Copper Treatment Increases Recombinant Baculovirus Production and Polyhedrin and p10 Expression

BioTechniques 23:728-735 (October 1997)

Lorene M. Lanier, Kathleen Storm, Alireza Shafaie and Loy E. Volkman

University of California
Berkeley, CA, USA

ABSTRACT

Treatment with 2 mM CuSO₄ was used to induce a *Drosophila melanogaster* metallothionein (*Mtn*) promoter that had been cloned into a recombinant baculovirus. Careful study revealed that the *Mtn* promoter functioned as an inducible, if somewhat "leaky" promoter within the context of baculovirus-infected cells. In the process of generating a recombinant-baculovirus, it was discovered that post-transfection treatment with copper resulted in a 10-fold increase in the production of recombinant virus. This effect on virus production was specific to transfection, as treatment of infected cells with copper did not increase the production of virus. Treatment of infected cells with copper did, however, extend the period of expression of the polyhedrin and p10 proteins by at least 12 h. These findings have practical applications for the production of recombinant baculoviruses and the subsequent expression of foreign proteins using baculovirus expression vectors.

INTRODUCTION

Autographa californica M nucleopolyhedrovirus (AcMNPV) is the most highly studied of the baculoviruses, a group of large, enveloped DNA viruses that infect primarily lepidopteran larvae. AcMNPV has two distinct phenotypes: one is embedded within a protective protein occlusion and the other remains non-occluded. Occlusion-derived virus (ODV) is responsible for the spread of infection between insects, while budded virus (BV) is responsible for spread of infection from cell to cell within an insect and in tissue culture (23). During the course of infection, BV is produced first, then nucleocapsids stop budding from the plasma membrane, remain within the nucleus and are enveloped and occluded there. Regulation of the transition from budded to occluded virus is not well understood. It is known, however, that two viral proteins, polyhedrin and p10, are highly expressed late in infection and are involved in the occlusion of ODV (15–17). Though highly expressed in infected cells, polyhedrin and p10 are not required for BV production. It is therefore possible to delete one or both of these genes and use their promoters to express foreign proteins in baculovirus expression vectors (10,24).

The studies reported herein were initiated to test the promoter of the *Drosophila melanogaster* metallothionein gene (*Mtn*) as an inducible promoter in a recombinant baculovirus. In general, *Mtn* promoters remain inducible in foreign cells, although their levels of expression may be altered (13). Although metallothionein promoters were originally identified by their sensitivity to metal exposure, it is now known that metallothionein promoters are induced in response to a va-

riety of stresses, including metals, heat, starvation and infection (1). In virally infected cells, controlled expression of foreign stress promoters is further complicated by the fact that the infection itself may induce a stress response that may in turn alter the infection cycle (4). The cellular stress response may lead to inhibition of viral replication (14) or may actually increase the production of progeny virus and exacerbate the cytopathic effects of infection (11,12). We were interested, therefore, in determining the effects of metal treatment on baculovirus-infected cells and on *Mtn* induction. Our results revealed that while the *Mtn* promoter was inducible, it also was nominally active in the absence of specific induction, thereby demonstrating the potential complications of using a stress-induced promoter to control protein expression within the context of viral infection. In addition, our results revealed that copper had unexpected effects on recombination frequency and temporal expression of specific viral proteins, findings which suggested a practical and inexpensive method of increasing both the production of recombinant BV and product yield when using baculovirus expression vectors.

MATERIALS AND METHODS

Construction of Transfer Vectors and Recombinant Baculovirus, AcMNPV-*MtnLacZ*

Plasmid DNA was prepared by alkaline lysis and polyethylene glycol (PEG) precipitation as described (18). Construction of pAcHspLacZ (also called pAcLV1; Reference 2) and pAcHspLacZ-ΔPhd (also called pAcDZ1; Reference 27) is described elsewhere. To construct the plasmid

Table 1. Effect of Copper on Polyhedra Formation

	Number of Polyhedra per Cell (mean $\pm$ SD)			
	36 hpi		72 hpi	
	-CuSO ₄	+CuSO ₄	-CuSO ₄	+CuSO ₄
All Cells	3.6 $\pm$ 5.4 <i>n</i> = 123	6.3 $\pm$ 6.5 <i>n</i> = 76	10.0 $\pm$ 6.7 <i>n</i> = 86	8.1 $\pm$ 5.4 <i>n</i> = 85
Cells with Polyhedra	8.1 $\pm$ 5.4 <i>n</i> = 123	9.6 $\pm$ 5.5 <i>n</i> = 50	10.0 $\pm$ 6.7 <i>n</i> = 86	8.1 $\pm$ 5.4 <i>n</i> = 185

pAcMtnLacZ, pAcDZ1 was digested with *Bam*HI and *Pst*I, the termini filled in with Klenow, and the *LacZ*-containing *Bam*HI-*Pst*I fragment was gel-purified using QIEX[®] (Qiagen, Chatsworth, CA, USA) following the manufacturer's instructions. The pNTP14 plasmid (13) was digested within the polylinker with *Bam*HI and *Xho*I, blunt-ended with Klenow and ligated to the purified *Bam*HI-*Pst*I *LacZ* fragment to create the plasmid #25 *Mtn*-*LacZ*. Meanwhile, the plasmid pAcEcoRI-I-*Mtn*#4 was created by ligating the Klenow-treated *Ssp*I-*Ssp*I fragment of pNTP14 (contains the entire metallothionein promoter, polylinker and polyadenylation signal) into the *Eco*RV site of pAcEcoRI-I. Finally, #25 *Mtn*-*LacZ* and pAcEcoRI-I-*Mtn*#4 were digested with *Bgl*II and *Pst*I and the metallothionein-*LacZ* cassette from #25 *Mtn*-*LacZ* was inserted in between the *Mtn* promoter and polyadenylation signal in the *Eco*RI-I fragment of

pAcEcoRI-I-*Mtn*#4 to produce pAcMtnLacZ. BacPAK[™] DNA (CLONTECH Laboratories, Palo Alto, CA, USA) and pAcMtnLacZ were co-transfected into Sf9 cells following standard protocols to generate AcMNPV-*Mtn*LacZ (10). AcMNPV-*Mtn*LacZ thus contains all the wild-type viral genes and the *LacZ* gene under the control of the *Mtn* promoter and polyadenylation signal upstream of the polyhedrin gene.

Test of Metal Toxicity and *Mtn* Induction

Spodoptera frugiperda Sf9 cells (CRL 1711; ATCC, Rockville, MD, USA), a clonal derivative of *Spodoptera frugiperda* Sf21 IPLB cells (20), were grown in Grace's medium (JRH Bioscience, Woodland, CA, USA) containing 10% fetal calf serum (FCS; Gemini Bioproducts, Calabasas, CA, USA) and 0.1% pleuronic-F68 (Sigma Chemical,

St. Louis, MO, USA). Log phase Sf9 cells were diluted to 2×10^5 cells/mL and aliquoted into 50-mL conical tubes. Metal treatments consisted of ZnCl₂, CuSO₄ or CdCl₂ at concentrations of 0.1 mM to 1 M. At 24-h intervals, aliquots were taken, and the cell concentration was determined. Trypan blue was used to determine cell viability. To assess *Mtn* inducibility, Sf9 cells were infected with AcMNPV-*Mtn*LacZ as described below for metabolic labeling. At the indicated hours post infection (hpi), medium was removed, and the cells were rinsed with cold phosphate-buffered saline (PBS) (18) and lysed directly in 2 $\times$ sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (18). Electrophoresis and immunoblotting was as described (18) using a 1:1000 dilution of mouse monoclonal antibody to β -galactosidase (β -gal) and a 1:3000 dilution of goat anti-mouse horseradish peroxidase-conjugated secondary antibody (Sigma Chemical). Signal was detected using the ECL[™] Chemiluminescence Reagent (Amersham, Arlington Heights, IL, USA).

Transfections and Recombinant BV Production

Transfections were done essentially as described (10). For each transfection, 2×10^6 IPLB Sf21 cells (20) in Grace's medium/10% FCS were plated onto 60-mm tissue culture dishes and allowed to adhere for 1 h. The medium was then removed and replaced with 750 mL of fresh medium. For each plate, 1 μ g of *Bsu*36I-digested BacPAK viral DNA and 2 μ g of the appropriate transfer vector were mixed in 750 μ L of transfection buffer (25 mM HEPES, pH 7.1, 140 mM NaCl, 125 mM CaCl₂) and added drop-wise to the plate with gentle rocking. The plates were incubated at 27°C for 4 h, then rinsed with fresh medium and fed with 1.5-mL fresh medium. After 7 days, the BV-containing medium was removed, floating cells were pelleted, and the BV titer was determined.

Determination of Virus Titer

Virus titers were determined using a sensitive and reproducible immunolog-

Figure 1. Effect of metal treatment on the growth of Sf9 cells. Log phase Sf9 cells were grown in the absence or presence of 1 mM ZnCl₂, 1 mM CuSO₄ or 0.1 mM CdCl₂. At the indicated hpi, aliquots were taken, and the viable cell density (cells/mL) was determined by trypan blue staining.

Research Reports

ical assay (22). Briefly, 30 μ L of Sf21 cells at 1×10^6 cell/mL were plated in each 5-mm well of a 12-well, acid-washed slide (Carson Optical, Peotone, IL, USA) and allowed to adhere for 1 h. Medium was then aspirated off and replaced with a 15- μ L drop of medium containing the appropriate dilution of BV (dilutions were 10^{-1} to 10^{-5} , depending on the hpi). Each dilution was placed in a minimum of 4 wells on two different slides (8 wells total). The slides were then incubated in a humidified incubator for 4 h, after which the inoculum was aspirated off and replaced with a drop (approximately 50 μ L) of 0.6% methylcellulose in Grace's medium/10% FCS with or without 2 mM CuSO_4 . After 72 h in a humidified 27°C incubator, the slides were processed and plaques visualized. First, the slides were overlaid for 1.5 min with fixative [45% (vol/vol) acetone, 25% (vol/vol) formaldehyde, 4.7 mM $\text{Na}_2\text{H}_2\text{PO}_4$, 2.4 mM KH_2PO_4], then rinsed for 5 min in Tris-buffered saline (TBS; Reference 18). After rinsing, the slides were blocked for 5 min with 1% normal goat serum (NGS) in TBS. The goat serum was then removed by aspiration and replaced with a 1:400 dilution of D6, a polyclonal antiserum to ODV (21). After 1 h in a humid chamber, the slides were rinsed for 5 min in TBS, overlaid with 0.5 mL of a 1:400 dilution of goat-anti-rabbit horseradish peroxidase-conjugated secondary antibody (Sigma Chemical) in TBS and incubated for another hour. Following incubation, the slides were rinsed twice and overlaid with 1 mg/mL 3,3-Diaminobenzidine (DAB; Sigma Chemical), 0.03% H_2O_2 in TBS for 15 min or until a visible precipitate developed. After the DAB signal was developed, the slides were rinsed twice, overlaid with X-gal in enhancement buffer (0.8 mg/mL 5-bromo-4-chloro-3-indoyl- β -D-galactopyranoside in 5 mM $\text{K}_4\text{Fe}(\text{CN})_6$, 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$, 2 mM MgCl_2 in distilled water) and incubated overnight to develop the β -gal (*LacZ*) signal. Slides were analyzed for DAB and *LacZ* signal to determine whether treatment was affecting expression from the *Mtn* promoter. The next morning, the slides were rinsed and mounted with glycerol to prevent drying. The slides were examined micro-

scopically and the plaques counted to determine the titer [titer = (No. plaques/0.015 mL) $\times$ dilution]. Titers are given as the average of 4–8 wells.

Virus Infection and Metabolic Labeling

For each time point, 1×10^6 Sf21 cells were plated in a 35-mm tissue culture dish and allowed to adhere for 1 h. The medium was then removed and replaced with 0.5 mL of medium containing third-passage, wild-type E2 strain AcMNPV (19) at a multiplicity of infection (MOI) of 0.1. The plates were rocked gently for 1 h, then the medium was replaced with 1 mL of fresh medium with or without 2 mM CuSO_4 . Cells were incubated at 27°C until 1 h before the indicated labeling time, at which time the medium was replaced with leucine-deficient medium (Sigma Chemical) supplemented with 10% complete medium, 2% dialyzed FCS and where indicated, 2 mM CuSO_4 . Medium removed from the cells was saved for titring. Cells were starved for 1 h, then 150 μ Ci of 2,3,4,5- ^3H -Leucine (ICN Biomedicals, Irvine, CA, USA) were added, and the incubation continued for 2 h. Thus, a time point at 12 hpi would be starved from 11–12 hpi and labeled from 12–14 hpi. After labeling, the medium was removed and discarded, and the cells were then gently rinsed with cold PBS and lysed directly in 1 $\times$ SDS-PAGE sample buffer (18). After boiling, aliquots were taken, and the incorporation (counts per million, [cpm]) was determined by scintil-

lation counting. Equal cpm of each sample was electrophoresed on a 15% SDS-PAGE gel. Immunoblotting and autoradiography were as previously described (8).

Polyhedra Counting

Individual acid-washed coverslips (22 mm²) were placed in each well of a 6-well tissue culture dish, 0.25 mL of medium containing 5×10^5 Sf21 cells was pipetted on top of each coverslip and the cells were allowed to adhere for 1 h. The medium was then removed and replaced with 0.25 mL of medium containing wild-type BV at an MOI 0.1. After 1 h, the inoculum was removed and replaced with 1.0 mL of medium with or without 2 mM CuSO_4 . At 36 or 72 hpi, the cells were prepared for immunofluorescence as described previously (8). Immunological detection of the major viral capsid protein, p39 (26), was used to verify infection, and the number of polyhedra was counted in all the cells in five randomly chosen areas on each coverslip.

RESULTS

Inducibility of the *Mtn* Promoter

The *Mtn* promoter is reported to be tightly regulated, even in non-*Drosophila* cells (13). We were interested in conditionally expressing an essential baculovirus gene and therefore required a promoter that was undetectable in the un-induced state and

Figure 2. *LacZ* expression in AcMNPV-*MtnLac*-infected cells. Log phase Sf9 cells were infected with the AcMNPV-*MtnLacZ* recombinant virus and grown in the presence (+) or absence (-) of 2 mM CuSO_4 . At the indicated hpi, cells were lysed directly into sample buffer, electrophoresed on a 10% SDS-PAGE gel and transferred to nitrocellulose. The immunoblot was probed using a rabbit anti- β -gal antibody and visualized by chemiluminescence.

strongly expressed in the induced state. Therefore, we initiated an investigation to determine whether the *Mtn* promoter would be suitable for our purposes. Because many of the metals that induce the *Mtn* promoter also can be toxic to cells, it was necessary to test several metals to determine which would be least toxic to Sf9 cells. Growth of uninfected cells was essentially normal in CuSO₄ but was inhibited by both ZnCl₂ and CdCl₂ (Figure 1). Next, we tested the ability of CuSO₄ to induce the *Mtn* promoter in cells infected with AcMN-PV-*MtnLacZ*, a recombinant baculovirus that contains the β-gal gene under the control of the *Mtn* promoter. Infections were done in the presence or absence of CuSO₄ at a variety of concentrations (data not shown). At various hpi, cells were harvested and analyzed by immunoblot. Optimal induction of *MtnLacZ* was achieved with 2 mM CuSO₄ at approximately 25 hpi (Figure 2). Note that very low levels of *LacZ* expression were detected in the absence of copper, indicating that the *Mtn* promoter was slightly “leaky”.

Effect of Copper on Recombinant BV Production

Recombinant viruses are produced by co-transfection of a transfer vector that contains the gene of interest with

linearized viral genomic DNA. Since our ultimate goal was to produce a recombinant virus that replaced an essential virus gene with a copy of the gene under control of the *Mtn* promoter, copper was included in all post-transfection steps in case the gene product was required early in the viral life cycle. We found that Sf9 cells treated with copper did not remain attached to the culture dish; because adherent cells were needed for transfection and determination of virus titers, these and subsequent experiments were conducted using Sf21 cells. Control transfections were done using BacPAK viral DNA and transfer vectors whose use did not result in the deletion of any viral genes and which contained the *LacZ* gene under the control of either the *Mtn* promoter (pAc*MtnLacZ*), the *Hsp70* promoter (pAc*HspLacZ*) or a transfer vector containing a *Hsp70* promoter whose use resulted in the deletion of the polyhedrin gene (pAc*HspLacZ*-Δ*Phd*). The pAc*HspLacZ*-Δ*Phd* transfer vector was tested to determine whether deletion of the polyhedrin gene (as occurs with many transfer vectors) would alter virus production or protein expression. After transfection, cells were rinsed and given fresh medium with or without 2 mM CuSO₄. All BV was harvested at 7 days post infection and titered both with and without copper (e.g., BV produced in the absence of copper was

Figure 3. Post-transfection production of recombinant baculovirus. Sf21 cells were transfected with linearized BacPAK DNA alone or with BacPAK DNA and one of the transfer vectors pAc*HspLacZ*, pAc*HspLacZ*Δ*Phd* or pAc*MtnLacZ*. After the initial transfection, cells were rinsed and incubated in the presence (+) or absence (-) of 2 mM CuSO₄. After 7 days, the medium was removed, and the titer of the recombinant, *LacZ*-expressing virus was determined. Error bars indicate 1 standard deviation.

Research Reports

titered both in the presence and absence of copper).

Surprisingly, there was a 10-fold increase in the production of recombinant BV when transfections were followed by incubation in the presence of copper (Figure 3). This was true regardless of the promoter used, indicating that the increased titers were not due to a specific effect of copper on the *Drosophila Mtn* promoter. Furthermore, the titers were the same whether or not the titering procedure itself was done in the presence of copper, indicating that the apparent increase in titer was not an artifact of the titering procedure (data not shown).

When transfected cells were grown in the presence of copper, the appearance of polyhedra was delayed 1–2 days, and there was an increase in the number of plaques that did not express *LacZ* (data not shown). DAB staining confirmed that these were indeed virus-infected cell plaques, suggesting that they resulted from aberrant recombination of the viral DNA and the transfer plasmid. Although the intensity of *LacZ* staining by *MtnLacZ* recombinant BV was greatly enhanced by induction, a faint blue signal was detected in the absence of induction, confirming the previous finding that the *Mtn* promoter is “leaky” when expressed on the viral genome in infected cells (data not shown). In contrast, the intensity of *LacZ* staining by *HspLacZ* and *HspLacZΔPhd* was unaffected by copper (data not shown). Finally, in the presence of copper, there was a detectable level of recombinant BV produced even when transfections were done with BacPAK DNA alone, suggesting that there was increased recombination of the linearized BacPAK DNA (Figure 3).

Effect of Copper Treatment on Protein Synthesis in Infected Cells

The observation that incubation with copper after transfection led to a delay in the production of polyhedra suggested that copper treatment might also affect viral protein synthesis. To test this hypothesis, infected cells were incubated in the presence or absence of 2 mM CuSO_4 and metabolically labeled at various times post infection. Because the results of the transfection studies in-

dicated that the effect of copper was independent of the type of transfer vector used, all infections were done with wild-type BV. Early in infection, copper treatment appeared to slightly decrease the levels of total protein synthesis (as determined by ^3H -Leucine incorporation, Figure 4). By 60 hpi, however, protein synthesis levels were higher in the presence of copper, and by 72 hpi, levels were 100-fold higher

(Figure 4). Autoradiography of labeled extracts supported these findings. In the presence of copper, there were slight delays in the shut off of host protein synthesis (as determined by the synthesis of higher molecular weight proteins) and in the expression of polyhedrin and p10 (Figure 5). Late in infection, however, copper treatment appeared to extend the expression of polyhedrin and p10 by at least 12 h (Figure 5).

Figure 4. Total protein synthesis in infected cells. Sf21 cells were infected with wild-type BV and grown in the presence (+) or absence (-) of 2 mM CuSO_4 . At the indicated hpi, cells were labeled for 2 h with 2,3,4,5- ^3H -Leucine then rinsed with PBS and lysed directly into SDS-PAGE sample buffer. The total protein synthesis was determined by the ^3H incorporation and is given in cpm/mL.

Figure 5. Protein synthesis in infected cells. Sf21 cells were infected with wild-type BV and grown in the presence (+) or absence (-) of 2 mM CuSO_4 . At the indicated hpi, cells were labeled for 2 h with 2,3,4,5- ^3H -Leucine then rinsed with PBS and lysed directly into SDS-PAGE sample buffer. Equivalent cpm of each sample were electrophoresed on a 15% SDS-PAGE gel and processed for autoradiography.

Research Reports

BV Production and Polyhedra after Infection in the Presence of Copper

The results of the previous experiments suggested that copper treatment led to a 10-fold increase in the production of recombinant BV after transfection and affected the synthesis and accumulation of viral proteins after infection. We expected, therefore that copper treatment also would affect the production of BV after infection. When infections were done with wild-type BV, copper treatment appeared to reduce the early release of BV (Figure 6), most likely due to the slight delay in the onset of viral protein synthesis. By 36 hpi, however, there was no significant difference in the titers of BV produced in the presence or absence of copper. Furthermore, copper treatment did not appear to affect the production of polyhedra in infected cells (Table 1). The fact that copper treatment delayed polyhedra formation in transfected cells, but not in infected cells, suggested that the effect on polyhedra formation was an indirect result of copper treatment.

DISCUSSION

Study of the *Drosophila Mtn* promoter revealed that it functioned as an inducible promoter when located in a baculovirus genome in infected cells. Optimal induction was achieved with 2 mM CuSO_4 at approximately 25 hpi. Low levels of *Mtn*-promoted/*LacZ* expression also could be detected in the absence of copper induction. Unfortunately, even this low level of un-induced *Mtn* expression made the promoter useless for our original purpose. Studying the effects of copper induction did, however, reveal several interesting phenomena. First, post-transfection treatment with copper resulted in a 10-fold increase in the production of recombinant BV, regardless of the transfer vector used. Second, the effect of copper on BV production was specific to transfection, as post-infection treatment with copper had little effect on BV production or polyhedra formation. Together, these results suggested that copper treatment affected recombination between the linearized viral DNA and the transfer vector. It is known that

metallothioneins are induced in response to DNA damage (6) and that the processes of DNA repair and recombination involve many of the same enzymes (5). Therefore, post-transfection treatment of cells with copper may have induced the DNA repair pathway of the host cell, thereby facilitating recombination, leading to increased production of recombinant BV.

Although copper treatment of infected cells did not affect BV production, treatment did affect the timing of viral protein synthesis. In the presence of copper, the shut off of host protein synthesis, the onset of viral protein synthesis and the expression of polyhedrin and p10 were all delayed, possibly because concentrations of CuSO_4 that were able to induce stress proteins also suppressed general protein synthesis (7). In contrast, copper treatment appeared to extend the period of synthesis of polyhedrin and p10. It is not altogether unexpected that copper treatment altered the pattern of viral protein expression. Metal exposure induces a variety of stress proteins, including metallothioneins, heat-shock proteins and ubiquitin (3,9), and metallothioneins can be induced by heat shock, starvation, and infection, among other

things (1). Therefore, it is possible that proper baculovirus infection involves or requires regulation of the cellular stress responses, including metallothionein induction. Treatment of infected cells with copper may have altered the ability of the virus to regulate the host stress response, which in turn may have affected the regulation of viral protein synthesis. Interestingly, it has been shown that treatment of AcMNPV-infected Sf21 cells with micromolar concentrations of AlCl_3 or ZnSO_4 increases both the yield of BV (3.6-fold) and polyhedra (1.2-fold; Reference 25). Whether treatment with micromolar concentrations of copper would have a similar affect on virus production remains to be determined.

The results of these studies have practical applications for the generation of recombinant baculoviruses. First, post-transfection treatment with 2 mM CuSO_4 may be a simple and inexpensive method for increasing the yield of recombinant BV. Because the copper is included after the transfection, it would be predicted to be effective regardless of the transfection method used. Second, copper treatment extended the expression of polyhedrin and p10. Since these are the viral promoters most

Figure 6. Post-infection production of budded virus. Sf21 cells were infected with wild-type BV and grown in the presence (+) or absence (-) of 2 mM CuSO_4 . At the indicated hpi, the medium was removed and the titer of budded virus was determined by immunoplaque assay. Titers are given in plaque-forming units (pfu)/mL and are the average of 4–8 values. Error bars indicate 1 standard deviation.

commonly used in baculovirus expression vectors, copper treatment may be useful for increasing the yield of foreign proteins expressed from these promoters.

ACKNOWLEDGMENTS

The authors thank Dr. Lauren N.W. Kam-Morgan for her contributions to the construction of AcMNPV-*MtnLacZ*. Financial support for these studies was provided by USDA Grant No. NRICG 94-37302-0566, by federal HATCH funds, and by NIH Training Grant No. GM07232.

REFERENCES

- Bremner, I. 1991. Nutritional and physiological significance of metallothionein. *Methods Enzymol.* 205:25-35.
- Engelhard, E.K., L.N. Kam-Morgan, J.O. Washburn and L.E. Volkman. 1994. The insect tracheal system: a conduit for the systemic spread of *Autographa californica* nuclear polyhedrosis virus. *Proc. Natl. Acad. Sci. USA* 91:3224-3227.
- Hatayama, T., Y. Tsukimi, T. Wakatsuki, T. Kitamura and H. Imahara. 1991. Different induction of 70,000-Da heat shock protein and metallothionein in HeLa cells by copper. *J. Biochem. (Tokyo)* 110:726-731.
- Jindal, S. and M. Malkovsky. 1994. Stress responses to viral infection. *Trends Microbiol.* 2:89-91.
- Kornberg, A. 1974. Repair, recombination, transformation, restriction, and modification, p. 607-650. *DNA Replication*. W.H. Freeman and Company, San Francisco.
- Kramer, M., C. Sachsenmaier, P. Herrlich and H.J. Rahmsdorf. 1993. UV irradiation-induced interleukin-1 and basic fibroblast growth factor synthesis and release mediate part of the UV response. *J. Biol. Chem.* 268:6734-6741.
- Lai, Y.K., C.H. Shen, T.J. Cheng, M.C. Hou and W.C. Lee. 1993. Enhanced phosphorylation of a 65 kDa protein is associated with rapid induction of stress proteins in 9L rat brain tumor cells. *J. Cell. Biochem.* 51:369-379.
- Lanier, L.M., J.M. Slack and L.E. Volkman. 1996. Actin binding and proteolysis by the baculovirus AcMNPV: the role of virion-associated V-CATH. *Virology* 216:380-388.
- Nemer, M., E. Rondinelli, D. Infante and A.A. Infante. 1991. Polyubiquitin RNA characteristics and conditional induction in sea urchin embryos. *Dev. Biol.* 145:255-265.
- O'Reilly, D.R., L.K. Miller and V.A. Luckow. 1992. *Baculovirus Expression Vectors: A Laboratory Manual*. W.H. Freeman and Company, New York.
- Oglesbee, M. and S. Krakowka. 1993. Cellular stress response induces selective intracellular trafficking and accumulation of morbillivirus major core protein. *Lab. Invest.* 68:109-117.
- Oglesbee, M.J., H. Kenney, T. Kenney and S. Krakowka. 1993. Enhanced production of morbillivirus gene-specific RNAs following induction of the cellular stress response in stable persistent infection. *Virology* 192:556-567.
- Otto, E., J.M. Allen, J.E. Young, R.D. Palmiter and G. Maroni. 1987. A DNA segment controlling metal-regulated expression of the *Drosophila melanogaster* metallothionein gene *Mtn*. *Mol. Cell. Biol.* 7:1710-1715.
- Pica, F., A. De Marco, F. De Cesare and M.G. Santoro. 1993. Inhibition of vesicular stomatitis virus replication by delta 12-prostaglandin J2 is regulated at two separate levels and is associated with induction of stress protein synthesis. *Antiviral Res.* 20:193-208.
- Quant-Russell, R.L., M.N. Pearson, G.F. Rohrmann and G.S. Beaudreau. 1987. Characterization of baculovirus p10 synthesis using monoclonal antibodies. *Virology* 160:9-19.
- Rohrmann, G., L. Yuen and B. Moss. 1986. Transcription of vaccinia virus early genes by enzymes isolated from vaccinia virions terminates downstream of a regulatory sequence. *Cell* 46:1029-1035.
- Russell, R.L., M.N. Pearson and G.F. Rohrmann. 1991. Immunoelectron microscopic examination of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus-infected *Lymantria dispar* cells: time course and localization of major polyhedron-associated proteins. *J. Gen. Virol.* 72:275-283.
- Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. *Molecular Cloning: Laboratory Manual*, 2nd ed. CSH Laboratory Press, Cold Spring Harbor, NY.
- Smith, G.E. and M.D. Summers. 1978. Analysis of baculovirus genomes with restriction endonucleases. *Virology* 89:517-527.
- Vaughn, J.L., R.H. Goodwin, G.J. Tompkins and P. McCawley. 1977. The establishment of two cell lines from the insect *Spodoptera frugiperda* (Lepidoptera; Noctuidae). *In Vitro* 13:213-217.
- Volkman, L.E. 1983. Occluded and budded *Autographa californica* nuclear polyhedrosis virus: immunological relatedness of structural proteins. *Virology* 124:221-229.
- Volkman, L.E. and P.A. Goldsmith. 1982. Generalized immunoassay for *Autographa californica* nuclear polyhedrosis virus infectivity in vitro. *Appl. Environ. Microbiol.* 44:227-233.
- Volkman, L.E., M.D. Summers and C.H. Hsieh. 1976. Occluded and nonoccluded nuclear polyhedrosis virus grown in *Trichoplusia ni*: comparative neutralization, comparative infectivity, and *in vitro* growth studies. *J. Virol.* 19:820-832.
- Webb, N.R. and M.D. Summers. 1990. Expression of proteins using recombinant baculoviruses. *Technique* 2:173-188.
- Weiss, S.A., G.C. Smith, J.L. Vaughn, E.M. Dougherty and G.J. Tompkins. 1982. Effect of aluminum chloride and zinc sulfate on *Autographa californica* nuclear polyhedrosis virus (ACNPV) replication in cell culture. *In Vitro* 18:937-944.
- Whitt, M.A. and J.S. Manning. 1988. A phosphorylated 34-kDa protein and a subpopulation of polyhedrin are thiol linked to the carbohydrate layer surrounding a baculovirus occlusion body. *Virology* 163:33-42.
- Zuidema, D., A. Schouten, M. Usmany, A.J. Maule, G.J. Belsham, J. Roosien, E.C. Klinge-Roode, J.W. van Lent and J.M. Vlak. 1990. Expression of cauliflower mosaic virus gene I in insect cells using a novel polyhedrin-based baculovirus expression vector. *J. Gen. Virol.* 71:2201-2209.

Received 31 January 1997; accepted 2 June 1997.

Address correspondence to:

Loy E. Volkman
251 Koshland Hall
University of California
Berkeley, CA 94720, USA
Internet:lvolkman@nature.berkeley.edu