sequences. Polymorphic positions included nucleotides 19, 21, and 26 within the 5′ noncoding region and positions 1136, 1142, 1143, 1145, 1155, and 1157 within the 3′ noncoding region. Sequence variability in the 20 terminal 5′ and 3′ nucleotides of the 13 S4 genes was not evaluated because all PCR products were prepared by using primers representing the D S4 gene sequence. All 16 S4 nucleotide sequences were conserved within the −1 to +3 region, which has been demonstrated to be important for translation initiation (28). Each S4 cDNA contained one long open reading frame predicted to encode 365 amino acids. The 13 predicted σ3 sequences are aligned in Fig. 1 with previously published sequences for the D, L, and J S4 genes (3, 22, 52).

S4 gene and σ3 protein sequence comparisons. Pairwise sequence comparisons were performed to ascertain the degree of homology between the 16 S4 genes and σ3 proteins (Fig. 2). The results of this analysis indicated that the S4 genes are between 77.4% (between J and D) and 99.8% (between c93 and c84) identical. The average level of homology between these genes was 82.4%. The J S4 gene showed the least homology with the other genes, having no more than 84.7% homology with any other S4 sequence. Because most of the nucleotide variability in the S4 genes occurred in the third codon positions, the 16 deduced σ3 proteins were more similar than the genes that encode them. The average degree of amino acid sequence identity between these strains was 96.2%. The J σ3 protein is the most divergent, having between 89.6 and 92% identity with the other σ3 proteins. Although the available data indicate that S4 genes are as conserved as S2 genes, the σ3

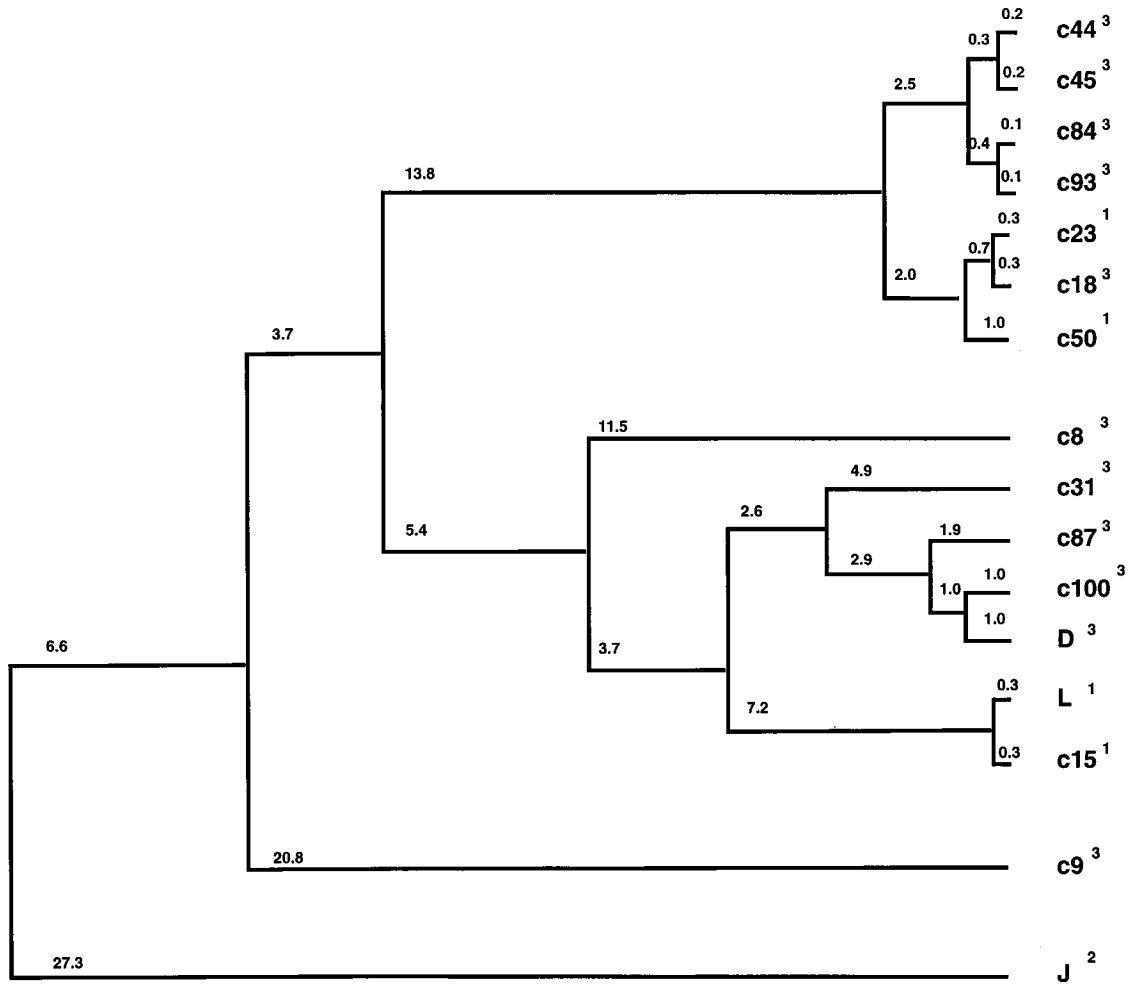

FIG. 4. Phylogenetic tree of S4 gene relationships constructed by the progressive sequence alignment program of Feng and Doolittle (18). Lengths of all horizontal lines are proportional to evolutionary distances. Lengths of all horizontal lines are proportional to evolutionary distances (also indicated by numeric branch lengths above each line). Lengths of vertical lines are arbitrary. The serotype of each strain (1, 2, or 3) is indicated as a superscript.

proteins were somewhat more variable than the σ2 proteins (7). This is not unexpected, since the position of σ3 in the outer capsid probably results in more immune selection on σ3 than on the core protein σ2. σ3 was considerably more conserved than the σ1 protein (6, 52). Selective pressures that might restrict variability within σ3 include the requirement to form stable interactions with other virion outer capsid proteins, including μ1/μ1C and λ2 (24).

We used the PLOTSIMILARITY program (12) to identify conserved regions within the 16 S4 gene sequences. This analysis revealed that sequences at the gene termini were significantly more conserved than those in the central portion of the gene (Fig. 3, top). The terminal sequence conservation was apparent in the 5′ ~250 nucleotides and the 3′ ~100 nucleotides and included third codon positions. Similar analysis of the deduced σ3 protein sequences (Fig. 3, bottom) revealed that protein sequence conservation within the proposed zinc finger may contribute to 5′ nucleotide sequence conservation between nucleotides 125 and 250 but that the sequence conservation within the 5′ ~125 and 3′ ~100 terminal nucleotides is not solely a consequence of σ3 amino acid sequence conservation.

Our analysis of S4 genes from 16 natural isolates and a previous analysis of 12 S2 genes (7) strongly indicate that there are structural or functional pressures to conserve both coding and noncoding nucleotides at reovirus gene termini. It has been suggested that the recognition sites for binding of reovirus polymerase proteins, components of the translational machinery, or viral proteins involved in gene segment assortment may involve higher-order RNA structures composed of the termini of reovirus RNAs (2, 7, 35, 36, 63). Studies of mutant M1 genes (63) and S2 genes from field isolates (7) have used available RNA folding algorithms to predict that portions of reovirus mRNAs could form stable terminal panhandle structures. Using the entire S4 sequence and a more refined version of this algorithm (MFOLD) (64), we did not consistently find conserved structures that pair 5′ and 3′ nucleotides when we examined multiple suboptimal folds of the 16 S4 gene sequences or the optimal MFOLD structures for the 9 other reovirus genes (data not shown). Experimental studies using either a reverse genetic approach or an in vitro replication system will be required in order to more precisely define the importance of the conserved terminal sequences for replication and assortment.

Conserved and variable domains of σ3 are predicted from tryptic peptide analysis (20, 21). When the 16 predicted σ3 sequences were aligned (Fig. 1), three conserved regions were clearly identified. Amino acids 31 to 73, which include the

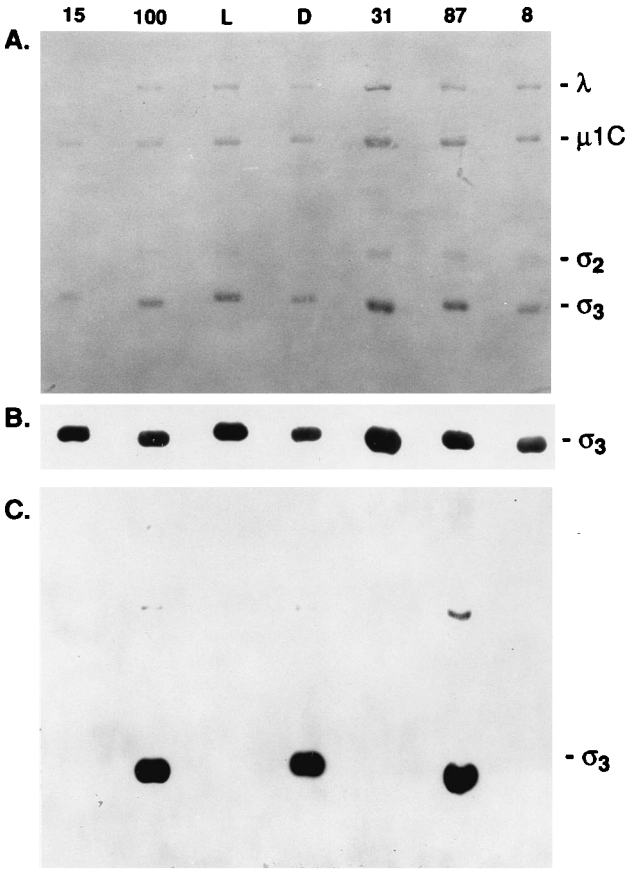

FIG. 5. The proteins of 2.5×10^{11} particles of reovirus strains c15, c100, L, D, c31, c87, and c8 were separated on 10% polyacrylamide gels (29) and transferred to nitrocellulose membranes in the presence of 25 mM Tris (pH 8.3)–192 mM glycine–20% methanol–0.01% sodium dodecyl sulfate (50, 57). Lanes containing 2×10^{12} D particles were run and stained with Coomassie blue to serve as protein markers (locations shown at right). (A) The presence of transferred reoviral proteins was determined by staining the membranes with 0.005% Ponceau S–0.17 M acetic acid. (B) One destained nitrocellulose membrane was blocked in a solution of 5% nonfat dry milk (Carnation) in TBS buffer (10 mM Tris [pH 8], 150 mM NaCl) and immunoblotted with rabbit antiserum prepared against L virions (provided by B. Fields). Membrane-bound antibody was detected by incubating the membranes with horseradish peroxidase-conjugated anti-immunoglobulin G and reagents that generated a chemiluminescent signal (Amersham, Arlington Heights, Ill.). (C) A membrane prepared in parallel was immunoblotted with monoclonal antibody 4F2 (provided by H. Virgin).

proposed zinc-binding site (Fig. 1, overlined) (38, 50), were highly conserved between strains. The σ3 molecules encoded by J and c9 each had a single amino acid change in this region. The proposed cysteine and histidine ligand-binding residues of the metal-binding motif were entirely conserved in all strains. The functional importance of the zinc finger in σ3 is unclear, but its rigid conservation suggests that it plays an important role in σ3 function. Zinc binding might be important in order for σ3 to assume its proper structure in the mature virion, for physiological interactions of σ3 with the other major outer capsid protein (μ1), or for other σ3 functions. Recent evidence indicates that mutations in the proposed zinc finger decrease the intracellular stability of σ3 (38).

The second conserved region in the σ3 sequence was found in the carboxy terminus and included sequences that are proposed to contribute to the dsRNA-binding activity of the molecule (41, 50). Within this long conserved region lie two basic amino acid motifs (residues 234 to 241 and 250 to 299) that are similar to the known dsRNA-binding site of PKR (41). The only substitutions in these motifs were conservative and did not disrupt the configuration of basic amino acids in the motif. The finding that σ3 binds dsRNA led to the suggestion that this activity of σ3 may influence cellular protein synthesis by sequestering dsRNA from PKR (26, 37). Given the high level of sequence conservation within the proposed dsRNA-binding regions, it is unlikely that inherent differences in dsRNA affinities of σ3 molecules are important for the strain differences in effects on cellular translation. The observed differences between reovirus strains with respect to inhibition of cellular protein synthesis could reflect the presence of different amounts of σ3 capable of binding dsRNA at critical times postinfection or modulation of σ3 activity by other proteins such as μ1 or cellular proteins.

The third conserved region of proposed functional importance (41) included amino acids 240 to 267, which were shown to be similar to the 65-kDa regulatory subunit of protein phosphatase 2A (41). Only two amino acid changes existed in this region, one a conservative change and the other at a residue that is not critical to the alignment.

We analyzed the 16 σ3 sequences at those positions altered in *ts*G453: an asparagine-to-lysine change at residue 16, a proline-to-serine change at residue 138, a methionine-to-isoleucine change at residue 141, and a glutamate-to-aspartate change at residue 229 (8). Of these changes, the asparagine-to-lysine change at residue 16 is predicted to be responsible for the temperature-sensitive phenotype because of its proximity to the zinc finger sequence (8). Our analysis indicated that all of the amino acid changes in *ts*G453 are unique to the mutant, suggesting that any one or more of the four changes may be involved in the temperature-sensitive phenotype.

Phylogenetic analysis of the S4 gene. To understand the evolutionary relationships between reovirus strains, a phylogenetic tree was constructed by using the progressive sequence alignment program of Feng and Doolittle (18). The predicted phylogram (Fig. 4) revealed four S4 gene lineages. The first lineage contained only the prototypic serotype 2 J strain; the second contained the only murine strain, serotype 3 c9; the third contained serotype 3 strains, including prototypic D and c8, c31, c87, and c100, and serotype 1 strains, including prototypic L and c15; and the fourth contained serotype 3 strains c18, c44, c45, c84, and c93 and serotype 1 strains c23 and c50. The tree topology was confirmed by using the maximum-likelihood phylogenetic inference package of Felsenstein (17) to perform bootstrap and resampling analysis (data not shown). This phylogenetic analysis demonstrated that the diversity within S4 genes of the serotype 3 strains is greater than that between serotype 3 and serotype 1 S4 genes. The phylogenetic analysis revealed no correlation between viral serotype and S4 gene relationships. The S4 genes from four serotype 1 strains were found in two different clades that each contained serotype 3 strains. These placements of serotype 1 S4 genes in clades which contain serotype 3 genes are consistent with tryptic peptide analysis of σ3 proteins from several of these strains (21).

The observation that the σ3-specific monoclonal antibody 4F2 detects molecules from serotype 3 strains significantly better than those from serotype 1 strains has been taken as evidence that σ3 contains "serotype-associated" determinants (59). It is hypothesized that these determinants reflect interactions between σ3 and σ1 that influence viral stability, viral survival, or optimal viral growth or transmission and that such interactions might provide selective pressure for coincident evolution of the reovirus S1 and S4 genes (59). Comparison of

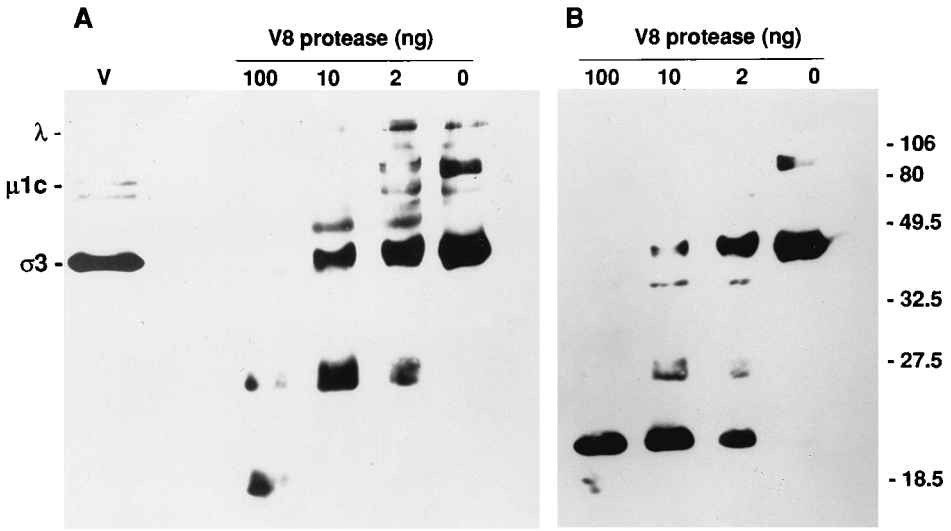

FIG. 6. (A) Purified σ3 from 2×10^{11} D virions was digested with 0, 2, 10, and 100 ng of V8 protease and immunoblotted as described previously (50). Reovirus peptides were detected by immunoblotting with monoclonal antibody 4F2. Membrane-bound antibody was detected by incubating the membrane with horseradish peroxidase-conjugated anti-immunoglobulin G and reagents that generate a chemiluminescent signal. The positions of the intact reovirus proteins are shown at the left. The C-terminal 16-kDa fragment of σ3 appears to migrate above the 18-kDa marker on this 15% gel. At high concentrations of protease, a small fragment of σ3 that reacts with 4F2 was evident. (B) Rabbit antiserum prepared against L virions was used to probe the nitrocellulose membrane to which the V8 protease digestion products of purified σ3 had been transferred (preparation was as described for panel A). The positions of molecular size markers (in kilodaltons) are on the right.

the S4 gene phylogram with that for S1 (10) revealed distinct evolutionary histories. This suggests that there is little or no selective pressure from the S1 gene segment on S4 gene evolution.

Comparison of phylograms for the S1, S2, and S4 genes from eight reovirus strains supports the hypothesis that reovirus evolution is characterized by cocirculation of multiple strains of the virus and free reassortment of segments between cocirculating strains. There is no evidence of host barriers to genome segment exchange for any of the genes examined thus far, although the S4 gene from the only murine isolate, c9, occupies a unique phylogenetic clade. To determine if this represents a host restriction, more murine field strains will have to be analyzed.

Delineation of the epitope recognized by monoclonal antibody 4F2. We used the primary σ3 sequence information to map the binding site of the σ3-specific monoclonal antibody, 4F2. Antibody 4F2 recognizes σ3 on immunoblots (58), suggesting that it likely recognizes a linear epitope. We used the immunoblotting technique to analyze the ability of 4F2 to recognize σ3 molecules from reovirus strains that belong to a single phylogenetic lineage (Fig. 4) containing the prototypic strains D and L. This analysis revealed that the σ3 molecules from strains D, c100, and c87 were recognized by 4F2, whereas those from c15, L, c31, and c8 were not recognized to the limits of resolution of the immunoblotting technique (Fig. 5). Immunoblot analysis of virions from reovirus strains J and strain c9 and a panel of fourteen serotype 1 isolates, including c15, c23, and c50, did not reveal other strains reactive with 4F2 (data not shown). These results predicted that a sequence important for avid 4F2 binding is unique to the σ3 molecules from strains D, c87, and c100.

Examination of the primary σ3 amino acid sequences revealed two regions in which the 4F2-reactive strains share amino acids that are distinct from the amino acids of 4F2-nonreactive strains (Fig. 1). An aspartate residue at position 116 and a glutamate residue at position 300 were unique to the 4F2-reactive σ3 proteins. To investigate which of these two unique regions is important for 4F2 recognition of σ3, we utilized staphylococcal V8 protease, which cleaves D σ3 between residues 217 and 218 to yield an amino-terminal 24-kDa fragment and a carboxy-terminal 16-kDa fragment (50). 4F2 detected the 24-kDa amino-terminal fragment of σ3 but not the 16-kDa carboxy-terminal fragment (Fig. 6A). Both amino- and carboxy-terminal fragments of σ3 were detected when the nitrocellulose was stripped and reprobed with polyclonal anti-reovirus serum (Fig. 6B). The results of this experiment indicated that the aspartate residue at position 116 of the D, c87, and c100 σ3 proteins is important for 4F2 recognition. This finding is consistent with structural predictions (data not shown) that suggested that the region including amino acid 116 is antigenic and likely to be exposed on the surface of the protein whereas that containing amino acid 300 is not (15, 27).

The interaction between 4F2 and σ3 has a serotype-specific effect on viral hemagglutination (HA) (59). 4F2 inhibits HA by L × D reassortant virions containing the D S1 gene but does not block HA by strains containing the L S1 gene. The strain of origin of the S4 gene does not affect HA inhibition by 4F2 (59). These data suggest that 4F2 may sterically inhibit HA by serotype 3 σ1 but not that by serotype 1 σ1. We propose that D σ1 assumes a conformation such that amino acids 198 to 204, which are known to be important for HA by σ1 (11), lie in close proximity to σ3 amino acid 116. This conformation allows 4F2-bound σ3 to effectively shield the D σ1 HA region. In contrast, the HA region of L σ1 may be more distant from the 4F2-binding region of σ3, either because it lies in a different region of primary sequence or because the L σ1 molecule assumes a different conformation.

The σ3 protein, even in the absence of 4F2, likely serves to partly shield the D HA region. The observation that D virions have an HA titer 30-fold lower than that of L virions whereas intermediate subviral particles (which lack σ3) prepared from D and L have similar HA titers (59) argues in favor of the hypothesis that a region of σ3 shields the D σ1 HA region better than the L σ1 HA region. A second σ3-specific monoclonal antibody, 8F12, also binds more avidly to D than to L

(59). This antibody inhibits HA by L preferentially (58). It will be of interest to identify the σ3 determinant critical for 8F12 binding, since this region(s) of σ3 would likely lie near residues important for L σ1 HA.

Nucleotide sequence accession numbers. The GenBank nucleotide sequence accession numbers for the clones described in this article are as follows: c100, U15072; c15, U15073; c18, U15074; c23, U15075; c31, U15076; c44, U15077; c45, U15078; c50, U15079; c84, U15080; c87, U15081; c8, U15082; c93, U15083; c9, U15084.

This work was supported by NIH award R29AI32139 to L.S. S.S. was supported by MSTP training grant T32GM08244, and Microbiology/Cancer Research training grant T32CA09138 from the NIH.

We thank Amy Schoenbaum for help in the preliminary stages of this project and Dave Sandvik for sequencing an isolate of the c87 S4 gene. Jean-Yves Sgro (University of Wisconsin, Madison) and Mark Dalton (Cray Research, Eagan, Minn.) provided support for computer analysis of reovirus RNA structures. We appreciate the technical and intellectual contributions of laboratory members Robert Anderson, Michael Chute, Jeff Ehnstrom, Pamela Skinner, and Deborah Shepard and of colleagues Max Nibert and Terence Dermody. We thank Deborah Shepard and Stephen Rice for critical reviews of the manuscript and Tim Leonard for photography. This work would not have been initiated without the advice and support of Bernard Fields.

REFERENCES

1. **Ahmed, R., P. R. Chakraborty, A. F. Graham, and B. N. Fields.** 1980. Genetic variation during persistent reovirus infection: presence of extragenically suppressed temperature-sensitive lesions in wild-type virus isolated from persistently infected cells. J. Virol. **34:**383–389.
2. **Anzola, J. V., Z. Xu, T. Asamizu, and D. L. Nuss.** 1987. Segment specific inverted repeats found adjacent to conserved terminal sequences in wound tumor virus genome and defective interfering RNAs. Proc. Natl. Acad. Sci. USA **84:**8301–8305.
3. **Atwater, J. A., S. M. Munemitsu, and C. E. Samuel.** 1986. Molecular complementary DNA cloning and nucleotide sequence of the reovirus serotype 1 Lang strain S4 messenger RNA which encodes the major capsid surface polypeptide sigma 3. Biochem. Biophys. Res. Commun. **136:**183–192.
4. **Bass, D. M., D. Bodkin, R. Dambrauskas, J. S. Trier, B. N. Fields, and J. L. Wolf.** 1990. Intraluminal proteolytic activation plays an important role in replication of type 1 reovirus in the intestines of neonatal mice. J. Virol. **64:**1830–1833.
5. **Bassel-Duby, R., D. R. Spriggs, K. L. Tyler, and B. N. Fields.** 1986. Identification of attenuating mutations on the reovirus type 3 S1 double-stranded RNA segment with a rapid sequencing technique. J. Virol. **60:**64–67.
6. **Cashdollar, L. W., R. A. Chmelo, J. R. Wiener, and W. K. Joklik.** 1985. Sequences of the S1 genes of the three serotypes of reovirus. Proc. Natl. Acad. Sci. USA **82:**24–28.
7. **Chappell, J. D., M. I. Goral, S. E. Rodgers, C. W. dePamphilis, and T. S. Dermody.** 1994. Sequence diversity within the reovirus S2 gene: reovirus genes reassort in nature, and their termini are predicted to form a panhandle motif. J. Virol. **68:**750–756.
8. **Danis, C., S. Garzon, and G. Lemay.** 1992. Further characterization of the ts453 mutant of mammalian orthoreovirus serotype 3 and nucleotide sequence of the mutated S4 gene. Virology **190:**494–498.
9. **Dermody, T. S.** 1994. Personal communication.
10. **Dermody, T. S., M. L. Nibert, R. Bassel-Duby, and B. N. Fields.** 1990. Sequence diversity in S1 genes and S1 gene translation products of 11 serotype 3 reovirus strains. J. Virol. **64:**4842–4850.
11. **Dermody, T. S., M. L. Nibert, R. Bassel-Duby, and B. N. Fields.** 1990. A sigma 1 region important for hemagglutination by serotype 3 reovirus strains. J. Virol. **64:**5173–5176.
12. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
13. **Drayna, D., and B. N. Fields.** 1982. Genetic studies on the mechanism of chemical and physical inactivation of reovirus. J. Gen. Virol. **63:**149–160.
14. **Dryden, K. A., G. Wang, M. Yeager, M. L. Nibert, K. M. Coombs, D. B. Furlong, B. N. Fields, T. S. Baker.** 1993. Early steps in reovirus infection are associated with dramatic changes in supramolecular structure and protein conformation: analysis of virions and subviral particles by cryoelectron microscopy and image reconstruction. J. Cell Biol. **93:**1023–1041.
15. **Emini, E. A., J. V. Hughes, D. S. Perhow, and J. Boger.** 1985. Induction of hepatitis A virus-neutralizing antibody by a virus-specific synthetic peptide. J. Virol. **55:**836–839.
16. **Fawcett, T. W., and S. G. Bartlett.** 1991. An efficient and reliable method for the elimination of artifact banding when sequencing double stranded templates, p. 19–20. Editorial comments, vol. 17. United States Biochemical Corporation, Cleveland.
17. **Felsenstein, J.** 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. **17:**368–376.
18. **Feng, D. F., and R. F. Doolittle.** 1987. Progressive sequence alignment as a prerequisite to correct phylogenetic trees. J. Mol. Evol. **25:**351–360.
19. **Fields, B. N., C. S. Raine, and S. G. Baum.** 1971. Temperature-sensitive mutants of reovirus type 3: defects in viral maturation as studied by immunofluorescence and electron microscopy. Virology **43:**569–578.
20. **Gentsch, J. R., and B. N. Fields.** 1981. Tryptic peptide analysis of outer capsid polypeptides of mammalian reovirus serotypes 1, 2, and 3. J. Virol. **38:**208–218.
21. **Gentsch, J. R., and B. N. Fields.** 1984. Genetic diversity in natural populations of mammalian reoviruses: tryptic peptide analysis of outer capsid polypeptides of murine, bovine, and human type 1 and 3 reovirus strains. J. Virol. **49:**641–651.
22. **Giantini, M., L. S. Seliger, Y. Furuichi, and A. J. Shatkin.** 1984. Reovirus type 3 genome segment S4: nucleotide sequence of the gene encoding a major virion surface protein. J. Virol. **52:**984–987.
23. **Giantini, M., and A. J. Shatkin.** 1989. Stimulation of chloramphenicol acetyltransferase mRNA translation by reovirus capsid polypeptide σ3 in cotransfected COS cells. J. Virol. **63:**2415–2421.
24. **Hrdy, D. B., L. Rosen, and B. N. Fields.** 1979. Polymorphism of the migration of double-stranded RNA segments of reovirus isolates from humans, cattle, and mice. J. Virol. **31:**104–111.
25. **Huismans, H., and W. K. Joklik.** 1976. Reovirus-coded polypeptides in infected cells: isolations of two monomeric polypeptides with high affinity for single-stranded and double-stranded RNA, respectively. Virology **70:**411–424.
26. **Imani, F., and B. L. Jacobs.** 1988. Inhibitory activity for the interferon-induced protein kinase is associated with the reovirus serotype 1 σ3 protein. Proc. Natl. Acad. Sci. USA **83:**7887–7891.
27. **Jameson, B. A., and H. Wolf.** 1988. The antigenic index: a novel algorithm for predicting antigenic determinants. Comput. Appl. Biosci. **4:**181–186.
28. **Kozak, M.** 1992. Regulation of translation in eukaryotic systems. Annu. Rev. Cell Biol. **8:**197–225.
29. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
30. **Lawrence, C. B., and D. A. Goldman.** 1988. Definition and identification of homology domains. Comput. Appl. Biosci. **4:**25–33.
31. **Lawrence, C. B., T. Shalom, and S. Honda.** 1991. EuGene: a comprehensive software package for nucleotide and protein sequence analysis for UNIX systems. Molecular Biology Information Resource, Baylor College of Medicine, Houston, Tex.
32. **Lawrence, C. B., T. Shalom, and S. Honda.** 1991. SAM: a software package for sequence assembly management for UNIX systems. Molecular Biology Information Resource, Baylor College of Medicine, Houston, Tex.
33. **Lee, P. W. K., E. C. Hayes, and W. K. Joklik.** 1981. Protein sigma 1 is the reovirus cell attachment protein. Virology **108:**156–163.
34. **Lemay, G., and S. Millward.** 1986. Expression of the cloned S4 gene of reovirus serotype 3 in transformed eukaryotic cells: enrichment of the viral protein in the crude initiation factor fraction. Virus Res. **6:**133–140.
35. **Li, J. K.-K, J. D. Keene, P. P. Scheible, and W. K. Joklik.** 1980. Nature of the 3′-terminal sequences of the plus and minus strands of the S1 gene of reovirus serotypes 1, 2 and 3. Virology **105:**41–51.
36. **Li, J. K.-K, P. P. Scheible, J. D. Keene, and W. K. Joklik.** 1980. The plus strand of reovirus gene S2 is identical with its *in vitro* transcript. Virology **105:**282–286.
37. **Lloyd, R. M., and A. J. Shatkin.** 1992. Translational stimulation by reovirus polypeptide σ3: substitution for VAI RNA and inhibition of phosphorylation of the α subunit of eukaryotic initiation factor 2. J. Virol. **66:**6878–6884.
38. **Mabrouk, T., and G. Lemay.** 1994. Mutations in a CCHC zinc-binding motif of the reovirus σ3 protein decrease its intracellular stability. J. Virol. **68:**5287–5290.
39. **Martin, P. E., and M. A. McCrae.** 1993. Analysis of the stimulation of reporter gene expression by the sigma 3 protein of reovirus in co-transfected cells. J. Gen. Virol. **74:**1055–1062.
40. **McCrae, M. A., and W. K. Joklik.** 1978. The nature of the polypeptide encoded by each of the 10 dsRNA segments of reovirus type 3. Virology **89:**578–593.
41. **Miller, J. E., and C. E. Samuel.** 1992. Proteolytic cleavage of the reovirus sigma 3 protein results in enhanced double-stranded RNA-binding activity: identification of a repeated basic amino acid motif within the C-terminal binding region. J. Virol. **66:**5347–5356.
42. **Morgan, E. M., and H. J. Zweerink.** 1974. Reovirus morphogenesis: core-like particles in cells infected at 39° with wild-type reovirus and temperature-sensitive mutants of groups B and G. Virology **59:**556–565.
43. **Mustoe, T. A., R. F. Ramig, A. H. Sharpe, and B. N. Fields.** 1978. A genetic map of reovirus. III. Assignment of dsRNA positive mutant groups A, B and G to genome segments. Virology **85:**545–556.
44. **Mustoe, T. A., R. F. Ramig, A. H. Sharpe, and B. N. Fields.** 1978. Genetics of reovirus: identification of the dsRNA segments encoding the polypeptides

of the μ and σ size classes. Virology **89:**594–604.
45. **Nibert, M. L., D. B. Furlong, and B. N. Fields.** 1991. Mechanisms of viral pathogenesis. Distinct forms of reoviruses and their roles during replication in cells and host. J. Clin. Invest. **88:**727–734.
46. **Rosen, L., and F. R. Abinanti.** 1960. Natural and experimental infection of cattle with human types of reovirus. Am. J. Hyg. **71:**424–429.
47. **Rosen, L., F. R. Abinanti, and J. F. Hovis.** 1963. Further observations on the natural infection of cattle with reoviruses. Am. J. Hyg. **77:**38–48.
48. **Rosen, L., J. F. Hovis, F. M. Mastrota, J. A. Bell, and R. J. Huebner.** 1960. Observations on a newly recognized virus (Abney) of the reovirus family. Am. J. Hyg. **71:**258–265.
49. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239:**487.
50. **Schiff, L. A., M. L. Nibert, M. S. Co, E. G. Brown, and B. N. Fields.** 1988. Distinct binding sites for zinc and double-stranded RNA in the reovirus outer capsid protein sigma 3. Mol. Cell. Biol. **8:**273–283.
51. **Schiff, L. A., M. L. Nibert, and B. N. Fields.** 1988. Characterization of a zinc blotting technique: evidence that a retroviral gag protein binds zinc. Proc. Natl. Acad. Sci. USA **85:**4195–4199.
52. **Seliger, L. S., M. Giantini, and A. J. Shatkin.** 1992. Translational effects and sequence comparisons of the three serotypes of the reovirus S4 gene. Virology **187:**202–210.
53. **Sharpe, A. H., and B. N. Fields.** 1982. Reovirus inhibition of cellular RNA and protein synthesis: role of the S4 gene. Virology **122:**381–391.
54. **Smith, R. E., H. J. Zweerink, and W. K. Joklik.** 1969. Polypeptide components of virions, top component and cores of reovirus 3. Virology **39:**791–810.
55. **St. Johnston, D., N. H. Brown, J. G. Gall, and M. Jantsch.** 1992. A conserved double-stranded RNA binding domain. Proc. Natl. Acad. Sci. USA **89:** 10979–10983.
56. **Sturzenbecker, L. J., M. Nibert, D. Furlong, and B. N. Fields.** 1987. Intracellular digestion of reovirus particles requires a low pH and is an essential step in the viral infectious cycle. J. Virol. **61:**2351–2361.
57. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
58. **Tyler, K. L., M. A. Mann, B. N. Fields, and H. W. Virgin IV.** 1993. Protective antireovirus monoclonal antibodies and their effects on viral pathogenesis. J. Virol. **67:**3446–3453.
59. **Virgin, H. W., IV, M. A. Mann, B. N. Fields, and K. L. Tyler.** 1991. Monoclonal antibodies to reovirus reveal structure/function relationships between capsid proteins and genetics of susceptibility to antibody action. J. Virol. **65:**6772–6781.
60. **Weiner, J. R., and W. K. Joklik.** 1988. Evolution of reovirus genes: a comparison of serotype 1, 2, and 3 M2 genome segments, which encode the major structural protein μ1C. Virology **163:**603–613.
61. **White, C. K., and H. J. Zweerink.** 1976. Studies on the structure of reovirus cores: selective removal of polypeptide λ2. Virology **70:**171–180.
62. **Wilson, G., and B. N. Fields.** Unpublished data.
63. **Zou, S., and E. G. Brown.** 1992. Identification of sequence elements containing signals for replication and encapsidation of the reovirus M1 genome segment. Virology **186:**377–388.
64. **Zuker, M.** 1989. On finding all suboptimal foldings of an RNA molecule. Science **244:**48–52.