JOURNAL OF VIROLOGY, Sept. 1991, p. 4598–4608
0022-538X/91/094598-11$02.00/0
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Vol. 65, No. 9

Sequence Analysis, Expression, and Deletion of a Vaccinia Virus Gene Encoding a Homolog of Profilin, a Eukaryotic Actin-Binding Protein

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Received 6 March 1991/Accepted 28 May 1991

A 4,500-bp *Bam*HI fragment, located within the *Hin*dIII A segment of the vaccinia virus genome, was found to contain eight potential coding regions for polypeptides of 78 to 346 amino acids. The open reading frames with 133, 346, and 125 codons were homologous to profilin (an actin-binding protein), 3-β-hydroxysteroid dehydrogenase, and Cu-Zn superoxide dismutase, respectively. Sequence alignments indicated that the vaccinia virus and mammalian profilins were more closely related to each other than to known profilins of other eukaryotes. The expression and possible role of the profilin homolog in the virus replicative cycle were therefore investigated. Antibody raised to *Escherichia coli* expressed vaccinia virus profilin was used to demonstrate the synthesis of the 15-kDa polypeptide at late times after vaccinia virus infection of mammalian cells. The protein accumulated in the cytoplasm, but only trace amounts remained associated with highly purified virions. The isolation of vaccinia virus mutants (in strains WR and IHD-J), with nearly the entire profilin gene replaced by the *E. coli gpt* gene, indicated that the protein is not essential for infectivity. The characteristic vaccinia virus-induced changes in actin fibers, seen by fluorescence microscopy, occurred in cells infected with the mutant. Moreover, the virus-encoded profilin homolog was not required for actin-associated events, including intracellular virus movement to the periphery of the cell, formation of specialized microvilli, or release of mature virions, as shown by electron microscopy and yields of infectious intra- and extracellular virus.

Vaccinia virus is the representative member of the *Poxviridae*, a family of complex DNA-containing viruses (reviewed in reference 32). Vaccinia virus replication occurs in the cytoplasm of the infected cell, where specialized areas called factories serve as sites for viral DNA synthesis and the initial steps in virion assembly (5, 7, 22, 31). As the maturing virions move toward the periphery of the cell, some are wrapped by Golgi-derived membranous structures (21, 30, 37). At late times after infection, mature virions appear in association with short, thickened actin fibers and specialized microvilli (18, 19, 44). The virions that exit through the plasma membrane and are recovered in the medium are referred to as extracellular enveloped virions (EEV) to contrast them with the intracellular naked virions (INV) (35). Both EEV and INV are infectious, and the ratios of the two vary greatly, depending on both the virus strain and cell type (36).

Vaccinia virus thus appears to use and modify actin, and possibly other components of the microfilament system, for morphogenesis and release of virions. Actin is a ubiquitous protein in eukaryotes that is important in cell shape, motility, cytokinesis, and intracellular movement of organelles. A number of actin-binding proteins, that may have roles in regulating the formation of actin filaments, have been characterized (39, 45, 49). One such protein, profilin, forms a 1:1 complex with actin monomers and affects the rate and extent of actin polymerization in vitro. Depending on the conditions, disruption of the profilin gene of *Saccharomyces cerevisiae* led to loss of viability (29) or aberrant cell size, shape, and actin localization (16).

While analyzing a previously unsequenced region of the vaccinia virus genome, we noted the presence of homologs of profilin, superoxide dismutase, and 3-β-hydroxysteroid dehydrogenase. In this report, we present the sequence of the DNA segment containing these genes, amino acid sequence alignments of the viral and cellular profilin homologs, evidence that the vaccinia virus profilin is expressed late in infection, and the isolation and characterization of virus mutants that have the profilin gene disrupted.

MATERIALS AND METHODS

DNA sequencing and analysis. The 4.5-kbp *Bam*HI fragment, derived from vaccinia virus DNA (strain WR), was inserted into plasmid pGEM 7Zf(−). For DNA sequencing, a number of nested deletion subclones were obtained by exonuclease III treatment (17). Sequencing of both DNA strands was carried out by the dideoxy-chain termination procedure (41), using the pGEM universal primers or other appropriate synthetic oligonucleotides.

DNA sequences were compiled and analyzed by using the Microgenie (Beckman Instruments, Inc.) and the University of Wisconsin Genetics Computer Group programs (11), respectively. DNA and protein sequence similarity searches were carried out with the FASTA and TFASTA programs (38).

Production of antiserum to the product of open reading frame (ORF) 2. The coding region of the vaccinia virus profilin homolog was amplified by the polymerase chain reaction (PCR), using the primers 5′-TATATAATAAATA CATATGGCTGAATGGCATAAAA-3′ and 5′-GGGGGGA TCCTTTTAATTACCAGTTGCGCG-3′ (restriction sites *Nde*I and *Bam*HI, respectively, are underlined). The PCR product was digested with *Nde*I and *Bam*HI and inserted into linearized plasmid pET-3c (46), thus putting the gene under the regulation of the bacteriophage T7 ϕ10 promoter. After introduction of the resulting plasmid in bacterial strain

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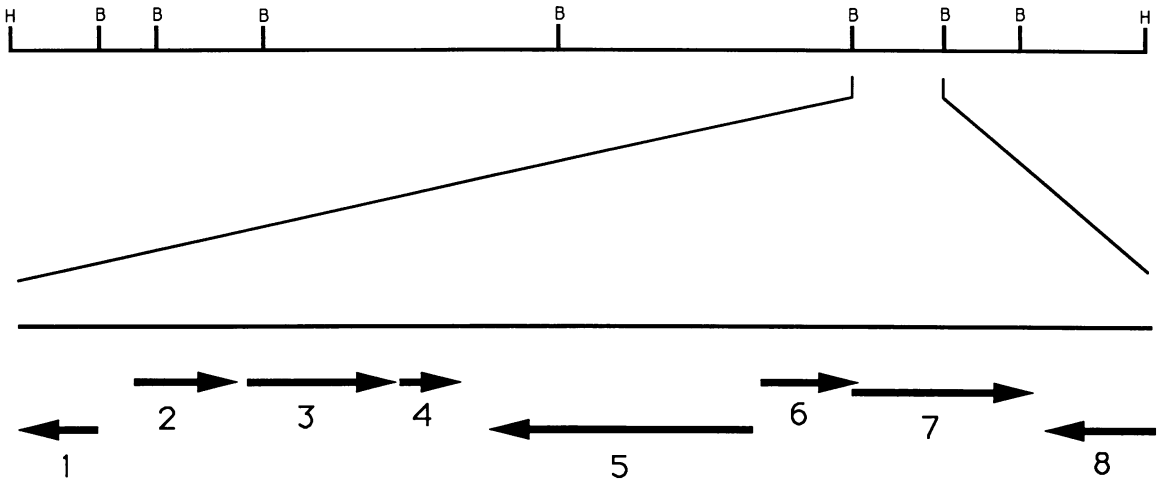

FIG. 1. Location of the sequenced region in the vaccinia virus genome. The *Hin*dIII A fragment of the vaccinia virus genome (10) is shown at the top. H, *Hin*dIII; B, *Bam*HI. Arrows at the bottom indicate the positions and directions of the numbered ORFs derived from the sequence in Fig. 2.

BL21(DE3) and induction of the expression of the T7 RNA polymerase, a polypeptide with an M_r of approximately 15,000 was synthesized to high levels. This largely insoluble polypeptide was treated with sodium dodecyl sulfate (SDS) and mercaptoethanol, purified by polyacrylamide gel electrophoresis (PAGE), eluted from the gel, emulsified with complete or incomplete Freund's adjuvant, and used to immunize rabbits.

Generation of deletion mutants. The flanks of the vaccinia virus profilin homolog gene were amplified by PCR, using the following oligonucleotides: 5′-TGCGCATGCGGATC CAAACTAGCAAACTTC-3′ (*Sph*I site underlined) and 5′-GAGCTCGAGCCATTCAGCCATTGTTATTTA-3′ (*Xho*I site underlined); and 5′-CTCGAGCTCCCGTGTACGCGCA ACTGGTA-3′ (*Sac*I site underlined) and 5′-CTTTAATGCA TATAATAGAATCGTATCC-3′ (*Nsi*I site underlined). The resulting PCR products were inserted in plasmid pGEM-*gpt* (described in Results). The final plasmid was used for transfection of vaccinia virus-infected CV-1 cells. Selection of gpt^+ virus was carried out as described previously (13).

Fluorescence microscopy. BSC-1 cells grown on coverslips were washed twice with phosphate-buffered saline (PBS) and fixed with 3.7% formaldehyde for 10 min at room temperature. After washing, the cells were permeabilized by using either acetone at −20°C or 0.1% Triton X-100 in PBS for five minutes. F-actin was visualized by using rhodamine-phalloidin (Molecular Probes, Eugene, Ore.).

The rabbit antiserum raised against the recombinant ORF 2 protein was incubated with fixed and permeabilized cells, that had been infected with an ORF 2 deletion mutant virus, to remove nonspecific antibody. This preadsorbed antiserum was then incubated with fixed and permeabilized vaccinia virus-infected cells. The bound antibody was visualized by staining with fluorescein-conjugated goat anti-rabbit immunoglobulin G.

Electron microscopy. RK13 cells were infected at a multiplicity of 10 PFU of virus per cell. At 16 h after infection, the medium was removed and the cells were fixed in 0.13 M sodium phosphate (pH 7.4)–2.5% glutaraldehyde for 2 h at 4°C. Subsequently, the cells were scraped and collected by centrifugation. Cells were postfixed in 1% osmium tetroxide and stained with uranyl acetate, embedded in Spurr's resin, sectioned, and examined in the electron microscope.

Nucleotide sequence accession number. The sequence data reported have been assigned GenBank accession number M72474.

RESULTS

Sequence of a 4.5-kbp segment of the vaccinia virus genome. At the start of this study, the sequence of only a small portion of the 45-kbp *Hin*dIII A restriction endonuclease fragment, which includes the central region of the approximately 200,000-bp vaccinia virus genome, had been reported (12). As part of our efforts to complete this analysis of the WR strain of vaccinia virus, the sequence of the 4.5-kbp *Bam*HI restriction endonuclease fragment, located between 9.3 and 13.8 kbp from the right *Hin*dIII site of the A fragment (Fig. 1), was determined. The sequence obtained was 4,487 nucleotides long, with a high A+T content (66%) typical of the vaccinia virus genome (Fig. 2).

Inspection of the sequence revealed the presence of eight putative protein-coding regions designated ORFs 1 to 8 (Fig. 1 and 2). ORFs 1, 5, and 8 are directed leftward, and the others are directed rightward. ORF 1 continues into the adjacent *Bam*HI fragment so that the full length is 121 codons. Likewise, ORF 8 abuts the right end of the *Bam*HI fragment and presumably starts in the contiguous DNA segment. The ORFs are closely spaced. The coding sequences of ORFS 6 and 7 overlap by 8 nucleotides; it is likely that the indicated ATG represents the genuine start of ORF 7, since the next ATG in frame is located more than 200 nucleotides downstream. The presence of only 7 bp between the TAA stop codon of ORF 3 and the ATG of ORF 4 suggests that the promoter element of the latter overlaps the coding sequence of the former. The 46 bp separating the oppositely oriented ORFs 5 and 6 may represent a bidirectional promoter element.

The DNA sequence was scanned for previously described vaccinia virus transcriptional motifs (33). The TAAAT motif, which is an essential element of vaccinia virus late promoters (9), was found close to the initiation codons of ORFs 2, 3, and 6, whereas ORFs 1, 4, 5, and 7 have A-rich sequences similar to the consensus for early promoters (8). In addition, the early transcription termination signal,

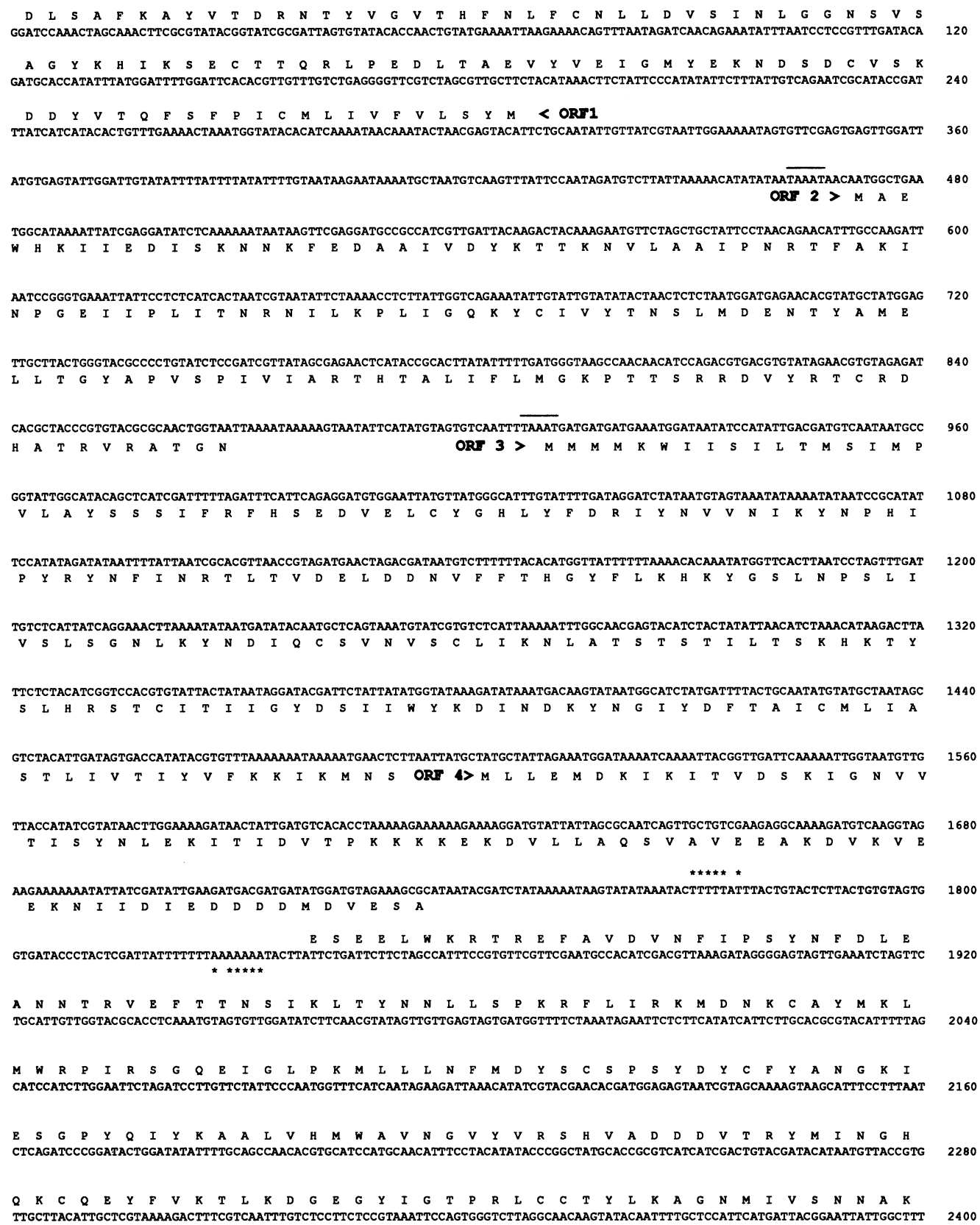

FIG. 2. DNA sequence. The sequence is shown in the 5′-to-3′ direction and from left to right according to the restriction endonuclease map of the viral genome. The translated amino acid sequence for each ORF is shown in the single-letter amino acid code. The translations of rightward and leftward ORFs are shown above and below the DNA sequence, respectively. TAAAT motifs close to the 5′ end of the ORFs are indicated by lines above the DNA sequence. Putative early termination signals are indicated by asterisks.

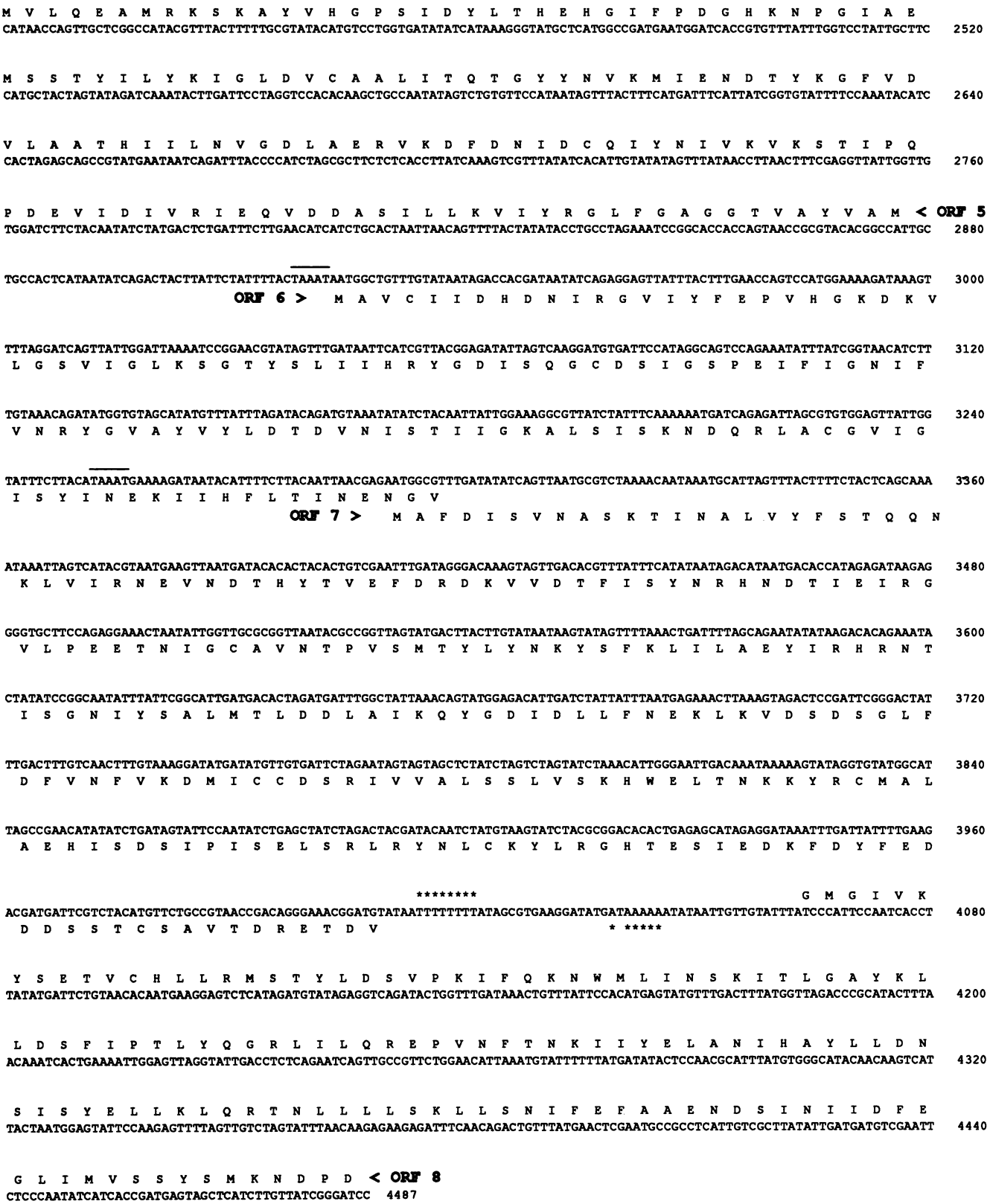

FIG. 2—*Continued.*

TABLE 1. ORFs found in the sequenced region

ORF	Position		Length (codons)	Similarity
	Start	End		
1	308	<1[a]	102[b]	
2	472	870	133	Profilin
3	911	1492	194	
4	1503	1736	78	
5	2877	1840	346	3-β-Hydroxysteroid dehydrogenase
6	2924	3298	125	Cu-Zn superoxide dismutase
7	3291	4010	240	
8	4486[a]	4046	141[b]	Vaccinia virus 33-kDa protein?

[a] End of the DNA sequence. These ORFs may extend to contiguous fragments.
[b] Maximum length of the ORF within the 4.5-kb *Bam*HI fragment.

TTTTTNT (51), was found within 40 nucleotides downstream of the 3′ end of ORFs 1, 4, 5, 7, and 8.

Amino acid sequence similarities. The amino acid sequence derived from each ORF was compared with sequences in the National Biomedical Research Foundation protein sequence data base (release 25.0) by using the FASTA program (38) and with sequences in the GenBank data base (release 64) by using the TFASTA program (38), which compares amino acid sequences to all six frames of the translated DNA sequences. This search rendered several interesting results, which are summarized in Table 1. ORFs 2, 5, and 6 showed 30 to 33% amino acid identity with cellular profilin, 3-β-hydroxysteroid dehydrogenase, and superoxide dismutase, respectively. ORF 5 exhibited about the same degree of similarity to a family of genes located partially within a repetitive element of fish lymphocystis disease virus, a member of the family *Iridoviridae* (42). ORF 8 gave the best match (22.6% identity), although of questionable significance, with a vaccinia virus 33-kDa protein of unknown function encoded within the *Hin*dIII D fragment (34, 50).

Comparison of vaccinia virus and cellular profilin homologs. A detailed comparison was made of the amino acid sequence derived from ORF 2 and those of cellular profilins. A multiple alignment of the vaccinia virus profilin sequence with sequences of the *S. cerevisiae* (15), acanthamoeba (2, 3), bovine (1), and human (23) profilins is presented in Fig. 3. Only one isoform (II) of the acanthamoeba profilin is shown, since the different ones share more than 80% of their residues. It is evident that only a small number of amino acids are common to all of the profilins. Pairwise alignments were made to quantitate the relationships between the members of the profilin family (Table 2), with the following

TABLE 2. Percent similarity between profilin sequences

Sequence	% Similarity to indicated sequence						
	Human	Bovine	Murine	Yeast	Acant[a] IA	Acant IB	Acant II
VV ORF2	32.1	32.1	32.1	19.7	10.5	12.1	14.1
Human		94.2	95.7	29.0	26.2	23.8	27.9
Bovine			95.7	30.7	26.2	26.2	28.7
Murine				29.8	22.9	23.8	27.0
Yeast					41.1	41.1	41.1
Acant IA						96.0	83.2
Acant IB							84.0

[a] Acant, acanthamoeba.

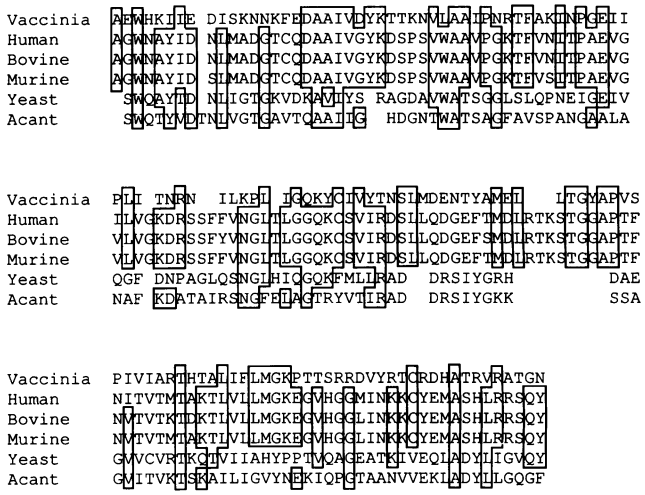

FIG. 3. Multiple alignment of the translated ORF 2 and profilin sequences. Sequences were aligned by introducing gaps to maximize the number of matches. Residues conserved in four or more of the sequences have been boxed. Acant, acanthamoeba.

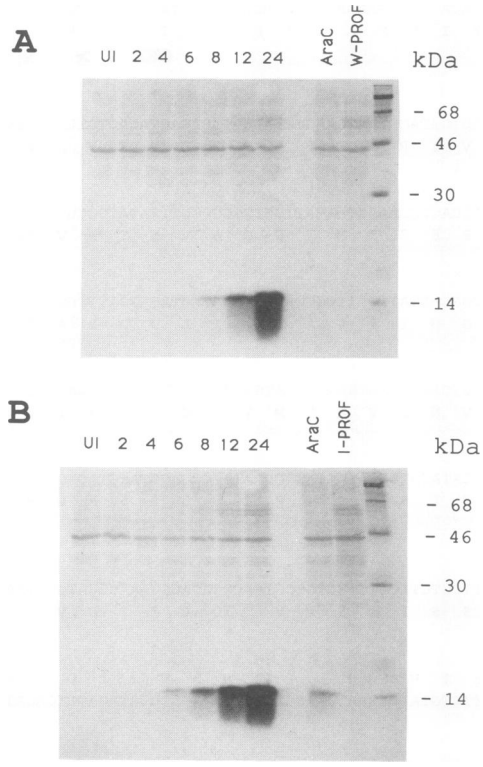

FIG. 4. Synthesis of the profilin homolog in vaccinia virus-infected cells. BSC-1 cells were infected with the WR (A) or IHD-J (B) strain of vaccinia virus. At the indicated times, the cells were lysed and the extracts were subjected to SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with antiserum to the vaccinia virus profilin followed by ^{125}I-staphylococcal protein A. Autoradiographs are shown. Lanes: UI, uninfected cell extracts; 4 to 24, cells harvested after the designated number of hours after infection; AraC, cells infected for 24 h in the presence of cytosine arabinoside (40 μg/ml); W-PROF and I-PROF, cells infected for 24 h with the ORF 2 deletion mutants of strains WR and IHD-J, respectively.

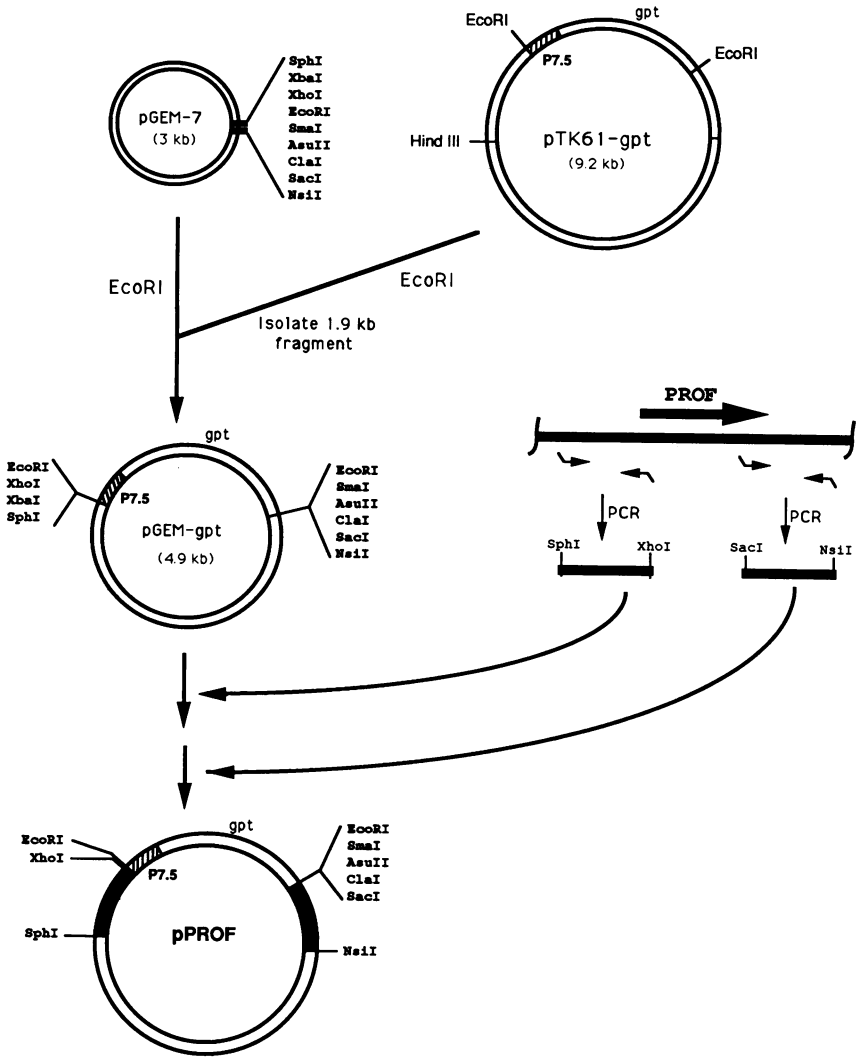

FIG. 5. Construction of plasmids to produce targeted deletions in the vaccinia virus genome. Unique restriction endonuclease sites and *Eco*RI sites are shown. The position of the profilin homolog gene (PROF) and the primers (bent arrows) used for PCR amplification are shown schematically. P7.5 represents a vaccinia virus early/late promoter (28). The PCR-amplified vaccinia virus sequences are depicted as black segments.

general conclusions: (i) the vaccinia virus profilin is more similar to the profilins of mammals (32.1% identity) than to those of yeasts (19.7%) or acanthamoebas (10.5 to 14.1%); (ii) mammalian profilins are more similar to vaccinia virus profilin than to those of either yeasts (29 to 30.7%) or acanthamoebas (22.9 to 28.7%); and (iii) yeast profilin is closer to acanthamoeba profilins (41.1%) than to those of mammals or vaccinia virus. Thus, on an evolutionary tree, the vaccinia virus sequence would branch out of the line leading to the mammalian profilins. An independent branch would lead to both the yeast and acanthamoeba profilins.

Expression of the vaccinia virus profilin gene. To determine whether the vaccinia virus profilin gene is expressed, specific antibody was needed. Accordingly, the ORF 2 was PCR amplified and inserted into an *Escherichia coli* inducible expression plasmid. Upon induction, an SDS-soluble polypeptide of apparent M_r 15,000 was detected as a prominent band on PAGE. This protein was eluted from the gel and used to immunize a rabbit. The resulting antiserum reacted with a polypeptide of about 15 kDa from vaccinia virus-infected but not uninfected cells (see below).

Since the intracellular movement and release of vaccinia virions appear to occur in association with actin, it seemed possible that these processes might be regulated by expression of vaccinia virus profilin. For this reason, we compared the synthesis of this protein in cells infected with the WR and IHD-J strains of vaccinia virus, which represent low and high yielders of EEV, respectively (36). In cells infected with either vaccinia virus strain, the 15-kDa protein was barely detectable between 4 and 6 h after infection, but it was prominent by 8 h and continued to accumulate between 12 and 24 h, thus providing a late expression pattern (Fig. 4). (The lanes in Fig. 4 that are labeled W-PROF and I-PROF contain proteins made in cells infected with deletion mutants and are described below.) The amounts of the 15-kDa protein made by the WR and IHD-J strains of vaccinia virus appeared to be quite similar. Small amounts of the protein were present in infected cells treated with cytosine arabino-

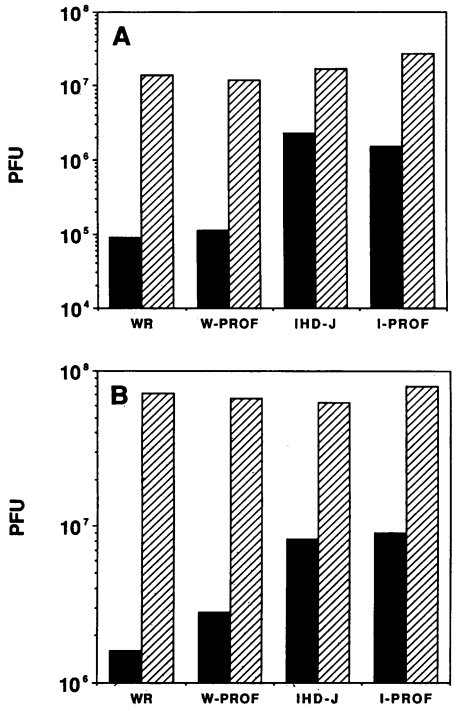

FIG. 6. Production of intra- and extracellular virus. BSC-1 (A) or RK13 (B) cells were infected with vaccinia virus strain WR or IHD-J or the derived profilin deletion mutant WR-PROF or IHD-PROF, as indicated. The 24-h yields of extracellular (filled bars) and intracellular (hatched bars) virus was determined by plaque assay on BSC-1 cells.

side, due either to incomplete inhibition of DNA replication by the inhibitor or to very low expression from parental DNA. The smear, present below the 15-kDa band (Fig. 4), was characteristic of this protein on PAGE immunoblots.

Immunoblots indicated that some of the 15-kDa protein remained associated with vaccinia virus (strain WR) that was purified from disrupted cells by sedimentation through a sucrose cushion and one sucrose gradient. After repeated gradient centrifugation, however, only trace amounts of the 15-kDa protein were detected by immunoblotting (data not shown).

Deletion of the profilin homolog. We wished to determine whether ORF 2 was essential for infectivity of vaccinia virus. The strategy followed to obtain a virus deletion mutant is outlined in Fig. 5. A cassette containing the vaccinia P7.5 promoter upstream of the *E. coli gpt* gene (13) was inserted into *Eco*RI-linearized pGEM-7 plasmid. The resulting plasmid, pGEM-*gpt*, has several unique restriction sites flanking the *gpt* cassette. Sequences from the flanks of the profilin homolog gene were PCR amplified so as to contain asymmetric restriction endonuclease sites *Sph*I-*Xho*I and *Sac*I-*Nsi*I compatible with those flanking *gpt*. After appropriate restriction endonuclease digestions and ligations, the final plasmid, pPROF, containing a copy of ORF 2 with the *gpt* cassette replacing codons 5 to 126, was used to transfect cells infected with either the WR or IHD-J strain of vaccinia virus. Recombinant viruses were isolated by three rounds of plaque purification in the presence of mycophenolic acid, which selects for the expression of *gpt* (13). The deletion and insertion of DNA were confirmed by Southern blotting using vaccinia virus and *gpt* DNA probes (not shown). The latter analysis confirmed the absence of either wild-type virus or single-crossover recombinants with intact profilin genes contaminating the isolated mutants.

The absence of expression of the profilin gene was demonstrated by PAGE and immunoblotting of extracts from cells at 24 h after infection with the deletion mutants. No immunoreactive 15-kDa protein was detected with either the WR or IHD-J strain mutants (Fig. 4). The ability to isolate such recombinant viruses, which formed normal-size plaques, was evidence that ORF 2 was not required for infectivity.

Characterization of the deletion mutants. As mentioned previously, the WR and IHD-J strains of vaccinia virus represent low and high yielders of EEV, respectively. The combination of IHD-J and rabbit kidney RK13 cells was reported to be an especially favorable combination for EEV production (36). We confirmed the higher percentage of EEV formed by IHD-J than by WR in both BSC-1 and RK13 cells (Fig. 6). However, lack of expression of the vaccinia virus profilin made no significant difference since the final yields of infectious INV and EEV produced by the deletion mutants, and their parental viruses were similar.

Fluorescence microscopy of actin fibers and vaccinia virus profilin in infected cells. Since profilins are thought to regulate actin polymerization, we were curious to compare the intracellular distribution of filamentous actin in cells infected with wild-type and mutant vaccinia viruses. In uninfected BSC-1 cells, actin appeared as long, slender stress fibers that stained with rhodamine-labeled phalloidin (Fig. 7A). In agreement with previous reports on studies using chicken embryo fibroblasts (19), changes in the actin cytoskeleton of BSC-1 cells were not apparent at early times after infection although cell rounding occurred in both wild-type- and mutant-infected cells. The characteristic short, thickened actin bundles described at late times after vaccinia virus infection of chicken embryo fibroblasts (19) also appeared in BSC-1 cells infected with wild-type and mutant viruses (Fig. 7C and E). Essentially similar results were obtained with use of antibody to actin instead of phalloidin (not shown).

The antiserum, raised against ORF 2 expressed in *E. coli*, was used to determine the intracellular localization of vaccinia virus profilin. The rabbit antiserum was first incubated with fixed and permeabilized cells that had been infected with the profilin deletion mutant so as to remove any nonspecific binding proteins. The preadsorbed antiserum was then incubated with similarly prepared uninfected cells or cells that were infected with wild-type or mutant virus. The bound antibody, visualized by staining with fluorescein-conjugated goat anti-rabbit immunoglobulin G, was diffusely distributed throughout the cytoplasm of cells infected with wild-type virus (Fig. 7D), but only faint staining was seen in uninfected (Fig. 7B) or mutant-infected (Fig. 7F) cells. There was no evident association of vaccinia virus profilin with actin filaments.

Electron microscopy. Transmission electron microscopy of wild-type and mutant virus-infected cell sections showed a normal distribution of immature and mature forms, with many of the latter near the periphery of the cytoplasm (Fig. 8). Thus, the absence of expression of vaccinia virus profilin had no apparent effect on morphogenesis or movement of mature particles.

Hiller and coworkers (19) previously showed, by scanning electron microscopy, that the majority of uninfected chicken embryo fibroblasts had relatively smooth surfaces with few 0.10- to 0.12-mm microvilli, although the latter were numerous in a minority of cells which were presumably in late G_2

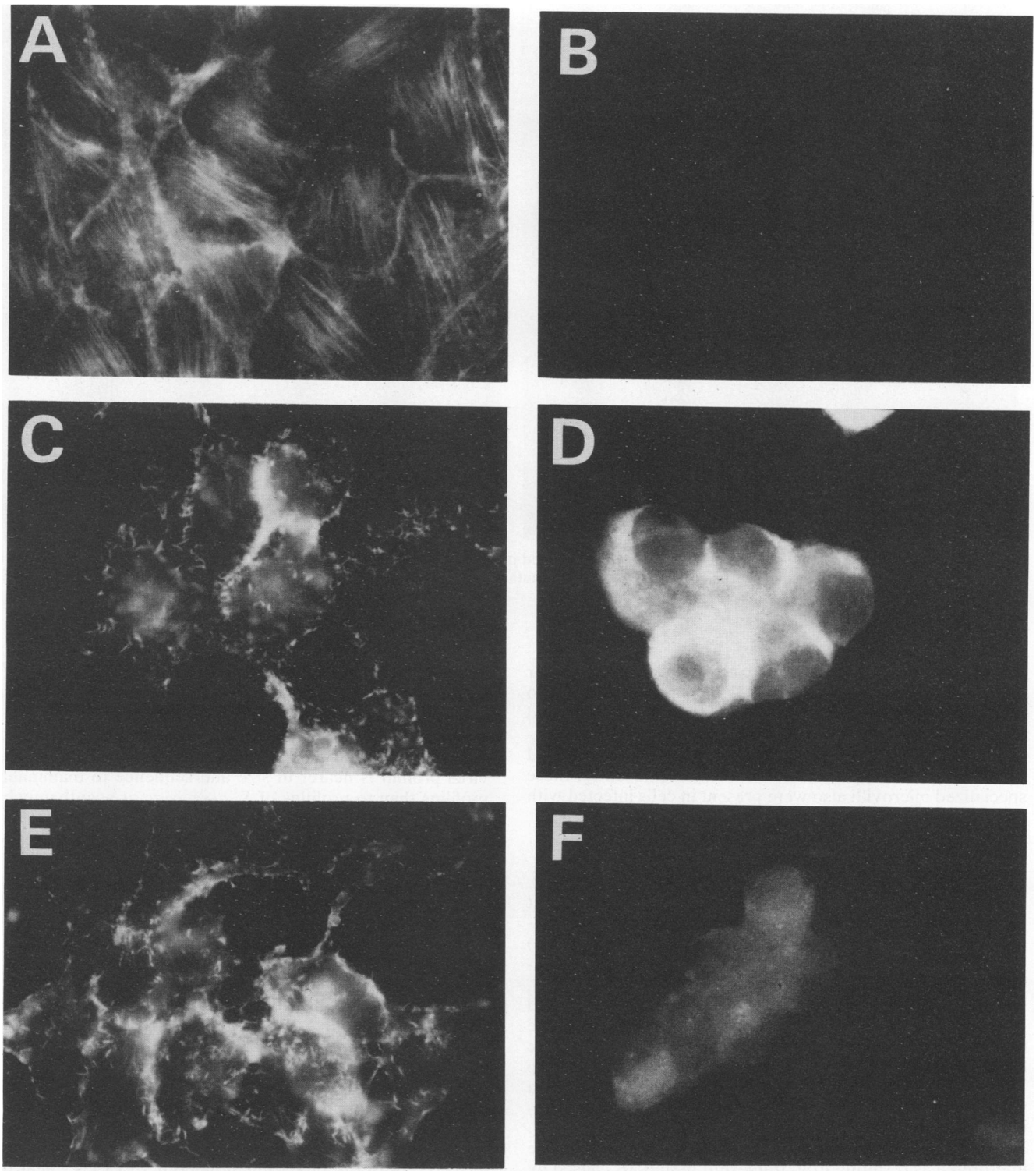

FIG. 7. Detection of actin and the vaccinia virus profilin homolog by fluorescence microscopy. BSC-1 cells were mock infected (A and B) or infected with vaccinia virus strain WR (C and D) or with the profilin deletion mutant of strain WR (E and F). Cells were fixed and permeabilized at 24 h after infection. (A, C, and E) Cells treated with rhodamine-phalloidin; (B, D, and F) cells treated with rabbit antiserum specific for the vaccinia virus profilin homolog and subsequently with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G.

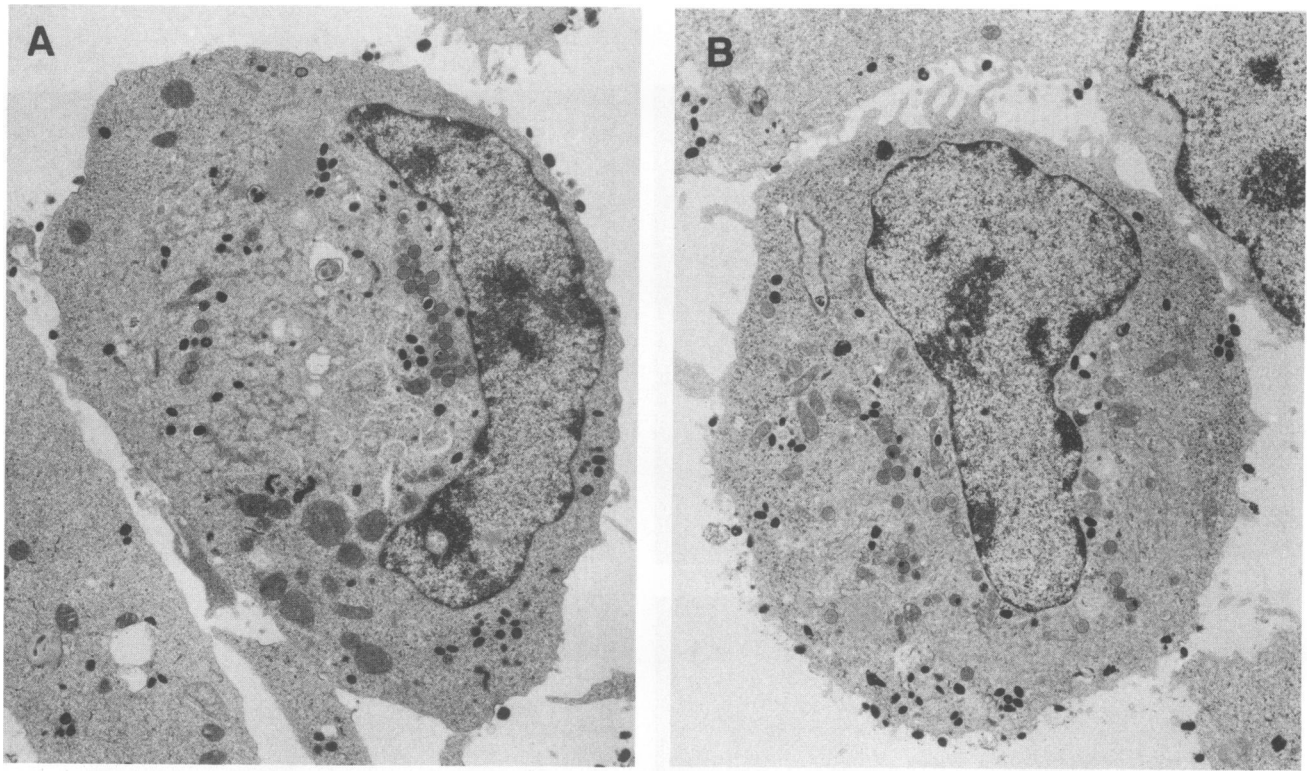

FIG. 8. Electron microscopy of cells infected with wild-type and mutant vaccinia virus. The electron micrographs show sections of cells that had been infected with vaccinia virus strain IHD-J (A) or a mutant of this strain with a deletion in ORF 2 (B). Cells were fixed at 16 h after infection. Magnification, ×7,900.

and M phases of the cell cycle. By contrast, at 9 to 16 h after vaccinia virus infection, nearly all of the chicken embryo fibroblasts had numerous microvilli that were distinguished from normal ones by their larger (0.30- to 0.35-mm) diameters. A similar difference between uninfected and infected BSC-1 cells was apparent (not shown). Significantly, these specialized microvilli also were present in cells infected with the profilin deletion mutant.

DISCUSSION

Remarkably, of the eight ORFs within a 4.5-kbp segment of the vaccinia virus strain WR genome, three (profilin, 3-β-hydroxysteroid dehydrogenase, and superoxide dismutase) have predicted amino acid sequence similarities to eukaryotic proteins. In each case, the degree of identity, 30 to 33%, is highly significant, strongly suggesting homology. Goebel et al. (14), in their recent report of the sequence of the Copenhagen (CH) strain of vaccinia virus, also noted these homologies. A computer-derived alignment of the 4.5-kbp DNA sequences of the WR and CH strains resulted in a 99.3% overall identity, with eight gaps of up to 16 nucleotides. Five of the gaps occurred in intergenic regions. Differences between three ORFs of WR and CH could be interpreted as resulting from frameshift mutations caused by deletions or additions of short DNA sequences. Thus, ORF 4, which is 78 codons in WR, has a 42-codon counterpart in CH that Goebel et al. (14) did not classify as a potential gene because of its small size. Similarly, the predicted protein products of ORFs 7 and 8 are, respectively, 26 and 7 residues longer in the WR sequence than are their counterparts in the CH sequence. ORFs 1, 2, 3, 5, and 6 are the same size in WR and CH, differing only by 3, 0, 1, 5, and 2 amino acid substitutions, respectively.

In this study, we focused on ORF 2, which is the homolog of profilin, a eukaryotic actin-binding protein. The vaccinia virus profilin is nearer in size and sequence to mammalian profilins than to profilins of *S. cerevisiae* or acanthamoeba. Likewise, the mammalian profilins are closer in size and sequence to the vaccinia virus profilin than to the other eukaryotic profilins. Thus, solely on a structural basis, the product of ORF 2 deserves to be placed in the profilin family. Members of this family vary in their affinities for actin, with the K_d for the actin-profilin complex ranging from 50 to 10 mM (24, 48). Cross-linking studies have demonstrated the formation of 1:1 heterodimers of a recombinant form of the vaccinia virus profilin with actin, but the specificity and affinity of this binding are still under investigation (27).

We have examined the synthesis of the vaccinia virus-encoded profilin and its possible role in the virus replication cycle. Antibody was made to the recombinant vaccinia virus profilin and used to demonstrate the appearance and accumulation of the predicted-size 15-kDa protein, mainly during the late phase (6 to 24 h) of infection. The presence of the TA AAT motif near the beginning of the ORF and the absence of the early transcription termination motif TTTTTNT near the end of the ORF are both consistent with late expression. Many of the major structural components of virions are also made at late times. Although some of the 15-kDa protein was detected on SDS-PAGE immunoblots of sucrose gradient-purified virions, most of it was removed upon repeated centrifugations, suggesting that it is not an integral compo-

nent of virions. It is possible that the vaccinia virus profilin is associated with residual loosely bound actin.

Previous observations regarding the formation of specialized actin bundles and microvilli in cells infected with vaccinia virus and the association of mature virions with these structures, apparently for their movement and ultimate release from cells (19, 44), led us to consider that the vaccinia virus-encoded profilin might influence these events. Both the time of synthesis of this viral protein and its homology with an actin regulatory protein were consistent with such a role. To investigate this possibility, we used two strains of vaccinia virus, WR and IHD-J, that are low and high yielders of EEV (36) and deleted the profilin gene from both. SDS-PAGE analyses indicated that similar amounts of profilin were made in cells infected with the two parental strains, whereas none was made in cells infected with either deletion mutant. Expression of the profilin gene was not essential for production of infectious virus in any of the cell lines tested. Indeed, in BSC-1 and RK13 cells, our deletion of the profilin gene had no significant effect on the yields of intra- or extracellular virus. Using a variety of techniques, including fluorescence, transmission electron, and scanning electron microscopy, we could discern no difference in wild-type and mutant virus-induced cell rounding, induction of actin bundles and specialized microvilli, in virus morphogenesis, movement of virus particles to the periphery of the cell, and release of extracellular virus. The simplest conclusion is that vaccinia virus profilin plays no role in any of these events, at least in the cell lines and under the culture conditions tested. It remains possible, however, that the vaccinia virus profilin might affect these processes under different conditions. Alternatively, the vaccinia virus profilin may have an entirely different role. In this regard, recent studies indicate that eukaryotic profilins interact with acidic phospholipids, such as phosphatidylinositol 4,5-bisphosphate (PIP_2) (25). Indeed, the affinity of platelet profilin for PIP_2 pentamers was found to be at least an order of magnitude higher than that for actin under physiological conditions (15). Profilin competes with phospholipase C for PIP_2 in vitro, leading Goldschmidt-Clermont et al. (15) to suggest that profilins may be negative regulators of the phosphoinositide signaling pathway in addition to their more established roles as inhibitors of actin polymerization. If vaccinia virus profilin has similar properties, inhibition of phospholipase C might reduce the host inflammatory and antiviral responses (4, 6, 20, 40) as well as autocrine effects of the vaccinia virus epidermal growth factor homolog (32). Such roles might explain our inability to detect an altered phenotype of the deletion mutants in cultured cells.

ACKNOWLEDGMENTS

We thank Laura M. Machesky and Thomas P. Pollard for communication of unpublished results and critical reading of the manuscript.

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