

Sequences of the *S1* genes of the three serotypes of reovirus

(cloning/homology/evolution/genetic relatedness/reading frames)

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ABSTRACT The *S1* genes of the three serotypes of reovirus have been cloned and sequenced. The *S1* genes encode protein $\sigma 1$, the protein against which serotype-specific neutralizing antibodies are directed; it is also the reovirus hemagglutinin and cell-attachment protein and is a major determinant of host range/tissue specificity and of the nature of the interaction of reovirus with cells of the immune system. The *S1* genes of serotypes 1, 2, and 3 are 1458, 1442, and 1416 nucleotides long, respectively. They possess untranslated regions 13, 13, and 12 nucleotides long at their 5' termini and 188, 229, and 36 nucleotides long at their 3' termini. They possess two open reading frames. The first starts with a "weak" initiation codon and extends for 418, 399, and 455 codons, respectively; this is the size expected for the $\sigma 1$ proteins. The other reading frame starts at a "strong" initiation codon about 70 residues downstream from the 5' terminus but extends for only about 120 codons, being terminated by 3 in-phase termination codons in all three genes. The proteins encoded by these short open reading frames are quite basic. The serotype 1 and 2 *S1* genes are much more closely related to each other (28% homology) than to the serotype 3 *S1* gene (5% and 9% homology, respectively). These figures are based on direct homology calculations, adjusted for 25% random coincidence. Serologic evidence and hydrophobicity profiles agree that the $\sigma 1$ proteins of serotypes 1 and 2 are much more closely related to each other (about 40% homology) than to that of serotype 3 (only about 20% homology). The fact that the serotype 1 and 2 *S1* genes are much more closely related to each other than to the serotype 3 *S1* gene is remarkable since for all other nine reovirus genes the serotype 1 and 3 genes are much more closely related to each other than to the serotype 2 gene. Mechanisms that may effect this remarkable evolutionary pattern are discussed.

There are three serotypes of reovirus. As judged by the ability of their double-stranded RNAs to hybridize with each other under standardized conditions, serotypes 1 and 3 are closely related (about 70%), whereas serotype 2 is related to serotypes 1 and 3 only to the extent of about 10% (1). The individual genes of the three serotypes vary in their relatedness from those that are most closely related, the 3 *L* genes, to the gene that has diverged to the greatest extent during evolution—namely, the *S1* gene, which encodes protein $\sigma 1$ (1). Protein $\sigma 1$ is not only the most type-specific of all reovirus proteins but is also functionally of great importance. It is the protein against which the major neutralizing antibodies are formed (2); it is the hemagglutinin (3); it is the viral cell-attachment protein (4); it is the major determinant of the nature of the interaction of reoviruses with cells, including cells of the immune system (5–7); and it also appears to be the reovirus protein that switches off host cell DNA synthesis (8).

Remarkably, protein $\sigma 1$ is present in reovirus particles to the extent of only 24 molecules that are located pairwise at

12 positions on the reovirus surface where the core projections or spikes protrude through the outer capsid shell (4). Not only is protein $\sigma 1$ only a minor reovirus particle component but it is also synthesized in infected cells in only small amounts; its mRNA is one of the most inefficiently translated reovirus mRNAs [>50 times less efficiently than the most efficiently translated reovirus mRNA—namely, s4 RNA (unpublished results)].

We have devised a technique for cloning reovirus genes into pBR322 (9). We present here the sequences of the *S1* genes of all three reovirus serotypes, together with the deduced sequences of the $\sigma 1$ proteins that they encode.

MATERIALS AND METHODS

The method used for growing the Lang strain of reovirus serotype 1, the D5/Jones strain of serotype 2, and the Dearing strain of serotype 3 was that described by Smith *et al.* (10). The procedures used for extracting reovirus genomic RNA, transcribing it into full-length cDNA, and cloning the double-stranded cDNA into pBR322 have been described (9, 11). Total genomic RNA of each serotype was used as the template for preparing cDNA. Before cloning, the populations of single-stranded cDNA molecules were enriched for full-length *S1* gene transcripts by electrophoresis in alkaline agarose gels (11). The cloned *S1* genes were sequenced by subcloning various restriction fragments into M13 mp8 and mp9 vectors (New England Biolabs) (12) and using the chain-terminator method of Sanger *et al.* (13). All regions of all 3 genes were sequenced in both directions.

RESULTS

Sequences of the 3 *S1* Genes. The sequences of the *S1* genes of reovirus serotypes 1–3 are presented in Fig. 1. The 3 genes are 1458, 1442, and 1416 base pairs (bp) long, respectively. The first initiation codons start at positions 14, 14, and 13, respectively, and are followed by open reading frames 1254, 1197, and 1365 bp long, capable of encoding proteins 418, 399, and 455 amino acids long, respectively (Table 1). These are the sizes expected of the $\sigma 1$ proteins, based on electrophoretic migration rates in NaDodSO₄/polyacrylamide gels.

There is another initiation codon, in a different reading frame, that starts at residues 75, 66, and 71, respectively, in all 3 *S1* genes (Fig. 1). These are "strong" initiation codons (14), with purines in positions –3 and +4; by contrast, the upstream codons are all "weak" (Table 1). However, the $\sigma 1$ proteins are translated from the first set of initiation codons and not from the second, because the latter are followed by reading frames that are only 119, 125, and 120 codons long, respectively. Interestingly, these short reading frames are all terminated by 3 in-phase termination codons; they are clearly highly conserved.

Abbreviation: bp, base pair(s).

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1 GCTATTGGCGGCT **ATG** GAT GCA TCT CTC ATT ACA GAG ATA CGG AAA ATA GTA CTC CAA CTA TCT GTA TCA AGC AAT GGC TCC CAG TCA AAA 91
 2 GCTATTGGCACTC **ATG** TCG GAT CTA GTG CAG CTC ATA AGA AGG GAG ATC TTA CTG TTA ACT GGG AAT GGA GAA TCA GCC AAC TGG AAA CAC 91
 3 GCTATTGGTCCG **ATG** GAT CCT CGC CTA CGT GAA GAA GTA GTA CGG CTG ATA ATC GCA TTA ACG AGT GAT AAT GGA GCA TCA CTG TCA AAA 90

1 GAA ATC GAG GAA ATC AAG AAA CAA GTC CAG GTC AAC GTT GAT GAT ATC AGG GCT GCC AAT ATT AAA CTC GAC GGA CTT GGA AGA CAG ATT 181
 2 GAG ATC GAG GAA ATT AAG AAA CAA ATT AAA GAC ATC TCT GCT GAT GTC AAC AGG ATC AGT AAC ATC GTT GAT TCA ATC CAA GGA CAA CTG 181
 3 GGG CTT GAA TCA AGG GTC TCG GCG CTC GAG AAG ACG TCT CAA ATA CAC TCT GAT ACT ATC CTC CGG ATC ACC CAG GGA CTC GAT GAT GCA 180

1 GCT GAC ATC AGC AAT AGC ATC TCA ACC ATT GAG TCA AGA TTG GGT GAG ATG GAT AAT CGA CTT GTG GGT ATC TGG AGT CAG GTC ACG CAA 271
 2 GGT GGA TTA TCT GTA CGC GTG TCA GCC ATT GAA TCG GGA GTT AGT GAG AAC GGC AAT CGA ATT GAT AGA CTC GAG CGA GAT GTC TCC GGC 271
 3 AAC AAA CGA ATC ATC GCT CTT GAG CAA AGT GGG GAT GAC TTG GTT GCA TCA GTC AGT GAT GCT CAA CTT GCA ATC TCC AGA TTG GAA AGC 270

1 TTA TCT AAC TCA GGT AGC CAG AAC ACT CAG AGC ATA TCC TCA TTG GGT GAC AGA ATC AAT GCT GTC GAA CCA CGA GTT GAC AGT CTG GAT 361
 2 ATA TCG GCT AGC GTT AGC GGA ATC GAT TCG GGT TTA TCC GAG CTG GGT GAC CGA GTC AAT GTT GCA GAA CAG CGA ATT GGC CAG TTG GAT 361
 3 TCT ATC GGA GCC CTC CAA ACA GTT GTC AAT GGA CTT GAT TCG AGT GTT ACC CAG TTG GGT GCT CGA GTG GGA CAA CTT GAG ACA GGA CTT 360

1 ACG GTC ACG TCT AAT CTC ACT GGA CGA ACA TCC ACT TTG GAG GCA GAT GTT GGA AGC TTA CGG ACA GAA CTA GCA GCG CTA ACA AGA CGG 451
 2 ACA GTC ACG GAT AAT CTC CTT GAG CJA GCA TCA AGA CTC GAA ACT GAT GTA TCA GCC ATT ACT AAT GAC CTT GGA TCA TTG AAT ACG AGG 451
 3 GCA GAG CTA CGC GTT GAT CAC GAC AAT CTC GTT GCG AGA GTG GAT ACT GCA GAA CGT AAC ATT GGA TCA TTG ACC ACT GAG CTA TCA ACT 450

1 GTG ACA ACT GAG GTT ACA AGG TTA GAT GGT CTA ATC AAT AGT GGC CAG AAT TCG ATT GGT GAG CTA TTC ACA AGA CTA TCC AAT GTG GAG 541
 2 GTG ACG ACT GAA TTG AAC GAT GTC CGC CAA ACT ATT GCT GCG ATA GAC ACG CTT CTA ACG ACA CTG GAG ACG AAT GCC GTG ACG TCG GTT 541
 3 CTG ACG TTA GCA GTA ACA TCC ATA CAA GCG GAT TTG GAA TCT AGG ATA TCC AGC AGA TCA GAG CGC ACG GCG GTC ACT AGC GCG GGA GCT CCC 540

1 ACG TCT ATG GTG ACG ACG GCT GGA CGG GGA CTG CAG AAA AAC GGA AAC ACC TTG AAC GTC ATT GTA GGT AAT GGA ATG TGG TTT AAT AGT 631
 2 GGT CAA GGG CTT CAG AAG ACT GGG AAC TCG ATT AAG GTT ATT GTG GGT ACG GGG ATG TGG TTC GAC CGC AAT AAT GTT CTG CAG TTA TTC 631
 3 CTC TCA ATC CGT AAT AAC CGT ATG ACC ATG GGA TTA AAT GAT GGA CTC ACG TTG TCA GGG AAT AAT CTC GCC ATC CGA TTG CCA GGA AAT 630

1 TCT AAT CAA TTG CAG CTC GAC CTT TCG GGG CAA TCA AAA GGG GTG GGA TTT GTC GGC ACA GGA ATG GTG GTT AAG ATT GAT ACT AAT TAT 721
 2 TTA TCG AAC CAG CAG AAA GGG TTG GGA TTC ATA GAC AAT GGA ATG GTA GTG AAA ATA GAT ACC CAG TAT TTC AGC TTC ACT AGC AAT GGC 721
 3 ACG GGT CTG AAT ATT CAA AAT GGT GGA CTT CAG TTT CGA TTT AAT ACT GAT CAA TTC CAG ATA GTT AAT AAT AAC TTG ACT CTC AAG ACG 720

1 TTT GCT TAC AAT AGT AAT GGA GAG ATT ACA TTG GTG AGT CAA ATG AAT GAA TTG CCA TCG CGC GTA TCA ACA CTG GAA TCA GCG AAA ATC 811
 2 AAC ATA ACT CTG AAC AAC AAC ATA AGT GGT CTG CCG GCG CGA ACA GGT TCC CTC GAG GCA TCT GGT ATC GAT GTG GTA GCG CCA CGC CTT 811
 3 ACT GTG TTT GAT TCT ATC AAC TCA AGG ATA GGC GCA ACT GAG CAA AGT TAC GTG CCG TCG GCA GTG ACT CCG TTG AGA TTA AAC AGT AGC 810

1 GAT TCA GTT TTA CCT CCA TTA ACC GTA CGC GAA GCG AGC GGC GTC GAT CCG ACT CTS AGC TTT GGT TAT GAT ACG AGC GAT TTT ACA ATC ATC 901
 2 GTG ATA CAG TCT ACT GGT AGC ACT GGT CTA CTG GGT CTC ATG TAC GAG GCT GTG GTC GTT ACT AAC CAC TTC GTC GTC ACA CTG AGA 901
 3 ACG AAG GTG CTG GAT ATG CTA ATA GAC AGT TCA ACA CTT GAA ATT AAT TCT AGT GGA CAG CTA ACT GTT AGA TCG ACA TCC CCG AAT TTG 900

1 AAC TCC GTA CTG TCG TTA CGG TCA GGT TTG ACT CTT CCG ACA TAC AGG TAC CCF CTG GAG CTC GAC ACA GCA AAT AAT AGA GGA CAG GTG 991
 2 AAT CGA TCG GTC ACG CCA ACA TTC AAG TTT CCT CTG GAG TTG AAT AGT GCT GAT AAC TCA GTG AGC ATT CAT AGA AAT TAC CGC ATT AGA 991
 3 AGG TAT CCG ATA GGT GAT GTT AGC GGC GGT ATC GGA ATG AGT CCA AAT TAT AGG TTT AGG CAG AGC ATG TGG ATA GGA ATT GTC TCG TAT 990

1 GCA CAT GGT TTT GGC ATG GCG ACG GGT ACT TGG ACG GGA CAA TTG CAA TAT CAG CAC CGA CAA TTG AGT TGG AGA GCA AAT GTC ACT TTG 1081
 2 CTT GGG CAA TGG TCA GGT CAA TTG GAA TAT CAC ACG CCG AGT TTG GGT TGG AAT GCT CCG GTC ACG GTT AAT TTG ATG GGA GTA GAC GAT 1081
 3 TCT GGT AGT GGG CTG AAT TGG AGG GTA CAG GTC AAC TCC GAC ATT TTT ATT GTA GAT GAT TAC ATA CAT ATA TGT CTT GCA GCT TTT GAC 1080

1 AAT TTG ATG AAG GTG GAT GAT TGG TTG GTG TTG AGC TTT TCT CAG ATG ACG ACT AAC TCA ATA ATG GCA GAT GGG AAA TTT GTG ATT AAT 1171
 2 TGG CTC ATT TTG AGT TTT ACT CCG TTT TCG ACG AGC GGC GAT CTT AGC GTC AGG AAA GTT TGT ATT GAA CTT GGT AAC TGG TTT GTC TCC 1171
 3 GGT TTC TCT ATA GCT GAC GGT GGA GAT CTA TCG TTG AAC TTT GTT ACC GGA TTG TTA CCA CCG TTA CTT ACA GGA GAC ACT GAG CCG GCT 1170

1 TTT GTG TCT GGG TTA TCT TCT GGA TGG CAG ACG GGG GAT ACT GAA CCA TCG TCA ACT ATT GAT CCA TTG CTA CGA CAT TTG CCG CCG TCC 1261
 2 AGG GTG GGC GAC TGG GAG TAC CGA GCC CTG GAC AAC TAC **TAA** CCCACTGTCAACGAGGTTTGTGCAATTTCAGTTTCATCAATGGGTGCATCTCGGCTAGACGGCT 1276
 3 TTT CAT AAT GAC GTG GTC ACA TAT GGA GCA CAG ACT GTA GCT ATA GGG TTG TCG TCG GGT GGT GCG CCT CAG TAT ATG ACT AAG AAT CTG 1260

1 AAT TTC **TAA** ATAACGGTCAACCGCATTGATGCGTTTGGATCATGGGAGTATGGAATGGACGGATGGAGAATTAGACATTAAGAATTATGGTGGCACATACACCGGTCACTACTCAAG 1377
 2 TTAGAATCTTTGGAGTCGAGAGTGAATGCCGGGAACATGAGATCAGCAATCATGGCGGAACATATACAGCGCATAACCAATGTCGACTGGGCGCGGATGACCAATTATGACCCATGT 1395
 3 TGG GTG GAG CAG TGG CAG GAT GGA GTA CTT CCG TTA CCT GTT GAG GCG GGT GGC TCA ATT ACG CAC TCA AAC AGT AAG TGG CCT GCC ATG 1350

1 TATATTGGGCTCGGTGGACCATGATGATCCATGCAATGTGAGGTGAATCTAGCGGCAATCGGCACAAGGGGTCAATCATC 1458
 2 CTGGCTGAGGATCCGGTGTCCACACGCGGCACTGGCACTCATC 1442
 3 ACC GTT TCG TAC CCG CGT AGT TTC ACG **TGA** GGATCAGACCACCCCGCGGCACTGGGCACTTTTCATC 1416

Fig. 1. Sequences of the serotype 1-3 *SI* genes of reovirus. The sequences are those of the plus (mRNA) strands.

Relatedness of the 3 *SI* Genes. The relatedness of the 3 *SI* genes was determined by assessment of base/base identities after locating possible deletions and insertions in each gene and matching cognate sequences. The results are presented

in Table 2. The *SI* genes of serotypes 1 and 2 are much more closely related to each other than to that of serotype 3; the absolute homologies of the *SI* gene of serotype 3 with those of serotypes 1 and 2 exceed the 25% random matching level

Table 1. Organization of the *SI* genes of the three reovirus serotypes

Sero-type	Length of 5'-untranslated sequences, bp	Sequences around first initiation codon	Length of long reading frame, bp	Position of second initiation codon	Sequences around second initiation codon	Length of short reading frame, bp	Length of 3'-untranslated sequences, bp
1	13	CCT ATG G	1254	75*	GCA ATG G	357	188
2	13	CTC ATG T	1197	66	GGA ATG G	375	229
3	12	CGG ATG G	1365	71	ATA ATG G	360	36

*This is the third initiation codon. The second initiation codon is followed by a termination codon 10 codons downstream.

Table 2. Relatedness of the 3 *S1* genes of reovirus

Serotypes	% homology		
	Long reading frame	Conserved portion of 3'-terminal untranslated region	$\sigma 1$ proteins
1:2	28	46	36
1:3	5	21	16
2:3	9	27	14

All values are corrected for random coincidences (25% for nucleic acid and 5% for protein sequences, respectively).

by only 5% and 9%, respectively. The homologies of the long and short open reading frames are similar. Interestingly, there is a rather highly conserved 96-bp sequence near the 3' terminus of the plus strand of all 3 genes (Fig. 2 and Table 2); for this sequence the direct homology values, uncorrected for random coincidences, range from almost 50% to >70%. The significance of this high degree of relatedness is not clear, especially since only in the serotype 3 gene is this sequence in the coding region.

Amino Acid Sequences of the Three $\sigma 1$ Proteins. The amino acid sequences of the three $\sigma 1$ proteins are presented in Fig. 3. There is significant homology between all these proteins provided that account is taken of several deletions/insertions. The sequences of the serotype 1 and 2 proteins are remarkably congruent; there is only one deletion or insertion of 7 amino acids starting at position 150 and two minor deletions/insertions near the COOH terminus. The sequence of the serotype 3 protein is only slightly less congruent; relative to the sequence of the serotype 1 protein, it contains an insertion of 4 amino acids at positions 33–36, a deletion of 14 amino acids starting at residue 153, and a deletion of 7 amino acids starting at position 316.

When cognate sequences are brought into apposition in this manner, the serotype 1 and 2 $\sigma 1$ proteins are 41% homologous with each other and 21% and 19%, respectively, with the $\sigma 1$ protein of serotype 3. Forty-three amino acids (slightly more than 10%) are shared by the $\sigma 1$ proteins of all three serotypes.

The amino acid compositions of the three $\sigma 1$ proteins are unremarkable. They contain about the same number of hydrophobic and uncharged polar amino acids and of basic and acidic amino acids. The serotype 1 $\sigma 1$ protein lacks cysteine, and the others contain only one molecule of cysteine. Thus, the pairs of $\sigma 1$ protein molecules that exist in reovirus particles are unlikely to be -SS-linked dimers.

The hydrophobicity profiles of the three $\sigma 1$ proteins are shown in Fig. 4. The serotype 1 and 2 $\sigma 1$ protein profiles are seen to be rather similar, but that for the serotype 3 protein is quite different, except in the terminal regions. It is conceivable that the domain for the conserved $\sigma 1$ function—namely, ability to bind to cells—is located in one or other of the terminal regions.

Molecular Properties of the Proteins Encoded by the Short Reading Frames. The short reading frames of the *S1* genes of serotypes 1, 2, and 3 are capable of encoding proteins that are 119, 125, and 120 amino acids long, respectively. These proteins are basic: they contain 29–35 lysine and arginine residues but only 10–13 aspartic and glutamic acid residues.

They contain 46–49 nonpolar and 39–44 uncharged polar amino acids. The proteins of serotypes 1 and 2, 1 and 3, and 2 and 3 share 35%, 17%, and 27% homology, respectively.

DISCUSSION

The *S1* gene is the reovirus gene that has diverged most markedly during evolution (1). The serotype 1 and 2 *S1* genes are still related, after taking account of sequence deletions and insertions, to the extent of 53%, but the serotype 3 *S1* gene is related to the other two to the extent of only 30% and 34%, respectively. After adjusting for a background of 25% for random matching, these values reduce to absolute homology values of 28%, 5%, and 9%, respectively.

These gene homology relationships are reflected in those of the three $\sigma 1$ proteins. The serotype 1 and 2 $\sigma 1$ proteins share 41% of their amino acid residues. Many of the amino acid substitutions are conservative and the hydrophobicity profiles of the two proteins are very similar. The serotype 3 $\sigma 1$ protein, on the other hand, is very different. It shares no more than 20% of its amino acids with either the serotype 1 or the serotype 2 $\sigma 1$ protein, and its hydrophobicity profile is significantly different from that of the other two.

The reading frames from which the $\sigma 1$ proteins are translated are clearly the long open reading frames that start at residue 13 or 14. The fact that the initiation codons at the beginning of these reading frames are weak is in accord with the fact that the *S1* mRNAs are translated poorly and that only small amounts of the $\sigma 1$ proteins are formed in infected cells (16). By contrast, the initiation codons that open up the short open reading frame are strong, and ribosome binding protection (17) and f-Met dipeptide formation experiments (18) indicate that they are functional. It should be possible to identify these proteins in infected cells, possibly by the use of antisera directed against certain regions of these putative proteins.

A curious feature of the $\sigma 1$ protein of serotype 3 is its length; it is the largest of the three $\sigma 1$ proteins, but in every NaDodSO₄/polyacrylamide system that has been used—in particular, in the phosphate-based system of Zweerink *et al.* (19) and in the Tris/glycine systems of Laemmli (20) and Maizel (21)—it migrates more rapidly than the $\sigma 1$ proteins of serotypes 1 and 2. In fact, in the Maizel system the serotype 3 $\sigma 1$ protein migrates faster than the serotype 3 $\sigma 2$ protein, the molecular weight of which is known to be about 38,000 (9); in the other two systems its molecular weight, compared with that of a variety of marker proteins, is about 42,000 (10, 19). However, its actual molecular weight is almost 48,000. It has been suggested that the $\sigma 1$ protein may be associated with the plasma membrane (22); but the $\sigma 1$ protein in mature reovirus particles does not appear to be a cleavage product of a precursor because the size of the free form of protein $\sigma 1$ in infected cells is the same as that of the form that exists in mature virus particles (19). Thus, the reason for the size discrepancy remains to be discovered.

The various monoclonal antibodies that have been raised against the $\sigma 1$ protein of reovirus serotype 3 differ in their relative ability to neutralize infectivity on the one hand and inhibit hemagglutination (that is, cell binding) on the other (23, 24). This suggests that the $\sigma 1$ proteins comprise at least two domains—one containing the dominant epitope and the

Serotype	Sequence	Length
1	AATGGACGGATGGAGAATTAGAGATTAAAGAATTATGGTGGCAGATACACGGTGCATCTCAAGTATATTGGGCTCCGTGGACGATCATGTATCCATG	1411
2	.G...AT.CC..G...C.....C.C...C...C..A....T..A.CG....CA.T..CG.C....G...AT...C...T.....C.....	1394
3	.G...CA.....T.C.TCG.T.ACGTGT.C.G..G..TGGC.CA.TTACG..CT.AA.CAGTA.G...C..G.CAT...CG.TTC...C..GC.	1367

FIG. 2. Sequences of conserved regions near the 3' termini of the plus strands of the 3 *S1* genes. Identity is indicated by periods. The sequences are 96 nucleotides long.

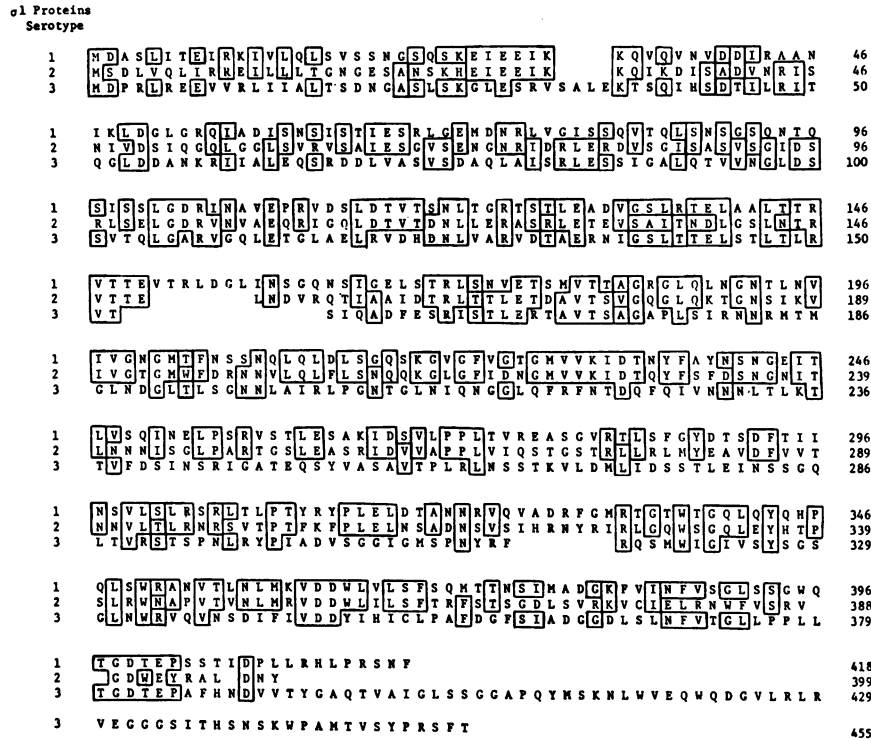


FIG. 3. Sequences of the $\sigma 1$ proteins of reovirus serotypes 1-3.

other containing the region with affinity for cell receptors. Further, the amino acid residues that are shared between the three $\sigma 1$ proteins tend to be clustered in the terminal regions (see Fig. 3); this is indicative of conservation of function and also suggests the existence of domains. Availability of the sequences of the three $\sigma 1$ proteins opens up several approaches for detailed studies concerning their functions.

A remarkable aspect of the 3 *S1* genes is the nature of their genetic relatedness pattern. The total genomes of serotypes 1 and 3 are far more closely related to each other than to that of serotype 2 (1). All individual genes show the same pattern: all individual genes of serotypes 1 and 3 are much more closely related to each other than to those of serotype 2. The only exceptions are the 3 *S1* genes for which the sequence

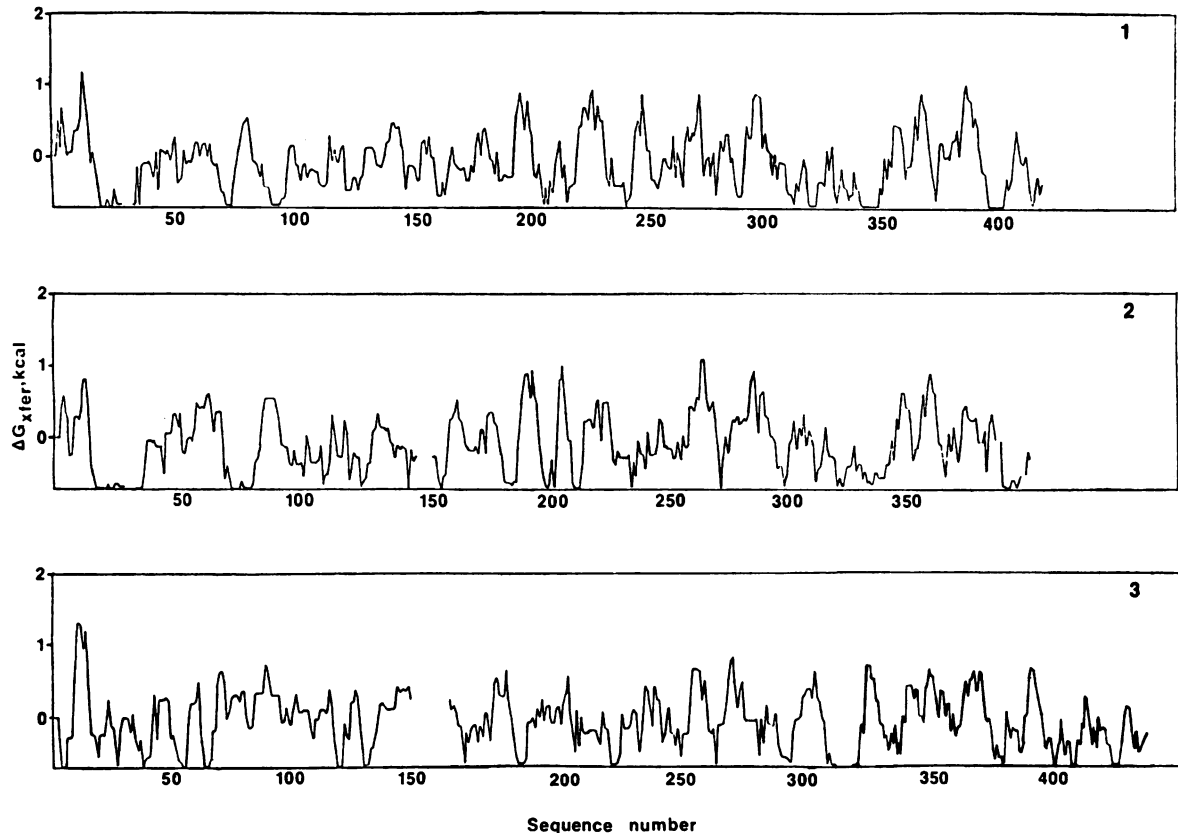


FIG. 4. Hydrophobicity profiles (15) of the $\sigma 1$ proteins of reovirus serotypes 1-3.

analysis presented here demonstrates that the genes of serotypes 1 and 2 are much more closely related to each other than to that of serotype 3. This agrees with the antigenic evidence that antibodies against serotype 3 protein $\sigma 1$ are absolutely specific, whereas those against serotype 1 and 2 $\sigma 1$ proteins are partly cross-reactive (25). Thus, 9 of the 10 reovirus genes show a serotype 1:3 relatedness pattern, whereas the *S1* gene exhibits serotype 1:2 relatedness. Perhaps the ready occurrence of gene reassortment is responsible for this pattern of evolution, for one possible explanation for it is that at some time during evolution the *S1* genes of serotypes 2 and 3 became associated with each other's (that is, the heterologous) gene pools. It is conceivable that the evolution of an ancestral reovirus did indeed proceed along three independent major pathways, yielding three independent gene pools (1), with the *S1* gene diverging most rapidly because of selective immunologic pressures on the one hand and tolerance of extensive structural diversification on the other (because protein $\sigma 1$ does not appear to be a true capsomer component, in contrast to the other two reovirus outer shell components, $\mu 1C$ and $\sigma 3$). Reassortants would be formed readily but be selected against because heterologous capsids would be less stable than homologous ones. At some relatively recent stage of evolution, exchange of serotype 2 and 3 *S1* genes might then have been favored by some selective mechanism like improved stability of heterologous $\sigma 1$ -capsid combinations caused by a structural modification, or the characteristics of the available immunologic pressures, perhaps modified by the acquisition of new host species.

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1. Gaillard, R. K. & Joklik, W. K. (1982) *Virology* **123**, 152-164.
2. Weiner, H. L. & Fields, B. N. (1977) *J. Exp. Med.* **146**, 1305-1310.
3. Weiner, H. L., Ramig, R. F., Mustoe, T. A. & Fields, B. N. (1978) *Virology* **86**, 581-584.
4. Lee, P. W. K., Hayes, E. C. & Joklik, W. K. (1981) *Virology* **108**, 156-163.
5. Weiner, H. L., Drayna, D., Averill, D. R., Jr., & Fields, B. N. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5744-5748.
6. Fontana, A. & Weiner, H. L. (1980) *J. Immunol.* **125**, 2660-2664.
7. Weiner, H. L., Greene, M. I. & Fields, B. N. (1980) *J. Immunol.* **125**, 278-282.
8. Sharpe, A. H. & Fields, B. N. (1981) *J. Virol.* **38**, 389-392.
9. Cashdollar, L. W., Esparza, J., Hudson, G. R., Chmelo, R., Lee, P. W. K. & Joklik, W. K. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7644-7648.
10. Smith, R. E., Zweerink, H. J. & Joklik, W. K. (1969) *Virology* **39**, 791-810.
11. Cashdollar, L. W., Chmelo, R., Esparza, J., Hudson, G. R. & Joklik, W. K. (1984) *Virology* **133**, 191-196.
12. Messing, J., Crea, R. & Seeburg, P. H. (1981) *Nucleic Acids Res.* **9**, 309-321.
13. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467.
14. Kozak, M. (1981) *Nucleic Acids Res.* **9**, 5233-5252.
15. Rose, G. D. & Roy, S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 4643-4647.
16. Zweerink, H. J. & Joklik, W. K. (1970) *Virology* **41**, 501-518.
17. Kozak, M. (1982) *J. Mol. Biol.* **156**, 807-820.
18. Cenatiempo, Y., Twardowski, T., Shoeman, R., Ernst, H., Brot, N., Weissbach, H. & Shatkin, A. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1084-1088.
19. Zweerink, H. J., McDowell, M. J. & Joklik, W. K. (1971) *Virology* **45**, 716-723.
20. Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685.
21. Maizel, J. V. (1971) *Methods Virol.* **5**, 177-246.
22. Kauffman, R. S., Lee, S. & Finberg, R. (1983) *Virology* **131**, 265-273.
23. Hayes, E. C., Lee, P. W. K., Miller, S. E. & Joklik, W. K. (1981) *Virology* **108**, 147-155.
24. Spriggs, D. R., Kaye, K. & Fields, B. N. (1983) *Virology* **127**, 220-224.
25. Gaillard, R. K. & Joklik, W. K. (1980) *Virology* **107**, 533-536.