JOURNAL OF VIROLOGY, Oct. 1990, p. 4842–4850
0022-538X/90/104842-09$02.00/0

Vol. 64, No. 10

Sequence Diversity in S1 Genes and S1 Translation Products of 11 Serotype 3 Reovirus Strains

TERENCE S. DERMODY,[1,2]†* MAX L. NIBERT,[1,3] RHONDA BASSEL-DUBY,[1]‡ AND BERNARD N. FIELDS[1,2,4]

Department of Microbiology and Molecular Genetics, Harvard Medical School,[1] Department of Medicine[2] and Department of Pathology,[3] Brigham and Women's Hospital, and Shipley Institute of Medicine,[4] Boston, Massachusetts 02115

Received 3 April 1990/Accepted 15 June 1990

The S1 gene nucleotide sequences of 10 type 3 (T3) reovirus strains were determined and compared with the T3 prototype Dearing strain in order to study sequence diversity in strains of a single reovirus serotype and to learn more about structure-function relationships of the two S1 translation products, σ1 and σ1s. Analysis of phylogenetic trees constructed from variation in the σ1-encoding S1 nucleotide sequences indicated that there is no pattern of S1 gene relatedness in these strains based on host species, geographic site, or date of isolation. This suggests that reovirus strains are transmitted rapidly between host species and that T3 strains with markedly different S1 sequences circulate simultaneously. Comparison of the deduced σ1 amino acid sequences of the 11 T3 strains was notable for the identification of conserved and variable regions of sequence that correlate with the proposed domain organization of σ1 (M. L. Nibert, T. S. Dermody, and B. N. Fields, J. Virol. 64:2976–2989, 1990). Repeat patterns of apolar residues thought to be important for σ1 structure were conserved in all strains examined. The deduced σ1s amino acid sequences of the strains were more heterogeneous than the σ1 sequences; however, a cluster of basic residues near the amino terminus of σ1s was conserved. This analysis has allowed us to investigate molecular epidemiology of T3 reovirus strains and to identify conserved and variable sequence motifs in the S1 translation products, σ1 and σ1s.

The study of sequence diversity among related virus strains has contributed to an understanding of viral evolution and has provided insight into the structure and function of viral proteins. Analysis of nucleotide sequence diversity in mammalian reovirus strains to date has been limited to gene segments from prototype strains of the three reovirus serotypes: type 1 Lang (T1L), type 2 Jones (T2J), and type 3 Dearing (T3D); however, many more strains are available for these types of studies. Reovirus isolates collected by Rosen during field investigations in the late 1950s and early 1960s include strains of each of the three serotypes (19, 34–36) and have been used in several studies of reovirus pathogenesis (20, 44). In this study we used 10 T3 field isolate strains, in addition to the prototype T3D strain, to examine sequence diversity in S1 genes and S1 translation products from strains of a single reovirus serotype.

The sequence of the S1 dsRNA gene segment has been determined for each of the prototype strains (3, 7, 11, 28, 30) and is bicistronic, encoding the σ1s protein (~14 kilodaltons) and the larger σ1 protein (~50 kilodaltons) in overlapping reading frames (12, 21, 37). The σ1s protein is a nonstructural protein of unknown function. The σ1 protein is the reovirus cell-attachment protein (25, 45, 55), hemagglutinin (32, 48), and determinant of the serotype-specific humoral immune response (47). The σ1 protein is also responsible for tissue- and cell-specific tropism (31, 38, 45, 46) and the pattern of viral spread (43).

* Corresponding author.
† Present address: Lamb Center for Molecular Pathogenesis, Division of Pediatric Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN 37232.
‡ Present address: Howard Hughes Medical Institute, Dallas, TX 75235.

Recent studies of σ1 have emphasized an understanding of its structure and the mechanism of its association with viral particles. The σ1 protein has the morphology of a fibrous tail (T) and a globular head (H) (2, 16, 17). The σ1 tail is proposed to be constructed from distinct domains having alternating α-helical coiled coil and cross-β sheet structures (30) and to play an important role in determining how σ1 is attached to virions (17). The σ1 head is thought to be formed predominantly from β-sheet structure mixed with loops (30) and to include sequences important for σ1 function as cell-attachment protein (29, 53, 56) and determinant of the type-specific humoral immune response (4). Domains predicted from sequence analysis are likely to correspond to morphologic regions [T(i), T(ii), T(iii), T(iv), and H] defined by computer-processed electron micrographs (16). These studies provide a framework with which to interpret findings concerning specific amino acid residues that are important for σ1 structure and function.

In this study, we determined the nucleotide sequences of the S1 genes of 10 T3 reovirus strains isolated from a variety of species and geographic locations. These sequences (and those of prototype strains) were compared in order to address the evolutionary relationships between the strains. The deduced amino acid sequences of S1 translation products σ1 and σ1s were also compared, and the findings were interpreted in light of previous work. This study represents the first examination of sequence diversity in reovirus strains of a single serotype and provides additional insight into structure-function relationships of the σ1 and σ1s proteins.

MATERIALS AND METHODS

Cells and viruses. Spinner-adapted mouse L929 (L) cells were grown in either suspension or monolayer cultures in Joklik-modified Eagle minimal essential medium (Irving Sci-

TABLE 1. Strains of T3 reovirus

T3 strain	Species	Site	Yr
Dearing (T3D)	Human	Ohio	1955
Clone 8 (T3C8)	Human	Tahiti	1960
Clone 9 (T3C9)	Murine	France	1961
Clone 18 (T3C18)	Bovine	Maryland	1961
Clone 31 (T3C31)	Bovine	Maryland	1959
Clone 43 (T3C43)	Bovine	Maryland	1960
Clone 44 (T3C44)	Bovine	Maryland	1960
Clone 45 (T3C45)	Bovine	Maryland	1960
Clone 84 (T3C84)	Human	Washington, D.C.	1957
Clone 87 (T3C87) (Abney)	Human	Washington, D.C.	1957
Clone 93 (T3C93)	Human	Washington, D.C.	1955

entific, Santa Ana, Calif.) that was supplemented to contain 5% fetal calf serum (Hyclone Laboratories, Logan, Utah), 2 mM glutamine, 1 U of penicillin per ml, and 1 μg of streptomycin per ml (Irving Scientific). Reovirus strains T1L and T3D are laboratory stocks. T3 strains isolated by Rosen (19, 34–36) that were used in this study are listed in Table 1. T3C87 is the T3 Abney strain (36). Second- and third-passage L-cell lysate stocks of twice-plaque-purified reovirus were used to make purified virion preparations (17).

SDS-PAGE. In preparation for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), virion samples were incubated in disruption buffer at pH 6.8 according to the method of Jayasuriya et al. (22). Electrophoresis of viral RNA was performed in 10% polyacrylamide gels for 400 mA-hours at constant current. Gels were stained with ethidium bromide, and gene segments were visualized with UV irradiation.

Sequencing dsRNA with primers. Genomic dsRNA was purified from virions according to the method of Nibert et al. (30). The dsRNA sequences were determined by using dideoxy sequencing reactions with oligonucleotide primers, reverse transcriptase (Boehringer-Mannheim Biochemicals, Indianapolis, Ind.), and [^{35}S]dATP (1,000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) according to the method of Bassel-Duby et al. (4). Oligonucleotide primers were made with an Applied Biosystems oligonucleotide synthesizer and purified by either ion-exchange high-pressure liquid chromatography or C-18 Sep-Pak column chromatography (Waters Associates, Milford, Mass.) (Applied Biosystems User Bull. 13:1–28, 1984). Initial primers were synthesized corresponding to the T3D S1 nucleotide sequence (3). Additional primers were synthesized corresponding to the S1 nucleotide sequences of individual strains at approximately 200 nucleotide intervals, allowing sufficient overlap for full-length sequence to be determined. Approximately 75% of the complementary strand sequence was determined for each S1 gene.

Comparison of S1 nucleotide and σ1 and σ1s amino acid sequences. Nucleotide sequence comparisons were facilitated by the multiple aligned sequence editor, MASE (13), from the Molecular Biology Computer Research Resource (Boston, Mass.). Amino acid sequence comparisons were facilitated by the global alignment program, GAP (10), from the University of Wisconsin Genetics Computer Group (Madison, Wis.).

Parsimony analysis of S1 nucleotide sequences. Phylogenetic trees were constructed from variation in the S1 nucleotide sequences by the maximum parsimony method (15) using the program PAUP (42) with the global branch-swapping option MULPARB. Phylogenetic trees were also constructed from variation in the S1 nucleotide sequences by the maximum parsimony method with the program DNAPARS included in the Phylogeny Inference Package, PHYLIP (14), and by the distance-linkage method with the program FITCH (PHYLIP) and data from the distance matrix algorithm DNADIST (PHYLIP). The alignment of S1 nucleotide sequences used in these analyses was derived from the alignment of the T1L, T2J, and T3D σ1 amino acid sequences determined by Nibert et al. (30).

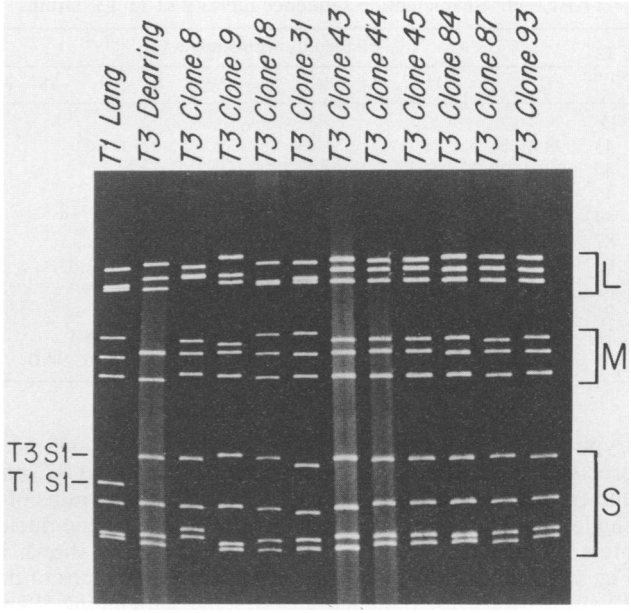

FIG. 1. SDS-PAGE of virions of reovirus T1L and 11 T3 strains. Equal amounts (5×10^{10} particles) of CsCl gradient-purified virions of reovirus strains T1L, T3D, T3C8, T3C9, T3C18, T3C31, T3C43, T3C44, T3C45, T3C84, T3C87, and T3C93 were disrupted in sample buffer for 5 min at 60°C prior to electrophoresis. Viral gene segments were separated in a SDS-polyacrylamide gel (10% acrylamide) and visualized by ethidium bromide staining and UV irradiation. The T1 and T3 S1 gene segments are indicated on the left and viral gene segment size classes are indicated on the right. L, Large; M, medium; S, small.

RESULTS

Diversity of T3 reovirus strains according to SDS-PAGE profiles of their dsRNA gene segments and structural proteins. The 10 dsRNA gene segments of mammalian reoviruses can be separated by SDS-PAGE (33) as shown in Fig. 1 for T1L and 11 T3 strains. S1 gene segment mobility is not entirely uniform among the T3 strains, varying most between T3C9 and T3C31; however, the S1 gene segment mobility of each of the T3 strains is closer to that of T3D than to that of T1L (19). The relative constancy of mobility of the S1 gene segment in the T3 strains is consistent with the fact that S1 encodes the type-specific protein, σ1 (19, 47). Gene segments other than S1 migrate in SDS-PAGE more similarly to those of T3D in some of the T3 strains, but more similarly to those of T1L in others. For example, the medium-class and large-class gene segments of T3C18 and T3C31 migrate very similarly to those of T1L.

Global comparisons of S1 nucleotide and σ1 and σ1s amino acid sequences. Variation in the σ1 proteins from different T3 strains was previously identified in studies of the neutralizing and hemagglutination-inhibiting activities of anti-σ1 monoclonal antibodies (6) and in studies of tyrosine-containing

TABLE 2. S1 nucleotide sequence identity of 11 T3 strains

T3 strain	% Identity with strain:										
	D	43	44	45	84	87	93	8	9	18	31
D											
43	98.4										
44	98.4	99.7									
45	98.3	99.6	99.6								
84	98.0	99.1	99.1	98.9							
87	98.4	99.2	99.2	99.0	98.8						
93	98.0	99.1	99.1	98.9	99.4	98.8					
8	84.3	84.3	84.3	84.2	84.0	84.3	83.9				
9	80.5	80.5	80.5	80.5	80.1	80.2	80.0	80.7			
18	79.6	79.8	79.9	79.6	79.5	79.6	79.2	80.2	90.8		
31	81.0	80.8	80.8	80.8	80.5	80.6	80.3	81.2	91.6	94.0	

tryptic peptides of σ1 (18). In order to ascertain more precisely the extent of variability that occurs in the S1 gene and the S1 translation products σ1 and σ1s from strains of a single reovirus serotype, we determined the S1 gene nucleotide sequences of the 10 T3 field isolate strains shown in Fig. 1 (data not shown). The S1 nucleotide sequences of the T3 strains were 1,416 nucleotides in length, including 5′ and 3′ noncoding sequences of 12 and 36 nucleotides, respectively. The sequences of the noncoding regions were identical to those of T3D (3, 7, 28).

In order to evaluate the degree of sequence similarity, we compared the S1 nucleotide sequences (Table 2) and the deduced σ1 and σ1s amino acid sequences (Table 3) of the T3 strains. The majority of the nucleotide differences were first or third base changes that produced no change in the σ1 and σ1s amino acid sequences. The S1 nucleotide and deduced σ1 amino acid sequences appear to fall into two groups with respect to degree of similarity. The first group includes very similar sequences from strains T3D, T3C43, T3C44, T3C45, T3C84, T3C87, and T3C93. The σ1 sequences of these strains have differences at only 19 amino acid positions; residues at the remaining 436 amino acid positions are identical (98 to 99% sequence identity). The σ1s sequences of these strains have differences at only 4 amino acid positions; residues at the remaining 116 amino acid positions are identical (97 to 100% sequence identity). The second group includes sequences from strains T3C9, T3C18, and T3C31. The σ1 sequences of these strains are less similar to one another (93 to 95% sequence identity) than are the σ1 sequences of strains in the first group. The σ1s sequences of these strains are more heterogeneous than their σ1 sequences and also are less similar to one another (87 to 88% sequence identity) than are the σ1s sequences of strains in the first group. Strain T3C8 does not appear to belong to either group by this analysis.

TABLE 3. σ1 and σ1s amino acid sequence identity of 11 T3 strains

T3 strain	% Identity[a] with strain:										
	D	43	44	45	84	87	93	8	9	18	31
D		98.9	98.7	98.2	98.5	98.7	98.0	91.4	86.6	87.3	88.1
43	97.5		99.3	98.9	99.1	99.3	98.7	91.2	86.4	87.0	87.5
44	97.5	100		98.7	98.9	99.1	98.5	91.0	86.4	87.0	87.5
45	97.5	100	100		98.5	98.7	98.0	90.6	86.2	86.4	87.3
84	96.7	99.2	99.2	99.2		98.9	99.1	90.8	85.9	86.6	87.0
87	98.3	99.2	99.2	99.2	98.3		98.5	91.0	86.2	86.8	87.3
93	97.5	100	100	100	99.2	99.2		90.3	85.5	86.2	86.6
8	79.2	80.0	80.0	80.0	79.2	79.2	80.0		87.5	87.7	88.4
9	77.5	76.7	76.7	76.7	75.8	75.8	76.7	77.5		93.0	94.1
18	71.7	72.5	72.5	72.5	71.7	71.7	72.5	74.2	86.7		94.7
31	79.2	80.0	80.0	80.0	79.2	79.2	80.0	79.2	87.5	88.3	

[a] σ1 amino acid sequence data are presented in the top right triangle, and σ1s amino acid sequence data are presented in the bottom left triangle.

To more accurately define the evolutionary relatedness of the different reovirus strains, we constructed phylogenetic trees using variation in the σ1-encoding nucleotide sequences of T1L, T2J, and the T3 strains. Variation in each codon position was determined by using the maximum-parsimony algorithm PAUP (42). Variation in the third codon position was found to provide the greatest resolution between individual strains and suggested that the strains form five distinguishable lineages (Fig. 2). Analyses of variation in the first and second codon positions assigned the strains to the same lineages, albeit with slightly different branch lengths and relationships between strains within each lineage (data not shown). The first lineage (Fig. 2, section A) includes T1L; the second (section B) includes T2J; the third (section C) includes T3D, T3C43, T3C44, T3C45, T3C84, T3C87, and T3C93; the fourth (section D) includes a single T3 strain, T3C8; and the fifth (section E) includes T3C9, T3C18, and T3C31. To determine whether reovirus strains could be assigned to these five lineages by other techniques, we used the maximum-parsimony algorithm DNAPARS and the distance-linkage method FITCH, with distance matrix data determined by DNADIST. Both techniques gave a branching order identical to that observed when variation in the third codon position was examined by using PAUP (data not shown).

The parsimony analysis showed that diversity between individual T3 strains appears to be as great as that between the T1 and T2 prototype strains. The most distantly related T3 strains, T3C18 and T3C84, vary in 260 third base positions; T1L and T2J vary in 259 third base positions. In this analysis, however, gap positions placed to align the T1L and T2J S1 sequences (30) were not scored. Thus, phylogenetic trees constructed from variation in the S1 nucleotide sequences may underestimate the extent of diversity between the T1L and T2J S1 sequences.

Regions of variable and conserved sequences in T3 σ1. Previous comparisons of the σ1 amino acid sequences of prototype strains of the three reovirus serotypes (T1L, T2J, and T3D) identified some regions in σ1 that are more conserved than others (30). In order to ascertain the distribution of variable and conserved regions in σ1 from strains of a single serotype, we examined the deduced σ1 amino acid sequences of the 11 T3 strains (Fig. 3). The length of σ1 is conserved in these strains (455 amino acids), and no gaps were needed to optimize the alignment of any of the sequences.

When the σ1 sequences of T3 strains from a single phylogenetic lineage (Fig. 2) were compared, the positions of variable amino acids were few and evenly scattered. When σ1 sequences of T3 strains from different lineages were compared, however, the positions of variable amino acids were more numerous and appeared to cluster, suggesting regions of hypervariability (e.g., amino acids 118 to 137, 172 to 184, 236 to 249, and 283 to 297). Conversely, several large regions of σ1 sequence were found to be conserved in all 11 T3 strains (e.g., amino acids 39 to 52, 73 to 85, 146 to 155, 185 to 194, 205 to 230, 307 to 322, 345 to 355, 364 to 388, and 419 to 434).

The locations of the variable and conserved regions of T3 σ1 sequence reflect in many cases the domain organization that is proposed for the σ1 protein based on analysis of the

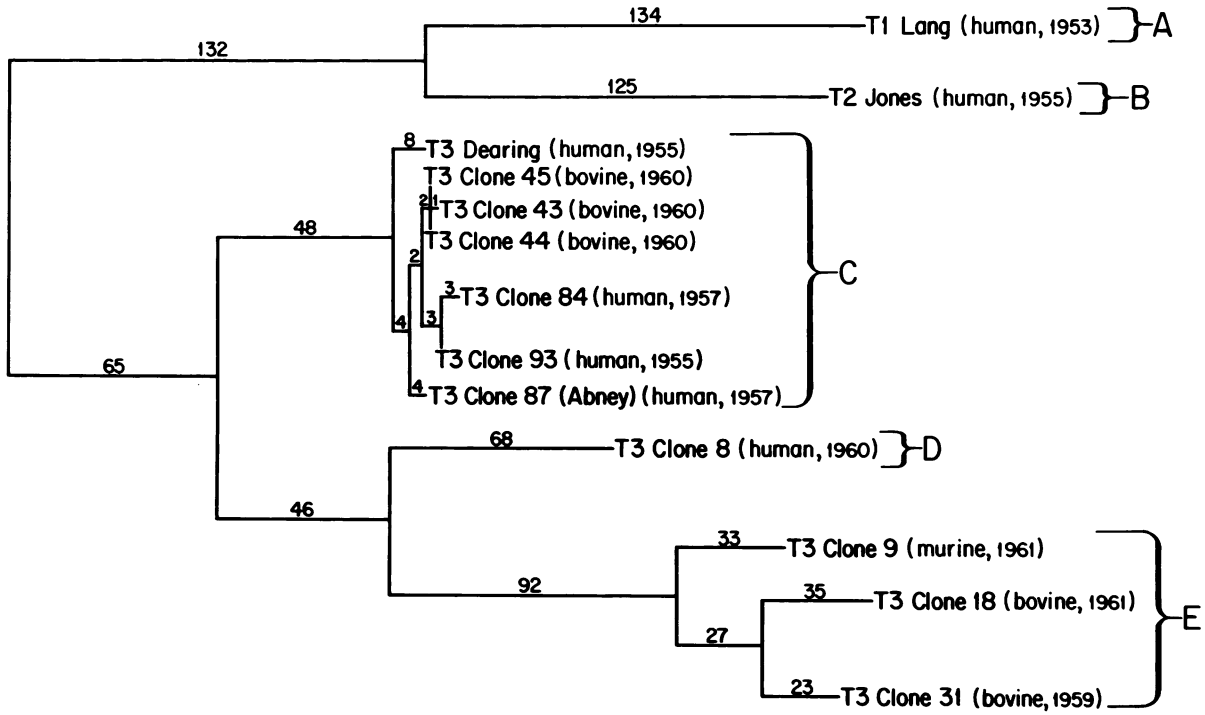

FIG. 2. Minimum-length evolutionary tree for reovirus strains constructed from variation in the σ1 third codon position by using PAUP (42). Lengths of the horizontal lines are proportional to the minimum number of single nucleotide changes required to generate the observed variation (also indicated by numeric branch lengths above each line). The length of vertical lines is arbitrary. The total number of sites examined was 478, of which 411 were variable. The minimum length of the tree was 855, and the consistency index was 0.810.

deduced σ1 amino acid sequences of prototype strains of the three reovirus serotypes (30) and are summarized in Fig. 4. Three of the variable regions occur at or near the junctions of sequence regions suggested to form discrete σ1 morphologic regions: amino acids 172 to 184 at the T(ii)-T(iii) junction, 236 to 249 at the T(iii)-T(iv) junction, and 283 to 297 near the T(iv)-H junction. Two of the conserved regions (amino acids 185 to 194 and 205 to 230) encompass most of the sequence region proposed to form morphologic region T(iii). Four of the other conserved regions (amino acids 307 to 322, 345 to 355, 364 to 388, and 419 to 434) occur in the region of sequence proposed to form morphologic region H (the σ1 head).

Conserved sequence patterns in T3 σ1. Repeating apolar residues in the deduced σ1 amino acid sequences of prototype reovirus strains T1L, T2J, and T3D have been proposed to be important for the structure of the σ1 tail (3, 16, 17, 30). As described below, this proposal is supported by the finding that the apolar repeats are well conserved in the 11 T3 σ1 sequences included in this study (Table 4).

A large region in the amino-terminal one-half of each prototype σ1 sequence is marked by heptad repeats of apolar residues (amino acids 28 to 173 in T3D). This region has been proposed to correspond to morphologic region T(ii) of the σ1 protein (3, 16, 17, 30). The heptad repeat pattern suggests that this region of sequence has an α-helical coiled-coil structure (27). In the 11 T3 σ1 sequences, apolar residues in heptad positions *a* and *d* are well conserved (Fig. 3, solid circles). Five amino acid positions in morphologic region T(ii) (39, 42, 67, 88, and 123) account for the majority of amino acid positions that are predicted to be occupied by apolar residues according to the repeat pattern but are in fact occupied by polar residues (Table 4). Acid-base pairs in the (*n*)*e* and (*n*+1)*g* positions of heptad repeats have been suggested to provide additional stability to α-helical coiled coils in several proteins (27, 41), including σ1 (17, 30). A total of five (*n*)*e*-(*n*+1)*g* acid-base pairs occur in the long heptad repeat region of T3D σ1 (pairs connected by underlines in Fig. 3). These five acid-base pairs are conserved in all T3 strains except T3C18, in which four of five are conserved (proline instead of arginine at position 59). The heptad repeat regions of some proteins include significant discontinuities in the repeat pattern, referred to as stutter (40) and skip (26) sequences. These sequences are also present in σ1 (30) and may serve to decrease the superhelical twist of the coiled coil either locally or over an extended region of protein structure (26, 40). Stutter and skip sequences are conserved in all T3 strains (Fig. 3, solid squares).

A region exhibiting a distinct repeating pattern of apolar residues occurs immediately carboxy terminal to the long heptad repeat region in the prototype σ1 sequences (amino acids 177 to 234 in T3D) and has been proposed to correspond to morphologic region T(iii) in the σ1 protein (30). Apolar residues in this region occur in small clusters that contain either two or three apolar residues which occur at every other amino acid position. These clusters are predicted to form short β-strands. The residues that occupy positions between the clusters are mostly polar and are predicted to form β-turns. Apolar repeat residues in this region are completely conserved in the T3 strains examined (Fig. 3, open circles).

Sequences proposed to correspond to morphologic region T(iv) in the prototype strains (amino acids 239 to 307 in T3D) have alternating apolar repeat patterns (heptad repeats and apolar clusters) and are predicted to form two short α-helical

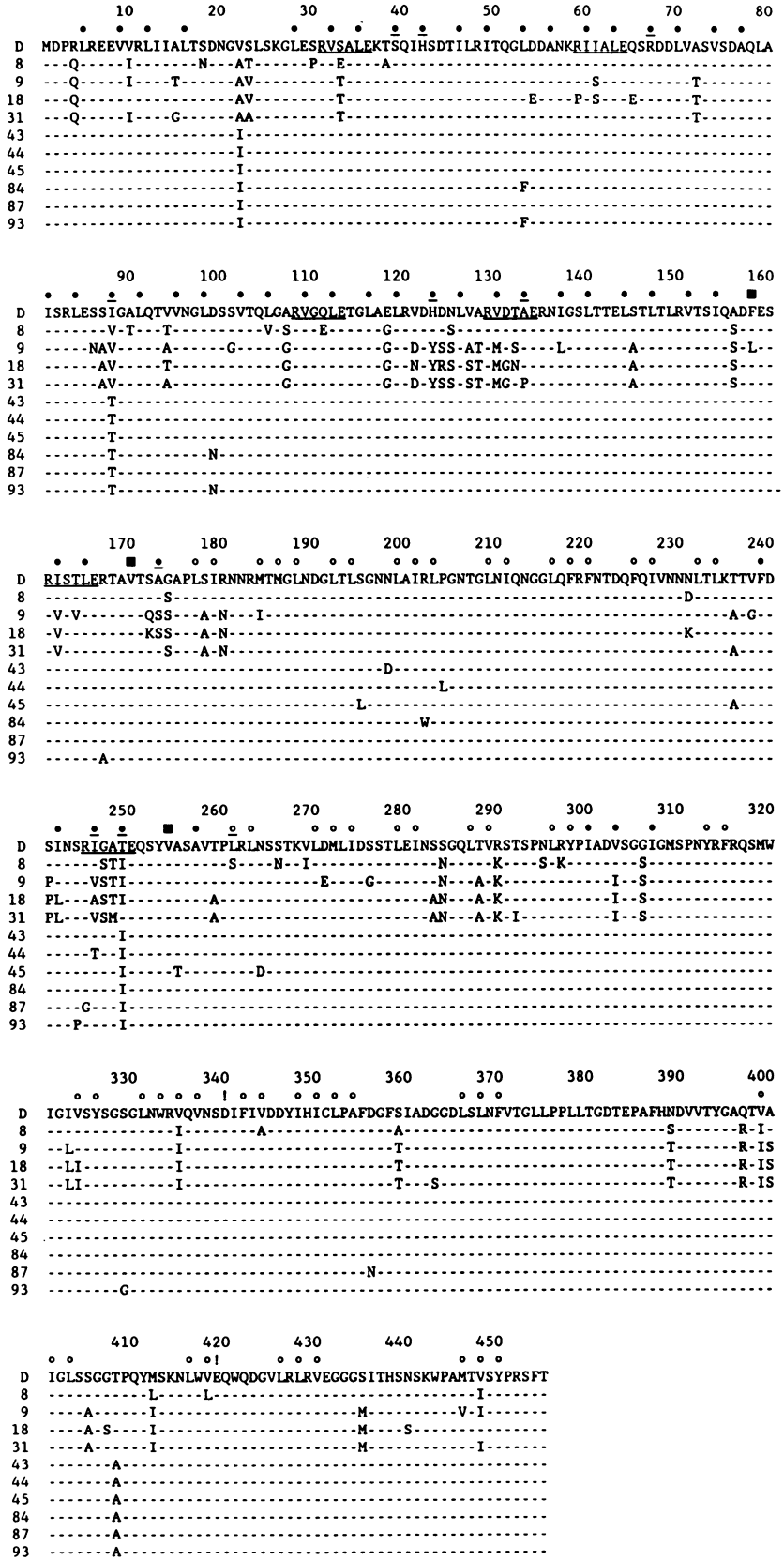
10 20 30 40 50 60 70 80
D MDPRLREEVVRLIIALTSDNGVSLSKGLESRVSALEKTSQIHSDTILRITQGLDDANKRIIALEQSRDDLVASVSDAQLA
8 ---Q-----I--------N---AT------P--E----A----------
9 ---Q-----I----T------AV---------T----------S----------T--------
18 ---Q----------------AV---------T----------E----P-S---E------T--------
31 ---Q-----I----G------AA---------T----------T--------
43 ----------I----------
44 ----------I----------
45 ----------I----------
84 ----------I----------F----------
87 ----------I----------
93 ----------I----------F----------
90 100 110 120 130 140 150 160
D ISRLESSIGALQTVVNGLDSSVTQLGARVGQLETGLAELRVDHDNLVARVDTAERNIGSLTTELSTLTLRVTSIQADFES
8 -------V-T---T----------V-S---E------G------S----------S----
9 -----NAV-----A------G-----G----------G--D-YSS-AT-M-S----L-------A----------S-L--
18 ------AV-----T----------G----------G--N-YRS-ST-MGN----------A----------S----
31 ------AV-----A----------G----------G--D-YSS-ST-MG-P----------A----------S----
43 -------T----------
44 -------T----------
45 -------T----------
84 -------T----------N----------
87 -------T----------
93 -------T----------N----------
170 180 190 200 210 220 230 240
D RISTLERTAVTSAGAPLSIRNNRMTMGLNDGLTLSGNNLAIRLPGNTGLNIQNGGLQFRFNTDQFQIVNNNLTLKTTVFD
8 ----------S----------D----------
9 -V-V-------QSS---A-N---I----------A-G--
18 -V---------KSS---A-N----------K----------
31 -V----------S---A-N----------A----
43 ----------D----------
44 ----------L----------
45 ----------L----------A----
84 ----------W----------
87 ----------
93 ------A----------
250 260 270 280 290 300 310 320
D SINSRIGATEQSYVASAVTPLRLNSSTKVLDMLIDSSTLEINSSGQLTVRSTSPNLRYPIADVSGGIGMSPNYRFRQSMW
8 ------STI----------S----N--I----------N-----K----S-K--------S----------
9 P----VSTI----------E----G-------N---A-K----------I--S----------
18 PL---ASTI---------A----------AN---A-K----------I--S----------
31 PL---VSM----------A----------AN---A-K-I----------I--S----------
43 --------I----------
44 -----T--I----------
45 --------I-----T--------D----------
84 --------I----------
87 ----G---I----------
93 ---P----I----------
330 340 350 360 370 380 390 400
D IGIVSYSGSGLNWRVQVNSDIFIVDDYIHICLPAFDGFSIADGGDLSLNFVTGLLPPLLTGDTEPAFHNDVVTYGAQTVA
8 ----------I--------A----------A----------S-------R-I-
9 --L----------I----------T----------T-------R-IS
18 --LI---------I----------T----------T-------R-IS
31 --LI---------I----------T---S----------T-------R-IS
43 ----------
44 ----------
45 ----------
84 ----------
87 ----------N----------
93 --------G----------
410 420 430 440 450
D IGLSSGGTPQYMSKNLWVEQWQDGVLRLRVEGGGSITHSNSKWPAMTVSYPRSFT
8 ----------L-----L----------I-------
9 ----A------I----------M----------V-I-------
18 ----A-S----I----------M----S----------
31 ----A------I----------M----------I-------
43 -------A----------
44 -------A----------
45 -------A----------
84 -------A----------
87 -------A----------
93 -------A----------

TABLE 4. Conservation of apolar residues in apolar repeat patterns proposed to be important for σ1 structure

Morphologic region[a]	Repeat pattern[b]	No. of aa positions occupied by apolar residues according to repeat pattern[c]	
		Expected	Observed
T(i) (aa 1–27)	Heptad	44	44
T(ii) (aa 28–173)	Heptad	462	412
T(iii) (aa 174–238)	Apolar clusters	209	209
T(iv) (aa 239–307)			
aa 239–256	Heptad	66	64
aa 257–299	Apolar clusters	121	120
aa 300–307	Heptad	33	33
H (aa 308–455)	Apolar clusters	308	308

[a] σ1 morphologic regions are as defined by Fraser et al. (16), and sequence regions are as defined by Nibert et al. (30). aa, Amino acid positions.

[b] Two types of apolar repeat patterns are observed in σ1: (i) heptad repeats in which apolar residues occur at positions *a* and *d* of the heptad and (ii) apolar clusters in which apolar residues occur in clusters of apolar residues separated by polar residues at every other position.

[c] Number of apolar residues (A, F, I, L, M, V, W, and Y) in repeat patterns in a given region of sequence multiplied by 11, the number of T3 σ1 amino acid sequences analyzed.

coiled coils separated by a small region of β-sheet (30). This region of sequence in the 11 T3 strains demonstrates a striking degree of variability; however, apolar repeat patterns are conserved in all of the T3 σ1 sequences (Fig. 3). An $(n)e$ and $(n+1)g$ acid-base pair is also conserved in all T3 strains except T3C87 (glycine instead of arginine at position 245). Lastly, a skip sequence is conserved in this region in the more amino terminal of the two α-helical coiled coils in the T3 strains.

Sequences proposed to form the σ1 head (morphologic region H) in the prototype strains (amino acids 308 to 455 in T3D) do not exhibit a regular apolar repeat pattern; however, clusters of apolar residues suggest that the dominant structural motif within the σ1 head is β-sheet (30). These apolar clusters are absolutely conserved in the T3 strains (Fig. 3, open circles). Additional conserved apolar residues (alanine 354 and valine 430) can be added to two of the clusters in these strains.

Regions of variable and conserved sequences in T3 σ1s. While a great deal is known about structure-function relationships of σ1, little is known about the other S1 translation product, σ1s. Therefore, in an attempt to learn more about this protein, we examined the deduced σ1s amino acid sequences of the 11 T3 strains (Fig. 5). The length of the σ1s protein is conserved in these strains (120 amino acids), and no gaps were needed to optimize the alignment of any of the sequences. Variability in the σ1s amino acid sequences of the T3 strains is more marked than the variability observed in their σ1 amino acid sequences, and the positions of variable amino acids appear to cluster (e.g., amino acids 3 to 32 and 99 to 117). A central region of σ1s (amino acids 58 to 84) was found to be more conserved than the remainder of the protein.

The observation of an amino-terminal cluster of basic residues in σ1s has led to the suggestion that the σ1s protein might interact with nucleic acid (21). Fifteen basic residues (K and R) are conserved in the σ1s proteins of the T3 strains (Fig. 5, solid diamonds), including a group of six basic residues near the amino terminus of the protein (amino acids 14, 15, 17, 18, 19, and 21). Differences in the 15 amino acid positions occupied by basic residues in all strains are limited to asparagine 81 in T3C8 and asparagine 114 in T3D and T3C18. Several strains have additional basic residues in their σ1s amino acid sequences not found in the majority of the other T3 strains (e.g., arginine 11, arginine 35, arginine 45, and arginine 58 in T3C18). Another interesting feature of the σ1s sequences of the T3 strains is the leucine-to-proline substitution at amino acid 20 in T3C8, T3C9, T3C18, and T3C31 and the reciprocal proline-to-leucine substitution at amino acid 31 in these strains. These amino acid changes are adjacent to the amino-terminal cluster of six basic residues conserved in the σ1s sequences and may be important for σ1s conformation in that region.

DISCUSSION

Sequence diversity among T3 S1 genes. In this study, we examined the diversity of S1 gene nucleotide sequences in strains of a single serotype (T3) in an attempt to understand evolutionary relationships between reovirus isolates. The S1 nucleotide sequences of 10 T3 field isolate strains are the same length as that of the T3D S1 sequence, and the 5′ and 3′ noncoding regions are identical in all T3 strains examined. The majority of nucleotide sequence variability is in the third base position of the S1 translation products, and the deduced amino acid sequences of σ1 are more conserved than those of σ1s.

Before this study, the analysis of nucleotide sequence diversity among reovirus strains had been limited to comparative studies of single gene segments from prototype strains of the three reovirus serotypes: T1L, T2J, and T3D. These studies have led to an understanding of gene segment sequence diversity between these strains, codon usage, and the size of noncoding regions. The S1 gene nucleotide sequence (7, 11, 30) appears to have diverged more than the S2 (T. S. Dermody, L. A. Schiff, K. M. Coombs, M. L. Nibert, and B. N. Fields, unpublished data), S3 (50), M2 (51), and L1 (52) sequences of these prototype strains. We found an extensive amount of sequence diversity in the S1 genes of T3 strains, which further suggests that S1 is the most divergent reovirus gene segment.

We used S1 gene sequence diversity to determine phylogenetic relationships between the reovirus prototype strains T1L, T2J, and T3D and 10 T3 field isolate strains. Analysis of phylogenetic trees constructed from variation in the S1 nucleotide sequences is notable for the division of these strains into five lineages (A through E). The grouping of these strains into lineages based on their S1 sequences does not appear to correlate with host species, geographic site, or date of isolation. Lineage C includes T3 strains that were isolated from both bovine and human sources in the eastern

FIG. 3. Alignment of σ1 amino acid sequences of reovirus T3 strains. The σ1 amino acid sequences were deduced from the S1 nucleotide sequences of strains T3D (D) and T3C8, T3C9, T3C18, T3C31, T3C43, T3C44, T3C45, T3C84, T3C87, and T3C93 (abbreviated by clone number). The single-letter amino acid code is used, and residues identical to T3D in the other T3 strains are indicated with dashes. Amino acid positions are numbered above the sequences. Positions of apolar residues that conform to a heptad repeat pattern (●), positions of apolar residues that conform to an alternating apolar-polar repeat pattern (○), and positions in the repeats at which apolar residues are not conserved in all sequences (● and ○) are indicated. Acid-base pairs in regions of sequence characterized by heptad repeats are underlined. ■, Conserved apolar residues at the amino-terminal boundaries of stutter and skip sequences; !, sites altered in neutralization-resistant variants of T3D (4).

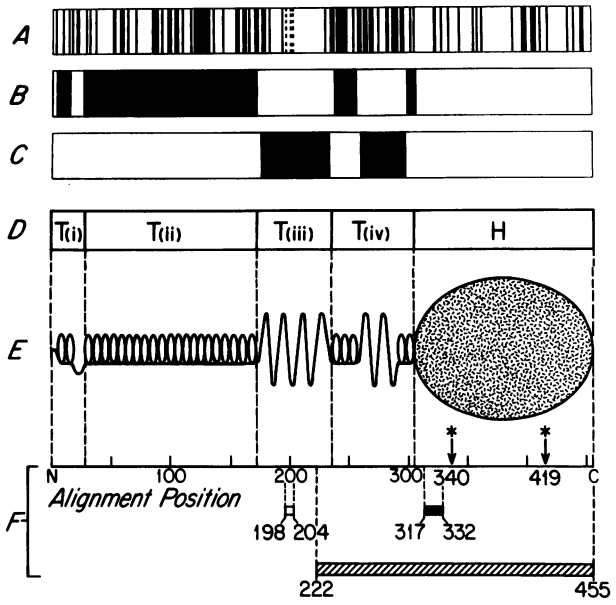

FIG. 4. Structure-function relationships of the T3 σ1 protein. (A) Positions of residues that differ from the prototype strain (T3D) in one or more of the other T3 strains are indicated by solid vertical lines. The hatched lines indicate amino acid positions at which unique residues are observed in hemagglutination-negative T3 strains (8). (B) Regions that have a heptad repeat pattern of apolar residues and are proposed to form α-helical coiled-coil structure. (C) Regions that have clusters of apolar residues in an alternating apolar-polar repeat pattern and are proposed to form β-sheet structure. (D) Morphologic regions of σ1 (16, 30). (E) Model of σ1 structure. Regions of proposed α-helical coiled-coil and β-sheet structure are depicted. Amino acid positions in the T3 σ1 sequence are shown at bottom and are in scale with each of the panels above. (F) Regions of sequence proposed to be important for hemagglutination (198 to 204) (8), cell attachment (222 to 455) (29), and neutralization (340 and 419) (4) and a region of sequence with similarity to a portion of an anti-idiotypic antibody raised against a T3 monoclonal antibody (317 to 332) (53).

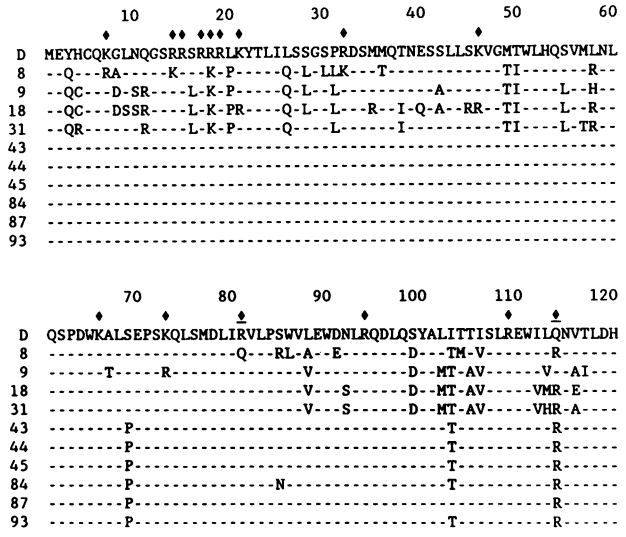

FIG. 5. Alignment of σ1s amino acid sequences of T3 reovirus strains. The σ1s amino acid sequences were deduced from the S1 nucleotide sequences of strains T3D (D), and T3C8, T3C9, T3C18, T3C31, T3C43, T3C44, T3C45, T3C84, T3C87, and T3C93 (abbreviated by clone number). The single-letter amino acid code is used, and residues identical to T3D in the other T3 strains are indicated by dashes. Alignment positions are numbered above the sequences. ◆, Conserved basic residues (K and R); ◆, positions at which basic residues are not conserved in all strains.

United States between 1955 and 1960. Lineage E also includes T3 strains isolated from different species (bovine and murine), as well as from different geographic sites (France and Maryland), between 1959 and 1961 (Fig. 2). Thus, reovirus strains appear to be transmitted rapidly between species. Furthermore, the markedly divergent S1 genes from bovine strains isolated in Maryland between 1959 and 1961 suggest that more than one S1 gene circulates in a given geographic locale at the same time. Given these findings, in addition to the limited time period over which the strains were collected, a linear relationship between S1 gene mutation rate and time cannot be determined from this analysis.

Studies of influenza B (54) and C (5) viruses suggest a precedent for the isolation of strains containing gene segments with divergent sequences from the same or different host species in a given geographic site at approximately the same time. The simultaneous circulation of influenza virus strains having divergent sequences has been used to explain an absence of sequence relatedness, based on host species, geographic site and date of isolation (1, 23, 54), similar to that observed for the T3 reovirus strains in this study. The maintenance of divergent genes in a given population at the same time is likely to be explained in part by reassortment of gene segments in viruses with segmented genomes, such as influenza virus (9, 24, 57) and reovirus (19, 33, 49). Reassortment of gene segments makes it possible for sequence divergence of any one gene segment to be independent of that of others. Therefore, the sequences of individual gene segments might be expected to vary without regard to species and site of origin if there are no host or geographic restrictions associated with that gene segment (i.e., host-range genes).

Conserved sequences in σ1. We have used comparative sequence analysis to investigate structure-function relationships of the σ1 protein. A model of reovirus T3 σ1 structure, correlated with sequence regions proposed to be important for several σ1 functions, is shown in Fig. 4. Protein sequence variability (Fig. 4A) is primarily concentrated in morphologic regions T(i), T(ii), and T(iv). Morphologic region T(iii), which corresponds to a proposed large cross-β region, is mostly conserved with the exception of unique residues in σ1 sequence of strains which show diminished capacities to agglutinate erythrocytes and to bind glycophorin (8). Morphologic region H, the σ1 head, is also a region in which greater sequence conservation is observed in the T3 strains. The deduced amino acid sequences of T1L and T2J σ1 also share greatest similarity in morphologic regions T(iii) and H (30). These observations suggest that σ1 morphologic regions T(iii) and H are under greater constraint than other σ1 regions to maintain their respective structures and/or functions.

Despite the variability observed in the 11 T3 σ1 amino acid sequences, repeat patterns of apolar residues proposed to be important for σ1 structure (30) are conserved (Table 4). Most of the sequence variability in the σ1 tail is observed in regions predicted to form α-helical coiled coil (Fig. 4B) rather than in regions predicted to form cross-β sheet (Fig. 4C). In addition to the importance of conserved apolar repeat patterns to σ1 structure, the importance of $(n)e$ and $(n+1)g$ acid-base pairs and stutter and skip sequences in

regions proposed to form α-helical coiled coil is emphasized by the conservation of these sequences in the T3 strains.

Several sequences within the σ1 head have been suggested to be important for the functions of serotype-specific neutralization and cell attachment. Variants of T3D that are resistant to neutralization by type-specific anti-σ1 monoclonal antibodies (39) have substitutions at amino acid positions 340 and 419 (4). These residues are absolutely conserved in the T3 strains in this study. A monoclonal anti-idiotypic antibody that was raised against the neutralizing monoclonal antibody G5, contains a region of amino acid sequence with marked similarity to residues 317 to 332 of T3D σ1 (53). A peptide corresponding to this region of similarity is able to block viral receptor recognition. This sequence is also conserved in the T3 strains with the exception of two conservative substitutions: leucine 323 in T3C9, T3C18, and T3C31 and isoleucine 324 in clones T3C18 and T3C31. Lastly, a region of striking sequence similarity between the T1L, T2J, and T3D σ1 proteins corresponds to amino acids 368 to 386 of the T3D σ1 amino acid sequence (30). This sequence is absolutely conserved in the T3 strains, again suggesting that it has structural or functional importance.

Conserved sequences in σ1s. We have also used comparative sequence analysis to study the σ1s protein. The σ1s sequences of the 11 T3 strains are more heterogeneous than their corresponding σ1 sequences. While the function of the σ1s protein is unknown, the large degree of primary sequence variability suggests that it may not be under the same structural constraints as other reovirus proteins. A large number of basic residues, including a cluster near the amino terminus of the protein, was found to be conserved in σ1s of the T3 strains. The observation of a cluster of basic residues in σ1s led Jacobs and Samuel (21) to propose that the σ1s protein might be capable of binding to nucleic acid. The potential importance of these residues to σ1s function is supported by the finding that this cluster of basic residues is conserved in the 11 T3 σ1s sequences.

Classification of reovirus strains. In a previous study, Hrdy et al. (19) grouped reovirus strains of all three serotypes according to the pattern of their gene segment mobility in SDS-PAGE (electropherotype). Although that grouping acknowledged the host species from which the strain was isolated [e.g., electropherotypic patterns B(bovine)T3a, H(human)T3a, and M(murine)T3a as represented by strains T3C18, T3C8, and T3C9, respectively], these authors concluded that the electropherotype of a reovirus strain does not unambiguously identify the host species or geographic site of its isolation. The T3 S1 nucleotide sequences reported in this study suggest a similar conclusion and also indicate that the previous grouping of T3 strains according to host species of isolation does not accurately reflect the relatedness of their S1 genes.

Reovirus strains are more generally classified according to serotype; however, serotype is a property determined by a single reovirus gene segment, S1 (47). As a consequence, although S1 gene nucleotide sequences from strains of one serotype are expected to be more closely related to each other than to S1 gene sequences from strains of another serotype, nucleotide sequences of other gene segments from these strains are likely to show relatedness patterns that are independent of serotype.

In this study of S1 gene sequence diversity in T3 reovirus strains, we made findings that contribute to an understanding of reovirus evolution and aspects of the structure and function of the σ1 and σ1s proteins. In a companion study, we have characterized a region of sequence important for hemagglutination by these T3 strains (8). These studies demonstrate the utility of comparative sequence analysis for the study of σ1 structure as well as σ1 function.

ACKNOWLEDGMENTS

We express our appreciation to Elaine Freimont and Richard van den Broek for expert technical assistance. We are grateful to Joseph Felsenstein, Randall Smith, and Temple Smith for assistance in the preparation and interpretation of phylogenetic trees. We also thank Man-Sung Co, Kevin Coombs, Lynda Morrison, Leslie Schiff, Kenneth Tyler, Herbert Virgin, and Greame Wilson for essential discussions and reviews of the manuscript.

This work was supported by Public Health Service research grant 5R37 AI13178-13 from the National Institute of Allergy and Infectious Diseases. Support to B.N.F. was also provided by the Shipley Institute of Medicine. M.L.N. was supported by National Research Service Award 5T32 GM07753-10 from the National Institute of General Medical Sciences. T.S.D. was the recipient of Physician-Scientist Award 5K11 AI00865 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Altmuller, A., W. M. Fitch, and C. Scholtissek.** 1989. Biological and genetic evolution of the nucleoprotein of human influenza A viruses. J. Gen. Virol. **70**:2111–2119.
2. **Banerjea, A. C., K. A. Brechling, C. A. Ray, H. Erikson, D. J. Pickup, and W. K. Joklik.** 1988. High-level synthesis of biologically active reovirus protein σ1 in a mammalian expression vector system. Virology **167**:601–612.
3. **Bassel-Duby, R., A. Jayasuriya, D. Chatterjee, N. Sonnenberg, J. V. Maizel, Jr., and B. N. Fields.** 1985. Sequence of reovirus haemagglutinin predicts a coiled-coil structure. Nature (London) **315**:421–423.
4. **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.
5. **Buonagurio, D. A., S. Nakada, W. M. Fitch, and P. Palese.** 1986. Epidemiology of influenza C virus in man: multiple evolutionary lineages and low rate of change. Virology **153**:12–21.
6. **Burstin, S. J., D. R. Spriggs, and B. N. Fields.** 1982. Evidence for functional domains of the reovirus type 3 hemagglutinin. Virology **117**:146–155.
7. **Cashdollar, L. W., R. A. Chmelo, J. R. Wiener, and W. K. Joklik.** 1985. The sequence of the S1 genes of the three serotypes of reovirus. Proc. Natl. Acad. Sci. USA **82**:24–28.
8. **Dermody, T. S., M. L. Nibert, R. Bassel-Duby, and B. N. Fields.** 1990. A σ1 region important for hemagglutination by serotype 3 reovirus strains. J. Virol. **64**:5173–5176.
9. **Desselberger, U., K. Nakajima, P. Alfino, F. S. Pedersen, W. A. Haseltine, C. Hannoun, and P. Palese.** 1978. Biochemical evidence that "new" influenza virus strains in nature may arise by recombination (reassortment). Proc. Natl. Acad. Sci. USA **75**:3341–3345.
10. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.
11. **Duncan, R., D. Horne, L. W. Cashdollar, W. K. Joklik, and P. W. K. Lee.** 1990. Identification of conserved domains in the cell atachment proteins of the three serotypes of reovirus. Virology **174**:339–409.
12. **Ernst, G., and A. J. Shatkin.** 1985. Reovirus hemagglutinin mRNA codes for two polypeptides in overlapping reading frames. Proc. Natl. Acad. Sci. USA **82**:48–52.
13. **Faulkner, D. V., and J. Jurka.** 1988. Multiple aligned sequence editor. Trends Biochem. Sci. **13**:321–322.
14. **Felsenstein, J.** 1989. PHYLIP 3.2 manual. University of California Herbarium, Berkeley, California.
15. **Fitch, W. M.** 1971. Toward defining the course of evolution: minimum change for a specific tree topology. Syst. Zool. **20**:406–416.
16. **Fraser, R. D. B., D. B. Furlong, B. L. Trus, M. L. Nibert, B. N.**

Fields, and A. C. Steven. 1990. Molecular structure of the cell-attachment protein of reovirus: correlation of computer-processed electron micrographs with sequence-based predictions. J. Virol. **64**:2990–3000.
17. **Furlong, D. B., M. L. Nibert, and B. N. Fields.** 1988. Sigma 1 protein of mammalian reoviruses extends from the surfaces of viral particles. J. Virol. **62**:246–256.
18. **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.
19. **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.
20. **Hrdy, D. B., D. N. Rubin, and B. N. Fields.** 1982. Molecular basis of reovirus neurovirulence: role of the M2 gene in avirulence. Proc. Natl. Acad. Sci. USA **79**:1298–1302.
21. **Jacobs, B. L., and C. E. Samuel.** 1985. Biosynthesis of reovirus-specified polypeptides: the reovirus S1 mRNA encodes two primary translation products. Virology **143**:63–74.
22. **Jayasuriya, A. K., M. L. Nibert, and B. N. Fields.** 1988. Complete nucleotide sequence of the M2 gene segment of reovirus type 3 Dearing and analysis of its protein product μ1. Virology **163**:591–602.
23. **Kawaoka, Y., S. Krauss, and R. G. Webster.** 1989. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. J. Virol. **63**:4603–4608.
24. **Laver, W. G., and R. G. Webster.** 1973. Studies on the origin of pandemic influenza. III. Evidence implicating duck and equine influenza viruses as possible progenitors of the Hong Kong strain of human influenza. Virology **51**:383–391.
25. **Lee, P. W. K., E. C. Hayes, and W. K. Joklik.** 1981. Protein σ1 is the reovirus cell attachment protein. Virology **108**:156–163.
26. **McLachlan, A. D., and J. Karn.** 1983. Periodic features in the amino-acid sequence of nematode myosin rod. J. Mol. Biol. **164**:605–626.
27. **McLachlan, A. D., and M. Stewart.** 1975. Tropomyosin coiled-coil interactions: evidence for an unstaggered structure. J. Mol. Biol. **98**:293–304.
28. **Nagata, L., S. A. Masri, and D. C. Mah.** 1984. Molecular cloning and sequencing of the reovirus (serotype 3) S1 gene which encodes the viral cell attachment protein sigma 1. Nucleic Acids Res. **12**:8699–8710.
29. **Nagata, L., S. A. Masri, R. T. Pon, and P. W. K. Lee.** 1987. Analysis of functional domains on reovirus cell attachment protein σ1 using cloned S1 deletion mutants. Virology **160**:162–168.
30. **Nibert, M. L., T. S. Dermody, and B. N. Fields.** 1990. Structure of the reovirus cell-attachment protein: a model for the domain organization of σ1. J. Virol. **64**:2976–2989.
31. **Onodera, T., A. Toniolo, U. R. Ray, A. B. Jenson, R. A. Knazek, and A. L. Notkins.** 1981. Virus-induced diabetes mellitus. XX. Polyendocrinopathy and autoimmunity. J. Exp. Med. **153**:1457–1473.
32. **Paul, R. W., and P. W. K. Lee.** 1987. Glycophorin is the reovirus receptor on human erythrocytes. Virology **159**:94–101.
33. **Ramig, R. F., R. K. Cross, and B. N. Fields.** 1977. Genome RNAs and polypeptides of reovirus serotypes 1, 2, and 3. J. Virol. **22**:726–733.
34. **Rosen, L., and F. R. Abinanti.** 1960. Natural and experimental infection of cattle with human types of reovirus. Am. J. Hyg. **71**:424–429.
35. **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.
36. **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.
37. **Sarkar, G., J. Pelletier, R. Bassel-Duby, A. Jayasuriya, B. N. Fields, and N. Sonenberg.** 1985. Identification of a new polypeptide coded by reovirus gene S1. J. Virol. **54**:720–725.
38. **Spriggs, D. R., R. T. Bronson, and B. N. Fields.** 1983. Hemagglutinin variants of reovirus type 3 have altered central nervous system tropism. Science **220**:505–507.
39. **Spriggs, D. R., and B. N. Fields.** 1982. Attenuated reovirus type 3 strains generated by selection of haemagglutinin antigenic variants. Nature (London) **297**:68–70.
40. **Steinert, P. M., A. C. Steven, and D. R. Roop.** 1985. The molecular biology of intermediate filaments. Cell **42**:411–419.
41. **Steven, A. C., B. L. Trus, J. V. Maizel, M. Unser, D. A. D. Perry, J. S. Wall, J. F. Hainfeld, and F. W. Studier.** 1988. Molecular substructure of a viral receptor-recognition protein: the gp17 tail-fiber of bacteriophage T7. J. Mol. Biol. **200**:351–365.
42. **Swafford, D. L.** 1985. Phylogenetic analysis using parsimony (computer program). Illinois Natural History Survey, Champagne, Illinois.
43. **Tyler, K. L., D. A. McPhee, and B. N. Fields.** 1986. Distinct pathways of viral spread determined by reovirus S1 gene segment. Science **233**:770–774.
44. **Tyler, K. L., H. W. Virgin IV, R. Bassel-Duby, and B. N. Fields.** 1989. Antibody inhibits defined stages in the pathogenesis of reovirus serotype 3 infection of the central nervous system. J. Exp. Med. **170**:887–900.
45. **Weiner, H. L., K. A. Ault, and B. N. Fields.** 1980. Interaction of reovirus with cell surface receptors. I. Murine and human lymphocytes have a receptor for the hemagglutinin of reovirus type 3. J. Immunol. **124**:2143–2148.
46. **Weiner, H. L., D. Drayna, D. R. Averill, Jr., and B. N. Fields.** 1977. Molecular basis of reovirus virulence: role of the S1 gene. Proc. Natl. Acad. Sci. USA **74**:5744–5748.
47. **Weiner, H. L., and B. N. Fields.** 1977. Neutralization of reovirus: the gene responsible for the neutralization antigen. J. Exp. Med. **146**:1305–1310.
48. **Weiner, H. L., R. F. Ramig, T. A. Mustoe, and B. N. Fields.** 1978. Identification of the gene coding for the hemagglutinin of reovirus. Virology **86**:581–584.
49. **Wenske, E. A., S. J. Chanock, L. Krata, and B. N. Fields.** 1985. Genetic reassortment of mammalian reoviruses in mice. J. Virol. **56**:613–616.
50. **Wiener, J. R., and W. K. Joklik.** 1987. Comparison of the reovirus type 1, 2, and 3 S3 genome segments encoding the nonstructural protein sigma NS. Virology **161**:332–339.
51. **Wiener, 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 capsid protein mu1C. Virology **163**:603–613.
52. **Wiener, J. R., and W. K. Joklik.** 1989. The sequences of the reovirus serotype 1, 2, and 3 L1 genome segments and analysis of the mode of divergence of the reovirus serotypes. Virology **169**:194–203.
53. **Williams, W. V., H. R. Guy, D. H. Rubin, F. Robey, J. N. Myers, T. Kieber-Emmons, D. B. Weiner, and M. L. Greene.** 1988. Sequences of the cell-attachment sites of reovirus type 3 and its anti-idiotypic/antireceptor antibody: modeling of their three-dimensional structures. Proc. Natl. Acad. Sci. USA **85**:6488–6492.
54. **Yamashita, M., M. Krystal, W. M. Fitch, and P. Palese.** 1988. Influenza B virus evolution: cocirculating lineages and comparison of evolutionary pattern with those of influenza A and C viruses. Virology **163**:112–122.
55. **Yeung, M. C., M. J. Gill, S. S. Alibhai, M. S. Shahrabadi, and P. W. K. Lee.** 1987. Purification and characterization of the reovirus cell attachment protein σ1. Virology **156**:377–385.
56. **Yeung, M. C., M. J. Gill, S. S. Alibhai, M. S. Shahrabadi, and P. W. K. Lee.** 1989. The cell attachment proteins of type 1 and type 3 reovirus are differentially susceptible to trypsin and chymotrypsin. Virology **170**:62–70.
57. **Young, J. F., and P. Palese.** 1979. Evolution of human influenza A viruses in nature: recombination contributes to genetic variation of H1N1 strains. Proc. Natl. Acad. Sci. USA **76**:6547–6551.