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Reovirus Outer Capsid Protein μ1 Induces Apoptosis and Associates with Lipid Droplets, Endoplasmic Reticulum, and Mitochondria

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The mechanisms by which reoviruses induce apoptosis have not been fully elucidated. Earlier studies identified the mammalian reovirus S1 and M2 genes as determinants of apoptosis induction. However, no published results have demonstrated the capacities of the proteins encoded by these genes to induce apoptosis, either independently or in combination, in the absence of reovirus infection. Here we report that the mammalian reovirus μ1 protein, encoded by the M2 gene, was sufficient to induce apoptosis in transfected cells. We also found that μ1 localized to lipid droplets, endoplasmic reticulum, and mitochondria in both transfected cells and infected cells. Two small regions encompassing amphipathic α-helices within a carboxyl-terminal portion of μ1 were necessary for efficient induction of apoptosis and association with lipid droplets, endoplasmic reticulum, and mitochondria in transfected cells. Induction of apoptosis by μ1 and its association with lipid droplets and intracellular membranes in transfected cells were abrogated when μ1 was coexpressed with α3, with which it is known to coassemble. We propose that μ1 plays a direct role in the induction of apoptosis in infected cells and that this property may relate to the capacity of μ1 to associate with intracellular membranes. Moreover, during reovirus infection, association with α3 may regulate apoptosis induction by μ1.

The mammalian orthoreoviruses (reoviruses) are nonenveloped viruses that carry 10 genomic segments of double-stranded RNA within two concentric capsid layers. Reovirus infection has become a well-studied viral model of apoptosis in a variety of cultured cells as well as in animals, where virus-induced apoptosis is a primary pathogenetic mechanism of tissue damage (reviewed in reference 17). Differences among reovirus strains in their capacities to induce apoptosis have allowed genetic determinants to be mapped to two viral genes: S1, encoding both outer capsid protein σ1 and nonstructural protein σ1s, and M2, encoding outer capsid protein μ1 (51, 60, 61).

Much work has focused on the role of the S1-encoded σ1 protein in reovirus-induced apoptosis. σ1 is a homotrimeric attachment protein (15, 56) that binds to junctional adhesion molecule A (JAM-A) and to α-linked sialic acid residues on the surface of susceptible cells (3, 4, 14, 46). Both of these binding events are required for apoptosis induction by type 3 (T3) reovirus strains (4, 19), and these findings have led to the proposal that binding of T3 reovirus σ1 to cell surface JAM-A...
interact with cellular membranes to effect penetration of a partially disassembled, subviral particle into the cytoplasm (7, 9, 10, 42, 44). Thus, a cleavage product of \( \mu \)1 might provide the postbinding signal required for induction of apoptosis during cell entry. Indeed, we have shown that a large fragment of virion-derived \( \mu \)1 enters the cytosol and nucleus of infected cells early in infection (11).

Although high-multiplicity infection by reovirus in cultured cells can induce apoptosis without the need for viral transcription or replication, the latter processes appear to be required for efficient apoptosis induction in cells infected at lower multiplicity (61). In addition, as reovirus-induced apoptosis occurs relatively late in the infectious cycle (17), it seems likely that replicative events are needed to amplify the proapoptotic signals that accompany cell entry by reovirus. Thus, de novo expression of the S1-encoded \( \mu \)1s that accompany cell entry by reovirus. Indeed, we have shown that a large fragment of virion-derived \( \mu \)1 enters the cytosol and nucleus of infected cells early in infection (11).

In this study, we tested the hypothesis that \( \mu \)1 can induce apoptosis independently of \( \sigma \)1 and \( \sigma \)1s when expressed in cultured cells. We show that \( \mu \)1 induced apoptosis and localized to lipid droplets, endoplasmic reticulum (ER), and mitochondria in transfected cells and had similar distributions in infected cells. We further show that regions encompassing two amphipathic \( \alpha \)-helices within a carboxyl (C)-terminal portion of \( \mu \)1 were key determinants of apoptosis induction and localization to lipid droplets, ER, and mitochondria. Apoptosis induction and localization to lipid droplets and intracellular membranes by \( \mu \)1 in transfected cells were inhibited by coexpression of its assembly partner \( \sigma \)3, suggesting that in infected cells the levels of free \( \mu \)1 may regulate apoptosis. Based on these findings, we conclude that the \( \mu \)1 protein and, in fact, specific small regions of this protein play a major role in apoptosis induction during reovirus infection.

**MATERIALS AND METHODS**

**Cells and viruses.** CHO and CV-1 cells were grown in Ham’s F-12 medium (CellGro) supplemented with 10% fetal bovine serum (HyClone), 100 U/ml penicillin, 100 \( \mu \)g/ml streptomycin, 1 mM sodium pyruvate, and nonessential amino acids (CellGro). Reoviruses T1L and T3D were laboratory stocks of the isolates previously identified as T1/human/Ohio/Lang/1953 and T3/human/Ohio/Dearing/1955, respectively, (27). The superscript N in T3DN differentiates the amino acids (CellGro). Reoviruses T1L and T3D were laboratory stocks of the isolate previously identified as T1/human/Ohio/Lang/1953 and T3/human/Ohio/Dearing/1955, respectively, (27). The superscript N in T3DN differentiates the Nibert laboratory from a T3D clone obtained from L. W. Cashdollar (Medical College of Wisconsin), denoted T3DC. The laboratory stock obtained from the Nibert laboratory from a T3D clone obtained from L. W. Cashdollar (Medical College of Wisconsin), denoted T3DC.

**Reagents.** Mouse MAbs to \( \mu \)1 (4A3) and \( \sigma \)3 (SC3) and rabbit polyclonal antiserum to \( \mu \)NS and reovirus cores have been described previously (8, 59, 62). MitoTracker Red CMXros (Molecular Probes), MAbs to human Golgin-97 (Molecular Probes), adipose differentiation-related protein (ADRP) (Progen), C63 (LAMP1) (BD Pharringen), calcinein (Affinity BioReagents), and protein disulfide isomerase (Molecular Probes) were gifts from L. W. Cashdollar (Medical College of Wisconsin), denoted T3DC.

**Plasmids and constructs.** The reovirus M2 and S4 genes from T1L were cloned into the pCI-neo vector and all PCR products were amplified with NheI and XhoI for cloning.

**TABLE 1. Primers used to prepare plasmids expressing M2 truncations**

<table>
<thead>
<tr>
<th>Construct</th>
<th>Primer sequence (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCI-M2(1–675)</td>
<td>TATGCTTAGCATGGGAACGCTTCTCTATC</td>
</tr>
<tr>
<td>pCI-M2(1–582)</td>
<td>TATGCTAGCAGTGGGAACGCTTCTCTATC</td>
</tr>
<tr>
<td>pCI-M2(43–582)</td>
<td>TTGTCTAGCATGCTGGAGGAGTACCATTG</td>
</tr>
<tr>
<td>pCI-M2(43–708)</td>
<td>TTGTCTAGCATGCTGGAGGAGTACCATTG</td>
</tr>
</tbody>
</table>

a Each construct was designed to contain the portion of the M2 gene encoding the indicated amino acid residues of \( \mu \)1. The pCI-neo vector and all PCR products were digested with NheI and XhoI for cloning.

b Forward primers are in the same orientation as the coding strand; reverse primers are in the reverse orientation to the coding strand. Restriction enzyme sites are italicized, and the inserted start and stop codons are underlined.
TABLE 2. Primers used to prepare plasmids expressing EGFP-M2 truncations

<table>
<thead>
<tr>
<th>Constructa</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>pEGFP-N-M2(1–41)</td>
<td>CGATCCTCGAAGGCAAGATGGGGAACGC</td>
<td>TATCAAGGATTTTCGCTATATTAGATTT</td>
</tr>
<tr>
<td>pEGFP-C-M2(582–708)</td>
<td>ATCACTGAGGAATTTGATATCTGATTC</td>
<td>AACTGGAAGTGTGGTACCGATATTC</td>
</tr>
<tr>
<td>pEGFP-M2(582–708)</td>
<td>ATTCGAGGATATTCAATCTCCAAAGGAAAT</td>
<td>TAAAGGCTTCCAGGTGATATACCGCCGCTTAA</td>
</tr>
<tr>
<td>pEGFP-C-M2(610–675)</td>
<td>ATTCGAGCAGATTCACCCGCAGACCGGTGATTT</td>
<td>TATACGTTTCAAAGGCGCTTGAAGATGCCGTCGTA</td>
</tr>
<tr>
<td>pEGFP-C-M2(582–611)</td>
<td>ATTCGAGGAATTTGATGTTACGATATTC</td>
<td>TAAAGCCTTAAATGTGAATTGATACCC</td>
</tr>
<tr>
<td>pEGFP-M2(582–643)</td>
<td>ATTCGAGGAATTTGATGTTACGATATTC</td>
<td>TAAAGGCTTCCAGGTGATATACCGCCGCTTAA</td>
</tr>
<tr>
<td>pEGFP-M2(582–675)</td>
<td>ATTCGAGGAATTTGATGTTACGATATTC</td>
<td>TAAAGGCTTCCAGGTGATATACCGCCGCTTAA</td>
</tr>
<tr>
<td>pEGFP-C-M2(582–675ΔH2)</td>
<td>(i) ACCCAACGAGAAGCGCGATCAC</td>
<td>(ii) ACCCAGGCAGATAACCTTACGTTAATATGG</td>
</tr>
<tr>
<td></td>
<td>(iii) TGAAGAAGGCGCTGGAAATGCGTCGAA</td>
<td>(iv) TTGAAAGGCGCTGGAAATGCGTCGAA</td>
</tr>
</tbody>
</table>

* Each construct was designed to express an EGFP fusion including the indicated amino acid residues of M2. The pEGFP-C1 and -N1 vectors and all PCR products were digested with XhoI and HindIII for cloning. Forward primers are in the reverse orientation to the coding strand. Restriction enzyme sites are in italics, and the inserted start and stop codons are underlined.

Induction of apoptosis in transfected cells expressing reovirus M2 protein. To test the hypothesis that M2 can induce apoptosis in cultured cells independently of σ1 and σ1s, we examined transfected cells that expressed this protein intracellularly. The M2-encoding M2 gene derived from T1L reovirus was cloned into the expression vector pCI-neo, under the control of a cytomegalovirus immediate-early promoter. Upon examining the M2-transfected CHO cells by phase-contrast and fluorescence microscopy, we saw that many of the cells expressing M2 had morphological changes characteristic of apoptosis: the cells were rounded up, partially detached from the coverslip, and/or spiculated, and DAPI staining revealed that many nuclei were small and had condensed, marginated chromatin (Fig. 1B). In addition, many of the cells expressing M2 had nuclear changes and/or activated caspase whose activation denotes commitment to apoptosis. Induction of apoptosis in transfected cells expressing reovirus M2 protein. To test the hypothesis that M2 can induce apoptosis in cultured cells independently of σ1 and σ1s, we examined transfected cells that expressed this protein intracellularly. The M2-encoding M2 gene derived from T1L reovirus was cloned into the expression vector pCI-neo, under the control of a cytomegalovirus immediate-early promoter. Upon examining the M2-transfected CHO cells by phase-contrast and fluorescence microscopy, we saw that many of the cells expressing M2 had morphological changes characteristic of apoptosis: the cells were rounded up, partially detached from the coverslip, and/or spiculated, and DAPI staining revealed that many nuclei were small and had condensed, marginated chromatin (Fig. 1A). In addition, many of the cells expressing M2 stained positive for activated caspase-3 (Fig. 1B), an effector caspase whose activation denotes commitment to apoptosis (48). At 48 h p.t., ~30% and ~20% of M2-transfected CHO and CV-1 cells, respectively, had nuclear changes and/or activated caspase-3 (Fig. 1C; also see Fig. 5C). From these data, we conclude that expression of M2 induces apoptosis in a subset of transfected cells.

Association of M2 with annular rings and tubulovesicular structures in transfected cells. We next examined the distribution of M2 in transfected cells by immunostaining with M2-specific antibodies.
specific MAbs. Despite the effects in the nucleus described above, the distribution of \( \mu_1 \) in M2-transfected CHO cells (Fig. 1A and B) and CV-1 cells (Fig. 2A and C) was largely cytoplasmic. Moreover, although much of \( \mu_1 \) appeared diffuse in the cytoplasm, two distinctive patterns suggested that it might also be associated with cytoplasmic organelles. First, in many M2-transfected CV-1 cells, \( \mu_1 \) localized to annular ring-like structures (Fig. 2A) which surrounded phase-dense globules (Fig. 2B). These structures may correspond to the “discrete particles” seen at lower magnification by Yue and Shatkin (65) following expression of \( \mu_1 \) in transfected HeLa cells. Second, though less frequently, \( \mu_1 \) staining of annular ring structures was often closely juxtaposed to ER, as shown by immunostaining for the integral ER membrane protein calnexin (Fig. 3C), and some \( \mu_1 \) staining appeared to colocalize directly with calnexin (Fig. 3C). The tubulovesicular distribution of \( \mu_1 \) was faint in both CV-1 and HeLa cells but was often more prominent in the HeLa cells. Although much of this tubulovesicular \( \mu_1 \) colocalized with the ER marker calnexin (Fig. 3C), some \( \mu_1 \) staining appeared to colocalize directly with calnexin (Fig. 3C). The annular ring pattern was the most common and prominent distribution of \( \mu_1 \) observed in M2-transfected cells.

**FIG. 1.** Apoptosis induction by \( \mu_1 \) in transfected cells examined by fluorescence microscopy. CHO cells were transfected with pEGFP-C1 to express EGFP, as a control, or pCI-M2(T1L) to express \( \mu_1 \). Cells were fixed at 48 h p.t. and then immunostained with anti-\( \mu_1 \) (MAb 4A3) and rabbit anti-activated caspase-3 serum followed by goat anti-mouse IgG conjugated to Alexa 488 and goat anti-rabbit IgG conjugated to Alexa 594. Nuclei were stained with DAPI. (A and B) Marginated chromatin of an apoptotic nucleus (A) and activated caspase-3 (B) are present in a representative \( \mu_1 \)-expressing cell. The inset in panel A shows a phase-contrast image. Scale bars, 5 \( \mu \)m. (C) Percentage of transfected cells showing apoptotic nuclear changes or activated caspase-3. At least 100 cells expressing either \( \mu_1 \) or EGFP were scored for the presence of apoptosis indicators. Means (\( \pm \) standard deviations) of three replicates are shown.

**FIG. 2.** Distributions of \( \mu_1 \) in transfected cells examined by fluorescence microscopy. CV-1 cells were transfected with pCI-M2(T1L) to express \( \mu_1 \), fixed at 24 h p.t., and then immunostained with anti-\( \mu_1 \) (MAb 4A3) followed by goat anti-mouse IgG conjugated to Alexa 488. Scale bars, 10 \( \mu \)m. (A) Annular ring distribution of \( \mu_1 \) (arrowheads; enlarged in inset). (B) Phase-dense globules in a phase-contrast image of the cells shown in panel A (arrowheads; enlarged in inset). (C) Tubulovesicular distribution of \( \mu_1 \) (arrowheads; enlarged in inset). This cell also contains \( \mu_1 \)-staining annular rings, which are indistinct and brighter as a result of the longer exposure needed to image the tubulovesicular structures.
FIG. 3. Subcellular localizations of µ1 in transfected cells examined by fluorescence microscopy. CV-1 cells transfected with pCI-M2(T1L) were fixed at 18 h p.t. and stained as described below for each row of panels. Right panels show colored merges of the different staining patterns, with labels in matching colors. Nuclei were stained with DAPI in each case. Scale bars, 10 µm. (A to C, C’ and F) After fixation, cells were immunostained with the following MAbs as markers for organelles—anti-golgin-97 for Golgi complex (A), anti-LAMP1 for lysosomes (B), anticalnexin for ER (C and C’), and anti-ADRP for lipid droplets (F)—followed by goat anti-mouse IgG conjugated to Alexa 594. Cells were then fixed again and immunostained with anti-µ1 (MAb 4A3) conjugated to Cy2. The area outlined in panel C is enlarged in panel C’ and shows colocalization between calnexin and µ1 (arrowheads). (D) Mitochondria were labeled with MitoTracker CMXros prior to fixation. After fixation, cells were immunostained with anti-µ1 (MAb 4A3) conjugated to Cy2. Arrowheads indicate areas of colocalization between µ1 and mitochondria. (E) After fixation, cells were immunostained with anti-µ1 (MAb 4A3) followed by goat anti-mouse IgG conjugated to Alexa 594. Neutral lipids were then stained with Bodipy 493/503.
(present in ~82% of transfected CV-1 cells). These rings surrounded lipid droplets that were labeled with Bodipy 493/503 (Fig. 3E) or Oil Red O (data not shown) dye, both of which stain for neutral fatty acids. Lipid droplets are storage organelles for cholesterol esters and triglycerides and are believed to be metabolic organelles involved in the synthesis and trafficking of cellular lipids (reviewed in reference 39). They are surrounded by a protein-encrusted monolayer of phospholipid (57), and in most cells the major protein associated with this monolayer is ADRP (38). Strong colocalization between $\mu_1$ and ADRP confirmed that $\mu_1$ was localizing to the periphery of lipid droplets (Fig. 3F).

The C-terminal, $\phi$ region of $\mu_1$ determines both targeting to intracellular membranes and induction of apoptosis in transfected cells. Three major regions of the $\mu_1$ protein, divided by proteolytic cleavage sites, are commonly identified: the amino (N)-terminal, myristoylated fragment $\mu_1$N (residues 2 to 41); the central fragment $\delta$ (residues 42 to 582); and the C-terminal fragment $\phi$ (residues 582 to 708) (40). In addition, pairwise combinations of these fragments can yield two other species: $\mu_1\delta$ (residues 2 to 582 plus the N-terminal, N-myristoyl group) and $\mu_1C$ (residues 42 to 708) (40). To identify the region(s) of $\mu_1$ responsible for its activities in the preceding Results sections, we constructed a series of $M_2$ gene truncations that encode the near-equivalents of each of these fragments (as some constructs have the addition of an initiating methionine residue at the N terminus). In addition, we prepared a construct to encode full-length $\mu_1$ lacking only residues 676 to 708 [$\mu_1(1–675)$], which are disordered in the $\mu_1\sigma_3$ crystal structure and genetically absent from the $\mu_1$ homologs of avian reoviruses and aquareoviruses (1, 37, 43, 68). Because none of the available anti-$\mu_1$ MAbs (62) recognize the $\mu_1$N or $\phi$ fragment, we prepared constructs to express EGFP-tagged versions of those regions. The constructs and results are summarized in Fig. 4A.

When expressed in transfected CHO cells, the three trunca-
tions lacking the \( \phi \) region (\( \mu 18, \delta, \) and \( \mu 1N-EGFP \)), as well as EGFP alone, were distributed diffusely through the cytosol and nucleus (Fig. 5A and data not shown). In contrast, the three truncations containing most or all of the \( \phi \) region (\( \mu 1(1–675), \mu 1C, \) and \( EGFP/\phi \)) were targeted to mitochondria, lipid droplets, and ER (Fig. 5B and data not shown). The association of EGFP/\( \phi \) with mitochondria was more prominent, and its association with lipid droplets less so (Fig. 5B), than seen with full-length \( \mu 1 \) (Fig. 3), \( \mu 1(1–675), \) or \( \mu 1C. \) EGFP/\( \phi \) was associated with lipid droplets in \( \sim25\% \) of transfected CHO cells compared to \( \sim87\% \) of CHO cells expressing full-length \( \mu 1. \) The reason(s) for these differences is not yet known. We
also tested truncated versions of \( \mu_1 \) for the capacity to induce apoptosis. At 48 h p.t. in CHO and CV-1 cells, only the truncations containing most or all of the \( \phi \) region [\( \mu_1(1–675) \), \( \mu_1C \), and EGFP/\( \phi \)], and not EGFP alone, induced apoptosis in a substantial percentage of cells, similarly to full-length \( \mu_1 \) (Fig. 5C).

Although the M2 gene we used for creating the \( \mu_1 \)-expressing constructs was derived from the TIL reovirus, TIL infection of CHO cells at an MOI of 100 induced a low level of apoptosis (~10% of infected cells) (Fig. 5C). This finding is in agreement with those of others showing that TIL is a poor inducer of apoptosis (51, 60). To address the possibility that virus strain differences in the \( \mu_1 \) protein were responsible for different levels of apoptosis induction, we examined the capacities of \( \mu_1 \) derived from the T3D\( ^N \) and T3D\( ^C \) reoviruses (44) to induce apoptosis in transfected CHO cells. We found no differences in the capacities of \( \mu_1 \) derived from the TIL, T3D\( ^N \), or T3D\( ^C \) strain to induce apoptosis; they induced apoptosis in ~27%, ~30%, and ~29% of transfected CHO cells, respectively, as assessed by nuclear changes. These findings were somewhat surprising, as previous genetic studies have shown that strain differences in the capacity to induce apoptosis are determined at least in part by the \( \mu_1 \)-encoding M2 gene (23, 50, 59). One likely explanation is that in the context of viral infection, \( \mu_1 \)-induced apoptosis is modulated indirectly by how, or the extent to which, it interacts with other viral factors in a strain-dependent manner (see Discussion).

In summary, we conclude that residues 582 to 675 in the \( \phi \) region of \( \mu_1 \) contain determinants for both inducing apoptosis and targeting to lipid droplets, ER, and mitochondria in transfected cells. Moreover, the determinants in the \( \phi \) region appear to be necessary and sufficient for the same activities exhibited by full-length \( \mu_1 \). Possible differences in the levels of apoptosis induced by the different \( \phi \)-containing proteins are addressed in the Discussion.

**Two regions encompassing amphipathic \( \alpha \)-helices in the \( \phi \) region of \( \mu_1 \) are major determinants for inducing apoptosis in transfected cells.** To identify specific determinants within the \( \phi \) region for inducing apoptosis in transfected CHO cells, we constructed a further series of truncation or deletion mutants for expressing this region fused to the N or C terminus of EGFP. The constructs and results are summarized in Fig. 4A. Considering that the weak capacity of EGFP to dimerize (67) might influence the proapoptotic activity of \( \phi \), we also prepared a FLAG epitope-tagged version of the \( \phi \) region (Fig. 4A). FLAG/\( \phi \) behaved like EGFP/\( \phi \) with regard to both induction of apoptosis and localization to intracellular membranes (Fig. 6A and data not shown), suggesting that EGFP was not involved in these activities.

As for further truncations in the \( \phi \) region, we first created a
immunostaining with an anti-FLAG MAb followed by goat anti-mouse IgG conjugated to Alexa 594. The FLAG-tagged construct was detected by immunostaining with rabbit anti-activated 

...was transfected with pEGFP-C-M2(610–675) showed limited activity at inducing apoptosis (Fig. 6A), suggesting that helix 3 may represent another determinant of apoptosis induction. However, a truncation designed to express a protein containing only helix 2 and helix 3 [construct pEGFP-C-M2(582–611)] showed limited activity at inducing apoptosis (Fig. 6A), suggesting that helix 3 may represent its proapoptotic activity predominantly in concert with helix 1. We obtained further evidence that the region encompassing helix 3 contributes to proapoptotic activ-

FIG. 6. Apoptosis induction and subcellular localization by ϕ truncation mutants in transfected cells examined by fluorescence microscopy. (A) CHO cells were transfected with the indicated constructs, fixed at 48 h p.t., and then immunostained with rabbit anti-activated caspase-3 polyclonal antibody followed by goat anti-rabbit IgG conjugated to Alexa 488. Cells were scored for caspase-3 activation as in Fig. 1. The means and standard deviations of three determinations are shown; Kruskal-Wallis (P) values of the group for CHO and CV-1 cells are 0.0014 and 0.01, respectively. (B) Immunoblots showing expression of ϕ and truncation constructs in CHO cells at 24 h p.t. in the absence or presence of the broad-spectrum caspase inhibitor z-VAD-fmk. Cells were transfected with 1 μg of each construct. Immediately after transfection, cells were treated with DMSO or 50 μM z-VAD-fmk. At 24 h p.t., cell lysates were collected, and samples were subjected to 10% or 15% SDS-PAGE, followed by protein transfer to nitrocellulose. Expression of ϕ constructs was detected with MAb anti-GFP (Clontech) followed by goat anti-mouse IgG conjugated to HRP. Expression of EGFP-fused constructs was detected with MAb anti-FLAG (Clontech) followed by goat anti-mouse IgG conjugated to Alexa 594. Right panels show colored merges of the different staining patterns, with labels in matching colors. Scale bars, 5 μm.

IgG conjugated to Alexa 488. Cells were scored for caspase-3 activation as in Fig. 1. The means and standard deviations of three determinations are shown; Kruskal-Wallis (P) values of the group for CHO and CV-1 cells are 0.0014 and 0.01, respectively. (B) Immunoblots showing expression of ϕ and truncation constructs in CHO cells at 24 h p.t. in the absence or presence of the broad-spectrum caspase inhibitor z-VAD-fmk. Cells were transfected with 1 μg of each construct. Immediately after transfection, cells were treated with DMSO or 50 μM z-VAD-fmk. At 24 h p.t., cell lysates were collected, and samples were subjected to 10% or 15% SDS-PAGE, followed by protein transfer to nitrocellulose. Expression of ϕ constructs was detected with MAb anti-GFP (Clontech) followed by goat anti-mouse IgG conjugated to HRP. Expression of EGFP-fused constructs was detected with MAb anti-FLAG (Clontech) followed by goat anti-mouse IgG conjugated to Alexa 594. Right panels show colored merges of the different staining patterns, with labels in matching colors. Scale bars, 5 μm.
ity, whereas the region encompassing helix 2 does not, by replacing the helix 2 region with a short linker in a protein otherwise containing the regions encompassing helix 1 and helix 3 [construct pEGFP-C-M2(582–675)]. Expression of this mutant induced high levels of apoptosis, similar to those induced by the 582-to-675 region containing all three helices (Fig. 6A). These results appear to identify the regions encompassing helix 1 and helix 3 as the minimal determinants for inducing maximal levels of apoptosis. The importance of the region encompassing helix 1 was further suggested by reduced levels of apoptosis induced by a mutant in which only the N-terminal three residues of this region were missing from full-length $\phi$ [construct pEGFP-C-M2(585–675)] (Fig. 6A) (see Discussion).

In summary, we conclude that regions encompassing the first and third amphipathic $\alpha$-helices of $\phi$ mediate its full proapoptotic activity. Moreover, including evidence from the previous section, these two helical regions of $\phi$ are probably responsible for the full proapoptotic activity of full-length $\mu_1$. We also note further evidence that residues 675 to 708 at the C terminus of $\mu_1$ and $\phi$ may serve to downregulate this activity.

**Steady-state levels of $\mu_1$ constructs and $\phi$ truncation mutants in transfected CHO cells differ in the presence or absence of the broad-spectrum caspase inhibitor z-VAD-fmk.** Although we were able to detect expression of all $\mu_1$ constructs and $\phi$ truncation mutants in transfected cells by fluorescence microscopy, we had limited success at detecting those constructs that induced apoptosis by immunoblotting. We also observed that constructs that induced apoptosis often appeared to have lower levels of relative fluorescence compared to constructs that did not induce apoptosis. As general translation is inhibited in cells undergoing apoptosis (28), we hypothesized that the proapoptotic constructs downregulated their own translation. To test this hypothesis, we compared expression of the different constructs in transfected CHO cells incubated with either the broad-spectrum caspase inhibitor z-VAD-fmk (50 $\mu$M) or DMSO control (Fig. 6B). We found that in the presence of z-VAD-fmk, those constructs that induced apoptosis had notably increased expression levels compared to untreated controls (e.g., compare expression of $\mu_1$, $\mu_1$C, and EGFP/$\phi$), whereas there was little change in the relative expression levels of those constructs that did not induce apoptosis. We conclude that in the presence of the broad-spectrum caspase inhibitor, most of the constructs had similar steady-state levels of expression. In addition, we were able to confirm that each construct was of the appropriate size.

**The regions of $\phi$ that induce apoptosis also determine targeting to intracellular membranes.** As described above, the full-length $\phi$ region fused to either EGFP or FLAG localized to lipid droplets, ER, and mitochondria (Fig. 5B and data not shown). The localizations of the various truncation and deletion mutants in the $\phi$ region are summarized in Fig. 4A. All mutants containing residues 582 to 611 localized to ER and mitochondria (Fig. 6C and data not shown), identifying this region encompassing helix 1 as a minimal determinant of these activities. Only mutants containing both the helix 1 and helix 3 regions, however, localized to lipid droplets (Fig. 6D). The truncation containing residues 610 to 675 did not associate with intracellular membranes, suggesting that the helix 3 region is not sufficient for membrane targeting and identifying the helix 1 and helix 3 regions together as minimal determinants for targeting to lipid droplets. In general, all mutants that targeted to ER and mitochondria also induced apoptosis. This correlation appeared weakest in the case of the 585-to-708 protein, which showed clear membrane targeting (data not shown) but induced lower levels of apoptosis. Targeting to lipid droplets, in contrast, did not correlate strongly with apoptosis induction. In summary, we conclude that similar regions of $\phi$ determine both membrane targeting and apoptosis induction and that localization to ER and/or mitochondria may be part of the mechanism by which these determinants induce apoptosis.

**Membrane association and apoptosis induction by $\mu_1$, but not $\phi$, are abrogated by coexpression of $\sigma_3$.** Previous reports have noted that coexpression of $\mu_1$ leads to a redistribution of $\sigma_3$ in cells (58, 64). We therefore hypothesized that coexpression of $\sigma_3$ would reciprocally lead to a redistribution of $\mu_1$. To test this hypothesis, we cotransfected $\mu_1$- and $\sigma_3$-expressing plasmids into CV-1 or CHO cells at different relative molar ratios. As a control, we cotransfected the $\mu_1$-expressing plasmid with one encoding the reovirus $\sigma_2$ protein. As the ratio of $\sigma_3$ to $\mu_1$ plasmid increased, the distribution of $\mu_1$ became more diffuse throughout the cytosol and less associated with lipid droplets, ER, or mitochondria (Fig. 7A). At a molar ratio of 1:4.1 (S4:2M), essentially all of $\mu_1$ was diffuse in the cytosol and the nucleus in the vast majority of transfected cells (data not shown). In contrast, coexpression of $\sigma_2$ with $\mu_1$ had no substantive effect on the subcellular distribution of $\mu_1$, i.e., $\mu_1$ remained strongly associated with intracellular membranes (Fig. 7B). We also found that as the $\sigma_3$ to $\mu_1$ ratio increased, apoptosis levels in CHO cells decreased, whereas $\sigma_2$ coexpression with $\mu_1$ had little or no effect on apoptosis levels (Fig. 7D, left panel). In other words, increasing $\sigma_3$, but not $\sigma_2$, had an increasingly antiapoptotic effect. The $\sigma_3$ and $\mu_1$ proteins are known to coassemble into soluble heterohexameric oligomers when coexpressed (37); thus, one possible explanation for these results is that progressive sequestration of $\mu_1$ into $\mu_1$:$\sigma_3$ heterohexamers decreases the amount of free $\mu_1$ able to associate with intracellular membranes and to induce apoptosis.

Another possible explanation for the preceding results for $\mu_1$:$\sigma_3$ coexpression is that the antiapoptotic effect of $\sigma_3$ is independent of its interaction with $\mu_1$. $\sigma_3$ is known to interact with double-stranded RNA and to prevent activation of protein kinase R, an effect that could be antiapoptotic (24, 64). To address the possibility that $\sigma_3$ inhibited apoptosis independently of its capacity to interact with $\mu_1$, we examined whether coexpression of $\sigma_3$ with $\phi$ [construct pEGFP-C-M2(582–708)] would abrogate the capacity of $\phi$ to associate with intracellular membranes and/or induce apoptosis. We reasoned that since $\phi$ lacks the vast majority of residues in $\mu_1$ that interact with $\sigma_3$ (37), $\sigma_3$ and $\phi$ should not interact upon coexpression and therefore any other antiapoptotic effect of $\sigma_3$ would be revealed. As a control, we coexpressed $\phi$ with the reovirus $\sigma_2$ protein. Coexpression of $\sigma_3$ or $\sigma_2$ with $\phi$ neither altered the intracellular distribution of $\phi$ (Fig. 7C) nor reduced the induction of apoptosis (Fig. 7D, right panel).

In summary, we conclude that coexpression of $\sigma_3$ with full-length $\mu_1$, as a function of relative levels of the two proteins, progressively abrogates $\mu_1$ association with intracellular membranes and induction of apoptosis, most likely because of $\mu_1$. 
plasmid DNA ratios of 1:2 and 2:1 (S4:M2). Cells were fixed at 48 h.p.i. and then immunostained with Cy2-conjugated anti-μ1 (4A3) and Alexa 594-conjugated anti-σ3 (MAb 5C3). Representative examples of the predominant distribution patterns are shown. Scale bars, 5 μm. (B) CV-1 cells were transfected with pCI-S2(T1L) to express σ2 plus pCI-M2(1–708) to express μ1 at a plasmid DNA ratio of 2:1 (S2:M2). Cells were fixed at 48 h.p.i. and then immunostained with Cy2-conjugated anti-μ1 (4A3) and rabbit anti-core serum (to detect μ1i in viral T1L-infected CV-1 cells by IF microscopy, we found that at 24 h postinfection (p.i.), in addition to localization to viral factories, μ1 localized to ring-like and tubulovesicular structures in a subset of infected cells (see Fig. 9). We discerned four patterns of μ1 distribution at 24 h p.i.: (i) diffuse through the cytosol (Fig. 8A), (ii) colocalized with μNS in viral factories (Fig. 8B), (iii) localized to ring-like structures (Fig. 8C), and (iv) localized to tubulovesicular structures (Fig. 8D). Many cells displayed more than one of these patterns (e.g., in Fig. 8B, diffuse and localized to viral factories). As with our findings in transfected cells, μ1 colocalized with markers for lipid droplets (Fig. 8E), ER (Fig. 8F), and mitochondria (Fig. 8G) in infected cells. We found similar distributions of μ1 in infected HeLa, CHO, and L929 cells (data not shown, but see Fig. 9). We conclude that μ1 localizes to lipid droplets, ER, and mitochondria in T1L-infected cells in addition to viral factories; thus, its distribution partially mirrors that seen in transfected cells.

Given the findings at 24 h p.i., we interpreted the different μ1 distribution patterns as representing a continuum that may vary with time p.i. In preliminary experiments, we noted that the distribution of μ1 was affected by the method used to permeabilize cellular membranes: methanol reduced the staining of μ1 with ring-like and tubulovesicular structures but increased its staining within viral factories, whereas 0.1% Triton X-100 had the opposite effects (data not shown). We there-
FIG. 8. Distribution patterns and subcellular localizations of \( \mu 1 \) in infected cells examined by fluorescence microscopy. CV-1 cells infected with T1L reovirus were fixed at 24 h p.i., and the distribution patterns of \( \mu 1 \) were detected by immunostaining with anti-\( \mu 1 \) (MAb 4A3) followed by goat anti-mouse IgG conjugated to Alexa 488. Four patterns of \( \mu 1 \) staining were detected as follows. (A) Diffuse. (B) Associated with viral factories (VF). Factories were detected by immunostaining with a rabbit polyclonal serum to \( \mu 1 \)NS followed by goat anti-rabbit IgG conjugated to Alexa 594. In this merged image, yellow indicates colocalization between \( \mu 1 \) (green) and \( \mu 1 \)NS (red). (C) Associated with annular ring-like structures (Rings). (D) Associated with tubulovesicular structures (TV). (E, F, and G) To ascertain the subcellular localization of \( \mu 1 \), fixed cells were first immunostained with anti-ADRP to detect lipid droplets (E) and anti-PDI to detect ER (F) followed by goat anti-mouse IgG conjugated to Alexa 594. Cells were then fixed again and immunostained with anti-\( \mu 1 \) (MAb 4A3) conjugated to Cy2. Alternatively, cells were first stained with MitoTracker CMXros to detect mitochondria (G), after which they were fixed and immunostained with anti-\( \mu 1 \) followed by goat anti-mouse IgG conjugated to Alexa 488. Nuclei were stained with DAPI. Arrowheads indicate areas of colocalization between \( \mu 1 \) and ER (F) or mitochondria (G). Scale bars, 5 \( \mu m \).
fore compared the distribution of \( \mu_1 \) in T1L-, T3DX-, and T3DC-infected L929 cells at 6, 12, 18, 24, and 48 h p.i. in cells permeabilized with either 0.1% Triton X-100 or 100% methanol (Fig. 9A). With all three strains, we found that \( \mu_1 \) was predominantly diffuse in the cytosol at 6 and 12 h p.i. regardless of the permeabilization method. Thereafter, in methanol-permeabilized cells, \( \mu_1 \) increasingly stained with viral factories and was seen associating with intracellular membranes (ring-like structures) only at the latest time point (48 h). In contrast, in Triton X-100-permeabilized cells, \( \mu_1 \) staining of intracellular membranes (tubulovesicular and ring-like structures) became visible at 12 to 18 h p.i. and increased to being seen in \( \sim 30\% \) of T1L+ cells, \( \sim 40\% \) of T3DX+, and \( \sim 87\% \) of T3DC-infected cells by 48 h p.i. We moreover noted that as \( \mu_1 \) became associated with viral factories and membrane structures, fewer cells had a diffuse distribution of \( \mu_1 \). From these results, we conclude that the pattern of \( \mu_1 \) distribution in infected cells changes as infection progresses, being initially diffuse and then becoming localized to viral factories and associated with ring-like and tubulovesicular structures from 12 to 18 h p.i. and beyond.

As we had found that coexpression of \( \mu_1 \) with \( \sigma_3 \) caused \( \mu_1 \) to redistribute from intracellular membranes to a predominantly diffuse distribution in cells and abrogated \( \mu_1 \)-induced apoptosis, we speculated that the association of \( \mu_1 \) with intracellular membranes in infected cells was related to apoptosis induction. In support of this hypothesis, we found that the level of apoptosis induced by the T1L, T3DX, and T3DC strains at 48 h p.i. in L929 cells (Fig. 9B) correlated with the percentage of infected cells in which \( \mu_1 \) associated with intracellular membranes (Fig. 9A, left panels).

DISCUSSION

Consistent with genetic reassortant studies showing that the reovirus M2 gene is a determinant of virus strain differences in the capacity to induce apoptosis during infection (51, 60, 61), we found that expression of the M2-encoded \( \mu_1 \) protein induced apoptosis in uninfected, transfected cells. Both S1 and M2 viral genes were previously identified as genetic determinants of strain differences in reovirus-induced apoptosis (60); however, since these earlier studies, most attention has been paid to the roles of the S1 gene products \( \sigma_1 \) and \( \sigma_1s \), particularly to receptor interactions by \( \sigma_1 \) with JAM-A and \( \alpha \)-sialic
acid and their possible importance for proapoptotic signaling (reviewed in reference 26). Recently, Danthi et al. showed that in CHO cells expressing the Fc receptor, but not JAM-A or sialic acid, infection and reovirus-induced apoptosis can occur when virion-associated σ1 is prebound with MAbs such that the Fc portion of the antibody mediates virus attachment (23). These authors also found that under such conditions the sole genetic determinant of apoptosis induction was the M2 gene. The results presented here extend and support those conclusions, and we therefore propose that the μ1 protein plays a more primary role in reovirus-induced apoptosis than was previously appreciated.

If sufficient numbers (a high MOI) of reovirus particles are added to cells, viral transcription or genome replication is not required for induction of apoptosis (19, 20, 61). Nevertheless, an unidentified postattachment or disassembly step is required (20). In this study, we found that the φ region of μ1 is necessary and sufficient for inducing apoptosis in transfected cells. During infectious entry, proteolytic processing of virion-associated μ1 within endo/lysosomes produces partially uncoated particles, ISVPs, which are primed for membrane penetration (reviewed in reference 10). The fragment region remains associated with ISVPs (41), and so it seems possible that particle-derived φ could be released into the cytosol after membrane penetration, where if present in sufficient concentrations, it could induce apoptosis. Our previous finding that the particle-derived δ fragment of μ1 is present in the cytosol and nucleus of the infected cell soon after penetration is consistent with this hypothesis (11). At lower MOI, it is possible that fragments of μ1 could be released into the cytosol in smaller amounts that do not directly induce apoptosis but instead prime the cells for apoptosis induction later in the infectious cycle.

In the current study, μ1 and all of its derived regions that induced apoptosis in transfected cells also associated with mitochondria and/or other cellular membranes. Several other viral proteins associate with mitochondrial membranes and induce apoptosis. These include human immunodeficiency virus type 1 Vpr, influenza A virus PB1-F2, and the human T-cell leukemia virus type 1 p13n protein accessory protein (16, 21, 33). All of these proteins have predicted amphipathic α-helical regions that are required for interactions with mitochondrial membranes. However, the mechanism(s) of apoptosis induction is not completely understood for any of these proteins. The Vpr and PB1-F2 proteins are thought to promote apoptosis by directly interacting with components of the mitochondrial permeability transition pore, thereby causing loss of the transmembrane potential with a resultant increase in mitochondrial outer membrane permeability (33, 66). However, both of these proteins may induce and/or promote apoptosis in other ways. Vpr interacts with the antiapoptotic protein HAX-1 on the outer mitochondrial membrane and may promote apoptosis by counteracting the HAX-1 antiapoptotic effect (63), and PB1-F2 may directly permeabilize mitochondrial and/or other cellular membranes by forming lipidic or proteolipidic pores (13). Previous authors have shown that mitochondrial apoptotic pathways are activated in reovirus-infected cells and have suggested that these pathways involve activation of caspase-8 and subsequent cleavage of the Bcl-2 family member Bid (35). However, it is possible that μ1 or μ1 fragments directly activate mitochondrial apoptotic pathways, and we are currently investigating this possibility.

We found that steady-state levels of μ1 and its constructs that induced apoptosis were much lower than those of constructs that did not induce apoptosis. Moreover, levels of the proapoptotic constructs were markedly increased by incubation of transfected cells with the broad-spectrum caspase inhibitor z-VAD-fmk (Fig. 6B). As apoptosis is reported to inhibit translation generally (28), one explanation for this finding is that μ1 induction of apoptosis resulted in inhibition of its own translation. Apoptosis induction appeared to be down-regulated by C-terminal residues 676 to 708 within either μ1 or φ. Interestingly, this polypeptide sequence contains a predicted PEST motif (PESTfind [http://emb1.bcc.univie.ac.at/content/view/21/45/]). PEST motifs are short regions of polypeptide sequence that are often found at the C terminus of proteins; are enriched in proline (P), glutamic acid (E), serine (S), and threonine (T) residues; and are usually flanked by basic residues. PEST sequences are thought to act as signals for rapid protein degradation (49). If this were true of μ1, it would explain the enhancement of apoptosis seen in constructs lacking this region. Avian reoviruses (ARVs) induce higher levels of apoptosis in infected cells at 24 h p.i. than do mammalian reoviruses (36). We have found that expression of the ARV-176 μB protein (the homolog of μ1) also robustly induces apoptosis in transfected cells (C. M. Coffey and J. S. L. Parker, unpublished data). We note that ARV μB lacks the C-terminal extension (68), including the putative PEST motif found in μ1, and we are currently investigating the role of this region of μ1 in protein stability.

EGFP fusions of the φ region tended to localize to mitochondrial membranes, weakly to ER membranes, and occasionally to lipid droplets in transfected cells (Fig. 8), contrasting with the primary localization of full-length μ1 to lipid droplets and less so to mitochondria and ER in both transfected and infected cells. Although both μ1 and φ induced
apoptosis in transfected cells, their differential localization patterns suggest that they may differ in their proapoptotic functions during reovirus infection. Caspase-3 and caspase-8 activation in cells infected with reovirus T3 Abney is biphasic, supporting the concept that two sequential proapoptotic signals may be present in infected cells (34). Kominsky et al. suggested that this biphasic pattern of caspase activation results from an initial tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-dependent activation of caspase-8 that leads to low-level activation of caspase-3 and cleavage of Bid, followed by more sustained activation of caspase-3 and caspase-8 that results from cleaved Bid-mediated activation of the intrinsic apoptotic pathway and release of cytochrome c and Smac/DIABLO from mitochondria (34, 35). This model proposes that during reovirus infection, TRAIL-dependent apoptotic pathways are activated before mitochondrial apoptotic pathways. Alternatively, it is possible that mitochondria become sensitized to TRAIL-dependent apoptosis before TRAIL secretion. The influenza A virus PB1-F2 protein, which has a localization pattern similar to that of $\phi$, is believed to sensitize transfected cells to tumor necrosis factor alpha-induced apoptosis by modulating mitochondrial membrane permeability (66). We speculate that $\phi$ and/or a related fragment of $\mu_1$ released into the cytosol of infected cells during membrane penetration sensitizes cells to apoptosis induction by TRAIL, perhaps by modulating mitochondrial membrane permeability.

As noted above, full-length $\mu_1$ localized primarily to lipid droplets in both transfected cells and infected cells. The hepatitis C virus (HCV) core protein is similarly localized (2, 6, 54). Moreover, expression of the HCV core protein causes apoptosis induction in some, but not all, transfected cells (6, 25, 29). The determinants of lipid droplet localization of the HCV core protein have been mapped to amphipathic $\alpha$-helices whose primary sequence is homologous to plant oleosins, which associate with lipid droplets (30). In addition, a 10-residue sequence at the C terminus of the HCV core protein mediates its localization to mitochondria (54). Our findings suggest that regions encompassing two amphipathic helices (residues 582 to 611 and 644 to 675) near the C terminus of $\mu_1$ are required for association with lipid droplets but that only the first of these regions is strictly required for mitochondrial localization (Fig. 6). The biological significance of associations by the HCV core protein and reovirus $\mu_1$ protein with lipid droplets remains uncertain. However, lipid droplets have recently been implicated as potential intracellular signaling platforms that might function analogously to lipid rafts on the plasma membrane (39). If this is the case, then it is possible that association of $\mu_1$ with lipid droplets may be important for activation of certain signaling pathways. In support of this idea, it is known that the M2 gene is the genetic determinant of strain differences in JNK activation during reovirus infection (18). Association of $\mu_1$ with lipid droplets may modulate its capacity to induce apoptosis. In support of this idea, we found that proteins EGFP/\(\phi\) (582–675) and EGFP/\(\phi\) (582–675ΔH2), which associated with mitochondria, ER, and lipid droplets, appeared to induce substantially higher levels of apoptosis than did EGFP/\(\phi\) (582–643) and EGFP/\(\phi\) (582–611), which associated only with mitochondria and ER.

It has been previously reported that coexpression of $\mu_1$ with the reovirus $\sigma_3$ protein modulates the distribution of $\sigma_3$ in transfected cells (58, 65). We have confirmed these findings and shown that coexpression of $\sigma_3$ with $\mu_1$ abrogates both the membrane association of $\mu_1$ and its capacity to induce apoptosis. We hypothesize that this is a result of coassembly of $\mu_1$/$\sigma_3$ heterohexamers. In our model, coexpression of $\mu_1$ with $\sigma_3$ leads to sequestration of $\mu_1$ from membranes as assembled $\mu_1$/$\sigma_3$ heterohexamers. Our finding that coexpression of $\sigma_3$ with the $\phi$ domain of $\mu_1$ does not abrogate the capacity of $\phi$ to induce apoptosis supports this hypothesis. Late in infection, $\mu_1$ is more often associated with intracellular membranes in cells infected with T3 viruses such as T3D than in those infected with T1 viruses such as T1L (Fig. 9A). This observation correlates with the increased capacity of the T3 viruses to induce apoptosis (60; also Fig. 9B). Schmechel et al. have proposed that differences in the affinity of $\sigma_3$ for $\mu_1$ regulate the subcellular distribution of $\sigma_3$, which in turn determines its capacity to bind double-stranded RNA and prevent activation of PKR (53). Similarly, we speculate that strain-dependent differences in the affinity of $\mu_1$ for $\sigma_3$ or in the kinetics of $\mu_1$/$\sigma_3$ heterohexamer assembly may in turn determine the levels of “free” $\mu_1$ and thus $\mu_1$-determined strain differences in proapoptotic activity. The capacity of $\sigma_3$ to interact with $\mu_1$ and to abrogate its proapoptotic activity in transfected cells is reminiscent of the control of proapoptotic Bcl-2 family members such as Bax and Bak by hetero-oligomerization with antiapoptotic members such as Bcl-X$_\text{L}$ and Bcl-2 (reviewed in reference 22). Relative levels of $\sigma_3$-bound versus free $\mu_1$ may thus be an important determinant of phenotypes and strain differences relating to apoptosis induction, as well as to inhibition of host translation, by reovirus.

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REFERENCES


anti-reovirus monoclonal antibodies and their effects on viral pathogenesis. 