

Association of Reovirus Outer Capsid Proteins $\sigma 3$ and $\mu 1$ Causes a Conformational Change That Renders $\sigma 3$ Protease Sensitive†

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

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

Received 27 June 1995/Accepted 12 September 1995

Association of the reovirus proteins $\sigma 3$ and $\mu 1$ influences viral entry, initiation of outer capsid assembly, and modulation of the effect of $\sigma 3$ on cellular translation. In this study, we have addressed whether structural changes occur in $\sigma 3$ as a result of its interaction with $\mu 1$. Using differences in protease sensitivity to detect conformationally distinct forms of $\sigma 3$, we showed that association of $\sigma 3$ with $\mu 1$ caused a conformational change in $\sigma 3$ that converted it from a protease-resistant to a protease-sensitive structure and occurred posttranslationally. The effect of $\mu 1$ on the structure of $\sigma 3$ was stoichiometric. Our results are consistent with a model in which $\sigma 3$'s association with $\mu 1$ shifts its function from translational control to assembly of an outer capsid in which $\sigma 3$ is folded into the protease-sensitive conformation that is required for its cleavage during the next round of infection.

The reovirus outer capsid is composed of 600 copies each of the $\sigma 3$ and $\mu 1$ proteins (8). In the capsid, $\mu 1$ exists primarily as a 72-kDa fragment, known as $\mu 1C$, that is generated by cleavage of $\mu 1$ near its amino terminus (15, 28, 40). The $\mu 1$ protein, which is myristoylated (28), is believed to mediate penetration of cellular membranes during entry (19, 26–28) and plays a role in transcriptase activation (5). The $\sigma 3$ protein, a 41-kDa zinc metalloprotein (30), occupies the outermost position on the viral capsid (8) and is implicated in viral stability (6, 7). During viral entry, $\sigma 3$ must be proteolytically removed from the virion (3, 35, 37). Within infected cells, $\sigma 3$ has been implicated in viral effects on cellular translation (31), and expression of $\sigma 3$ in transfected cells stimulates translation of a cotransfected reporter gene (11). The $\sigma 3$ protein binds double-stranded RNA (dsRNA) (12), and it is believed that the ability to sequester dsRNA and inhibit the interferon-induced, dsRNA-activated inhibitory kinase (PKR) mediates $\sigma 3$'s stimulatory effect on translation (13).

The association of $\mu 1$ and $\sigma 3$ plays an important role in the functions of these proteins. Studies in which the M2 and S4 genes, encoding $\mu 1$ and $\sigma 3$, respectively (20, 25), were cotransfected into cells demonstrate that the presence of $\mu 1$ eliminates the ability of $\sigma 3$ to stimulate translation, suggesting that the formation of $\sigma 3$ - $\mu 1$ protein complexes interferes with $\sigma 3$'s ability to bind dsRNA and inhibit the PKR pathway (38). Association of $\sigma 3$ and $\mu 1$ also is required for outer capsid assembly. A temperature-sensitive mutant with a lesion that maps to the S4 gene (24) produces only core particles at the nonpermissive temperature (9, 23), indicating that the ability of $\mu 1$ to form the icosahedral lattice of the outer capsid (8) is dependent on the formation of $\sigma 3$ - $\mu 1$ complexes. Despite the evidence that the association of $\sigma 3$ and $\mu 1$ has important functional consequences, it is not known if this association causes structural changes in either protein.

In this study, we examined the structural consequences of the association of $\mu 1$ with $\sigma 3$. We expressed ^{35}S -labeled $\sigma 3$ by in vitro transcription and translation from plasmid pCITE-2b-S4T3D, which was constructed by subcloning a cDNA copy of the T3D S4 gene (17) into pCITE-2b (Novagen, Madison, Wis.). In vitro transcription was carried out by the Ribomax (Promega) method. In vitro translation was carried out with the Flexi Rabbit Reticulocyte Lysate System (Promega).

Analysis of the translation product by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (18) and fluorography revealed a single, ^{35}S -labeled protein band with the same mobility as virion $\sigma 3$ (data not shown). To confirm that translated $\sigma 3$ was not misfolded, we looked at the ability of seven anti- $\sigma 3$ monoclonal antibodies to precipitate the protein. Experimental evidence indicates that only one of the seven antibodies recognizes a linear determinant maintained in denatured $\sigma 3$, whereas the other six antibodies recognize epitopes that are dependent on protein conformation (39). Proteins were immunoprecipitated, using a previously described procedure (39), from a buffer consisting of 0.1 M NaCl, 1 mM EDTA, 10 mM Tris (pH 7.5), and 1% Nonidet P-40 (14). Translated $\sigma 3$ was recognized by all seven anti- $\sigma 3$ antibodies but not by a monoclonal antibody directed against $\mu 1$ (data not shown).

Our finding that conformation-dependent monoclonal antibodies directed against virion $\sigma 3$ recognize translated $\sigma 3$ indicates that elements of native tertiary structure are retained in translated $\sigma 3$. The $\sigma 3$ in virions is known to be protease sensitive (2, 16, 32). Early in infection, virions must be converted to intermediate subviral particles (ISVPs) by the proteolytic removal of $\sigma 3$ and carboxy-terminal cleavage of $\mu 1$ and $\mu 1C$ into two smaller fragments (3, 26, 35–37).

To determine whether $\sigma 3$ is folded into a protease-sensitive conformation during or just after translation or whether this happens upon capsid assembly, we examined the protease sensitivity of translated and virion $\sigma 3$. Folded domains of proteins are generally insensitive to protease digestion, whereas polypeptide segments that link folded domains are often susceptible. Changes in protease sensitivity reflect alterations in protein conformation (4). Purified ^{35}S -labeled virions, obtained as previously described (10), and translated $\sigma 3$ were

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

† Dedicated to the memory of Bernard N. Fields, who continues to serve as a spiritual mentor.

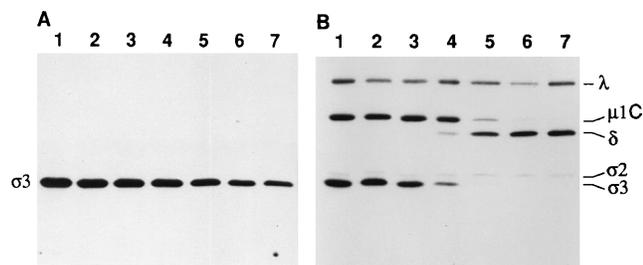


FIG. 1. Proteinase K digestion of translated $\sigma 3$ and virions. Translated $\sigma 3$ (98,000 trichloroacetic acid-precipitable ^{35}S cpm) and T3D virions (280,000 ^{35}S cpm) were digested with 20 μg of proteinase K per ml for 2 h at 30°C. Samples were analyzed by SDS-PAGE (10% resolving gel) and fluorography. Digestion samples of translated $\sigma 3$ and virions are shown in panels A and B, respectively. Lane 1 contains the untreated control. Samples were removed at 0 (immediately after protease addition and incubation at 30°C; lane 2), 10 (lane 3), 30 (lane 4), 60 (lane 5), 90 (lane 6), and 120 (lane 7) min.

digested in virion storage buffer (10) with 20 μg of proteinase K (Sigma Chemical Co.) per ml for 2 h at 30°C. Reticulocyte lysate was added to all samples, and unlabeled virions were added to translated samples to balance the protein concentration. Samples were removed at intervals, and the digestion was stopped by the addition of 1 mM phenylmethylsulfonyl fluoride (Gibco BRL). Samples were analyzed by SDS-PAGE and fluorography (Fig. 1).

We found that translated $\sigma 3$ (Fig. 1A) was considerably less protease sensitive than virion $\sigma 3$ (Fig. 1B). A similar result was obtained if digestion was performed with trypsin (data not shown). Translated $\sigma 3$ could be completely converted to a protease-sensitive form by the addition of 0.5% SDS, demonstrating that it was not misfolded to such a degree that it was permanently resistant to protease (data not shown). Incubation of translated $\sigma 3$ and virions in the absence of protease did not affect the quantity of $\sigma 3$ (data not shown), indicating that no contaminating proteases were present in the samples.

The finding that translated $\sigma 3$ was less protease sensitive than virion $\sigma 3$ suggested that $\sigma 3$ undergoes a conformational change subsequent to its translation. To investigate directly whether translated $\sigma 3$ would assume a protease-sensitive conformation upon binding to the viral particle, we developed an assay based on the finding that $\sigma 3$ from infected cells can recoat subviral particles (1). ^{35}S -labeled infected cell extracts were made by using a previously described procedure (12), with the following modifications. Monolayers of L cells were infected with T3D at a multiplicity of infection of 50, and at 12 h postinfection, 100 μCi of ^{35}S -label per ml was added for 2 h at 37°C. ISVPs were obtained as described by Nibert and Fields (26). To recoat ISVPs, 2.5 ml of extract was incubated with 2×10^{12} ISVPs for 1 h at room temperature and sedimented in a preformed CsCl gradient (1.25 to 1.45 g/cm^3) in a Beckman 50.1 rotor at 35,000 rpm for 3 h at 4°C. The gradient was fractionated, and fractions containing the radioactive peak corresponding to re-coated ISVPs were pooled and concentrated. The re-coated ISVPs were analyzed by SDS-PAGE. Proteins of the ISVP were visualized by staining the gel (data not shown), and labeled protein bound to the ISVP was visualized by fluorography (Fig. 2A). This analysis confirmed the finding of Astell and colleagues (1) that $\sigma 3$ from reovirus-infected cell extracts (Fig. 2A, lane 1) can recoat ISVPs (Fig. 2A, lane 3).

Next, we examined the ability of translated $\sigma 3$ to recoat ISVPs. Radiolabeled, translated $\sigma 3$ was mixed with 5×10^{10} ISVPs in virion storage buffer, and the re-coated particles were purified and analyzed as described above. Like $\sigma 3$ from in-

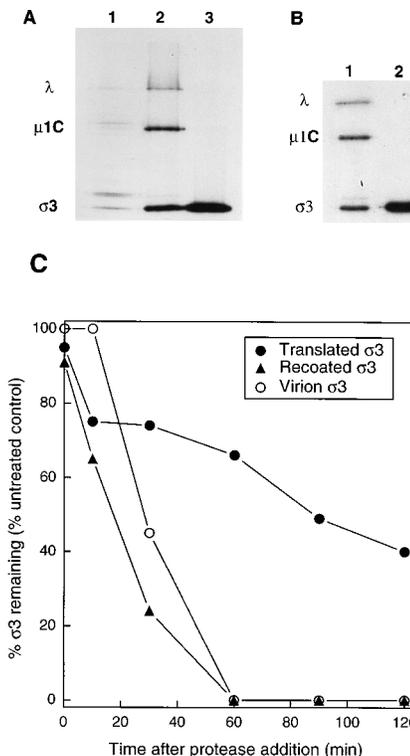


FIG. 2. Proteinase K digestion of translated $\sigma 3$, re-coated ISVPs, and virions. (A) ^{35}S -labeled, reovirus-infected cell extract (lane 1) was incubated with ISVPs. The resulting particles were purified and analyzed by SDS-PAGE (10% resolving gel) and fluorography (lane 3). An untreated sample of ^{35}S -labeled virions was also analyzed (lane 2). The positions of the viral proteins are indicated on the left. (B) Untreated ^{35}S -labeled virions (lane 1) and ISVPs re-coated with ^{35}S -labeled, translated $\sigma 3$ (lane 2) were analyzed by SDS-PAGE (10% resolving gel) and fluorography. Positions of viral proteins are indicated on the left. (C) ISVPs re-coated with translated $\sigma 3$ (280,000 ^{35}S cpm), translated $\sigma 3$ (98,000 trichloroacetic acid-precipitable ^{35}S cpm) from the sample used for ISVP re-coating, and virions (280,000 ^{35}S cpm) were digested with 20 μg of proteinase K per ml for 2 h at 30°C. Samples were removed at intervals and analyzed by SDS-PAGE (10% resolving gel) and fluorography. Bands representing $\sigma 3$ were quantitated by densitometry.

fectured cells, translated $\sigma 3$ re-coated ISVPs (Fig. 2B, lane 2). This finding provided further evidence that translated $\sigma 3$ shares functional properties with native $\sigma 3$ from infected cells. Re-coating was not stoichiometric with either translated $\sigma 3$ or $\sigma 3$ from infected cell extract.

Using ISVPs re-coated with translated $\sigma 3$, we assessed whether association of $\sigma 3$ with the viral particle altered its conformation. We used the proteinase K digestion assay to compare the protease sensitivities of translated $\sigma 3$, $\sigma 3$ in virions, and translated $\sigma 3$ bound to ISVPs. Bands representing $\sigma 3$ were quantitated by densitometry. Densitometric analysis was performed on a Macintosh IIsi computer using the public domain NIH Image program (written by Wayne Rasband at the National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161, part no. PB93-504868). The percentage of $\sigma 3$ that remained over time was calculated and is represented graphically in Fig. 2C. Association with the ISVP rendered translated $\sigma 3$ protease sensitive to the same degree as virion $\sigma 3$. These results indicated that $\sigma 3$ undergoes a conformational change upon association with the ISVP and provided evidence that the protease-resistant conformation of newly translated $\sigma 3$ is not irreversible.

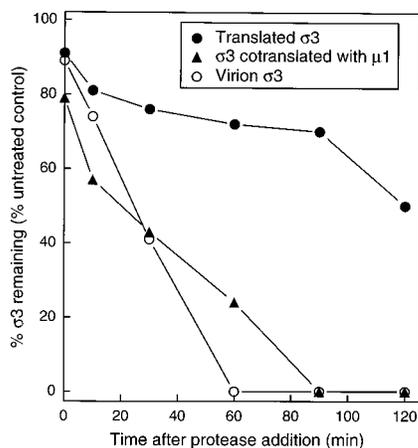


FIG. 3. Proteinase K digestion of translated $\sigma 3$, cotranslated $\sigma 3$ and $\mu 1$, and virions. Translated $\sigma 3$ (98,000 trichloroacetic acid-precipitable ^{35}S cpm), cotranslated $\sigma 3$ and $\mu 1$ (280,000 trichloroacetic acid-precipitable ^{35}S cpm), and virions (280,000 ^{35}S cpm) were digested with 20 μg of proteinase K per ml for 2 h at 30°C. Samples were analyzed by SDS-PAGE (10% resolving gel) and fluorography. Bands representing $\sigma 3$ were quantitated by densitometry, and the percentage of $\sigma 3$ remaining, relative to the level in the untreated control, was calculated and represented graphically. The time zero value for $\sigma 3$ cotranslated with $\mu 1$ was lower than the time zero values for virion and translated $\sigma 3$ as a result of the presence of truncated products of $\mu 1$ that interfered with quantitation of intact $\sigma 3$. These truncated $\mu 1$ products were completely digested upon addition of protease (data not shown).

In the ISVP, the most exterior major protein is $\mu 1$ (8), which has been cleaved into three fragments (26, 28, 36, 40). Thus, the conformational change that occurs when $\sigma 3$ binds to the ISVP is likely to have resulted from interaction with the cleaved form of $\mu 1$, which has been shown by cryoelectron microscopy to largely retain the conformation of $\mu 1$ and $\mu 1\text{C}$ found in virions (8). To determine whether the conformational change in $\sigma 3$ could be mediated by soluble, intact $\mu 1$, we compared the protease sensitivity of $\sigma 3$ cotranslated with $\mu 1$ with that of $\sigma 3$ translated alone and $\sigma 3$ in virions. To generate $\mu 1$ by *in vitro* transcription and translation, we introduced a cDNA copy of the T3D M2 gene (15) into pCITE-2b. Cotranslation of $\sigma 3$ with $\mu 1$ resulted in $\sigma 3$ that was as protease sensitive as virion-bound $\sigma 3$ (Fig. 3), suggesting that association of $\sigma 3$ with $\mu 1$ is sufficient to confer a conformational change on $\sigma 3$. The conformational change in $\sigma 3$ can be detected in cotranslation samples within 15 min of the initiation of translation, the earliest time point assayed (data not shown). Cotranslation of $\sigma 3$ with chloramphenicol acetyltransferase did not produce $\sigma 3$ that was protease sensitive, demonstrating that this finding was specific for $\mu 1$ (data not shown). Protease digestion and subsequent immunoprecipitation of $\sigma 3$ and $\mu 1$ from infected cell extracts revealed that at late times during infection, $\sigma 3$ is similarly protease sensitive (data not shown).

Our results suggested that association of $\sigma 3$ and $\mu 1$ as early as 15 min following the initiation of translation mediates a conformational change in $\sigma 3$. To determine whether $\mu 1$ could mediate a change in preexisting $\sigma 3$, the protease digestion was repeated with $\sigma 3$ and $\mu 1$ that were translated separately and then mixed for 1 h at 30°C. Mixing of $\sigma 3$ and $\mu 1$ rendered $\sigma 3$ protease sensitive (data not shown), indicating that a conformational change in $\sigma 3$ could be mediated by $\mu 1$ posttranslationally. To determine whether the $\mu 1$ -mediated change in $\sigma 3$ involved formation of a $\mu 1$ - $\sigma 3$ complex, we used monoclonal antibodies to immunoprecipitate $\sigma 3$ and $\mu 1$ that had been

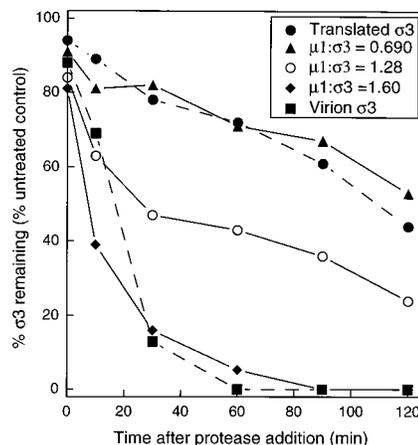


FIG. 4. Proteinase K digestion of translated $\sigma 3$, cotranslated $\sigma 3$ and $\mu 1$ at various ratios, and virions. For this experiment, all translated samples were labeled with [^{35}S]methionine. Translated $\sigma 3$ (98,000 trichloroacetic acid-precipitable ^{35}S cpm), cotranslated $\sigma 3$ and $\mu 1$ (280,000 trichloroacetic acid-precipitable ^{35}S cpm), and virions (280,000 ^{35}S cpm) were digested with 20 μg of proteinase K per ml for 2 h at 30°C. Samples were analyzed by SDS-PAGE (10% resolving gel) and fluorography. Bands representing $\sigma 3$ were quantitated by densitometry, and the percentage of $\sigma 3$ remaining, relative to the level in the untreated control, was calculated and represented graphically. For the cotranslation samples, the $\sigma 3$ and $\mu 1$ bands in the untreated control were quantitated by densitometry, corrected for numbers of methionines in each protein, and divided to obtain a ratio. The positive and negative controls, virions and translated $\sigma 3$, respectively, are indicated by dashed lines.

cotranslated or individually translated and mixed. Cotranslated and mixed samples were immunoprecipitated in a buffer containing 0.05% Nonidet P-40 to maintain protein complexes. Our analysis indicated that both the $\mu 1$ -specific monoclonal antibody 10H2 and the $\sigma 3$ -specific monoclonal antibody 4F2 coprecipitated $\sigma 3$ and $\mu 1$ (data not shown). Thus, for both cotranslated and mixed $\sigma 3$ and $\mu 1$, the conformational change in $\sigma 3$ was associated with $\sigma 3$ - $\mu 1$ complex formation.

In the outer capsid, $\sigma 3$ and $\mu 1$ are present in a 1:1 stoichiometry (15), and it has been suggested that formation of a complex containing one molecule each of $\sigma 3$ and $\mu 1$ blocks the ability of $\sigma 3$ to bind dsRNA (38). To determine whether a 1:1 complex of $\sigma 3$ and $\mu 1$ is required to confer a conformational change on $\sigma 3$, we cotranslated $\sigma 3$ and $\mu 1$ at different relative ratios and examined the protease sensitivity of the resulting $\sigma 3$. Densitometric analysis of the $\sigma 3$ and $\mu 1$ bands was performed. Values representing band intensity were corrected for the relative number of methionine residues in each protein to determine the ratios of $\mu 1$ to $\sigma 3$. We found that as the number of $\mu 1$ molecules increased relative to the number of $\sigma 3$ molecules, $\sigma 3$ became more protease sensitive (Fig. 4). Thus, the ability of $\mu 1$ to confer protease sensitivity on $\sigma 3$ is dependent on their relative stoichiometries and appears to require at least equal numbers of $\sigma 3$ and $\mu 1$ molecules. Limitations in quantitation by densitometry do not allow us to precisely determine the relative amounts of $\sigma 3$ and $\mu 1$ in our cotranslated samples. However, our finding suggests that higher-order structures are being formed when a ratio of 1:1 or higher is achieved. Such structures may be required for $\mu 1$ to efficiently confer a conformational change on $\sigma 3$ and could serve to initiate the assembly of the outer capsid.

To determine whether the $\mu 1$ -mediated conformational change folds $\sigma 3$ into a conformation similar to that of $\sigma 3$ in virions, we examined the intermediate peptides formed upon proteinase K digestion of TIL $\sigma 3$ in virions, on recoated

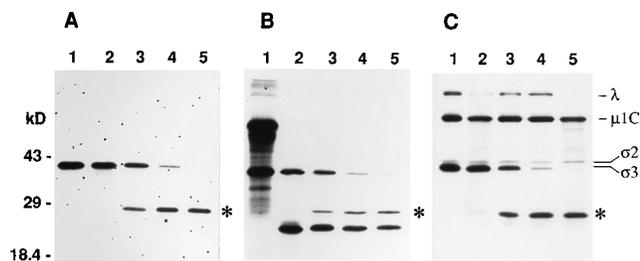


FIG. 5. Proteinase K digestion of T1L σ 3-coated ISVPs (A), T1L σ 3 cotranslated with μ 1 (B), and T1L virions (C). Samples (280,000 ^{35}S cpm) were digested with 100 μg of proteinase K per ml for 1 h at 5°C, and samples were analyzed by SDS-PAGE (10 and 12% resolving gels) and fluorography. In each panel, lane 1 contains the untreated control and lanes 2 through 5 contain samples removed at 0, 10, 30, and 60 min, respectively. The positions of protein size standards are indicated on the left, and the positions of the viral proteins are indicated on the right. Each asterisk marks the position of the 26- to 28-kDa peptide fragment produced by protease digestion. In the cotranslation sample (B), a predominant μ 1 band, as well as two minor truncated forms of μ 1, are produced by *in vitro* translation of the M2 gene. The additional band seen upon digestion of cotranslated σ 3 and μ 1 was generated from digestion of μ 1 (data not shown).

ISVPs, and cotranslated with μ 1. Limited proteolysis of gel-purified σ 3 or bacterially expressed σ 3 with V8 protease produces an amino-terminal 24-kDa fragment and a carboxy-terminal 16-kDa fragment (22, 29, 30). Digestion of virions with other proteases generates similarly sized fragments of σ 3 (29). Intermediate digestion products of σ 3 from T1L virions are relatively stable, whereas those produced from T3D are transient (29). T1L σ 3 was prepared by *in vitro* transcription and translation from plasmid pCITE-2b-S4T1L. The results are shown in Fig. 5. The protease-sensitive form of σ 3 on ISVPs and in cotranslated samples was degraded by proteinase K to yield the same approximately 26- to 28-kDa peptide seen when virion σ 3 was digested (asterisks in Fig. 5). These results suggest that association of μ 1 with soluble σ 3 folds σ 3 into the same protease-sensitive conformation in which it is found on virions.

In this report, we have demonstrated that binding of μ 1 to σ 3 results in a conformational change in σ 3 that converts it from a protease-resistant form to a protease-sensitive form. Monoclonal antibodies directed against two nonlinear epitopes bind the *in vitro*-translated, protease-resistant form of σ 3, as well as the protease-sensitive conformation of the protein found on virions (39) and in complexes containing translated σ 3 and μ 1 (34). These results suggest that the conformational change is a subtle one, rather than a gross rearrangement of σ 3's tertiary structure. Information currently available regarding σ 3's structure suggests a molecular mechanism for this conformational change. A number of studies have identified a region near the center of the σ 3 protein that is hypersensitive to proteolytic cleavage (22, 29, 30). These data, along with cryoelectron microscopy analyses (8), suggest that the tertiary structure of σ 3 consists of two domains joined by a polypeptide hinge. Our results suggest that the hinge region in newly translated σ 3 is inaccessible to proteases and that the μ 1-mediated conformational change in σ 3 affects the hinge region, causing it to become accessible to proteases.

Proteolytic removal of σ 3 is an early, essential step in the reovirus life cycle (37). We have demonstrated that association with soluble μ 1 converts σ 3 to a protease-sensitive structure. We have also used limited proteolysis to provide evidence that soluble σ 3 complexed with μ 1 is folded into the same conformation as σ 3 in virions. Our results suggest that the σ 3- μ 1

interaction is important for folding σ 3 into a conformation that allows its proteolytic cleavage during entry. Similarly, studies showing that extragenic revertants of mutations in the M2 gene map to the S4 gene suggest that the σ 3- μ 1 association is important for μ 1's ability to function during entry (9, 21, 24). Thus, association of σ 3 and μ 1 in a specific conformation appears to be important for the formation of an outer capsid that is competent for entry.

Our studies are consistent with a model in which formation of a 1:1 complex of σ 3 and μ 1 and the subsequent conformational change in σ 3 serve as a functional switch that converts σ 3's function from translational control to assembly of the outer capsid onto nascent core particles. Association of σ 3 and μ 1 folds σ 3 in progeny virions into a conformation that renders it susceptible to proteolysis during the next round of infection. Our findings raise the interesting possibility that the negative effect of the M2 gene on the translational effects of the S4 gene (38) is the result of the conformational change in σ 3 rather than competition by μ 1 for the dsRNA-binding site on σ 3. Recent studies from our laboratory suggest that μ 1 and dsRNA do not share identical binding sites on σ 3 (33). Ongoing studies on the effect of μ 1 on dsRNA binding by σ 3 will further our understanding of the significance of σ 3- μ 1 interactions during reovirus infection.

We thank Ken Tyler and Skip Virgin for the gifts of monoclonal antibodies; Michael Chute for technical assistance; Kay Faaberg, Max Nibert, Stephen Schmechel, and Peter Southern for helpful discussions and technical advice; Max Nibert, Stephen Rice, and Stephen Schmechel for critical reviews of the manuscript; and Tim Leonard for photography.

This work was supported by Public Health Service grant R29 AI32139 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 Microbiology/Cancer Research training grant 5T32CA09138 from the National Cancer Institute.

REFERENCES

- 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.
- 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.
- Chang, C. T., and H. J. Zweerink. 1971. Fate of parental reovirus in infected cell. *Virology* **46**:544-555.
- Creighton, T. E. 1984. *Proteins: structures and molecular properties*. W. H. Freeman & Company, New York.
- Drayna, D., and B. N. Fields. 1982. Activation and characterization of the reovirus transcriptase: genetic analysis. *J. Virol.* **41**:110-118.
- Drayna, D., and B. N. Fields. 1982. Biochemical studies on the mechanism of chemical and physical inactivation of reovirus. *J. Gen. Virol.* **63**:161-170.
- Drayna, D., and B. N. Fields. 1982. Genetic studies on the mechanism of chemical and physical inactivation of reovirus. *J. Gen. Virol.* **63**:149-160.
- 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.
- 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.
- Furlong, D. B., M. L. Nibert, and B. N. Fields. 1988. Sigma 1 protein of mammalian reoviruses extends from the surfaces of viral particles. *J. Virol.* **62**:246-256.
- Giantini, M., and A. J. Shatkin. 1989. Stimulation of chloramphenicol acetyltransferase mRNA translation by reovirus capsid polypeptide sigma 3 in cotransfected COS cells. *J. Virol.* **63**:2415-2421.
- 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.
- 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.
14. **Jaguk, R.** 1987. Characterization of *in vitro* translation products. Methods Enzymol. **152**:296–304.
 15. **Jayasuriya, A. K., M. L. Nibert, and B. N. Fields.** 1988. Complete nucleotide sequence of the M2 gene segment of reovirus type 3 dearing and analysis of its protein product mu 1. Virology **163**:591–602.
 16. **Joklik, W. K.** 1972. Studies on the effect of chymotrypsin on reovirions. Virology **49**:700–801.
 17. **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.
 18. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
 19. **Lucia-Jandris, P., J. W. Hooper, and B. N. Fields.** 1993. Reovirus M2 gene is associated with chromium release from mouse L cells. J. Virol. **67**:5339–5345.
 20. **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.
 21. **McPhillips, T. H., and R. F. Ramig.** 1984. Extragenic suppression of temperature-sensitive phenotype in reovirus: mapping suppressor mutations. Virology **135**:428–439.
 22. **Miller, J. E., and C. E. Samuel.** 1992. Proteolytic cleavage of the reovirus sigma 3 protein results in enhanced double-stranded RNA-binding activity: identification of a repeated basic amino acid motif within the C-terminal binding region. J. Virol. **66**:5347–5356.
 23. **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.
 24. **Mustoe, T. A., R. F. Ramig, A. H. Sharpe, and B. N. Fields.** 1978. A genetic map of reovirus. III. Assignment of the double-stranded RNA mutant groups A, B, and G to genome segments. Virology **85**:545–556.
 25. **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.
 26. **Nibert, M. L., and B. N. Fields.** 1992. A carboxy-terminal fragment of protein $\mu 1/\mu 1C$ is present in infectious subviral particles of mammalian reoviruses and is proposed to have a role in penetration. J. Virol. **66**:6408–6418.
 27. **Nibert, M. L., D. B. Furlong, and B. N. Fields.** 1991. Mechanisms of viral pathogenesis. Distinct forms of reoviruses and their roles during replication in cells and host. J. Clin. Invest. **88**:727–734.
 28. **Nibert, M. L., L. A. Schiff, and B. N. Fields.** 1991. Mammalian reoviruses contain a myristoylated structural protein. J. Virol. **65**:1960–1967.
 29. **Schiff, L. A., and M. L. Nibert.** Unpublished data.
 30. **Schiff, L. A., M. L. Nibert, M. S. Co, E. G. Brown, and B. N. Fields.** 1988. Distinct binding sites for zinc and double-stranded RNA in the reovirus outer capsid protein sigma 3. Mol. Cell. Biol. **8**:273–283.
 31. **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.
 32. **Shatkin, A. J., and A. J. LaFiandra.** 1972. Transcription by infectious subviral particles of reovirus. J. Virol. **10**:698–706.
 33. **Shepard, D. A., J. E. Ehnstrom, P. J. Skinner, and L. A. Schiff.** Mutations in the zinc-binding motif of the reovirus capsid protein $\sigma 3$ eliminates its ability to associate with capsid protein $\mu 1$. Submitted for publication.
 34. **Shepard, D. A., J. G. Ehnstrom, and L. A. Schiff.** Unpublished data.
 35. **Silverstein, S. C., C. Astell, D. H. Levin, M. Schonberg, and G. Acs.** 1972. The mechanisms of reovirus uncoating and gene activation in vivo. Virology **47**:797–806.
 36. **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.
 37. **Sturzenbecker, L. J., M. Nibert, D. Furlong, and B. N. Fields.** 1987. Intracellular digestion of reovirus particles requires a low pH and is an essential step in the viral infectious cycle. J. Virol. **61**:2351–2361.
 38. **Tillotson, L., and A. J. Shatkin.** 1992. Reovirus polypeptide $\sigma 3$ and N-terminal myristoylation of polypeptide $\mu 1$ are required for site-specific cleavage to $\mu 1C$ in transfected cells. J. Virol. **66**:2180–2186.
 39. **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.
 40. **Zweerink, H. J., and W. K. Joklik.** 1970. Studies on the intracellular synthesis of reovirus-specified proteins. Virology **41**:501–518.