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# Computational Sequence Analysis of Mammalian Reovirus Proteins

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**Abstract.** In the present study, computer-assisted searches for sequence similarities were performed with amino acid sequences from mammalian reovirus proteins. These analysis revealed that many proteins of reovirus are partially similar to known viral or cellular proteins. Consensus sequences have been identified that are in accordance with already suspected functions of reovirus proteins. The analysis has also revealed unexpected similarities of some reovirus proteins with specific classes of proteins which sequences are present in the databases. This could suggest yet unidentified activities for some of the reovirus proteins.

**Key words:** reovirus, computer analysis

## Introduction

Mammalian reoviruses are members of the *Reoviridae* family and infect a variety of mammalian species but have not yet been clearly associated with any serious human disease (1). Despite this low pathogenicity in humans, reoviruses have been extensively used as model system and have provided numerous insights in the study of transcription, translation and virus-cell interactions. These nonenveloped viruses contain 10 segments of genomic double-stranded RNA (dsRNA) enclosed in two concentric icosahedral capsids made of eight viral proteins (1). The reovirus inner capsid consists of three major proteins (λ1, λ2, and σ2) and two minor proteins (λ3 and μ2) while the outer capsid is composed primarily of three proteins (σ1, σ3, and μ1C). Recent structural studies have demonstrated that most of λ2 resides within the outer capsid and suggest that λ2 can also be considered as an outer capsid protein bound to the inner capsid (2–4). A schematic representation of the position of the various proteins in both outer and inner capsid is shown in Fig. 1. The reovirus genome also encodes three nonstructural proteins (σNS, μNS, and σ1S) that are not incorporated into mature virions but likely mediate various functions during viral multiplication (1).

Three serotypes of mammalian reoviruses have been identified by seroneutralization and hemagglutination-inhibition assays (1). All of the genes from prototype strain Dearing (serotype 3; T3D) and many of the genes from prototype strains Lang (serotype 1; T1L) and Jones (serotype 2; T2J) have now been sequenced either from cDNAs or directly from genomic RNA (1). Homologous gene segments from the three prototypes are similar in size and nucleotide sequence, although the S1 genes exhibit greater variability than the other 9 genes (5). This divergence in S1 sequence is in accordance with the observation that the σ1 protein, encoded by the S1 gene, is the principal antigen against which type-specific neutralizing antibodies are directed (6,7).

Due to the segmented nature of their genome, reoviruses have been extensively studied by genetic analysis (reviewed in 8). Analysis of gene reassortants generated upon co-infection with two different viral strains has thus allowed the assignment of certain functions to specific viral proteins. In the past few years, rapid accumulation of nucleic acids and deduced amino acids sequences in databases has led

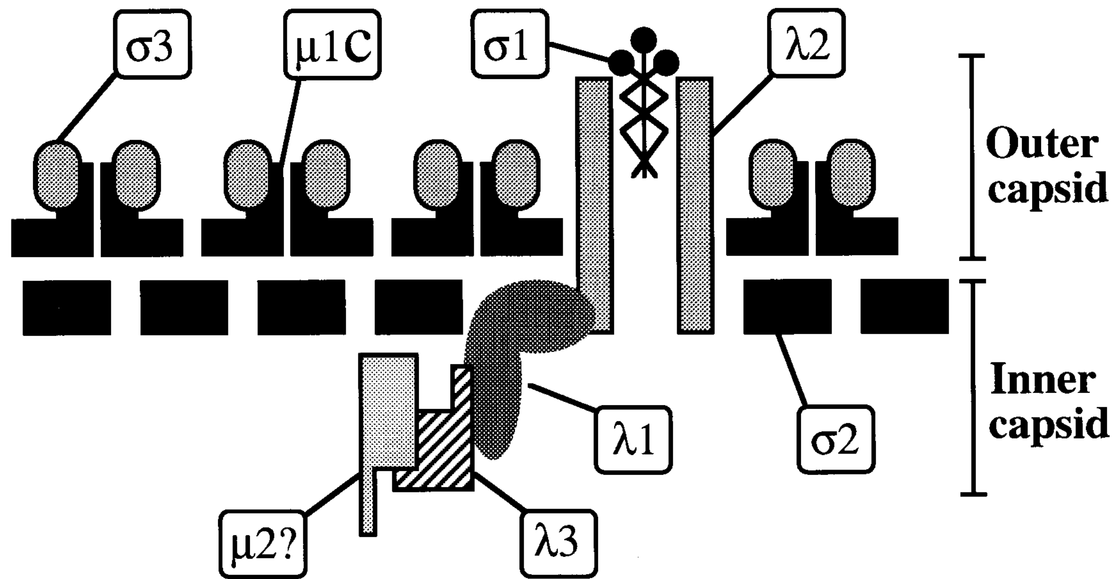


*Fig. 1.* Schematic representation of the position of the various mammalian reovirus proteins in the outer and inner capsid. Proteins are not drawn to scale.

to important progress in structure-function studies of proteins from various sources. Although the nucleotide sequence of all ten reovirus genes has been determined, this information has only been partly exploited to increase our understanding of the structural and functional properties of reovirus proteins. In the present study, computer-assisted searches for sequence similarities were performed on all eleven proteins from mammalian reoviruses. These searches were performed using several programs to identify global or local regions of significant similarity between reovirus proteins and other amino acid sequences. These analysis revealed that many proteins of reoviruses are partially similar to known viral or cellular proteins. The results obtained with 7 of the 11 reovirus proteins are systematically presented, compared with known or suspected functions of these proteins, and significance of these results is briefly discussed in the context of reovirus multiplication.

## Methods

Amino acid sequences were obtained from the SWISS-PROT and Genbank databases that are combined in the nonredundant sequence database at the National Center for Biotechnology Information (NCBI). A list of sequences used in this study, and their identification number, is presented in Table 1. Database searches for sequence similarity were performed using the BLASTP program and the BLOSUM62 matrix for comparisons of amino acids residues (9,10). The BLASTP program seeks local, as opposed to global alignments, to detect relationships among sequences which share only isolated regions of similarity. The BLOSUM62 matrix was constructed by Henikoff et al. (10) and is based on substitution patterns within ungapped local alignments of short regions of related sequences.

Multiple alignments of amino acids sequences were generated using the Clustal V algorithm (11). The probability that matches occurred due to chance (e.g., $P = 0.05$ signifies that there is a 5% chance that the same match could occur between random sequences of the same size) is also included for some of the generated alignments. Finally, the ProDom protein domain database (Release 34.1) was also used to search for homologous domains in the SWISS-PROT database using the DOMAINER algorithm (12,13).

## σ3

The σ3 protein, in association with μ1C, is the main component of reovirus outer capsid. It has been shown

*Table 1.* Protein sequences used in this study

| Designation[a] | Protein | Origin | ID Number[b] |
|---|---|---|---|
| Sigma 3 T3D | Sigma 3 | Reovirus type 3 strain Dearing | VSI3_REOVD |
| Sigma 3 T2J | Sigma 3 | Reovirus type 2 strain Jones | VSI3_REOVJ |
| Sigma 3 T1L | Sigma 3 | Reovirus type 1 strain Lang | VSI3_REOVL |
| 2A Cox B1 | Protease 2A | Coxsackievirus B1 | POLG_COXB1 |
| 2A Bov entero | Protease 2A | Bovine enterovirus strain VG-5-27 | POLG_BOVEV |
| 2A Cox A9 | Protease 2A | Coxsackievirus A9 strain Griggs | POLG_CXA9 |
| 2A Cox B3 | Protease 2A | Coxsackievirus B3 | POLG_COXB3 |
| 2A Cox B4 | Protease 2A | Coxsackievirus B4 | POLG_COXB4 |
| 2A Cox B5 | Protease 2A | Coxsackievirus B5 | POLG_COXB5 |
| 2A Swine Ves H | Protease 2A | Swine vesicular disease virus strain H/3 ′76 | POLG_SVDVH |
| 2A Swine Ves U | Protease 2A | Swine vesicular disease virus strain UKG/27/72 | POLG_SVDVU |
| 2A Entero 70 | Protease 2A | Human enterovirus 70 | POLG_HUEV7 |
| 2A Cox A21 | Protease 2A | Coxsackievirus A21 | POLG_CXA21 |
| 2A Cox A24 | Protease 2A | Coxsackievirus A24 | POLG_CXA24 |
| 2A Polio 1 M | Protease 2A | Poliovirus type 1 strain Mahoney | POLG_POL1M |
| 2A Polio 1 S | Protease 2A | Poliovirus type 1 strain Sabin | POLG_POL1S |
| 2A Polio 2 L | Protease 2A | Poliovirus type 2 strain Lansing | POLG_POL2L |
| 2A Polio 2 W | Protease 2A | Poliovirus type 2 strain W-2 | POLG_POL2W |
| 2A Polio 3 | Protease 2A | Poliovirus type 3 strain 23127 | POLG_POL32 |
| 2A Polio 3 L | Protease 2A | Poliovirus type 3 strain P3/Leon/37 | POLG_POL3L |
| 2A Rhino 14 | Protease 2A | Human rhinovirus 14 | POLG_HRV14 |
| 2A Rhino 1B | Protease 2A | Human rhinovirus 1B | POLG_HRV1B |
| 2A Rhino 2 | Protease 2A | Human rhinovirus 2 | POLG_HRV2 |
| 2A Rhino 89 | Protease 2A | Human rhinovirus 89 | POLG_HRV89 |
| 2A Echo 11 | Protease 2A | Echovirus 11 strain Gregory | POLG_EC11G |
| RIP C Phy am | Antiviral protein C | *Phytolacca americana* | RIPC_PHYAM |
| RIP S Phy am | Antiviral protein S | *Phytolacca americana* | RIPS_PHYAM |
| RIP A Phy am | Antiviral protein alpha | *Phytolacca americana* | RIPA_PHYAM |
| RIP 6 Sapof | Ribosome inactivating protein saporin-6 | *Saponaria officinalis* | RIP6_SAPOF |
| RIP 2 Sapof | Ribosome inactivating protein saporin-2 | *Saponaria officinalis* | RIP2_SAPOF |
| RIP 0 Diaca | Antiviral protein DAP-30 | *Dianthus caryophillus* | RIP0_DIACA |
| RIP 2 Momba | Ribosome inactivating protein momordin II | *Momordica balsamina* | RIP2_MOMBA |
| RIP A Lufcy | Ribosome inactivating protein luffin-alpha | *Luffa cylindrica* | RIPA_LUFCY |
| RIP T Triki | Ribosome inactivating protein alpha-trichosanthin | *Trichosanthes kirilowii* | RIPT_TRIKI |
| RIP S Triki | Ribosome inactivating protein karasurin | *Trichosanthes kirilowii* | RIPS_TRIKI |
| RIP B Lufcy | Ribosome inactivating protein luffin-B | *Luffa cylindrica* | RIPB_LUFCY |
| RIP 1 Momch | Ribosome inactivating protein momordin I | *Momordica balsamina* | RIP1MOMCH |
| RICI Ricco | Ricin | *Ricinus communis* | RICI_RICCO |
| AGGL Ricco | Agglutinin | *Ricinus communis* | AGGL_RICCO |
| ABRC Abrpr | Abrin-C | *Abrus precatorius* | ABRC_ABRPR |
| ABRA Abrpr | Abrin-A | *Abrus precatorius* | ABRA ABRPR |
| Sigma 1 T3D | Sigma 1 | Reovirus type 3 strain Dearing | VSI1_REOVD |
| Lysozyme SF6 | Lysozyme | Bacteriophage SF6 | LY_BPSF6 |
| Sigma 1 T2J | Sigma 1 | Reovirus type 2 strain Jones | VSI1_REOVJ |
| Sigma 1 T1L | Sigma 1 | Reovirus type 1 strain Lang | VSI1_REOVL |
| Mu NS T3D | Mu NS | Reovirus type 3 strain Dearing | VM3_REOVD |

*Table 1. (Continued)*

| Designation[a] | Protein | Origin | ID Number[b] |
|---|---|---|---|
| Beta myosin human | Myosin Heavy chain, cardiac muscle beta-isoform | *Homo sapiens* (Human) | MYSB_HUMAN |
| Embryo myosin human | Myosin Heavy chain, fast skeletal muscle embryonic | *Homo sapiens* (Human) | MYSE_HUMAN |
| Alpha myosin human | Myosin Heavy chain, cardiac muscle alpha-isoform | *Homo sapiens* (Human) | MYSA_HUMAN |
| Embryo myosin rat | Myosin Heavy chain, fast skeletal muscle embryonic | *Rattus norvegicus* (Rat) | MYSE_RAT |
| Alpha myosin rat | Myosin Heavy chain, cardiac muscle alpha-isoform | *Rattus norvegicus* (Rat) | MYSA_RAT |
| Beta myosin rat | Myosin Heavy chain, cardiac muscle beta-isoform | *Rattus norvegicus* (Rat) | MYSB_RAT |
| Myosin chick | Myosin Heavy chain, fast skeletal muscle embryonic | *Gallus gallus* (Chicken) | MYSE_CHICK |
| Cytokeratin I human | Cytokeratin I | *Homo sapiens* (Human) | K2C1_HUMAN |
| Cytokeratin 6D human | Cytokeratin 6D | *Homo sapiens* (Human) | K2CD_HUMAN |
| Cytokeratin I mouse | Cytokeratin I | *Mus musculus* (Mouse) | K2C1_MOUSE |
| Cytokeratin 8 bovine | Cytokeratin 8 | *Bos taurus* | K2C8_BOVIN |
| Keratin IX. laevis | Cytokeratin I | *Xenopus laevis* | K2C1_XENLA |
| Keratin IIX. laevis | Cytokeratin II | *Xenopus laevis* | K2C2_XENLA |
| Lambda 3 T3D | Lambda 3 | Reovirus type 3 strain Dearing | VL3_REOVD |
| Lambda 3 T2J | Lambda 3 | Reovirus type 2 strain Jones | VL3_REOVJ |
| Lambda 3 T1L | Lambda 3 | Reovirus type 1 strain Lang | VL3_REOVL |
| Bovine rotavirus | RNA-directed RNA polymerase | Simian rotavirus SA11 | RRO_ROTS1 |
| Bluetongue | RNA-directed RNA polymerase | Bluetongue virus serotype 10 | RRPL_BTV10 |
| S. cerevisiae LA virus | RNA polymerase | S. cerevisiae virus L-A | Genbank 557596 |
| Yellow fever virus | Polyprotein | Yellow fever virus strain Pasteur 17D-204 | POLG_YEFV1 |
| West Nile virus | Polyprotein | West Nile virus | POLG_WNV |
| Dengue fever 2 | Polyprotein | Dengue fever virus type 2 strain Jamaica | POLG_DEN2J |
| Japanese encephalitis | Polyprotein | Japanese encephalitis virus strain JaOArS982 | POLG_JAEVJ |
| Poliovirus 1M | Polyprotein | Poliovirus type 1 strain Mahoney | POLG_POL1M |
| EMC virus | Polyprotein | Encephalomyocarditis virus strain EMC-D | POLG_EMCVD |
| Rhinovirus 14 | Polyprotein | Human rhinovirus 14 | POLG_HRV14 |
| Foot and mouth | Polyprotein | Foot and mouth disease virus A | POLG_FMDV1 |
| Coxsackievirus B1 | Polyprotein | Coxsackievirus B1 | POLG_COXB1 |
| Hepatitis A | Polyprotein | Hepatitis A virus strain 18F | POLG_HPAV8 |
| Tobacco mosaic | RNA-directed RNA polymerase | Tobacco mosaic virus | RRPO_TMV |
| Alfalfa mosaic | 90 kDa protein | Alfalfa mosaic virus | V90K_AMVLE |
| Brome mosaic | Protein 2A | Brome mosaic virus | V2A_BMV |
| Cucumber mosaic | Protein 2A | Cucumber mosaic virus strain FNY | V2A_CMVFN |
| Sindbis virus | Polyprotein | Sindbis virus | POLN_SINDV |
| Semliki Forest | Nonstructural polyprotein | Semliki Forest virus | POLN_SFV |
| Ross River virus | Polyprotein | Ross River virus | POLN_RRVT |
| Lambda 1 T3D | Lambda 1 | Reovirus type 3 strain Dearing | VL1_REOVD |
| Human eIF-4A | Eukaryotic initiation factor 4A | *Homo sapiens* (Human) | IF41_HUMAN |
| NDH II bovine | ATP-dependent RNA helicase | *Bos taurus* | RNHA_BOVIN |
| NDH II Human | ATP-dependent RNA helicase | *Homo sapiens* (Human) | RNHA_HUMAN |
| Vaccinia 18R | Helicase (NPH-II) | Vaccinia virus strain WR | VI08_VACCV |
| Fowlpox 18R | Helicase | Fowlpox virus | |
| Human p68 | p68 | *Homo sapiens* (Human) | P68_HUMAN |
| Dengue fever 4 | Polyprotein | Dengue fever virus type 4 | POLG_DEN4 |

*Table 1. (Continued)*

| Designation[a] | Protein | Origin | ID Number[b] |
|---|---|---|---|
| RAD3 S. cerevisiae | Yeast DNA repair helicase RAD3 | *Saccharomyces cerevisiae* | RAD3_YEAST |
| Vaccinia | mRNA capping enzyme | Vaccinia virus | MCEL_VACCV |
| Rabbit fibroma | mRNA capping enzyme | Rabbit fibroma virus | MCEL_SFVKA |
| Variola major | mRNA capping enzyme | Variola major virus | MCEL_VARV |
| African Swine | mRNA capping enzyme | African Swine fever virus | MCE_ASFB7 |
| CET1 | mRNA capping enzyme (β subunit) | *Saccharomyces cerevisiae* | CET1_YEAST |
| Mu 2 T3D | Mu 2 | Reovirus type 3 strain Dearing | VM1_REOVD |
| Human ADA | Adenosine deaminase | *Homo sapiens* (Human) | ADA_HUMAN |
| Mouse ADA | Adenosine deaminase | *Mus musculus* (Mouse) | ADA_MOUSE |
| E. coli ADA | Adenosine deaminase | *Escherichia coli* | ADD_ECOLI |
| E. coli ADEC | Adenine deaminase | *Escherichia coli* | ADEC_ECOLI |
| B. subtilis ADEC | Adenine deaminase | *Bacillus subtilis* | ADEC_BACSU |
| Rat DRADA | Double-stranded RNA specific adenosine deaminase | *Rattus norvegicus* (Rat) | DSRA_RAT |
| Human DRADA | Double-stranded RNA specific adenosine deaminase | *Homo sapiens* (Human) | DSRA_HUMAN |
| Rat RED1 | Double-stranded RNA specific editase | *Rattus norvegicus* (Rat) | RED1_RAT |
| Lambda 2 T3D | Lambda 2 | Reovirus type 3 strain Dearing | MCE_REOVD |
| Bluetongue 11 | VP4 core protein | Bluetongue virus serotype 11 | VP4_BTV11 |
| Bluetongue 13 | VP4 core protein | Bluetongue virus serotype 13 | VP4_BTV13 |
| Bluetongue 10 | VP4 core protein | Bluetongue virus serotype 10 | VP4_BTV10 |
| Bluetongue 17 | VP4 core protein | Bluetongue virus serotype 17 | Genbank 387917 |
| Simian rotavirus | VP3 core protein | Simian rotavirus strain SA11 | VP3_ROTSI |
| Porcine rotavirus | VP3 core protein | Porcine rotavirus C strain Cowden | VP3_ROTPC |
| Human rotavirus | Guanylyltransferase | Human rotavirus C | NA[c] |
| Bovine rotavirus | VP3 core protein | Bovine rotavirus group C | Genbank 1353254 |
| S. cerevisiae | Yeast ABD1 protein | *Saccharomyces cerevisiae* | ABD1_YEAST |
| Dengue fever 2P | Polyprotein | Dengue fever virus type 2 strain PR159/S1 | POLG_DEN2P |
| Dengue fever 1 | Polyprotein | Dengue fever virus type 1 strain Singapore S275/90 | POLG_DEN1S |
| Dengue fever 3 | Polyprotein | Dengue fever virus type 3 | POLG_DEN3 |
| Parainfluenza 3 HW | Hemagglutinin-neuraminidase | Parainfluenza virus type 3 strain 64179 | HEMA_PI3HW |
| Parainfluenza 3 HV | Hemagglutinin-neuraminidase | Parainfluenza virus type 3 strain Tex/12677/83 | HEMA_PI3HV |
| Parainfluenza 3 HT | Hemagglutinin-neuraminidase | Parainfluenza virus type 3 strain Tex 545/80 | HEMA_PI3HT |
| Parainfluenza 3 HX | Hemagglutinin-neuraminidase | Parainfluenza virus type 3 strain Wash/1511/73 | HEMA_PI3HX |
| Parainfluenza 3 HU | Hemagglutinin-neuraminidase | Parainfluenza virus type 3 strain Tex 9305/82 | HEMA_PI3HU |
| Parainfluenza 3 HA | Hemagglutinin-neuraminidase | Parainfluenza virus type 3 strain Aus/124854/74 | HEMA_PI3HA |
| Parainfluenza 3 H4 | Hemagglutinin-neuraminidase | Parainfluenza virus type 3 strain 47885 | HEMA_PI3H4 |
| Parainfluenza 3 B | Hemagglutinin-neuraminidase | Bovine parainfluenza virus type 3 | HEMA_PI3B |
| Sendai virus H | Hemagglutinin-neuraminidase | Sendai virus strain Harris | HEMA_SENDH |
| Sendai virus F | Hemagglutinin-neuraminidase | Sendai virus strain Fushimi | HEMA_SENDF |
| Sendai virus J | Hemagglutinin-neuraminidase | Sendai virus strain HVJ | HEMA_SENDJ |
| Sendai virus Z | Hemagglutinin-neuraminidase | Sendai virus strain Z | HEMA_SENDZ |
| Sendai virus 5 | Hemagglutinin-neuraminidase | Sendai virus strain Z host mutant | HEMA_SEND5 |
| Parainfluenza 1 HW | Hemagglutinin-neuraminidase | Parainfluenza virus type 1 | HEMA_PI1HW |

*Table 1. (Continued)*

| Designation[a] | Protein | Origin | ID Number[b] |
|---|---|---|---|
| | | strain Washington/1957 | |
| NDV J | Hemagglutinin-neuraminidase | Newcastle disease virus strain IBA/85 | HEMA_NDVJ |
| NDV L | Hemagglutinin-neuraminidase | Newcastle disease virus strain LAS/46 | HEMA_NDVL |
| NDV H4 | Hemagglutinin-neuraminidase | Newcastle disease virus strain B1-Hitchner/47 | HEMA_NDVH4 |
| NDV TG | Hemagglutinin-neuraminidase | Newcastle disease virus strain Texas G.B./48 | HEMA_NDVTG |
| NDV B | Hemagglutinin-neuraminidase | Newcastle disease virus strain Beaudette C/45 | HEMA_NDVB |
| NDV U | Hemagglutinin-neuraminidase | Newcastle disease virus strain Ulster | HEMA_NDVU |
| NDV Q | Hemagglutinin-neuraminidase | Newcastle disease virus strain Queensland | HEMA_NDVQ |
| NDV D | Hemagglutinin-neuraminidase | Newcastle disease virus strain D26/76 | HEMA_NDVD |
| NDV M | Hemagglutinin-neuraminidase | Newcastle disease virus strain Miyadera/51 | HEMA_NDVM |
| NDV I | Hemagglutinin-neuraminidase | Newcastle disease virus strain Italien | HEMA_NDVH3 |
| NDV H3 | Hemagglutinin-neuraminidase | Newcastle disease virus strain HER/33 | |
| NDV C | Hemagglutinin-neuraminidase | Newcastle disease virus strain CHI/85 | HEMA_NDVC |
| NDV A | Hemagglutinin-neuraminidase | Newcastle disease virus strain Australia-Victoria | HEMA_NDVA |
| Parainfluenza 4 HA | Hemagglutinin-neuraminidase | Parainfluenza virus type 4A strain Toshiba | HEMA_PI4HA |
| Simian virus 5 | Hemagglutinin-neuraminidase | Simian paramyxovirus SV5 isolate human/LN | HEMA_SV5LM |

[a]Designations used in the figures.
[b]ID, identification number from the SWISS-PROT database unless otherwise stated.
[c]NA, not available.

that assembly of the outer capsid requires the cleavage of the primary translation product μ1 to generate μ1C (2). Proteolytic processing is a common mechanism used by numerous viruses for the generation of functional viral proteins. Such cleavage can be mediated by cellular enzymes but viral-encoded proteases are most often involved (14). Studies have demonstrated that the σ3 protein is required for μ1 to μ1C cleavage and that no other viral product appears to be necessary for this cleavage to occur (15). It has previously been noted that the amino-terminal domain of σ3 from all three reovirus serotypes possesses a short amino acid sequence similar to a conserved region of picornaviral proteases (16). Computer-assisted sequence analysis revealed that the reovirus CGGAVVCMH sequence is similar to the consensus CGGxUxCxH sequence present in protease 2A of poliovirus, rhinovirus, enterovirus, swine vesicular disease virus, and coxsackieviruses (Fig. 2); amino acid residues (cysteine and histidine) directly involved in the picornaviral catalytic activity were identified as part of this consensus sequence (17–19). Alignment of σ3 amino acid sequence with the corresponding region of picornaviral proteases revealed the presence of bulky aliphatic residues (I, L, V, M) near the CGGxUxCxH sequence in all these proteins. The presence of a conserved glycine residue, 2–3 amino acids following the CGGxUxCxH consensus sequence, was also revealed by this alignment (Fig. 2). The reovirus σ3 protein thus shares between 24 and 28% amino acid identity with the protease 2A of picornaviruses over a 25-amino acid domain and a 44–48% similarity over the same domain (residues belonging to the following groups were considered similar: [I, L, V, M]; [R, K];[D, E]; [S, T], [Y, F]). Despite this strong similarity with picornaviral proteases, cotransfection experiments clearly demonstrated that the proteolytic consensus sequence in σ3 is not directly responsible for μ1 cleavage (20); however, it still needs to be determined if σ3 can exert a proteolytic activity on another yet unidentified substrate.

In addition to its structural role, it has been suggested that σ3 exerts a role in the modulation of

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                    37                            68
Sigma 3 T3D         SAQPDMMVCGGAVVCMH-CLGVVGSLQRKLKHL
Sigma 3 T2J         SAQPDMMVCGGAVVCMH-CLGVVGSLQRKLKHL
Sigma 3 T1L         SAQPDMMVCGGAVVCMH-CLGVVGSLQRKLKHL

2A Cox B1           AGFSEPGDCGGILRCEHGVVGIVTMGGEGVVGF
2A Bov entero       IGFAEPGDCGGLLRCEHGVMGILTVGGGDHVGF
2A Cox A9           AGFSEPGDCGGILRCEHGVIGIVTMGGEGVVGF
2A Cox B3           AGFSEPGDCGGILRCEHGVIGIVTMGGEGVVGF
2A Cox B4           TGFSEPGDCGGILRCEHGVIGLVTMGGEGVVGF
2A Cox B5           AGFSEPGDCGGILRCEHGVIGLVTMGGEGVVGF
2A Swine Ves H      AGFAEPGDCGGILRCQHGVIGIVTMGGEGVVGF
2A Swine Ves U      AGFAEPGDCGGILRCQHGVIGIVTVGGEGVVGF
2A Entero 70        MGPCQPGDCGGLLVCSHGVIGLVTAGGEGIVAF
2A Cox A21          CGFAEPGDCGGILRCTHGVIGIITAGGEGIVAF
2A Cox A24          MGFAEPGDCGGILRCNHGVMGIVTAGGNGIVAF
2A Polio 1 M        HGFESPGDCGGILRCHHGVIGIITAGGEGLVAF
2A Polio 1 S        HGFASPGDCGGILRCHHGVIGIITAGGEGLVAF
2A Polio 2 L        HGFASPGDCGGILRCQHGVIGIITAGGEGLVAF
2A Polio 2 W        HGFASPGDCGGILRCQHGVIGIITAGGEGLVAF
2A Polio 3          HGFASPGDCGGILRCQHGVIGIITAGGEGLVAF
2A Polio 3 L        HGFASPGDCGGILRCQHGVIGIVTAGGEGLVAF
2A Rhino 14         VGPAEPGDCGGILRCIHGPIGLLTAGGSGYVCF
2A Rhino 1B         EGPCEPGDCGGKLLCRHGVIGIITAGGEGHVAF
2A Rhino 2          EGPCEPGDCGGKLLCKHGVIGIVTAGGDNHVAF
2A Rhino 89         EGPCEPGDCGGKLLCKHGVIGMITAGGEGHVAF
2A Echo 11          AGFSEPGDCGGILRCEHGVIGIVTMGGEGVVGF

Consensus               DxxxCGGxUxCxHxxUGUUxxxxxxU
                        E
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*Fig. 2.* Alignment of the amino acid sequences of mammalian reovirus σ3 protein from reovirus serotype 1 (T1L), serotype 2 (T2J), and serotype 3 (T3D) with segments of picornaviral proteases. Identical or similar amino acids residues between σ3 and the picornaviral proteases are shown in bold; residues belonging to the following groups were considered similar: [I, L, V, M]; [D, E]; [S, T]. The consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D σ3 protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue.

inhibition of host-cell protein synthesis consecutive to reovirus infection; differences in the extent of inhibition observed upon infection by different viral strains map to the S4 gene, which encodes the σ3 protein (21). The mechanism of inhibition remains largely unknown but several studies indicated that σ3 can downregulate the dsRNA-activated protein kinase (PKR) probably by sequestering dsRNA (22–24). A role for free σ3, rather than σ3 complexed with μ1/μ1C, has been proposed for this downregulation (15,22,23,25). Activation of PKR normally results in phosphorylation of eIF-2α and inhibition of translation initiation (26). However, a proteolytic degradation of translational initiation factors could also be responsible for the inhibition of host-cell protein synthesis as observed during the multiplication of other viruses (27). A similar mechanism has been previously suggested for reovirus, although experimental evidence is lacking. The cleavage of some component of the cellular transcriptional machinery by σ3 thus remains an attractive possibility.

In addition to the sequence analogy with picornaviral proteases, computer-assisted analysis of the T3D σ3 protein sequence further revealed four regions similar to the ribosome-inactivating protein of *Phytolacca americana*, the common pokeberry (Fig. 3a). Over these regions that span 61 amino acids, 38% of the residues are identical and 16% are scored as

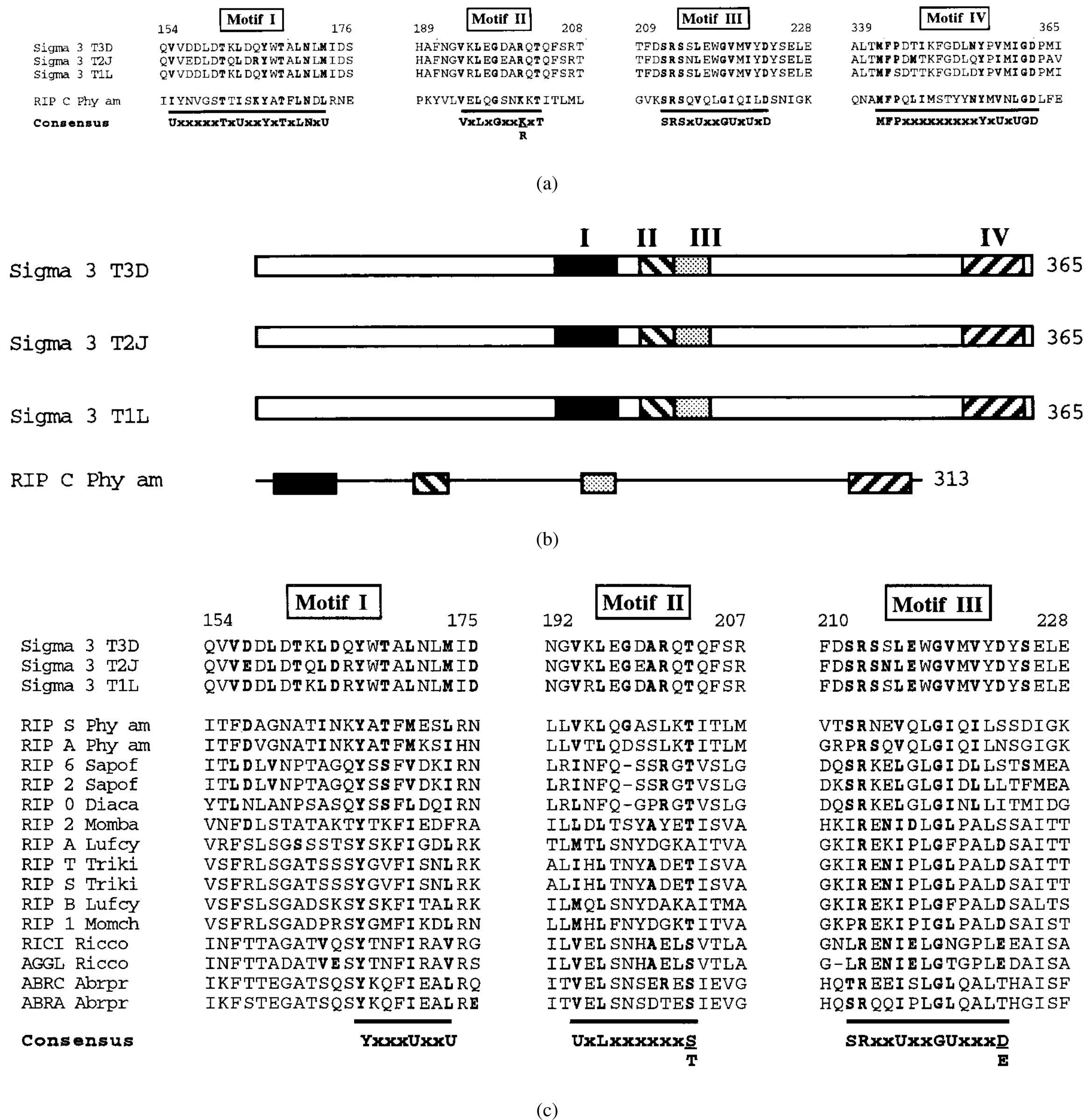


*Fig. 3.* Sequence similarity between mammalian reoviruses σ3 protein and ribosome-inactivating protein of plants. (a) Alignment of the amino acid sequences of the σ3 protein from reovirus T1L, T2J, and T3D with segments of the ribosome-inactivating protein of *Phytolacca americana*. Identical or similar amino acids residues are shown in bold; residues belonging to the following groups were considered similar: [I, L, V, M]; [D, E]; [K, R]; [S, T]. Conserved segments are identified above the alignment and the consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D σ3 protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue. (b) Diagram representing the conserved segments between the σ3 protein from reovirus T1L, T2J, T3D and the ribosome-inactivating protein of *Phytolacca americana*. The sizes of the conserved segments and the distance between them are drawn to scale. The length of the respective proteins is also indicated. (c) Alignment of the amino acid sequences of the σ3 protein from reovirus T1L, T2J, and T3D with segments of the ribosome-inactivating protein of various plants.

similar by the BLOSUM62 matrix for a total similarity of 54%. These regions encompassed a large portion of the ribosome-inactivating protein and are arranged in the same order in the C-terminal half of σ3 (Fig. 3b). Analysis of the σ3 protein from reovirus T1L and T2J revealed the conservation of the first three regions while the last region, located at the extreme C-terminal end, is more divergent (Fig. 3a). The ribosome-inactivating protein of *Phytolacca americana* possesses an rRNA N-glycosidase activity which catalyzes the hydrolysis of the N-glycosidic bond at a specific adenosine residue of the 28S rRNA (28). *In vitro* experiments have also shown that the protein can inhibit protein synthesis (29). A closely related group of plant proteins that can also cleave the rRNA component of ribosomes was also identified (Fig. 3c). All these proteins share similarities with the σ3 protein of mammalian reoviruses (27–38%); the second and third regions exhibit the most similarities with the reovirus σ3 protein while the fourth region is absent from these related plant proteins. The exact significance of σ3 resemblance to this whole class of proteins able to cleave rRNA is unknown but could be interpreted as a further indication that σ3 plays an important role in translational regulation via an interaction with the cellular machinery.

Finally, a region of similarity between the reovirus σ3 protein and the 65-kDa regulatory subunit of protein phosphatase 2A (PP2A) has been previously reported (30). Upon binding of the catalytic subunit, PP2A is activated and involved in dephosphorylation of eukaryotic initiation factor eIF-2α, leading to an increase of protein synthesis (31). By directly binding to the catalytic subunit of PP2A, it was suggested that σ3 might modulate the enzymatic activity of PP2A (30). This is yet another evidence that the σ3 protein is involved in the regulation of translation during the later stages of reovirus infection (32). However, such similarity between σ3 and PP2A was not detected using the BLASTP program in the present study. Further work will be needed to establish if such a short sequence similarity has any significance.

## σ1

The σ1 protein is responsible for the attachment of the virus at the surface of susceptible cells via interaction with the cellular receptor (1). The σ1 protein is a minor component of the outer capsid and can assume an extended conformation in which it appears as a long fiber projecting from the particle surface at the 12 vertices of the icosahedral capsid (1). Thin-section electron micrographs of cells soon after reovirus infection revealed particles associated with clathrin-coated pits or vesicles near the plasma membrane, suggesting that uptake from the cell surface consecutive to receptor binding occurs by receptor-mediated endocytosis (33,34). As with other non-enveloped viruses, the mechanism by which reoviruses cross the endosomal membrane barrier during entry into cells is still poorly understood. It has been suggested that a direct effect of viral components can result in local disruption of the membrane bilayer to allow the entry in the cytoplasm; alternatively, a more specific porelike structure might be formed by viral proteins within the membrane (33,35–37). During the natural course of gastro-intestinal reovirus infection, intermediate subviral particles (ISVPs) are apparently formed by the action of intestinal proteolytic enzymes (38). These particles differ notably from the virions in protein composition and conformation since most of the outer capsid is removed (1). It has been noted that when similar particles are generated *in vitro*, virions can directly penetrate through the plasma membrane, given they have been treated with chymotrypsin, thus bypassing endocytosis (33,35). The δ protein, a cleavage product of outer capsid protein μ1C, seems to be implicated in mediating interactions with membranes: a strain difference in the capacity of ISVPs to mediate $^{51}$Cr release from L cells via cell lysis or permeabilization was mapped to the M2 gene, which encodes μ1 and therefore μ1C and δ (39).

Database searches for sequence similarity revealed that the N-terminal region of the reovirus T3D σ1 protein harbors a striking degree of identity (98.7%) to the lysozyme protein of the double-stranded DNA bacteriophage SF6 (Fig. 4). This 316 amino acids bacteriophage protein is identical, except for four amino acids residues, to the N-terminal region of reovirus T3D σ1 protein. Such high levels of identity between two proteins of unrelated viruses is very unusual but very significant at the $P = 6.2 \times 10^{-198}$ level. The SF6 lysozyme is able to hydrolyze the 1,4-β-linkages between N-acetyl-D-glucosamine and N-acetylmuramic acid in peptidoglycan heteropolymers of the bacterial cell wall, although the role of this enzyme in penetration and/or lysis of susceptible bacterial cells has not been clearly established (40). The σ1 proteins from reovirus T1L and T2J harbor

```
                                                       * *
Sigma 1 T3D      1 MDPRLREEVVRLIIALTSDNGASLSKGLESRVSALEKTSQIHSDTILRIT  50
Lysozyme SF6       MDPRLREEVVRLIIALTSDNGASLSKRLQSRVSALEKTSQIHSDTILRIT

Sigma 1 T3D     51 QGLDDANKRIIALEQSRDDLVASVSDAQLAISRLESSIGALQTVVNGLDS 100
Lysozyme SF6       QGLDDANKRIIALEQSRDDLVASVSDAQLAISRLESSIGALQTVVNGLDS

Sigma 1 T3D    101 SVTQLGARVGQLETGLADVRVDHDNLVARVDTAERNIGSLTTELSTLTLR 150
Lysozyme SF6       SVTQLGARVGQLETGLADVRVDHDNLVARVDTAERNIGSLTTELSTLTLR

                                                  *
Sigma 1 T3D    151 VTSIQADFESRISTLERTAVTSAGAPLSIRNNRMTMGLNDGLTLSGNNLA 200
Lysozyme SF6       VTSIQADFESRISTLERTAVTSAGAPLSIRNNRITMGLNDGLTLSGNNLA

Sigma 1 T3D    201 IRLPGNTGLNIQNGGLQFRFNTDQFQIVNNNLTLKTTVFDSINSRIGATE 250
Lysozyme SF6       IRLPGNTGLNIQNGGLQFRFNTDQFQIVNNNLTLKTTVFDSINSRIGATE

                                              *
Sigma 1 T3D    251 QSYVASAVTPLRLNSSTKVLDMLIDSSTLEINSSGQLTVRSTSPNLRYPI 300
Lysozyme SF6       QSYVASAVTPLRLNSSTKVLDMLIDMSTLEINSSGQLTVRSTSPNLRYPI

Sigma 1 T3D    301 ADVSGGIGMSPNYRFRQSMWIGIVSYSGSGLNWRVQVNSDIFIVDDYIHI 350
Lysozyme SF6       ADVSGGIGMSPNYRFR

Sigma 1 T3D    351 CLPAFDGFSIADGGDLSLNFVTGLLPPLLTGDTEPAFHNDVVTYGAQTVA 400

Sigma 1 T3D    401 IGLSSGGAPQYMSKNLWVEQWQDGVLRLRVEGGGSITHSNSKWPAMTVSY 450

Sigma 1 T3D    451 PRSFT                                              455
```

*Fig. 4.* Amino acid sequence alignment of reovirus T3D σ1 protein and the lysozyme protein of bacteriophage SF6. Asterisks (*) show nonidentical amino acid residues. The positions of the aligned amino acid sequences are also indicated. The alignment was performed as described under Methods.

divergent forms of the protein, since σ1 is the principal antigen against which the serotype-specific humoral response is directed (6,7), and thus possess reduced but still significant identity with the SF6 lysozyme. The σ1 protein from reovirus T1L showed a 25.3% identity and a total similarity of 42.1% while the σ1 protein from reovirus T2J harbors a 25.6% identity and a total similarity of 41.5% (Fig. 5a). These results revealed a statistically significant amino acid match between the σ1 protein from serotypes T1L and T2J and the lysozyme protein of bacteriophage SF6 at the $P = 8.7 \times 10^{-12}$ and $1.3 \times 10^{-9}$ levels, respectively. Despite this divergence between serotypes, all σ1 proteins share conserved regions that are similar to the C-terminal half of the bacteriophage SF6 lysozyme (Fig. 5b). The effect of lysozymes on mammalian cells has not been thoroughly examined but it has been reported that lysozymes can decrease DNA replication of cultured cells (41), as also observed during reovirus infection (42). It might be significant that this effect of reovirus was mapped to the S1 gene, which encodes the σ1 protein (43). Inhibition of DNA synthesis during reovirus infection occurs through a membrane-linked signalling pathway, suggesting that interaction at the membrane surface is required (44).

Recent experiments revealed that recombinant σ1 protein, expressed in *Pichia pastoris* yeast cells, possesses a glycosyl hydrolase activity against various glycoside substrates of lysozyme such as β-N-acetyl-D-glucosamine (Bisaillon and Lemay, unpublished results). Although reovirus virions appear to be devoid of such hydrolytic activity, infectious subviral particles (ISVPs), generated *in*

```
Sigma 1 T2J      1   M-SDLVQLIRREILLLTGNGESANSKH---EIEEIKKQIKDISADVNRIS   46
Sigma 1 T1L      1   MDASLITEIRKIVLQLSVSSNGSQSK----EIEEIKKQVQVNVDDIRAAN   46

Lysozyme SF6     1   MDPRLREEVVRLIIIALTSDNGASLSKRLQSRVSALEKTSQIHSDTILRIT  50

Sigma 1 T2J     47   NIVDSIQGGQLGGLSVRVSAIESGVSENGNRIDRLERDVSGISASVSGIDS  96
Sigma 1 T1L     47   IKLDGLGRQIADISNSISTIESRLGEMDNRLVGISSQVTQLSNSVSQNTQ  96

Lysozyme SF6    51   QGLDDANKRIIALEQSRDDLVASVSDAQLAISRLESSIGALQTVVNGLDS  100

Sigma 1 T2J     97   RLSELGDRVNVAEQRIGQLDTVTDNLLERASRLETEVSAITNDLGSLNTR  146
Sigma 1 T1L     97   SISSLGDRINAVEPRVDSLDTVTSNLTGRTSTLEADVGSLRTELAALTTR  146

Lysozyme SF6   101   SVTQLGARVGQLETGLADVRVDHDNLVARVDTAERNIGSLTTELSTLTLR  150

Sigma 1 T2J    147   LTTE-------LNDVRQTIAAIDTRLTTLETDAVTSVGQGLQKTGNSIKV  189
Sigma 1 T1L    147   VTTEVTRLDGLINSGQNSIGELSTRLSNVETSMVTTAGRGLQKNGNTLNV  196

Lysozyme SF6   151   VTS--------------IQADFESRISTLERTAVTSAGAPLSIRNNRITM  186

Sigma 1 T2J    190   IVGTGNWFDRNNVLQLFLSNQQKGLGFIDNGNVVKIDTQYFSFDSNGNIT  239
Sigma 1 T1L    197   IVGNGNWFNSSNQLQLDLSGQSKGVGFVGTGNVVKIDTNYFAYNSNGEIT  246

Lysozyme SF6   187   GLNDGLTLSGNN-LAIRLPGNT-GLNIQNGGLQFRFNTDQFQIVNNNLTL  234

Sigma 1 T2J    240   LNNNISGLPARTGSLEASRIDVVAPPLVIQSTGSTRLLRLNYEAVDFVVT  289
Sigma 1 T1L    247   LVSQINELPSRVSTLESAKIDSVLPPLTVREASGVRTLSFGYDTSDFTII  296

Lysozyme SF6   235   KTTVFDSINSRIGATEQSYVASAVTPLRLNS--STKVLDMLIDMSTLEI-  281

Sigma 1 T2J    290   NNVLTLRNRSVTPTFKFPLELNSADNSVSIHRNYRIRLGQWSGQLEYHTP  339
Sigma 1 T1L    297   NSVLSLRSRLTLPTYRYPLELDTANNRVQVADRFGMRTGTWTGQLQYQHP  346

Lysozyme SF6   282   NSSGQLTVRSTSPNLRYPI-------------------------------  300

Sigma 1 T2J    340   SLRWNAPVTVNLMRVDDWLILSFTRFSTSGILASGKFVLNFVTGLSPGWA  389
Sigma 1 T1L    347   QLSWRANVTLNLMKVDDWLVLSFSQMTTNSIMADGKFVINFVSGLSSGWQ  396

Lysozyme SF6   301   -----ADVSGGI-------------------------------GMSPNYR  314

Sigma 1 T2J    390   TGSTEPSTTTNPLSTTFAAIQFINGSSRVDAFRILGVAEWNAGELEITNH  439
Sigma 1 T1L    397   TGDTEPSSTIDPLSTTFAAVQFLNNGQRIDAFRIMGVSEWTDGELEIKNY  446

Lysozyme SF6   315   --------------------------------------------------  314

Sigma 1 T2J    440   GGTYTAHTNVDWAPMTIMYPC-LG  462
Sigma 1 T1L    447   GGTYTGHTQVYWAPMTIMYPCNVR  470

Lysozyme SF6   315   ----------------------FR  316
```

(a)

*Fig. 5.* Sequence similarity between the mammalian reovirus σ1 protein and the lysozyme protein of bacteriophage SF6. (a) Alignment of the amino acid sequences of σ1 protein from reovirus T1L and T2J with the lysozyme protein of bacteriophage SF6. Identical or similar amino acids residues between σ1 and the lysozyme protein are shown in bold. Residues belonging to the following groups were considered similar: [I, L, V, M]; [R, K]; [D, E]; [S, T], [Y, F]. The positions of the aligned amino acid sequences are also indicated. The alignment was performed as described under Methods. (b) Diagram representing the conserved segments between the σ1 protein from reovirus T1L, T2J, T3D and the lysozyme protein of bacteriophage SF6. The sizes of the conserved segments and the distance between them are drawn to scale. The length of the respective proteins is also indicated.

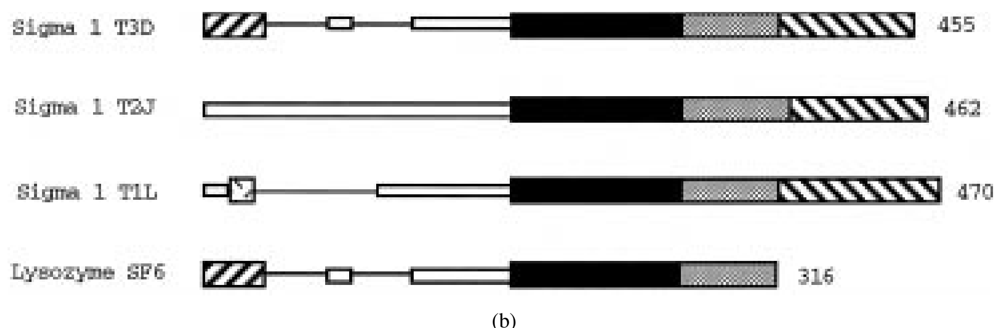


*Fig. 5. (Continued)*

*vitro* by partial uncoating using chymotrypsin digestion of purified virions, are able to hydrolyze the glycoside substrates (Bisaillon and Lemay, unpublished results). The conformational changes induced in σ1 upon removal of the outer capsid (45) are probably required to expose the helical amino-terminal portion of the protein that is buried deeper in the virion and that harbors most similarity to lysozyme. Activation of such a lysozyme-like activity upon partial uncoating by intestinal enzymes could be involved in degradation of sugar moieties in mucins covering epithelial cells, thus facilitating viral infection. Interestingly, it has been recently shown that mucin covering epithelial cells can inhibit adenovirus and rotavirus infection (46,47); evolution of a mechanism for degradation of mucin layers could thus present a selective advantage to the virus. In fact, such a mucin-degrading enzyme has been demonstrated both *in vitro* and *in vivo* for baculovirus; *in vivo* degradation of mucin was correlated with the enhancement of baculovirus infection in insects (48).

## μNS

The μNS protein is one of the three reovirus nonstructural proteins that are synthesized during viral multiplication but apparently never incorporated into mature virions (1). Experimental studies have shown that μNS is associated with elements of the cytoskeleton and that a specific monoclonal antibody can react not only with μNS but also with elements of the cytoskeleton (49). Early microscopic studies also suggested that reoviruses might use the cytoskeleton to facilitate genome replication or maturation since viral particles were observed in close proximity to microtubules and intermediate filaments (50). The full significance of the interactions of μNS with the cytoskeleton is not clear but suggests that μNS might serve to anchor the structures involved in viral genome synthesis and assembly to the cell matrix (49). In addition, reovirus mRNAs bind to μNS shortly after synthesis, even before these RNAs associate with other viral proteins (51). The formation of these initial RNA-protein complexes could be among the first steps on the pathway of reovirus morphogenesis which may proceed in association with the cytoskeleton.

It has been previously noted that the deduced amino acid sequence of μNS from T3D possesses a high potential of adopting α-helical secondary structure and similarity with myosin (52). As expected, computer-assisted protein database screening did reveal similarities with myosins from human, rat, and chick embryo (Fig. 6). It was found that the amino acid sequence of μNS shares between 20 and 24% identity and 34% similarity with these myosins from different organisms over a 41-amino acid domain. The same region of μNS also shares some common region with human, mouse, bovine, and *Xenopus laevis* cytoskeleton keratin protein (Fig. 6). However, these similarities with keratin proteins are not as high (15–20% identity and 27–32% similarity) than those observed with the various myosins. Altogether, these observations suggest that the μNS protein harbors structural similarities with many cytoskeletal proteins and could represent a potential support during reovirus genome replication or assembly of virions.

## μ2

The μ2 protein is a minor component of the inner capsid (1). The role of μ2 during reovirus infection remains unknown but it has been reported that

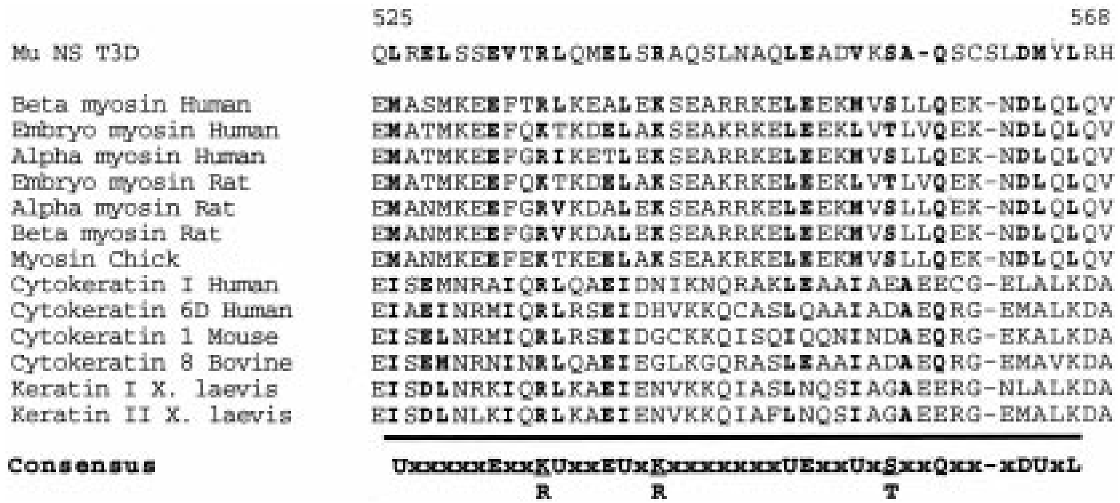


*Fig. 6.* Alignment of the amino acid sequence of mammalian reovirus μNS protein from reovirus T3D with regions of cytoskeletal proteins. Identical or similar amino acids residues between μNS and any of the cytoskeletal proteins are shown in bold; residues belonging to the following groups were considered similar: [I, L, V, M]; [R, K]; [D, E]. The consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D μNS protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue.

thermosensitive μ2 mutants fail to synthesize dsRNA at nonpermissive temperature (52,53). Furthermore, temperature optimum of transcription maps to the M1 gene encoding the μ2 protein further suggesting a role for this protein in synthesis of viral nucleic acids (53). Sequence analysis of μ2 reveals that the central portion of the protein shares some similarities with enzymes involved in nucleic acids metabolism, namely adenosine deaminases from *E. coli*, mouse and human (Fig. 7a). This 25-amino acid domain harbors a 44% similarity with the human and mouse adenosine deaminases while the *E. coli* enzyme displays a little less similarity with the reovirus μ2 protein (40% similarity). Another region of the μ2 protein harbors similarities with an adenine deaminase from *Bacillus subtilis* and with a probable adenine deaminase from *E. coli* (Fig. 7b). It was observed that the primary sequence of μ2 shares 39% amino acid identity with the putative *E. coli* deaminase and a 48% similarity over a region of 44 amino acids. This last region also shares some common residues with the recently isolated dsRNA adenosine deaminase (DRADA) protein from human and rat and with dsRNA-specific editase (RED1) protein from rat. Analysis of the resulting alignment revealed that μ2 shares two other similar regions with the human DRADA (Fig. 7b). Over these three domains encompassing 95 amino acids, 25 of the residues (26%) were identical with the human DRADA protein and 9 (10%) were scored as similar by the BLOSUM62 matrix for a total similarity of 36%. The rat RED1 protein also shares 29% identity and 33% similarity over these two regions. These last two cellular enzymes (human DRADA and rat RED1) are involved in conversion of adenosine to inosine in double-stranded secondary structures present on mRNA molecules (54,55). This mechanism is responsible for RNA editing, a biochemical process involving the modification of mRNAs, since inosine residues are ''read'' as guanosine during translation (56).

Interestingly, RNA editing is thought to be involved in biased hypermutation of vesicular stomatitis virus (57). A similar phenomenon was also described in measles virus isolated from patients with subacute sclerosing panencephalitis (58). Adenosine to guanosine hypermutations were also found in two escape mutants of another paramyxovirus, the human respiratory syncitial virus, and in the U3 region of an avian lymphomatous retrovirus (59,60). Hepatitis deltavirus (HDV) nucleotide 1012 is also edited from uridine to cytidine in 10–40% of the RNA genomes during replication (61). Although reoviruses commonly cause lytic infections, they can also produce nonlytic, persistent infections in a variety of cultured cells (62–64). However, the

*Fig. 7.* Sequence similarity between the μ2 protein of mammalian reovirus and the adenosine deaminases from various species. (a) Alignment of the amino acid sequence of μ2 protein from reovirus T3D with the adenosine deaminase proteins. Identical or similar amino acids residues between μ2 and the adenosine deaminase proteins are shown in bold; residues belonging to the following groups were considered similar: [I, L, V, M]; [S, T]; [Y, F]; [D, E]. The consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D μ2 protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue. (b) Alignment of the amino acid sequence of mammalian reovirus μ2 protein from serotype T3D with other adenine or adenosine deaminase proteins.

detailed mechanisms responsible for the establishment and maintenance of persistent infections are poorly understood but clearly involve mutations in the virus leading to a decrease in cellular injury that commonly accompanies lytic infections. It is possible to speculate a role of high mutation rate in viral persistence as in measles virus-induced subacute sclerosing panencephalitis (57,58). Current evidence is that mutations in specific viral genes have also a primary effect in the establishment of persistence (65–68). Obviously, if reovirus encodes a protein with adenosine deaminase activity, this could contribute to a more rapid modification of the information encoded by viral mRNAs and accumulation of mutations. The presence of an actual editing mechanism in reovirus, and its putative role in viral persistence, certainly deserves to be investigated.

## λ2

The reovirus λ2 protein forms the spikes projecting from the inner capsid, and observations of viral particles by cryoelectron microscopy have confirmed that a significant portion of λ2 is exposed at the surface of virions (2,3,69). Reovirus inner capsids incubated with [α-$^{32}$P]GTP were shown to form covalent λ2-GMP complexes, consistent with the

activity of λ2 as a guanylyltransferase (70,71). Guanylyltransferase activity is responsible for the formation of mRNA cap structure via the transfer of a GMP moiety, derived from GTP, to diphosphorylated mRNA molecules resulting from the action of RNA 5′-triphosphatase on nascent mRNA molecules (72,73). The λ2 protein, expressed using a vaccinia virus vector, was actually shown to mediate the transfer of GMP to appropriate acceptor molecules (72,74).

Determination of the primary structure of various guanylyltransferases from many viruses has allowed a better knowledge of the active site and catalytic mechanism of these enzymes. A lysine-containing motif, KxDG, is conserved among guanylyltransferases encoded by DNA viruses (vaccinia virus, Shope fibroma virus, African swine fever virus) and the yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* (75,76). This motif is also conserved at the active site of polynucleotide ligases which, like guanylyltransferases, catalyze their reaction via the formation of a covalent Lys-nucleoside monophosphate intermediate (77). In addition to the active guanylyltransferase site (KxDG), other conserved sequence elements were identified in guanylyltransferases from DNA viruses and yeasts (76). These elements were shown to be essential for capping enzyme function *in vivo* (76). Although the reovirus λ2 protein covalently binds GTP, it lacks these conserved motifs. A similar situation is observed in rotavirus VP3 and bluetongue virus VP4 proteins, all viral guanylyltransferases from viruses of the *Reoviridae* family. These capping enzymes apparently evolved differently from other viral and cellular guanylyltransferases, despite their identical enzymatic functions. The Lys(226) residue of the reovirus λ2 protein KPTNG sequence has been identified as the GMP attachment site (71); this sequence differs from the consensus signature of DNA viruses (KxDG) but is related to the murine rotavirus SA-11 VP3 protein sequence and bluetongue virus VP4 (KxT(x)G) (Fig. 8a). The presence of bulky aliphatic residues (I, L, V, M) following the KxT(x)G consensus sequence was also revealed by this alignment (Fig. 8a). Furthermore, another motif (YxR) is conserved between the reovirus λ2 protein (YVRKN), the VP3 protein of the murine rotavirus SA-11 (YYRYN) and the bluetongue virus VP4 protein (YKRKM). Mutagenesis of these residues might eventually reveal important amino acids involved in guanylate binding or GMP transfer from the enzyme to the acceptor mRNA molecules. This alignment also showed that the reovirus λ2 protein shares a higher degree of similarity with the guanylyltransferase of bluetongue virus (41% similarity) than with guanylyltransferase from human and simian rotavirus (22 and 34% similarity, respectively) over these two domains of 32 amino acids.

In addition to guanylyltransferase activity, a role has been previously suggested for the λ2 protein as a methyltransferase to produce the classical methylated 5′ cap structure ($^{m7}$Gppp$^{m}$GpC) found at the 5′ end of reovirus mRNAs. This is supported by the observation that λ2 is the only reovirus protein labeled by 8-azido-S-adenosyl[$^{35}$S]methionine, an analog of the methyl donor *S*-adenosylmethionine (78). However, isolated λ2 failed to methylate the cap of reovirus mRNA; interaction with other proteins in the virus core may be required for the protein to exert its activity (74). A limited sequence similarity was previously found between the central region of λ2 and other methyltransferases from viral and cellular sources (79). The amino acid sequences of different viral and cellular methyltransferases are widely variable but a conserved sequence element, the G-loop UU[D/E]UoxG can be identified; where U designates a bulky aliphatic amino acid residue, o represents a small residue (G, A, or S), and x is any amino acid (80). The importance of the G-loop in binding of the methyl donor has been suggested by mutational analysis of a DNA methyltransferase, an unrelated enzyme that nevertheless uses *S*-adenosylmethionine as a methyl donor for its transfer to DNA (81). Analysis of the reovirus λ2 protein revealed that a sequence very similar to the G-loop, except for the last amino acid residue, is found in the C-terminal portion of the λ2 protein of reovirus (Fig. 8b). This sequence harbors a 36% identity and a 50% similarity with the yeast ABD1 methyltransferase over a 22-amino acid domain. This domain is also similar to other methyltransferases of viral origins (27–41% similarity, Fig. 8b). Interestingly, our analysis of λ2 sequence showed that it also harbors amino acids similarity with 3 additional conserved motifs (UxY, UxxTxxxxD/ExxxxU, ExGxxU) shared by the methyltransferases of DNA viruses (vaccinia, Shope fibroma virus and African swine fever virus) and *S. cerevisiae* (82) (Fig. 8c). The λ2 protein displays 33% and 46% similarity with the *S. cerevisiae* and African Swine fever virus methyltransferases over these four domains encompassing 61

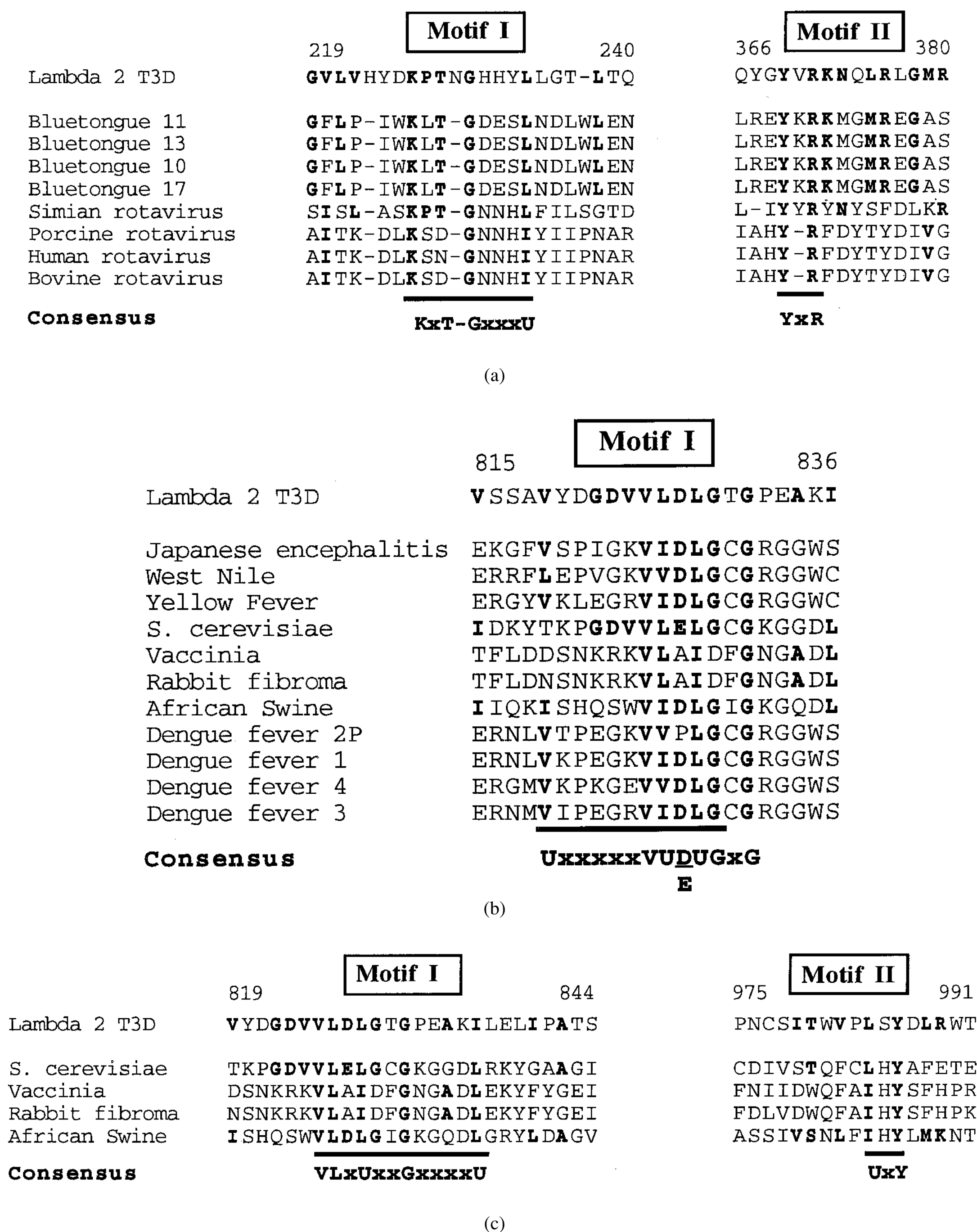


*Fig. 8.* Sequence similarity between the λ2 protein of mammalian reovirus and various viral and yeast proteins. (a) Alignment of the amino acid sequence of λ2 protein from reovirus T3D with viral guanylyltransferase proteins. Identical or similar amino acids residues are shown in bold; residues belonging to the following groups were considered similar: [I, L, V]; [D, E]; [S, T]; [K, R]. The consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D λ2 protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue. (B) alignment of the amino acid sequence of λ2 protein from reovirus T3D with viral and yeast methyltransferase proteins. The conserved segment is shown above the alignment and corresponds to motif I. (C) Alignment of the amino acid sequence of λ2 protein from reovirus T3D with selected viral and yeast methyltranferase proteins. Additional conserved motifs are indicated above the alignment.

```
                    Motif III                    Motif IV
                    1132              1149       1237             1253
Lambda 2 T3D        AQLDFTIAGTDVDITVNP           EWAVKESGNTICILNSQ

S. cerevisiae       GHFFGTIPDSEFIRYKLN           RSLADEYGLELVSQMPF
Vaccinia            GKVLITTMDGDKLSKLTD           VRVFNEYGFVLVDNVDF
Rabbit fibroma      GKVLITTMDGDLLSQLTD           TKIFSEYGFELIDCVHF
African Swine       GMVWFTTMLGEQVLELLH           IKIFKHHGFSLVQKQSF

Consensus             UxxTxxxxDxxxxU               ExGxxU
                              E
```

(c)

*Fig. 8. (Continued)*

amino acids. Mutational analysis of the vaccinia virus enzyme has previously revealed that the His-Tyr dipeptide in motif II is likely a component of the active site since a double amino acid substitution in this motif abolishes the methyltransferase activity (83). Furthermore, a single substitution of the tyrosine

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                       291                                            338
Lambda 2 T3D           YVGEDSLTYRLGV-LSLLATNGYQLA-RPIPRQLTNRWLSSFVSQIMSDG

Parainfluenza 3 HW     SHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-SSGIEDIVLDI
Parainfluenza 3 HV     SHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-SSGIEDIVLDI
Parainfluenza 3 HT     SHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-SSGIEDIVLDI
Parainfluenza 3 HX     SHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-SSGIEDIVLDI
Parainfluenza 3 HU     SHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-SSGIEDIVLDI
Parainfluenza 3 HA     SHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-SSGIEDIVLDI
Parainfluenza 3 H4     SHTFNINDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-SSGIEDIVLDI
Parainfluenza 3 B      THTFNIDDNRKSCSLALLNTDVYQLCSTPKVDERSDYA-STGIEDIVLDI
Sendai virus H         SHTYDINDNRKSCSVVATGTRGYQLCSMPTVDERTDYS-SDGIEDLVLDV
Sendai virus F         SHTYDINDNRKSCSVVATGTRGYQLCSMPTVDERTDYS-SDGIEDLVLDV
Sendai virus J         SHTYDINDNRKSCSVVATGTRGYQLCSMPTVDERTDYS-SDGIEDLVLDV
Sendai virus Z         SHTYDINDNRKSCSVVATGTRGYQLCSMPTVDERTDYS-SDGIEDLVLDV
Sendai virus 5         SHTYDINDNRKSCSVVATGTRGYQLCSMPTVDERTDYS-SDGIEDLVLDV
Parainfluenza 1 HW     SHTYDINDNRKSCSVIAAGTRGYQLCSLPTVNETTDYS-SEGIEDLVFDI
NDV J                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYK-SVTPTSMVHGR
NDV L                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SAVPTRMAHGR
NDV H4                 SINLDDTQNRKSCSVSATPLGCDMLCSKATETEEEDYN-SAVPTRMVHGR
NDV TG                 SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SAVPTLMVHGR
NDV B                  SISLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SAVPTLMAHGR
NDV U                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SAVPTSMVHGR
NDV Q                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SAIPTSMVHGR
NDV D                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SAIPTSMVHGR
NDV M                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SVTPTSMVHGR
NDV I                  SINLDDNQNRKSCSVSATPLGCDMLCSKITETEEEDYS-SVTPTSMVHGR
NDV H3                 SINLDDNQNRKSCSVSATPLGCDMLCSKITETEEEDYS-SVTPTSMVHGR
NDV C                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYK-SVTPTSMVHGR
NDV A                  SINLDDTQNRKSCSVSATPLGCDMLCSKVTETEEEDYN-SVIPTSMVHGR
Parainfluenza 4 HA     SHYLNDGRNRKSCSVVAVPDGCLRNCVTMTKNETENFK-DLNWQHNYLHT
Simian virus 5         TLYLSDGVNRKSCSISTVPGGCMMYCFVSTQPERDDYF-SAAPPEQRIII

Consensus                   DxxxxRxxxxUxxxxxxxxxxLxxxxxxxxxxxxxxSxxxxxUU
```

*Fig. 9.* Alignment of the amino acid sequence of λ2 protein from reovirus T3D with segments of viral hemagglutinin-neuraminidase proteins. Identical or similar amino acids residues between λ2 and the hemagglutinin-neuraminidase proteins are shown in bold; residues belonging to the following groups were considered similar: [I, L, V, M]; [S, T]; [K, R]. The consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D λ2 protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue.

residue of motif IV to an alanine causes a dramatic reduction of the methyltransferase activity (83). The significance of these methyltransferase consensus motifs still needs to be established but their presence certainly makes the λ2 protein a very good candidate to exert the methyltransferase activity required for reovirus mRNA cap synthesis. Interestingly, a recent study showed that a truncated λ2 protein, encompassing the first 3 methyltransferase motifs, retains all activities required to add the methylated cap structure found on reovirus mRNA molecules (84). The methyltransferase domain, being located carboxy-terminal to the guanylyltransferase motif, is also consistent with the known orientation of that protein where the carboxy terminal portion is located at the outside of the virion (84).

Analysis of the λ2 sequence from reovirus serotype 3 also unexpectedly revealed a similarity with a region known to be important for the hemagglutination process in viral hemagglutinins (Fig. 9). This was noted with viral hemagglutinin (HA) or hemagglutinin-neuraminidase (HN) proteins from many serotypes of Sendai virus, Newcastle disease virus, and parainfluenza viruses. Analysis of influenza A and mumps virus HA protein also revealed some similarities with λ2 but to a lesser extent. Mammalian reoviruses can actually bind erythrocytes and cause hemagglutination (1). Differences in the ability of the three reovirus serotypes to agglutinate bovine and human erythrocytes was mapped to their S1 genes, encoding the σ1 protein (85,86). Purified σ1 was actually shown to cause hemagglutination of erythrocytes (87,88). However, in contrast with λ2, comparison of the primary structure of σ1 with various viral hemagglutinin (HA) proteins reveals no significant sequence similarities. Previous analysis of a Sendai virus thermosensitive mutant (*ts*271) provided opportunity to localize a region involved in hemagglutinating activity of the paramyxovirus HN protein (89). The HN glycoprotein of this mutant is able to agglutinate erythrocytes and infect host cells at 30°C but not at 38°C while its neuraminidase activity is unaffected by the increase in temperature (89). Sequence analysis of the HN gene of *ts*271 revealed two closely located amino acid substitutions at position 262 and 264 (89). This finding suggests that the erythrocyte binding site in the sequence of the HN protein is located at, or close to, amino acid 260–270; this is actually the region of the protein that harbors most resemblance with the reovirus λ2 C-terminal 1238–1250 region and with other viral hemagglutinins (Fig. 9). Over this region of 45 amino acids, the reovirus λ2 protein harbors a 29% identity and 36% similarity with the HN protein of parainfluenza viruses. A 22% identity and 31% similarity to the HN protein of Sendai viruses was also observed with this alignment. Overall, it is tempting to speculate that λ2 could serve as a second viral attachment protein on the surface of erythrocytes under certain conditions. Such a phenomenon was observed in coronaviruses: studies have demonstrated that binding of the coronavirus E3 glycoprotein to 9-*O*-acetylated neuraminic acid on erythrocytes results in hemagglutination while coronaviruses that lack this hemagglutinin (E3) can still attach on the surface of the host cell via their large E2 spike protein (90–92). The localization of the putative hemagglutination domain of λ2, at the carboxy-terminal end close to the tip of the molecule and to the σ1 protein, is also consistent with its interaction with cell surfaces.

## λ3

The λ3 protein is a minor component of the inner capsid and has been shown to interact with the λ1 and λ2 proteins (93). Genetic studies have indicated that λ3 may be the viral transcriptase since differences in optimal pH values for transcription among different serotypes were assigned to the L1 gene encoding λ3 (94,95). More recently, biochemical evidence has shown that the purified protein possesses poly(C)-dependent poly(G) polymerase activity (93). These findings strongly suggest that λ3 harbors the catalytic site of the reovirus RNA-dependent RNA polymerase although the protein appears to be unable by itself to transcribe its natural dsRNA substrate (93).

Many primary structures of viral RNA polymerases have been deduced from sequencing of viral genomes. One conserved region (GDD motif) was found in various RNA polymerases from single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA) viruses (96–98). This remarkable sequence conservation between ssRNA and dsRNA viruses may reflect evolution from a common ancestor or convergent evolution to a highly favored structure. The λ3 protein from all three reovirus serotypes harbors this conserved GDD sequence (Fig. 10). Analysis of the primary structure of viral RNA polymerases also showed that two bulky aliphatic

| | Motif I | Motif II | Motif III |
|---|---|---|---|
| | 586 600 | 676 686 | 729 740 |
| Lambda 3 T3D | ISACDASITW--DFFLS | MTTTFPSGSTA | YVCQGDDGLMII |
| Lambda 3 T2J | ISACDASITW--DFFLS | MTTTFPSGSTA | YVCQGDDGLMII |
| Lambda 3 T1L | ISACDASITW--DFFLS | MTTTFPSGSTA | YVCQGDDGLMII |
| Bovine rotavirus | VLYTDVS-QW--DSSQH | QYGAVASGEKQ | IRVDGDDNYAVL |
| Bluetongue | KTTFDAYIRL--DESER | LIDTHLSGENS | EQYVGDDTLFYT |
| S. cerevisiae LA virus | NMMLDGASSFCFDYDDF | LQGTLLSGWRL | SVHNGDDVMISL |
| Yellow fever virus | FYADDTA-GW--DTRIT | RRDQRGSGQVV | MAVSGDDCVVRP |
| West Nile virus | VYADDTA-GW--DTRIT | REDQRGSGQVV | MAVSGDDCVVKP |
| Dengue fever 2 | MYADDTA-GW--DTRIT | RRDQRGSGQVG | MAISGDDCVVKP |
| Japanese encephalitis | MYADDTA-GW--DTRIT | REDQRGSGQVV | MAISGDDCVVKP |
| Poliovirus type 1 | LFAFDYT-GY--DASLS | VKGGMPSGCSG | MIAYGDDVIASY |
| EMC virus | VYDVDYS-NF--DSTHS | ITGGLPSGCAA | VLSYGDDLLVAT |
| Rhinovirus 14 | LMAFDYS-NF--DASLS | VEGGMPSGCSG | ILAYGDDLIVSY |
| Foot and mouth | VWDVDYS-AF--DANHC | VEGGMPSGCSA | MISYGDDIVVAS |
| Coxsackievirus B1 | LVAFDYS-GY--DASLS | VRGGMPSGCSG | MIAYGDDVIASY |
| Hepatitis A | GLDLDFS-AF--DASLS | VCGSMPSGSPC | ILCYGDDVLIVF |
| Tobacco mosaic | VLELDIS-KY--DKSQN | IWYQRKSGDVT | GAFCGDDSLLYF |
| Alfalfa mosaic | FKEIDFS-KF--DKVLD | VDFQRRTGDAL | VVASGDDSLIGT |
| Brome mosaic | FLEADLS-KF--DKSQG | VSFQRKTGDAF | AIFSGDDSLIIS |
| Cucumber mosaic | CLEIDLS-KF--DKSQG | ISFQRRTGDAF | LLFSGDDSLAFS |
| Sindbis virus | VLETDIA-SF--DKSQD | FGAMMKSGMFL | AAFIGDDNIIHG |
| Semliki Forest | VLETDIA-SF--DKSQD | FGAMMKSGMFL | AAFIGDDNIVHG |
| Ross River virus | VLETDIA-SF--DKSQD | FGAMMKSGMFL | AAFIGDDNIVHG |
| **Consensus** | **UxxxDxS-xx--DxxUS** | **UxxxxxSG** | **UxxGDDxUU** |
| | **T T** | **T** | |

*Fig. 10.* Alignment of the amino acid sequences of λ3 protein from reovirus T3D, T2J, and T1L with segments of viral polymerase proteins. Identical or similar amino acids residues between λ3 and the viral polymerase proteins are shown in bold; residues belonging to the following groups were considered similar: [I, L, V, M]; [S, T]; [F, Y]. Conserved segments are identified above the alignment and the consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D λ3 protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue.

amino acid residues (I, L, V, M) are conserved following the consensus GDD sequence (Fig. 10). Other conserved regions have also been reported in viral RNA polymerases from many viruses and include an acidic motif in the N-terminal region of the polymerases with two strongly conserved aspartate residues (motif I) and a third characteristic serine-glycine motif (motif II) located near the GDD consensus sequence considered as motif III (94). Secondary structure predictions for motif II indicated a turn at the serine-glycine both preceded and followed by a beta sheet structure (99). Inspection of the resulting alignment revealed that these additional motifs (motifs I and II) are present as well in reovirus λ3 protein. However, the significance of these λ3 sequences has not yet been studied. It can be seen from this alignment that the reovirus λ3 protein shares many identical and similar residues with these RNA virus polymerases and particularly with members of the *Picornaviridae* family (25–33% identity and 42–50% similarity) over these three domains that span 36 amino acid residues.

## λ1

The λ1 protein, a major component of the reovirus inner capsid, has been shown to exhibit an affinity for double-stranded and single-stranded nucleic acids (100,101). Recent gene reassortment analysis and biochemical studies have shown that λ1 is responsible for the nucleoside triphosphate phosphohydrolase (NTPase) activity present in reovirus inner capsid (102,103). It was also demonstrated that λ1 can unwind double-stranded nucleic acids molecules, a reaction which requires the presence of nucleoside triphosphates (NTPs) or deoxynucleoside triphosphates (dNTPs) (102). These findings strongly support

the idea that λ1 participates as an helicase during transcription of the viral genome. Nucleic acid helicases unwind double-stranded DNA and/or RNA, a process energetically coupled to the hydrolysis of NTPs or dNTPs, and play a key role in nucleic acids replication, transcription, splicing, translocation, recombination and repair (104–107). Helicases of prokaryotic, eukaryotic and viral origin have been isolated and classified into defined superfamilies (108–113). These proteins are characterized by conserved motifs designated I–VI and numerous mutational studies have demonstrated that these motifs are required for helicase activity (114–119). Motifs I and II are very well conserved and correspond to the A and B consensus sequences of a nucleotide-binding domain (120). Superfamily II includes an expanding group of DNA and RNA helicases which harbor a DEAD/H sequence in motif

| | Motif I | Motif II | Motif III |
|---|---|---|---|
| | 1 16 | 95 108 | 150 162 |
| Lambda 1 T3D | MKRIPRKTKGKSSGKG | HSEAKDEADEATKK | MSTRIAEATSAIV |
| eIF-4A Human | VIAQAQSGTGKTATFA | KMFVLDEADEMLSR | TQVVLLSATMPSD |
| RNA Helicase I HeLa | VIIRGATGCGKTTQVP | SHVIVDEIHERDIN | VRIVFMSATIDTS |
| NDH II Vaccinia | VVIIRGAGCGKTTQVP | SHVIVDEIHERDIN | VRIVLMSATIDTS |
| 18R Vaccinia | VVLTGGTGVGKTSQVP | GTLIIDEVHEHDQI | DSMFLMTATLEDD |
| 18R Fowlpox | IVVTGSTGIGKTSQLP | NILLVDEIHEHDRI | HSLVLMSATLEDD |
| p68 Human | MVGVAQTGSGKTLSYL | TYLVLDEADRMLDM | RQTLMWSATWPKE |
| Yellow fever | TVLDFHPGAGKTRRFL | EVIIMDEAHFLDPA | SATILMTATPPGT |
| West Nile | TVLDLHPGAGKTRKIL | NLFIMDEAHFTDPA | AAAIFMTATPPGT |
| Dengue fever 2 | TIMDLHPGAGKTKRYL | NLIIMDEAHFTDPA | AAGIFMTATPPGS |
| Dengue fever 4 | TIMDLHPGAGKTKRIL | NLIVMDEAHFTDPS | AAAIFMTATPPGA |
| Japanese encephalitis | TVLDLHPGSGKTRKIL | NLFVMDEAHFTDPA | AAAIFMTATPPGT |
| RAD3 S. cerevisiae | SILEMPSGTGKTVSLL | SIVIFDEAHNIDNV | SSVIITSGTISPL |
| **Consensus** | **GKS/T** | **DEA** | **UxxAT** |

| | Motif IV | Motif V | Motif VI |
|---|---|---|---|
| | 220 230 | 338 352 | 427 438 |
| Lambda 1 T3D | ITEFISSWQNH | LLNTETLSV-RGANPL | VR-NRVGRFDRAQ |
| eIF-4A Human | AVIFINTRRKV | RVLITTDLLARGIDVQ | GRGGRFGRKGVAI |
| RNA Helicase I HeLa | VLVFLPGWNLI | KVILSTNIAETSITIN | QRKGRAGRSTAGF |
| NDH II Vaccinia | VLVFLPGWNLI | KIILSTNIAETSITIN | QRKGRAGRVRPGF |
| 18R Vaccinia | GIVFVASVAQC | SIIISTPYLESSVTIR | QRKGRVGRVNPGT |
| 18R Fowlpox | GILFLASVSQC | CILVSTPYLESSITIR | QRKGRVGRVSKGI |
| p68 Human | TIVFVETKRRC | PILIATDVASRGLDVE | HRIGRTARSTKTG |
| Yellow fever | TAWFLPSIRAA | DFILATDIAEMGANLC | QRRGRIGRNPNRD |
| West Nile | TVWFVPSVKMG | DFVYTTDISEMGANFK | QRRGRIGRNPSQV |
| Dengue fever 2 | TVWFVPSIKTG | DFVVTTDISEMGANFK | QRRGRIGRNPRNE |
| Dengue fever 4 | TVWFVPSIKAG | DFVVTTDISEMGANFR | QRRGRIGRNPAQE |
| Japanese encephalitis | TVWFVASVKMG | DFVITTDISEMGANFG | QRRGRVGRNPNQV |
| RAD3 S. cerevisiae | MVVFFPSYLYM | GAILLSVARGEGIDFQ | QCLGRVLRGKDDY |
| **Consensus** | **FUxS/T** | **S/TxxxxxGAN** | **RxxRUGR** |

(a)

*Fig. 11.* Sequence similarity between the λ1 protein of reovirus T3D and various human, viral and yeast proteins. (a) Alignment of the amino acid sequence of λ1 protein from reovirus T3D with helicase proteins. Identical or similar amino acids residues are shown in bold; residues belonging to the following groups were considered similar: [I, L, V, M]; [S, T]; [K, R]; [D, E]. Conserved segments are identified above the alignment and the consensus pattern is shown below the alignment which was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D λ1 protein are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue. (b) Alignment of the amino acid sequence of λ1 protein from reovirus T3D with viral RNA 5′-triphosphatase proteins. Conserved segments are also identified above the alignment.

| | Motif I | Motif II |
|---|---|---|
| | 1089 1105 | 1130 1141 |
| Lambda 1 T3D | AAPMIRDETGLMVPFEG | KTGE-LRIR-IEM |
| Vaccinia | NHPKSRPNTSLEIEFTP | VNGEILKPR-IDK |
| Rabbit fibroma | NHPKSKPNPSLEFEIIT | ATGELIKPR-IDK |
| Variola major | NHPKSRPNTSLEIEFTP | VNGEILKPR-IDK |
| African Swine | SAKLAAFKTLLFDTLLF | SVLVRLKNRTTFR |
| West Nile | CFDGPRTNTILEDNNEV | GERKILRPRWADA |
| Japanese encephalitis | CFDGPRTNAILEDNTEV | GERKILKPRWLDA |
| Dengue fever 4 | CFTGERNNQILEENMEV | GEKKKLRPRWLDA |
| Yellow fever | CFEGPEEHEILNDSGET | GAKKPLRPRWCDE |
| **Consensus** | **RxxTxLxxxxE**<br>**K** | **LRxRxU**<br>**K** |

(b)

*Fig. 11. (Continued)*

II (121). The sequences present in motifs III, IV and V are less strictly conserved and their roles are not clearly defined while motif VI is supposed to be involved in the binding of nucleic acids given its high content in positively charged amino acids (121).

Sequence comparisons revealed that λ1 possesses two nucleotide binding motifs normally present in NTPase: a GKS/T sequence at the extreme N-terminal region and a DEAD motif. Furthermore, although the λ1 protein does not share any significant overall similarities to other proteins, it possesses the characteristic motifs found in the DEAD subfamily of the DNA/RNA helicase superfamily II (Fig. 11a). All these motifs are located in the amino-terminal third of λ1, a region previously assigned to the affinity of the protein for nucleic acids (100,101). The λ1 protein shares many similar amino acid residues with helicases from human (30–38% similarity), poxviruses (33–36% similarity), flaviviruses (29–32% similarity), and *S. cerevisiae* (28% similarity) over these six domains encompassing 69 amino acids.

It was recently demonstrated that, in addition to being an NTPase/helicase, the reovirus λ1 protein possesses an RNA 5′-triphosphatase activity (122); this activity is likely responsible for the first step in the formation of the cap structure at the 5′-end of reovirus early mRNAs. Few primary structures of proteins possessing an RNA 5′-triphosphatase activity are actually known. Studies have shown that both the NS3 protein of the West Nile virus and the D1 subunit of the vaccinia virus capping enzyme possess such an activity (123,124). It has been noted that the LRPR amino acid sequence found in the West Nile Virus protein is related to the vaccinia virus D1 subunit sequence LKPR (123). Since no actual structure-function studies have been performed, the importance of this motif (LK/RxR) remains purely speculative although a similar motif (LRIR) is also present in the reovirus λ1 protein (Fig. 11b). Furthermore, another somewhat degenerate motif (RxxTxL) is present on the vaccinia virus capping enzyme D1 subunit (RPNTSL), West Nile virus NS3 (RTNTIL) and reovirus λ1 protein (RDETGL) (Fig. 11b). These two motifs are also found on various putative RNA 5′-triphosphatases of other flaviviruses and DNA viruses for which no activity has been actually demonstrated. Interestingly, a substitution of the glutamate residue in this latter motif of the vaccinia virus capping enzyme inactivates the triphosphatase but does not affect the guanylyltransferase activity present on the same polypeptide chain (125). This supports the idea that these consensus motifs have a functional significance, although further studies will be needed to firmly establish their exact nature and importance.

The primary structure of the RNA 5′-triphosphatase from *S. cerevisiae* has been recently determined (126). Interestingly, sequence analysis revealed that the central region of the yeast protein CET1 (305 amino acids) shares 36% similarity with the reovirus λ1 protein (Fig. 12a). Furthermore, the yeast protein also harbors a 52% similarity with a 21 amino acids region of the λ1 protein, identified as the putative nucleotide binding site, that could be involved in the RNA 5′-triphosphatase activity

```
Lambda 1 T3D   498   PSVNRLMPYRISNAERQISQIIRIMNIGNNATVIQPVLQDISVLLQRISP   547
CET1           140   PSDDSIHQNSKSDEEQ------RIPKQGNEGNIASNYITQVPLQKQK---   180

Lambda 1 T3D   548   LQIDPTIISNTMSTVSESTTQTLSPASSILGKLRRSNSDFSSFRVALAGW   597
CET1           181   -QTEKKIAGNAVGSVVKKEEEANAAVDNIFEEKATLQSKKNNIKRDLE--   227

Lambda 1 T3D   598   LYNGVVTTVIDDSSYPKDGGSVTSLENLWDFFILALALPLTTDPCAPVKV   647
CET1           228   -----VLNEISASSKPSKYKNVPIWAQKWKPTIKALQSINVKD----LKI   268

Lambda 1 T3D   648   FMTLANMMVGFETIPMDNQIYTQSRRASAFSTPHTWPRCFMNIQLISPID   697
CET1           269   DPSFLNI------IPDDD--LTKSVQDWVYATIYSI--------------   296

Lambda 1 T3D   698   APILRQWAEIIHRYWPNPSQIRYGAPNVFGSANLFTSPEVLLLPIDHQPA   757
CET1           297   APELRSFIELEMKFGVI---IDAKGPD---RVNPPVSSQCVFTELDAH--   338

Lambda 1 T3D   758   NVTTPTLDFTNELTNWRARVCELMKNLVDNQRYQPGWTQSLVSSMRGTLD   797
CET1           339   --LTPNID------------ASLFKEL---SKYIRG-----ISEVTENTG   366

Lambda 1 T3D   798   KLKLIKSMTPMYLQQLAPVELAVIAPMLPFPPFQVPYVRLDRDRVPTMVG   847
CET1           367   KFSIIESQT---------------------------------RDSVYRVGL   384

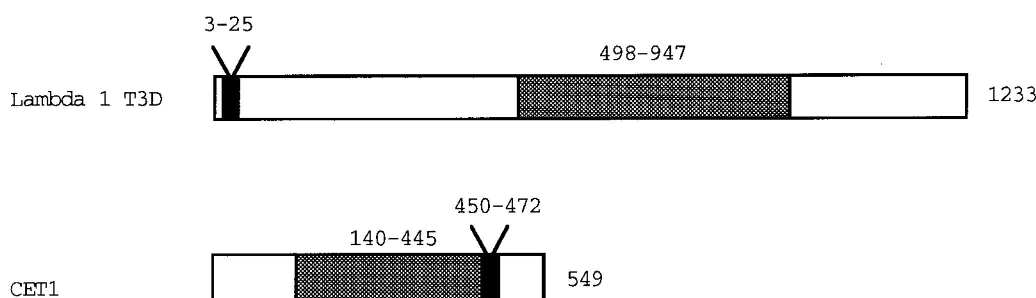
Lambda 1 T3D   848   VTRHSRDTITQPALSLSTTNTTVGVPLALDARAITVALLSGKYPPDLVTN   897
CET1           385   STQRPR------FLRMSTDIKTGRVGQFIEKRHVAQLLL---YSPK----   421

Lambda 1 T3D   898   VWYADAIYPMYADTEVFSNLQRDMITCEAVQTLVTLVAQISETQYPVDRY   947
CET1           422   ----DSY-----DVKISLNLELPVPDND-----------------PPEKY   445
```

(a)

```
Lambda 1 T3D   3     RIPRKTKGKSSGKGNDSTERADD   25
CET1           450   PISERTKDRVSYIHNDSCTRIDI   472
```

(b)




(c)

*Fig. 12.* Sequence similarity between the λ1 protein of reovirus T3D and the *S. cerevisiae* RNA 5′-triphosphatase. (a) Alignment of the amino acid sequence of λ1 protein from reovirus T3D with the yeast CET1 protein. Identical or similar amino acids residues are shown in bold; residues belonging to the following groups were considered similar: [I, L, V]; [D, E]; [S, T]; [K, R]. The alignment was performed as described under Methods. Positions of amino acid residues corresponding to the reovirus T3D λ1 and the yeast CET1 proteins are also indicated. Abbreviations: U, bulky aliphatic residues; x, any amino acid residue. (b) Alignment of the putative nucleotide-binding site of the λ1 protein from reovirus T3D with the yeast CET1 protein. (c) Diagram representing the conserved segments between the λ1 protein from reovirus T3D and the RNA 5′-triphosphatase (CET1) protein of *S. cerevisiae*. The sizes of the conserved segments and the distance between them are drawn to scale. The length of the respective proteins is also indicated.

(102,127) (Fig. 12b). However, the importance of these regions still needs to be investigated.

## Conclusions

Computer-assisted comparisons of reovirus proteins with available databases has revealed various amino acids motifs. Among these motifs, consensus sequences have been identified that are in accordance with already suspected functions of reovirus proteins: RNA polymerase, guanylyltransferase, RNA triphosphatase, and helicase activity. There is little doubt that future functional studies will take advantage of the identification of these consensus sequences that represent putative targets for site-directed mutagenesis experiments. This analysis has also revealed unexpected similarities of some reovirus proteins with specific classes of proteins present in the databases. This suggests yet unidentified activities for some of the reovirus proteins. Among most potentially significant findings are the strong similarities noted between σ1 and SF6 lysozyme and the similarity of a short region of λ2 with viral hemagglutinins. Further work is obviously needed to determine if these sequence similarities actually reflects functions of reovirus proteins. However, the similarities between reovirus proteins whose functions are known and proteins exhibiting similar properties, does support the idea that sequence similarities, although not necessarily extensive, could reflect actual function of reovirus proteins. Exponential increase in the number of sequences found in databases and the development of increasingly powerful tools for their analysis should certainly further contribute to our knowledge of reovirus proteins functions.

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