Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 409–413, January 1990
Microbiology

Identification of a viral gene encoding a ubiquitin-like protein

(baculovirus/virus–host interaction/gene expression)

LINDA A. GUARINO

Department of Entomology, Institute of Biosciences and Technology, Texas Agricultural Experiment Station and Texas A&M University, College Station, TX 77843

Communicated by Max D. Summers, September 29, 1989 (received for review August 22, 1989)

ABSTRACT **The baculovirus *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV, which is representative of the *M*NPV subtype in which the virions may contain many nucleocapsids within a single viral envelope) encodes a protein, v-ubi, that has 76% identity with the eukaryotic protein ubiquitin. Transcriptional mapping indicated that the gene for v-ubi was transcribed during the late phase of viral infection. Two transcriptional start sites potentially encoding v-ubi were identified. Both sites were contained within a sequence motif common to baculovirus late genes. A recombinant virus, AcUbi-βGal, encoding a ubiquitin-β-galactosidase fusion protein was constructed to monitor the temporal regulation of v-ubi gene during viral infection. The fusion protein was expressed maximally at 14–18 hr postinfection, consistent with its classification as a late protein. The amount of ubiquitin-β-galactosidase fusion protein that accumulated in AcUbi-βGal-infected cells by 48 hr postinfection was ≈14% of the level of β-galactosidase that was synthesized under control of the polyhedrin promoter. Transcriptional analysis confirmed that synthesis of the fusion protein was directed by the v-ubi gene promoter. AcUbi-βGal also produced normal levels of authentic viral ubiquitin message. Southern blot analysis of AcUbi-βGal and 15 additional isolates revealed that the fusion sequences had not recombined at the ubiquitin locus. A polyubiquitin gene was isolated and sequenced from *Spodoptera frugiperda*, a lepidopteran host cell line for Ac*M*NPV. The predicted amino acid sequence of the product of the host gene is identical to animal ubiquitin.**

Ubiquitin is a small eukaryotic protein involved in a number of basic cellular processes (1, 2). The amino acid sequence of ubiquitin is highly conserved, differing by only three amino acids between animals, yeast, and plants. Ubiquitin is abundant in cells, both free and covalently joined to an array of acceptor proteins. All of the known functions of ubiquitin are mediated through conjugation of ubiquitin to an acceptor protein via an isopeptide bond between the C-terminal glycine residue of ubiquitin and the ε-amino group of a lysine residue in the acceptor protein. A major function of ubiquitin is to mark proteins for selective elimination. Several ubiquitin molecules are attached sequentially to these "targeted" proteins to form branched ubiquitin–ubiquitin conjugates in which the C-terminal Gly-76 of one ubiquitin is joined to the internal Lys-48 of an adjacent ubiquitin (3). The attachment of a multiubiquitin chain is apparently essential for the degradation of a variety of proteins via the ubiquitin-dependent protease.

Another role that has been suggested for ubiquitin is the reversible attachment of ubiquitin to a protein, which modulates protein function without targeting the protein for degradation. Stable ubiquitin acceptors include histones H2A and H2B (4), actin (5), the growth hormone receptor (6), and the lymphocyte homing receptor (7). The existence of these stable ubiquitin–protein conjugates is apparently explained by the observation that these proteins are monoubiquitinated and therefore do not activate the ubiquitin-dependent protease.

Ubiquitin is encoded by multiple genes in all organisms examined to date; these genes direct the synthesis of polyubiquitin or ubiquitin fusion proteins. The polyubiquitin genes contain tandem arrays of ubiquitin coding regions with repeat lengths varying from 5 in yeast (8) to 18 in *Drosophila* (9). Stop codons are not present between the coding units, and cleavage at the Gly-Met bonds between each ubiquitin molecule generates monomers from a polyubiquitin translation product. The single ubiquitin coding regions are fused to carboxyl-terminal extensions of 52 or 76–80 amino acids. Expression of the fusion proteins and polyproteins is differentially regulated in yeast cells and probably in other organisms as well. In normally growing cells, most ubiquitin is generated from the fusion proteins. The polyubiquitin gene is dispensable in growing cells but is essential during stress as the main source of ubiquitin (8).

It recently has been reported that the extension proteins are ribosomal proteins (10, 11), and the association of the extension proteins with ubiquitin facilitates ribosome assembly. This observation suggests that an additional function of ubiquitin may be to serve as a "molecular chaperone" for the incorporation of specific proteins into cellular structures (10).

Ubiquitin molecules are covalently linked to tobacco mosaic virus coat protein subunits (12). Quantitative analysis indicated that approximately one subunit per virion is ubiquitinated. Recently, 18 viruses from several different virus families were examined for the presence of ubiquitinated proteins in purified virions (13). The majority of these viruses were found to contain proteins that cross-reacted with affinity-purified anti-ubiquitin antibody. The function of these ubiquitinated proteins in the virus is unknown; but their widespread occurrence indicates that ubiquitin may play a role in viral life cycles. Alternatively, ubiquitination of viral proteins could be a common host response to the stress of virus infection.

This analysis included a member of the Baculoviridae. Baculoviruses are complex DNA viruses that infect invertebrate organisms (14). These viruses have the potential to encode ≈100 proteins. In this report, we show that one of the proteins, v-ubi, encoded by the baculovirus *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV, which is representative of the *M*NPV subtype in which virions may contain many nucleocapsids within a single viral envelope) shares 76% identical amino acids with animal ubiquitin, while the amino acid sequence of ubiquitin from a lepidopteran host

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ac*M*NPV, *Autographa californica* nuclear polyhedrosis virus, which is representative of the *M*NPV subtype in which the virions may contain many nucleocapsids within a single viral envelope; v-ubi, viral ubiquitin-like protein.

cell line is identical with the animal protein. Analysis of infected cell proteins indicated that v-ubi is highly expressed during the late phase of viral infection.

MATERIALS AND METHODS

DNA Sequencing. The nucleotide sequence of the *Pst* I K fragment of Ac*M*NPV* was determined by a combination of subcloning restriction fragments and making nested deletions with exonuclease III (15, 16). Host ubiquitin genes were selected from a λ phage EMBL3 library of *Spodoptera frugiperda* DNA by using a *Drosophila* ubiquitin probe (9) and standard cloning techniques (17). *Xho* I fragments of ubiquitin monomers were subcloned into phage M13 and sequenced. Sequences were compiled and analyzed by using the programs of Devereaux *et al.* (18).

Construction of Viral Mutants. The transfer plasmid pUbi-βGal was constructed by cloning a *Sal* I fragment of pMC1871 (19) into the *Afl* II site of *Pst* I K fragment after repair of both ends with the Klenow fragment of *Escherichia coli* DNA polymerase I by standard cloning techniques (17). Viral recombinants were selected after cotransfection of *S. frugiperda* cells with pUbi-βGal and viral DNA (20).

Analysis of Viral Infected Cells. *S. frugiperda* cells were infected and labeled as described (21). Intracellular protein extracts were prepared by treating the cells for 20 min on ice with extraction buffer [50 mM Tris·HCl, pH 8.0/100 mM NaCl/1% Nonidet P-40/1% Empigen BB (Albright & Wilson, Whitehaven, U.K.)], microcentrifuging the extract for 10 min, and harvesting the supernatant fluid. Extracts were mixed with an equal volume of 2× sodium dodecyl sulfate (SDS) sample buffer (0.125 M Tris, pH 6.8/4% SDS/2% 2-mercaptoethanol/20% glycerol) and analyzed on 8% polyacrylamide gels. For immunoblot (Western) analysis, proteins were electrophoretically transferred to nitrocellulose sheets with a semidry apparatus according to the recommendations of the manufacturer (American Bionetics, Hayward, CA). After transfer, the nitrocellulose sheet was treated with blocking buffer and probed with mouse monoclonal anti-β-galactosidase IgG. The blot was subsequently probed with alkaline phosphatase-conjugated goat anti-mouse IgG and developed with a standard alkaline phosphatase color reaction. Total cell RNA was purified from cells infected with wild-type virus at 6 and 18 hr postinfection or with the AcUbi-βGal fusion virus at 18 hr postinfection. RNA was hybridized with an *Afl* II–*Nsi* I fragment 5′-end-labeled at the *Afl* II site or with an *Eco*RI–*Nsi* I fragment 5′-end-labeled at the *Eco*RI site. The RNA isolation, end-labeling, and S1 nuclease analysis were conducted as described (22). Assays for β-galactosidase activity were performed by using a modification of the method of Zamn and Fowler (23). A unit of β-galactosidase is the amount of enzyme that produces 1 nmol of *o*-nitrophenol per min at 28°C at pH 7.0.

RESULTS

A map of the Ac*M*NPV *Pst* I K fragment (21.0–23.5 map units) is presented in Fig. 1*A*. This region of the viral genome contains the gene for the 39-kDa protein ("39K gene") that has been used extensively for mapping immediate early viral regulatory genes (22). Sequence analysis of DNA flanking *Pst* I K fragment revealed the presence of a small open reading frame located downstream and on the opposite strand of the 39K gene. A search of the National Biomedical Research Foundation protein bank showed 76% identity with animal ubiquitin. This viral gene encoding ubiquitin-like v-ubi encodes 77 amino acids rather than the 76 residues found in mature ubiquitin. The products of yeast, chicken, and *Drosophila* polyubiquitin genes contain extra C-terminal residues following glycine-76 (8, 9, 24).

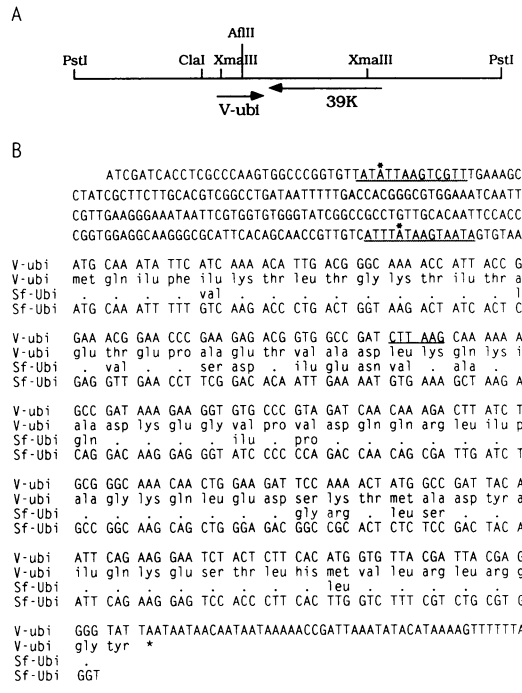

FIG. 1. Nucleotide sequence of the Ac*M*NPV ubiquitin gene and the predicted amino acid sequence of v-ubi. (*A*) Map of the *Pst* I K fragment of the Ac*M*NPV genome. The location and direction of transcription of the genes encoding the 39-kDa protein and v-ubi are indicated. (*B*) Nucleotide sequence and predicted amino acid sequences of v-ubi and *S. frugiperda* ubiquitin (Sf-Ubi). The coding region and flanking sequences are shown for v-ubi; the sequence of a single *Xho* I monomer is shown for ubiquitin. Identical residues in both sequences are indicated by a dot in the *S. frugiperda* sequence, while differences are indicated by the correct amino acids. The *Afl* II restriction site in the viral DNA that was used for S1 nuclease mapping and cloning of the β-galactosidase gene is underlined. The transcriptional start sites are indicated by asterisks, and the sequence homologous to the conserved sequence found in baculovirus late genes is indicated by a dotted underline.

The nucleotide sequence and deduced amino acid sequence of this region of *Pst* I K fragment is presented in Fig. 1*B*. The predicted amino acid sequence for v-ubi differs from the animal ubiquitin sequence at 18 residues in addition to the C-terminal tyrosine. Ten of the substitutions are conservative with respect to the side group, and the numbers of acidic and basic residues are the same as in animal ubiquitin. Two features known to be important for ubiquitin function are conserved in the viral protein. The lysine-48 residue, necessary for branched-chain multiubiquitin adducts (3), is present. The glycine-glycine dipeptide (positions 75–76) is also conserved. This sequence is essential for cleavage of the terminal residue and conjugation to acceptor proteins (25).

The presence of ubiquitin from lepidopteran insects has not been reported previously. To determine whether the viral ubiquitin sequence was virus-specific or whether it was derived from its lepidopteran host, the nucleotide sequence of the host ubiquitin gene was determined. Ubiquitin genes were selected from a phage λ EMBL3 library of *S. frugiperda* DNA by using a *Drosophila* ubiquitin probe (9). Both strongly and weakly hybridizing plaques were detected. DNA was purified from the strongly hybridizing plaques and subjected to partial restriction digestion with *Xho* I, which cuts each ubiquitin monomer once (Fig. 2*A*). This number of bands in the partial digest indicated that the 228-bp ubiquitin-

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M30305 for the viral ubiquitin sequence and M30306 for the host ubiquitin sequence).

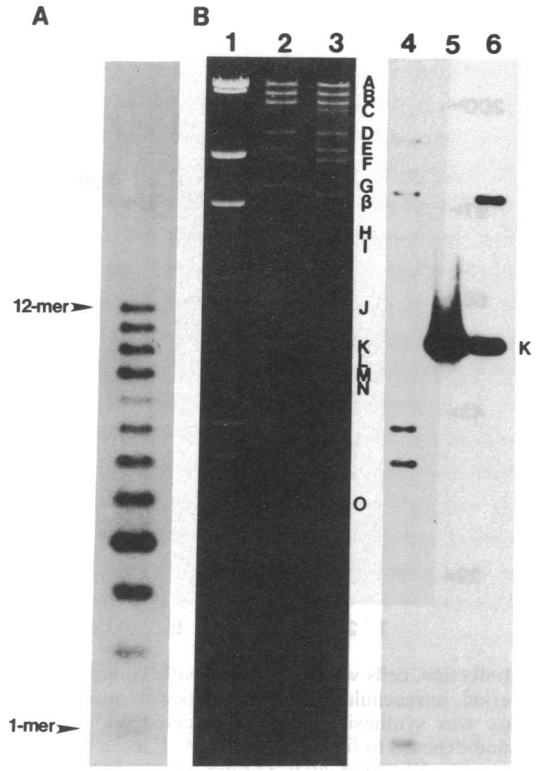

FIG. 2. Southern blot analysis of DNA encoding *S. frugiperda* polyubiquitin and v-ubi. (*A*) Tandem ubiquitin-encoding repeats in *S. frugiperda* polyubiquitin gene. A phage λ genomic clone containing the *S. frugiperda* polyubiquitin gene was partially digested with *Xho* I. The DNA was separated on a 0.8% agarose gel and blotted onto nitrocellulose. The filters were hybridized with a *Drosophila* ubiquitin probe (9). The positions of the 228-base-pair (bp) ubiquitin monomer and the ubiquitin 12-mer are indicated. (*B*) Southern blot analysis of wild-type Ac*M*NPV and AcUbi-βGal. Viral DNA was digested with *Pst* I and separated on a 0.8% agarose gel, stained with ethidium bromide, and visualized with UV light (lanes 1–3). The gel was blotted onto nitrocellulose, and the filter was hybridized with a 1.5-kilobase (kb) *Xma* III subfragment of *Pst* I K fragment encoding V-ubi (lanes 4–6). The locations of the viral *Pst* I fragments and the Ubi-βGal fusion sequences are indicated on the right. Lanes: 1 and 4, *Hin*dIII fragments of phage λ DNA; 2 and 5, wild-type Ac*M*NPV DNA; 3 and 6, AcUbi-βGal DNA.

coding region is repeated 12 times in tandem. Several ubiquitin monomers were subcloned and sequenced. The nucleotide sequence of the monomers varied, especially at the third codon position; however, the predicted amino acid sequences of the repeats were identical to each other and to that of animal ubiquitin (Fig. 1*B*).

Eukaryotic organisms contain at least three different genes that encode polyubiquitin and ubiquitin fusion proteins. To determine whether the Ac*M*NPV genome contained additional ubiquitin-like genes, a 1.5-kb *Xma* III fragment of the plasmid *Pst* I K fragment containing the v-ubi coding region was used to probe *Pst* I-digested viral DNA. The autoradiogram revealed a single radioactive band that migrated with *Pst* I K fragment, indicating that the virus contains only one gene with homology to ubiquitin (Fig. 2*B*).

To determine whether the viral ubiquitin gene was transcribed in infected cells, S1 nuclease analysis was performed with RNA purified from Ac*M*NPV-infected cells at 6 and 18 hr postinfection. Two protected fragments were detected with 18-hr RNA, none with 6-hr RNA (Fig. 3). To precisely map the transcriptional start sites, the protected fragments were analyzed in lanes adjacent to a Maxam–Gilbert (26) sequencing ladder of the probe. The corresponding transcriptional start sites are indicated in Fig. 1. The 5′ proximal AUG

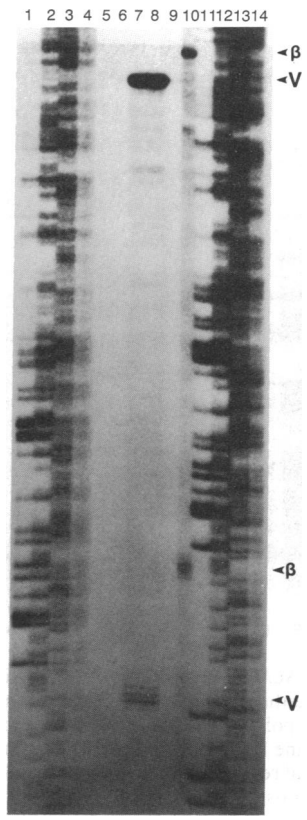

FIG. 3. S1 nuclease protection analyses of v-ubi transcripts. RNA was purified from *S. frugiperda* cells infected with wild-type virus at 0 (lane 5), 6 (lane 6), or 18 hr (lane 7) or from cells with AcUbi-βGal (lane 8) and hybridized with a the *Afl* II–*Nsi* I probe, specifically 5′-end-labeled at the *Afl* II site. A set of Maxam–Gilbert (26) sequencing ladders of the same fragment is shown in lanes 1–4, (corresponding to GATC). RNA was purified from cells infected with AcUbi-βGal and purified at 0 (lane 9) or 18 hr (lane 10) and hybridized with an *Eco*RI–*Nsi* I fusion-specific probe. The corresponding sequencing ladder is presented in lanes 11–14 (corresponding to GATC). The positions of the S1 nuclease-protected fragments is indicated on the right.

for both transcripts is the initiation codon for v-ubi. The major site of transcription initiation is located 197 nucleotides upstream of the methionine codon. The minor site is 18 nucleotides upstream of the initiation codon. Both promoter regions are contained within conserved motifs located near the transcriptional start sites of highly expressed baculovirus late genes (27).

To investigate whether the gene for v-ubi translated in infected cells, a recombinant virus containing the β-galactosidase gene under the control of the v-ubi gene was produced. A fragment encoding β-galactosidase was inserted in frame with the coding region at the *Afl* II site indicated in Fig. 1. The resulting construct should encode β-galactosidase with 26 amino acids of v-ubi fused to the N terminus. This plasmid (pUbi-βGal) contained 1.2 kb of ubiquitin flanking sequence upstream and 2.0 kb downstream of the fusion gene. *S. frugiperda* cells were cotransfected with pUbi-βGal and viral DNA. Progeny virus was plaque-purified in the presence of the chromogenic substrate Bluo-gal (Bethesda Research Laboratories). A Ubi-βGal recombinant was used to monitor the time of expression of v-ubi during virus infection. *S. frugiperda* cells were infected with wild-type (Ac*M*NPV) virus or one of the Ubi-βGal recombinants (AcUbi-βGal). At the indicated times postinfection, cells were pulse-labeled with [^{32}S]methionine for 4 hr. As a control, nonfused β-galactosidase was expressed from the polyhedrin promotor by infection of *S. frugiperda* cells with VL720-βGal (28), and cells were radiolabeled from 44 to 48 hr postinfection. Protein extracts were analyzed on duplicate SDS/polyacrylamide gels (Fig. 4). One gel was dried and exposed to film (Fig. 4*A*). A radiolabeled protein that migrated more slowly than nonfused β-galactosidase was detected in cells infected with AcUbi-βGal but not in cells infected with wild-type virus. Synthesis of the Ubi-βGal fusion protein was first detected at 8–12 hr postinfection, was maximal at 14–18 hr postinfection, and declined at later times. This pattern of synthesis is characteristic of late or γ-phase genes. The other gel was transferred to nitrocellulose

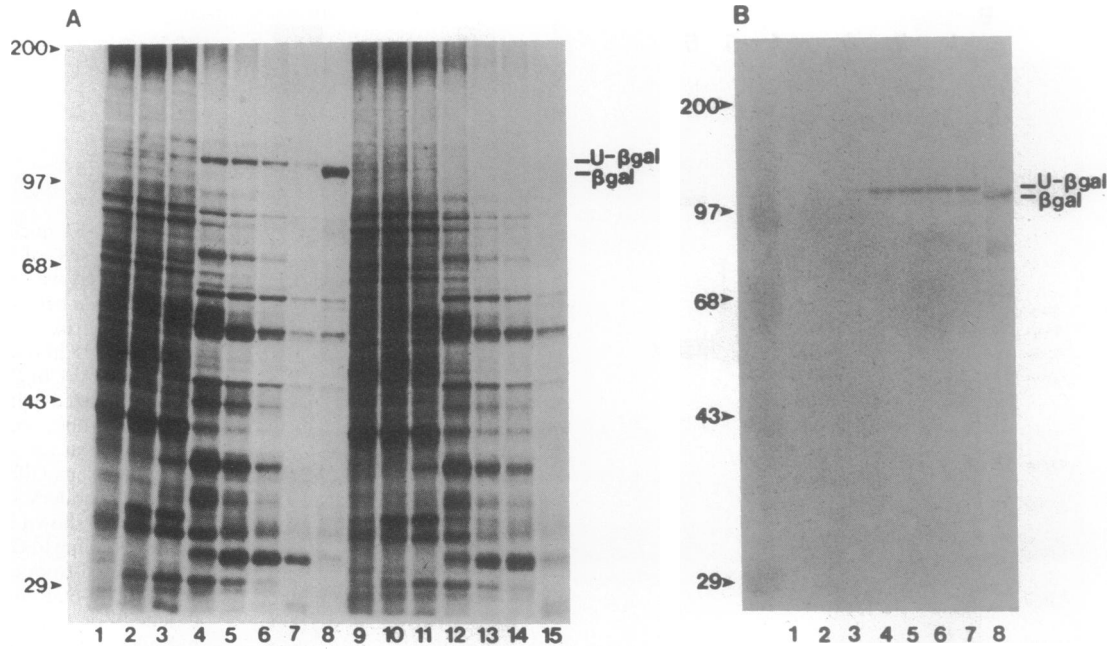

FIG. 4. Temporal expression of Ac*M*NPV ubiquitin. At the indicated times postinfection, cells were pulse-label with Tran^{35}S-labeled (ICN; a mixture of ^{35}S-labeled cysteine/methionine) for 4 hr. At the end of the labeling period, intracellular protein extracts were prepared. Extracts were analyzed on duplicate SDS/polyacrylamide gels. Nonfused β-galactosidase was synthesized in cells infected with the Ac*M*NPV recombinant pVL720-βGal. Each lane represents 1×10^4 cells. One gel was dried and exposed to film as shown in *A*. Lanes: 1–7, time course of cells infected with the AcUbi-βGal recombinant; 8 cell extracts 48 hr postinfection with pVL720-βGal; 9–15 time course of cells infected with wild-type Ac*M*NPV. Labeling was postinfection from 0 to 4 hr (lanes 1 and 9), from 4 to 8 hr (lanes 2 and 10), from 8 to 12 hr (lanes 3 and 11), from 14 to 18 hr (lanes 4 and 12), from 22 to 26 hr (lanes 5 and 13), from 32 to 36 hr (lanes 6 and 14), and from 44 to 48 hr (lanes 7 and 15). The positions of radiolabeled molecular markers (Bethesda Research Laboratories) run in the left-most lane are indicated on the left. The other gel was used for immunoblot analysis as shown in *B*. The bands corresponding to the ubiquin-β-galactosidase fusion protein (U-βgal) and nonfused β-galactosidase (βgal) are indicated on the right. Lanes 1–8 contain the same samples are presented in *A*. The positions of prestained molecular markers analyzed in the left-most lane are indicated on the left.

and probed with anti-β-galactosidase (Fig. 4*B*). Immunoblot analysis indicated a major reactive species in the AcUbi-βGal-infected cells migrating more slowly than nonfused β-galactosidase produced under the control of polyhedrin. The fusion protein was detectable at a low level in the 12-hr time point and remained at a maximum, constant level from 18 to 48 hr postinfection. Densitometric analysis of the separate immunoblot containing serial dilutions of both extracts indicated that the amount of β-galactosidase produced under control of the v-ubi gene promoter was ≈14% of that synthesized in VL720-βGal-infected cells under the control of the polyhedrin promoter at 48 hr postinfection. This result was confirmed by enzymatic analysis of β-galactosidase in extracts of cells infected with both recombinants. Cells infected with VL720-βGal produced 0.29 units of β-galactosidase per 10^6 cells, while AcUbi-βGal-infected cells produced 0.035 units per 10^6 cells.

To confirm that the message encoding the fusion protein was expressed under the control of the ubiquitin promoter, RNA was purified from cells infected with AcUbi-βGal at 18 hr postinfection and subjected to S1 nuclease analysis by using a probe specific for the fusion sequences (Fig. 4). As predicted by the cloning strategy, the fragments protected by the Ubi-βGal probe were 19 nucleotides longer than the fragments protected by the v-ubi probe. Comparison of the sequencing ladder indicated that the messages initiated at the same site as authentic v-ubi mRNA. The recombinant cells also expressed authentic v-ubi mRNA, as detected by S1 nuclease analysis. Expression of the β-galactosidase-ubiquitin fusion protein strongly suggests that v-ubi mRNA is translated in infected cells.

Restriction analysis of the genomic DNA AcUbi-βGal indicated that fusion sequences had not recombined into the ubiquitin locus of the virus (Fig. 2*B*). Instead, the DNA had inserted into the region of the *Pst* I G fragment, which has previously been shown to be hypermutable (29). Four additional recombinants were purified as blue-plaque viruses, and three recombinants were identified by using radioactive probes to detect the β-galactosidase gene sequence. Southern blot analysis of these viruses also indicated that recombinants had not deleted v-ubi gene (data not shown). To increase the efficiency of allelic recombination, the *Pst* I K fragment containing the Ubi-βGal sequences was cloned into pXho I H fragment (19.1–24.1 map units). This plasmid, pXho I-H/Ubi-βGal, contains 3.7 kb upstream and 3.8 kb downstream of v-ubi gene. After cotransfection with viral DNA, eight additional recombinant viruses were plaque-purified. Southern blot analysis of these recombinants again indicated that recombination had not occurred in the v-ubi locus (data not shown).

DISCUSSION

It has recently been shown that ubiquitin is covalently linked to coat protein subunits of several different plant and animal viruses (13). The function of these ubiquitinated proteins is unknown. However, their apparently widespread occurrence suggests that ubiquitin may play a role in virus life cycles or in virus–host interactions. This manuscript showing that a ubiquitin variant is encoded by the baculovirus Ac*M*NPV strengthens this hypothesis. Although the primary sequence of v-ubi differs from canonical ubiquitin, it is possible that v-ubi retains some or all of the functions normally associated with ubiquitin. Most of the amino acid substitutions are conservative with respect to the side group, and many of the residues known to be important for function have been conserved.

Two genes encoding ubiquitin-like proteins have been described (Fig. 5). One example is GDX, a constitutively expressed gene located on the human X chromosome (30).

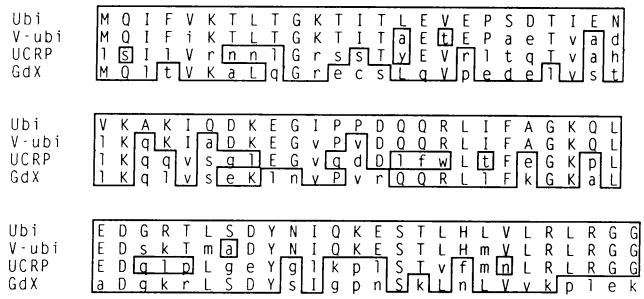

FIG. 5. Sequence comparison of ubiquitin-like proteins. The amino acid sequence of animal ubiquitin (Ubi) is compared to that for Ac*M*NPV ubiquitin (V-ubi), the carboxyl domain of the interferon-induced homologue of ubiquitin (UCRP), and the human X chromosome protein (GdX). The alignment was done by using the programs of Devereaux *et al.* (18). Capitol letters indicate identical amino acids, while lower-case letters indicate substitutions relative to the animal sequence. The identical and conserved amino acids are boxed.

GDX encodes a protein of 157 amino acids. The amino-terminal 76 amino acids of GDX are 43% identical with ubiquitin; the homology is 57% if conservative substitutions are considered. Another example of a ubiquitin-like protein is the interferon-induced protein UCRP (31). This 15-kDa protein consists of two domains, both of which have homology with ubiquitin. Only the carboxyl-terminal domain is presented in Fig. 4. This region of UCRP is 28% identical or 57% homologous with ubiquitin. The functions of these ubiquitin-like proteins and v-ubi are unknown. However, it may be of interest to note that identical substitutions are found in four residues in GDX, UCRP, and v-ubi. Three of these substitutions are conservative substitutions: valine for Ile-23 and leucine for Val-26 and Ile-36. The fourth, glutamine for Ala-28, is not conservative with respect to side group. Structure–function analysis of canonical ubiquitin and the variants may yield clues as to their functions. Because of amino acid substitutions in the Gly-Gly dipeptide, it is unlikely that the GDX and UCRP proteins are processed to yield monomers of ubiquitin. However, v-ubi is expected to be a substrate for the enzymes that normally process ubiquitin fusion proteins to yield a monomer of ubiquitin (32).

In an attempt to determine whether v-ubi was essential in the virus life cycle, the bacterial gene for β-galactosidase was cloned into the v-ubi locus in the plasmid pPst I K fragment. Sixteen recombinant viruses were selected according to standard procedures. However, restriction enzyme and Southern blot analyses revealed that correct allelic replacement had not occurred; instead the transfer vector had integrated into a region of the viral genome previously shown to be hypermutable (29). This technique of producing mutants by using β-galactosidase has been useful for several genes of Ac*M*NPV (20, 33). If mutants can be selected, it indicates that the gene in question is not essential for virus growth in tissue culture. Although not definitive, inability to select for ubi$^-$ mutants by this method suggests that the gene for v-ubi is an essential gene.

The function of v-ubi is currently unknown. Proteins expressed during the late phase of baculovirus infection are primarily viral structural proteins. It is possible that the role of this protein is to serve as a molecular chaperone for the incorporation of viral proteins into particles. This would be analogous to the proposed role of ubiquitin in the assembly of ribosomes (10). Another possibility is that v-ubi plays a role in the inhibition of host transcription and translation that occurs during the late phase of infection. The mechanism for this inhibition is unknown, but it is conceivable that the virus could mediate these effects through inhibition of the host ubiquitin system or ubiquitination of host regulatory genes.

I thank Donald Jarvis, Gerald Kovacs, Raymond Mernaugh, and Max Summers for helpful discussion; Milton Zaitlin for communication of results prior to publication; and Melinda Smith and Dong Wen for excellent technical assistance. This work was supported by the National Science Foundation (DMB-8804732) and the Texas A&M Center for Advanced Invertebrate Molecular Sciences.

1. Rechsteiner, M. A., ed. (1988) *Ubiquitin* (Plenum, New York).
2. Schlesinger, M. & Hershko, A., eds. (1988) *The Ubiquitin System* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
3. Chau, V., Tobias, J. W., Bachmair, L., Marriott, D., Ecker, D. J., Gonda, D. K. & Varshavsky, A. (1989) *Science* **243,** 1576–1583.
4. Bonner, W. M., Hatch, C. L. & Wu, R. S. (1988) in *Ubiquitin*, ed. Rechsteiner, M. (Plenum, New York), pp. 157–172.
5. Ball, E., Karlik, C. C., Beall, C. J., Saville, D. L., Sparrow, J. C., Bullard, B. & Fryberg, E. A. (1987) *Cell* **51,** 221–228.
6. Leung, D. W., Spencer, S. A., Cachaines, G., Hammonds, R. G., Collins, C., Henzel, W. J., Barnard, R., Waters, M. J. & Wood, W. I. (1987) *Nature (London)* **330,** 537–543.
7. St. John, T., Gallatin, W. M., Siegelman, M., Smith, H. T., Fried, V. A. & Weissman, I. L. (1986) *Science* **221,** 845–850.
8. Ozkaynak, E., Finley, D., Solomon, M. J. & Varshavsky, A. (1987) *EMBO J.* **6,** 1429–1439.
9. Lee, H., Simon, J. A. & Lis, J. T. (1988) *Mol. Cell. Biol.* **8,** 4727–4735.
10. Finley, D. A., Bartel, B. & Varshavsky, A. (1989) *Nature (London)* **338,** 394–401.
11. Redman, K. L. & Rechsteiner, M. (1989) *Nature (London)* **338,** 438–440.
12. Dunigan, D. D., Dietzgen, R. G., Schoelz, J. E. & Zaitlin, M. (1988) *Virology* **165,** 310–312.
13. Hazelwood, D. & Zaitlin, M. (1989) *Virology*, in press.
14. Granados, R. R. & Federici, B. A. (1986) *The Biology of Baculoviruses* (CRC, Boca Raton, FL).
15. Sanger, F., Nicklen, S. & Coulsen, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
16. Yanisch-Peron, C., Viera, J. & Messing, J. (1985) *Gene* **33,** 103–109.
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
18. Devereaux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12,** 387–396.
19. Shapiro, S. K., Chou, J., Richard, F. V. & Casadaban, M. J. (1983) *Gene* **25,** 71–82.
20. Summers, M. D. & Smith, G. E. (1987) *Tex. Agric. Exp. Stn. Bull.* **1555.**
21. Jarvis, D. L. & Summers, M. D. (1989) *Mol. Cell. Biol.* **9,** 214–223.
22. Guarino, L. A. & Summers, M. D. (1986) *J. Virol.* **57,** 563–571.
23. Zamm, I. & Fowler, A. (1970) *The Lactose Operon* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
24. Bond, U. & Schlesinger, M. J. (1985) *Mol. Cell. Biol.* **5,** 949–9456.
25. Bachmair, A., Finley, D. & Varshavsky, A. (1986) *Science* **234,** 179–186.
26. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
27. Rhormann, G. F. (1986) *J. Gen. Virol.* **67,** 1499–1513.
28. Luckow, V. L. & Summers, M. D. (1989) *Virology* **170,** 31–39.
29. Kumar, S. & Miller, L. K. (1987) *Virus Res.* **7,** 335–349.
30. Toniolo, D., Persico, M. & Alcalay, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 851–855.
31. Haas, A. L., Ahrens, P., Bright, P. M. & Ankel, H. (1987) *J. Biol. Chem.* **262,** 11315–11323.
32. Bachmair, A., Finley, D. & Varshavsky, A. (1986) *Science* **234,** 179–186.
33. Crawford, A. M. & Miller, L. K. (1988) *J. Virol.* **62,** 2773–2781.