JOURNAL OF VIROLOGY, Mar. 1996, p. 2065–2068
0022-538X/96/$04.00+0
Copyright © 1996, American Society for Microbiology
Vol. 70, No. 3

Mutations in the Zinc-Binding Motif of the Reovirus Capsid Protein σ3 Eliminate Its Ability To Associate with Capsid Protein μ1

DEBORAH A. SHEPARD, JEFFREY G. EHNSTROM, PAMELA J. SKINNER, AND LESLIE A. SCHIFF*

Department of Microbiology, University of Minnesota, Minneapolis, Minnesota

Received 26 September 1995/Accepted 21 November 1995

Reovirus capsid protein σ3 binds both double-stranded RNA (dsRNA) and zinc. Previous studies have revealed that the amino-terminal zinc finger is not required for the ability of σ3 to bind dsRNA. We expressed wild-type and mutant σ3 molecules by in vitro transcription/translation to evaluate the importance of the zinc finger for other functions of σ3. σ3 molecules with mutations in the zinc finger did not form complexes with capsid protein μ1 but bound dsRNA more efficiently than wild-type σ3 did. In contrast, a dsRNA-binding mutant was unimpaired in its ability to associate with μ1. Studies with σ3 fragments support these findings and indicate that sequences critical for σ3's interaction with μ1 lie in the amino terminus of the molecule. Our finding that μ1 and dsRNA do not compete for identical binding sites on σ3 has implications for its function as a translational regulator in infected cells.

The major capsid protein σ3 is the 41-kDa product of the reovirus S4 gene (17, 20). The predicted σ3 amino acid sequence contains an amino-terminal zinc finger motif, and atomic absorption analysis indicates that each molecule of virion-associated σ3 contains a single zinc atom (22). A second biochemical activity associated with the σ3 protein is the ability to bind double-stranded RNA (dsRNA) (9). A number of protein-blotting studies have shown that sequences in the carboxy-terminal half of σ3 can bind dsRNA (18, 22) and that the carboxy terminus contains a basic motif similar to the known dsRNA-binding site of the interferon-induced, dsRNA-dependent protein kinase, PKR (18). Mutagenesis of some residues within this motif abrogates σ3's dsRNA-binding activity (4).

Experimental evidence suggests that, in addition to its structural role in the outer capsid, σ3 is involved in the regulation of translation within infected cells (23). Further, expression of S4 cDNA in transfected cells stimulates the translation of nonviral mRNAs (8). These activities have been proposed to be related to the ability of σ3 to bind dsRNA and inhibit the activation of PKR (10). The biological function of the amino-terminal zinc finger in σ3 is less clear. Studies with proteolytic fragments and truncation mutants suggest that the zinc finger is not required for dsRNA binding (18, 22). Transfection studies with point mutants suggest that one function of the zinc finger is to influence the intracellular stability of σ3 (15).

In the viral outer capsid and the infected cell, σ3 exists in close association with the myristoylated polypeptide, μ1 (14, 28). Within transfected cells, this protein interaction has been shown to be required for the assembly-related, amino-terminal endoproteolytic cleavage of μ1 (14, 29, 32). Studies with temperature-sensitive mutants support the hypothesis that the σ3-μ1 association is a required step in the assembly of outer capsids onto progeny core-like particles (6, 19). Tillotson and Shatkin have suggested that formation of σ3-μ1 complexes may also interfere with the dsRNA-binding function of σ3 because the ability of σ3 to influence translation in transfected cells is eliminated when σ3 and μ1 are coexpressed (29).

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

We are interested in defining the molecular determinants involved in the interaction of σ3 with its functionally important ligands and in understanding the significance of those interactions for the viral life cycle. In this study, we used an in vitro system of protein expression to identify σ3 sequences important for its interaction with μ1 and to investigate the contribution of the amino-terminal zinc-binding motif to the ligand-binding activities of σ3.

Studies in which mutant S4 genes were analyzed in cotransfected COS cells suggest that specific amino-terminal residues in σ3 may be important for interactions with μ1, but the interpretation of these studies is complicated by the fact that coexpression of μ1 with σ3 affects the accumulation and the stability of the μ1/μ1C protein (16). We first used a biochemical approach to identify the region(s) of σ3 which determines its ability to bind μ1. Cleavage of σ3 with staphylococcal V8 protease between residues 217 and 218 yields an amino-terminal 24-kDa fragment and a carboxy-terminal 16-kDa fragment (22) (Fig. 1A). When a nitrocellulose filter containing these σ3 fragments was probed with ^{35}S-labeled, translated μ1, we found that μ1 recognized intact σ3 and the amino-terminal fragment of σ3 but not the carboxy-terminal fragment (Fig. 1B). Thus, the amino-terminal portion of σ3 appears to be required for σ3-μ1 interaction. Interestingly, we found that μ1 recognized one or more of the λ proteins and the core protein σ2. This finding is consistent with a current model of reovirus structure in which μ1 and core protein λ2 form the icosahedral shell of the outer capsid and interact with nodules (composed of λ1 and σ2) that protrude from the core shell (5).

A well-characterized structural determinant within the amino terminus of σ3 is the CCHC zinc finger motif (15, 22). To investigate the contribution of this motif to the functions of σ3, we used the Altered Sites Mutagenesis System (Promega) to substitute serine residues for the cysteines at positions 51 (C51S) and/or 54 in the reovirus type 3 Dearing (T3D) σ3 sequence. DNA fragments containing the mutations were subcloned into the in vitro transcription/translation vector pCITE2b-S4 (25). The plasmids pCITE2b-S4, pCITE2b-S4[C51S], pCITE2b-S4[C54S], and pCITE2b-S4[C51,54S] encoded wild-type (WT) σ3, σ3[C51S], σ3[C54S], and σ3[C51,54S], respectively, downstream of the encephalomyocarditis virus internal ribosome entry site in pCITE2b (Novagen, Madison, Wis.). A point mutation (K293T), which ab-

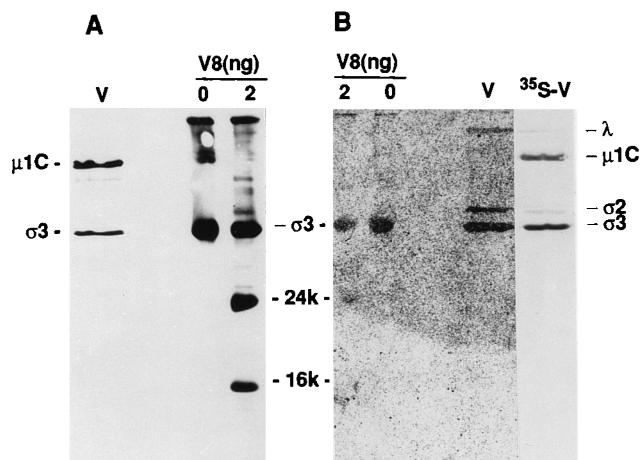

FIG. 1. Protein blot of σ3 proteolytic fragments. Gel-purified T3D σ3 from 2×10^{11} virions was digested in the absence or presence of 2 ng of V8 protease and transferred to nitrocellulose as described previously (22). (A) Rabbit antiserum prepared against virions was used to detect the reovirus peptides. Antibody bound to the nitrocellulose was detected by horseradish peroxidase-conjugated anti-immunoglobulin G and enhanced chemiluminescence detection reagents (Amersham International, Arlington Heights, Ill.). The positions of intact reovirus proteins (lane V) are indicated. (B) A 50-µl µ1 translation reaction mixture was diluted into 20 ml of Tris-buffered saline (pH 7.6)–0.05% Tween (TBST) and used to probe reovirus peptides for 1 h at room temperature. The blot was washed with TBST, coated with En^3Hance spray (NEN, Boston, Mass.), and exposed to film. The unlabeled virus in the lane labeled V was probed with µ1. The [^{35}S]methionine-labeled virus in the lane labeled ^{35}S-V was transferred to nitrocellulose, and the positions of the viral proteins are indicated.

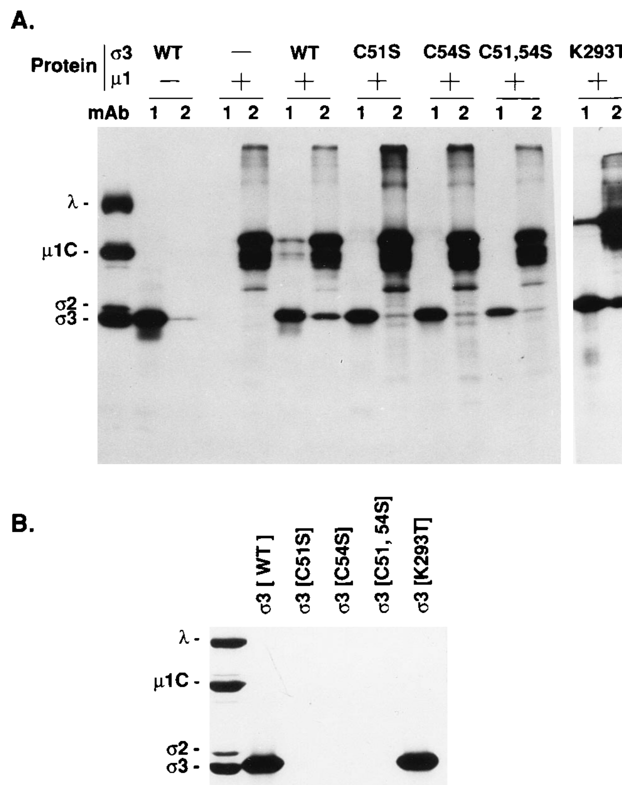

FIG. 2. Ability of σ3 mutants to form complexes with µ1. (A) Cotranslation reaction mixtures containing µ1 and WT σ3 or mutant σ3 (200,000 trichloroacetic acid-precipitable ^{35}S cpm) or control proteins that were translated individually (100,000 trichloroacetic acid-precipitable ^{35}S cpm) were immunoprecipitated with the σ3-specific monoclonal antibody 4F2 (lanes 1) or the µ1-specific monoclonal antibody 10H2 (lanes 2) in a buffer consisting of 0.1 M NaCl, 1 mM EDTA, 10 mM Tris [pH 7.5], and 0.05% Nonidet P-40. Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography. The leftmost lane contains ^{35}S-labeled virions to identify the positions of the viral proteins. (B) ^{35}S-labeled WT and mutant σ3 were prepared by in vitro transcription/translation and incubated with 5×10^{10} purified reovirus type 1 Lang ISVPs for 1 h at room temperature in virion storage buffer. Samples were purified by CsCl gradient centrifugation and concentrated. Untreated ^{35}S-labeled virions (far left) and unlabeled ISVPs recoated with ^{35}S-labeled, in vitro-translated WT σ3 or mutant σ3 were analyzed by SDS-PAGE and fluorography. The positions of viral proteins are indicated on the left.

rogates σ3's dsRNA-binding activity (4), was introduced into pCITE2b-S4 by PCR-mediated mutagenesis to generate pCITE2b-S4[K293T]. We also introduced a cDNA copy of the T3D M2 gene, encoding µ1 (17, 20), into pCITE2b. In vitro transcription was carried out according to the Ribomax method (Promega), and the resultant mRNAs were translated in vitro by using the Flexi Rabbit Reticulocyte Lysate System (Promega). In vitro transcription/translation with the pCITE2b-M2 and pCITE2b-S4 plasmids yielded appropriately sized protein products that reacted with µ1- and σ3-specific monoclonal antibodies, respectively (data not shown).

To determine whether σ3's amino-terminal zinc finger is an important structural determinant for the interaction of σ3 and µ1, we used a coimmunoprecipitation assay. WT or mutant S4 mRNAs were cotranslated with M2 mRNA, and the resultant proteins were subjected to immunoprecipitation (30) in a buffer designed to maintain protein complexes (11). The results of our analysis are shown in Fig. 2A. Complexes between WT σ3 and µ1 were evident when the cotranslated proteins were immunoprecipitated with monoclonal antibody 4F2 (directed against σ3) or 10H2 (directed against µ1). In contrast, immunoprecipitation of cotranslated µ1 and σ3[C51S], σ3[C54S], or σ3[C51,54S] with either 4F2 or 10H2 demonstrated that the zinc mutants did not form complexes with µ1 to a significant degree. In addition to analyzing σ3 zinc mutants, we evaluated the µ1-binding activity of σ3[K293T], a σ3 mutant defective in dsRNA binding (4). Evidence in the literature suggests that σ3 can bind either dsRNA or µ1, but not both, suggesting that their binding sites might overlap (29). Interestingly, we found that σ3[K293T] efficiently formed complexes with µ1, indicating that this amino acid substitution in the dsRNA-binding region does not affect the ability of σ3 to form a stable complex with µ1 (Fig. 2A).

To determine whether the failure of the σ3 zinc mutants to form complexes with µ1 was simply a consequence of the coimmunoprecipitation assay conditions, we used a subviral particle-binding assay to analyze their ability to complex with particle-bound µ1 fragments. This assay is based on the observation that σ3 from infected cell extracts can bind to intermediate subviral particles (ISVPs) (1). ISVPs are stable particles that can be generated by treatment of purified virions with trypsin or chymotrypsin (3, 12, 24). ISVPs differ from virions in that they have a cleaved form of µ1 and an extended form of the cell-attachment protein σ1 and are completely lacking σ3 (7, 12, 24). To assess the ability of mutant σ3 molecules to interact with ISVPs, purified ISVPs were recoated with translated WT or mutant σ3 as described previously (25). Proteins of the ISVP were visualized by staining the gel with Coomassie brilliant blue (data not shown), and the labeled protein bound to the ISVP was visualized by fluorography (Fig. 2B). WT σ3 and σ3[K293T] stably associated with ISVPs. In contrast, none of the σ3 zinc mutant proteins bound to these particles. Together these experiments demonstrated that the σ3 zinc mutants are defective in their ability to associate with outer capsid protein µ1.

The results of our studies with σ3 fragments and site-di-

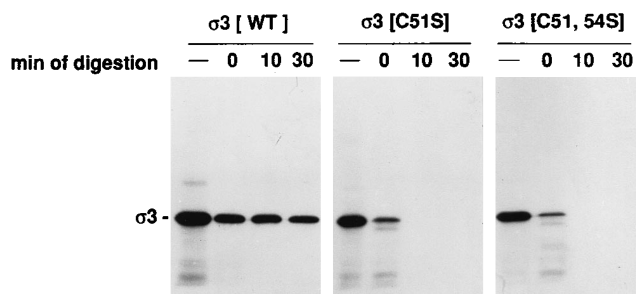

FIG. 3. Stability of σ3 mutants in vitro. The indicated samples of in vitro-translated σ3 (98,000 trichloroacetic acid-precipitable ^{35}S cpm each of WT σ3, σ3[C51S], and σ3[C51,54S]) were digested with 20 μg of proteinase K per ml for 30 min at 30°C in virion storage buffer. Digestion was stopped by the addition of 1 mM phenylmethylsulfonyl fluoride. Samples were analyzed by SDS-PAGE and fluorography. The lane labeled − contains an untreated control removed from the sample prior to the addition of protease. Samples were removed at 0, 10, and 30 min, as indicated above the lanes.

rected point mutants strongly suggest that the ability to coordinate a zinc atom contributes to μ1-ligand-binding activity associated with the amino terminus of σ3. The μ1-binding determinant could specifically involve sequences within the zinc finger, or, more likely, our findings could reflect a significant contribution of the zinc finger to the conformation of native σ3. To test the hypothesis that the zinc mutants have an altered conformation relative to WT σ3, we used a protease digestion assay that has been described previously (25) to analyze the protease sensitivity of translated WT and mutant σ3. The results of this experiment (Fig. 3) illustrate that the σ3 zinc mutants were significantly more protease sensitive than WT σ3. WT σ3 was relatively stable throughout the 30-min digestion period shown in this figure; between 45 and 60% of the input WT σ3, measured by densitometry, was still detectable after 2 h under these digestion conditions (data not shown). In contrast, σ3[C51S] and σ3[C51,54S] were extremely protease sensitive, as evidenced by both the significant decrease in recovery of full-length protein immediately following proteinase K addition (time zero) and by the complete loss of labeled protein after incubation for 10 min. WT and mutant σ3 proteins were stable in the absence of protease for the 30-min incubation period (data not shown). Similar results were obtained for the mutant σ3[C54S] (data not shown).

Our results suggested that the conformation of σ3 molecules with amino acid substitutions in the zinc finger was altered so as to render them protease sensitive. Such a conformational alteration may contribute to the inability of the zinc mutants to bind μ1. The conclusion that the zinc mutants are altered in conformation was supported by the observation that six conformation-dependent, σ3-specific monoclonal antibodies, which recognize WT σ3 in immunoprecipitation assays but not in immunoblotting assays (30), failed to immunoprecipitate the σ3 zinc mutants (data not shown). The epitopes recognized by these antibodies have not been mapped, but these results suggest that they may include amino-terminal sequences. Monoclonal antibody 4F2 recognizes an epitope in the amino-terminal half of the σ3 molecule (13). In contrast to the finding with conformation-dependent monoclonal antibodies, our analysis indicated that 4F2 immunoprecipitates WT and mutant σ3 molecules with equal efficiency (data not shown). Monoclonal antibody 4F2 reacts with denatured σ3 on Western blots (immunoblots) (30), suggesting that it recognizes a linear epitope that is maintained in the σ3 zinc mutants.

Our finding that σ3[K293T], which is defective in dsRNA binding (4), binds μ1 as well as WT σ3 does strongly suggests that the sites on σ3 that mediate interaction with μ1 and dsRNA are not identical. If the binding sites of μ1 and dsRNA on σ3 are indeed not identical and the molecular determinants responsible for dsRNA binding lie in the carboxy terminus of the protein (4, 18, 22), then the σ3 zinc mutants should be unimpaired in their ability to bind dsRNA. To investigate this hypothesis, samples with equal numbers of input counts from translation reaction mixtures of WT or mutant σ3, in which all proteins were shown to be synthesized to equivalent levels (Fig. 4A), were incubated with poly(IC)-agarose beads in a batch-binding assay according to the method of Siomi et al. but modified to include 60 μl of poly(IC)-agarose per sample (27). As a negative control, we used translated μ1. The results (Fig. 4B) demonstrate that the three σ3 zinc mutants, σ3[C51S], σ3[C54S], and σ3[C51,54S], have increased affinity for dsRNA relative to WT σ3. No binding was detected by the control protein μ1. Thus, mutations in the amino-terminal zinc finger that eliminated the ability of σ3 to bind μ1 apparently increase its ability to bind dsRNA.

In the absence of other data, these results would be consistent with an hypothesis that the amino terminus of σ3 contains latent dsRNA-binding sites which are revealed when the molecule cannot coordinate zinc. This hypothesis is unlikely, however, given previous work indicating that carboxy-terminal cleavage fragments (22) and amino-terminal deletion mutants (18) have an increased capacity to bind dsRNA in blotting assays. Therefore, it is more likely that the conformation assumed by the amino terminus of σ3 when it coordinates zinc is one that is critical for the interaction with μ1 and also attenuates the dsRNA-binding activity associated with the carboxy terminus of σ3.

Our results provide evidence for an important functional property associated with the amino-terminal domain of σ3. The ability of σ3 to associate with μ1 appears to depend on the native conformation of the amino terminus of σ3, which in turn is dependent on sequences in the zinc finger. Like the zinc finger motifs of the polyomavirus large T antigen and the

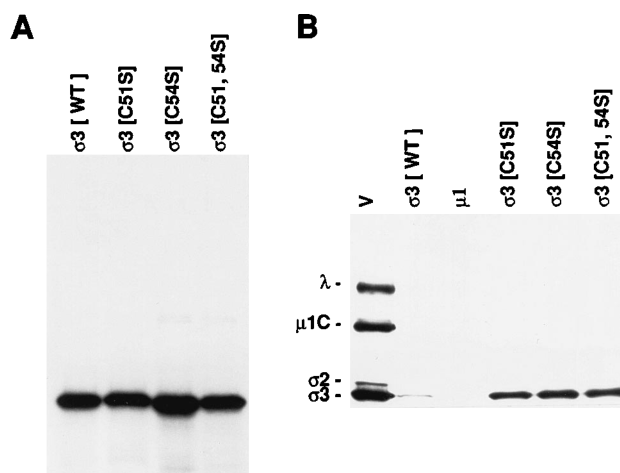

FIG. 4. dsRNA-binding activity of σ3 mutants. (A) ^{35}S-labeled proteins were prepared by in vitro transcription/translation from the pCITE2b vector. Approximately 2% of the total volume of each translation reaction mixture of WT σ3 and σ3 zinc mutants was analyzed by SDS-PAGE and fluorography to confirm that the proteins were translated to equivalent levels. (B) Samples with 100,000 trichloroacetic acid-precipitable input counts were diluted into binding buffer [10 mM Tris HCl (pH 7.4), 2.5 mM $MgCl_2$, 0.5% Triton X 100, 0.25 M NaCl], incubated with 60 μl poly(IC)-agarose for 1 h at 4°C, and subjected to five washes in binding buffer. Bound protein was eluted by boiling in Laemmli sample buffer, and half the sample was analyzed by SDS-PAGE and fluorography. Lane V, untreated [^{35}S]methionine-labeled virus.

adenovirus E1A protein, that of σ3 may represent a means to facilitate important protein-protein interactions (21, 31). In addition, whereas it may not mediate nucleic acid-binding like the prototypic transcription factor IIIA-like zinc finger motif, which serves to fold that protein into a conformation that can bind nucleic acid (2), our experimental evidence indicates that the zinc finger does influence the dsRNA-binding activity of σ3. Although the conformation of the σ3 zinc finger mutants is altered so that they no longer bind μ1, they bind dsRNA more efficiently than does WT σ3. This finding suggests that a native σ3 conformation is not required for dsRNA binding and is consistent with other data from our laboratory indicating that small carboxy-terminal deletions in σ3 do not impair dsRNA binding while they do result in enhanced protease sensitivity (26). Our findings conflict, however, with those of Denzler and Jacobs (4), who find that small amino-terminal or carboxy-terminal deletions in σ3 eliminate dsRNA-binding activity. The reason for this inconsistency is not clear but may reflect differences in the assay conditions and/or the source of protein that influence protein conformation.

It has been suggested that the binding sites on σ3 for μ1 and dsRNA overlap such that σ3-μ1 complexes cannot bind dsRNA (29). This hypothesis arose from the finding that co-expression with μ1 interferes with the ability of σ3 to stimulate reporter gene expression (29) and the observation that σ3-μ1 protein complexes are never isolated from extracts of infected cells on poly(IC) columns that effectively purify free σ3 (9). We have shown that σ3[K293T], the mutant which is defective in dsRNA binding (4), efficiently binds μ1 and that the σ3 zinc mutants bind dsRNA but not μ1. Furthermore, Tillotson and Shatkin have described a μ1 mutant that binds σ3 but does not inhibit the ability of σ3 to stimulate reporter gene expression (29). Taken together, these results strongly suggest that the binding sites on σ3 for μ1 and dsRNA are not identical. Two hypotheses could explain the failure of σ3-μ1 complexes to bind dsRNA. Binding of μ1 by σ3 could interfere with σ3's dsRNA binding in a steric manner. In fact, cryoelectron microscopy data indicate that the σ3-μ1 interface is extensive (5). Thus, whereas our work and the work of others (16) suggest that sequences required for binding μ1 may be localized to the amino terminus of σ3, a more extensive region of contact with μ1 may obscure σ3's dsRNA-binding sequences in the carboxy terminus of the protein. Alternatively, as recent data from our laboratory indicate, the σ3-μ1 interaction induces a conformational change in σ3, which may in turn render σ3 unable to bind dsRNA (25). Further experiments to test the ability of μ1 to block dsRNA binding by σ3 are ongoing in our laboratory.

D.A.S. and J.G.E. contributed equally to this work.

This work was supported by Public Health Service grant R29AI32139 to L.A.S. from the National Institute of Allergy and Infectious Diseases. D.A.S. was supported by Public Health Service award 1F32AI0902001A1 from the National Institute of Allergy and Infectious Diseases and Cancer Research training grant 5T32CA09138 from the National Cancer Institute.

We thank Stephen Schmechel for construction of the K293T mutant, Brent Huberty for assistance in construction of some of the plasmids, Michael Chute for sequence analysis of mutant clones, and Tim Leonard for photography. Skip Virgin and Ken Tyler graciously provided monoclonal antibodies, and Bernie Fields provided polyclonal anti-reovirus serotype 1 Lang antiserum. Stephen Schmechel, Stephen Rice, and Max Nibert provided critical reviews of the manuscript.

REFERENCES

1. **Astell, C., S. C. Silverstein, D. H. Levin, and G. Acs.** 1972. Regulation of the reovirus RNA transcriptase by a viral capsomere protein. Virology **48:**648–654.
2. **Berg, J. M.** 1986. Potential metal binding domains in nucleic acid binding proteins. Science **232:**485–487.
3. **Borsa, J., T. P. Copps, M. D. Sargent, D. G. Long, and J. D. Chapman.** 1973. New intermediate subviral particles in the in vitro uncoating of reovirus virions by chymotrypsin. J. Virol. **11:**552–564.
4. **Denzler, K. L., and B. L. Jacobs.** 1994. Site-directed mutagenic analysis of reovirus sigma 3 protein binding to dsRNA. Virology **204:**190–199.
5. **Dryden, K. A., G. Wang, M. Yeager, M. L. Nibert, K. M. Coombs, D. B. Furlong, B. N. Fields, and T. S. Baker.** 1993. Early steps in reovirus infection are associated with dramatic changes in supramolecular structure and protein conformation: analysis of virions and subviral particles by cryoelectron microscopy and image reconstruction. J. Cell Biol. **122:**1023–1041.
6. **Fields, B. N., C. S. Raine, and S. G. Baum.** 1971. Temperature-sensitive mutants of reovirus type 3: defects in viral maturation as studied by immunofluorescence and electron microscopy. Virology **43:**569–578.
7. **Furlong, D. B., M. L. Nibert, and B. N. Fields.** 1988. σ1 protein of mammalian reoviruses extends from the surfaces of viral particles. J. Virol. **62:**246–256.
8. **Giantini, M., and A. J. Shatkin.** 1989. Stimulation of chloramphenicol acetyltransferase mRNA translation by reovirus capsid polypeptide σ3 in cotransfected COS cells. J. Virol. **63:**2415–2421.
9. **Huismans, H., and W. K. Joklik.** 1976. Reovirus-coded polypeptides in infected cells: isolation of two native monomeric polypeptides with affinity for single-stranded and double-stranded RNA, respectively. Virology **70:**411–424.
10. **Imani, F., and B. L. Jacobs.** 1988. Inhibitory activity for the interferon-induced protein kinase is associated with the reovirus serotype 1 sigma 3 protein. Proc. Natl. Acad. Sci. USA **85:**7887–7891.
11. **Jagus, R.** 1987. Characterization of *in vitro* translation products. Methods Enzymol. **152:**296–304.
12. **Joklik, W. K.** 1972. Studies on the effect of chymotrypsin on reovirions. Virology **49:**700–801.
13. **Kedl, R., S. Schmechel, and L. Schiff.** 1995. Comparative sequence analysis of the reovirus S4 genes from 13 serotype 1 and serotype 3 field isolates. J. Virol. **69:**552–559.
14. **Lee, P. W. K., E. C. Hayes, and W. K. Joklik.** 1981. Characterization of anti-reovirus immunoglobulins secreted by cloned hybridoma cell lines. Virology **108:**134–146.
15. **Mabrouk, T., and G. Lemay.** 1994. Mutations in a CCHC zinc-binding motif of the reovirus σ3 protein decrease its intracellular stability. J. Virol. **68:**5287–5290.
16. **Mabrouk, T., and G. Lemay.** 1994. The sequence similarity of reovirus σ3 protein to picornaviral proteases is unrelated to its role in μ1 viral protein cleavage. Virology **202:**615–620.
17. **McCrae, M. A., and W. K. Joklik.** 1978. The nature of the polypeptide encoded by each of the ten double-stranded RNA segments of reovirus type 3. Virology **89:**578–593.
18. **Miller, J. E., and C. E. Samuel.** 1992. Proteolytic cleavage of the reovirus σ3 protein results in enhanced double-stranded RNA-binding activity: identification of a repeated basic amino acid motif within the C-terminal binding region. J. Virol. **66:**5347–5356.
19. **Morgan, E. M., and H. J. Zweerink.** 1974. Reovirus morphogenesis: core-like particles in cells infected at 39 with wild-type reovirus and temperature-sensitive mutants of groups B and G. Virology **59:**556–565.
20. **Mustoe, T. A., R. F. Ramig, A. H. Sharpe, and B. N. Fields.** 1978. Genetics of reovirus: identification of the dsRNA segments encoding the polypeptides of the m and s size classes. Virology **89:**594–604.
21. **Rose, P. E., and B. S. Schaffhausen.** 1995. Zinc-binding and protein-protein interactions mediated by the polyomavirus large T antigen zinc finger. J. Virol. **69:**2842–2849.
22. **Schiff, L. A., M. L. Nibert, M. S. Co, E. G. Brown, and B. N. Fields.** 1988. Distinct binding sites for zinc and double-stranded RNA in the reovirus outer capsid protein σ3. Mol. Cell. Biol. **8:**273–283.
23. **Sharpe, A. H., and B. N. Fields.** 1982. Reovirus inhibition of cellular RNA and protein synthesis: role of the S4 gene. Virology **122:**381–391.
24. **Shatkin, A. J., and A. J. LaFiandra.** 1972. Transcription by infectious subviral particles of reovirus. J. Virol. **10:**698–706.
25. **Shepard, D. A., J. G. Ehnstrom, and L. A. Schiff.** 1995. Association of reovirus outer capsid proteins σ3 and μ1 causes a conformational change that renders sigma 3 protease sensitive. J. Virol. **69:**8180–8184.
26. **Shepard, D. A., J. G. Ehnstrom, and L. A. Schiff.** Unpublished data.
27. **Siomi, H., M. C. Siomi, R. L. Nussbaum, and G. Dreyfuss.** 1993. The protein product of the fragile X gene, FMR1, has characteristics of an RNA-binding protein. Cell **74:**291–298.
28. **Smith, R. E., H. J. Zweerink, and W. K. Joklik.** 1969. Polypeptide components of virions, top component and cores of reovirus type 3. Virology **39:**791–810.
29. **Tillotson, L., and A. J. Shatkin.** 1992. Reovirus polypeptide σ3 and N-terminal myristoylation of polypeptide μ1 are required for site-specific cleavage to μ1C in transfected cells. J. Virol. **66:**2180–2186.
30. **Virgin, H. W., M. A. Mann, B. N. Fields, and K. L. Tyler.** 1991. Monoclonal antibodies to reovirus reveal structure/function relationships between capsid proteins and genetics of susceptibility to antibody action. J. Virol. **65:**6772–6781.
31. **Webster, L. C., and R. P. Ricciardi.** 1991. *trans*-dominant mutants of E1A provide genetic evidence that the zinc finger of the *trans*-activating domain binds a transcription factor. Mol. Cell. Biol. **11:**4287–4296.
32. **Zweerink, H. J., and W. K. Joklik.** 1970. Studies on the intracellular synthesis of reovirus-specified proteins. Virology **41:**501–518.