JOURNAL OF VIROLOGY, Oct. 1988, p. 3668–3674
0022-538X/88/103668-07$02.00/0

Vol. 62, No. 10

Inducible Expression of Herpes Simplex Virus Type 2 Glycoprotein Gene gG-2 in a Mammalian Cell Line

HENRY K. SU AND RICHARD J. COURTNEY*

Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, Louisiana 71130-3932

Received 9 May 1988/Accepted 1 July 1988

The gG-2 glycoprotein gene of herpes simplex virus type 2 (HSV-2) was cloned into the mammalian expression vector pMSG under the control of the inducible mouse mammary tumor virus promoter. Transfection of this cloned gG-2 construct into NIH 3T3 cells resulted in the stable expression of gG-2 upon induction with dexamethasone. In addition, the 104,000-molecular-weight (104K) and 72K gG-2 precursors as well as the 34K secreted component were generated in the transformed cells. The synthesis of gG-2 in these transformed cells appeared to follow the same cleavage-processing pathway as gG-2 synthesis during an HSV-2 infection. These results indicate that the processing of gG-2 can occur in the absence of an HSV-2 infection.

Several antigenically distinct herpes simplex virus type 2 (HSV-2) glycoproteins are present on both the virus-infected cell membranes and the virus envelope. These include glycoproteins gB-2, gC-2, gD-2, gE-2, gG-2 (36) and possibly gI-2, an additional glycoprotein encoded by the US7 open reading frame of the HSV-2 genome (18). The synthesis of glycoproteins gB-2, gC-2, gD-2, and gE-2 appears to parallel that of their well-characterized counterpart glycoproteins in HSV type 1 (HSV-1) (36). Unlike these glycoproteins, gG-2 differs from its HSV-1 counterpart in several aspects. Both gG-1 and gG-2 were initially mapped to the unique short region of the HSV genome (1, 26, 27, 30, 34, 35) and were later identified as the US4 gene products of their respective viral genomes (12, 28). However, DNA sequence analysis revealed that the gG-2 gene, which encodes a 699-amino-acid protein, contains an additional 1,460-nucleotide region absent from the smaller gG-1 gene, which encodes a 238-amino-acid protein (28). Clear homology between the gG-1 and gG-2 molecules is restricted to the carboxyl-terminal portion that includes the putative transmembrane anchor domain (28). In addition, gG-2 undergoes a unique cleavage event during its synthesis (3, 37); the processing of gG-1 synthesis does not include such a cleavage step (1, 12). Our studies have shown that gG-2 is synthesized as a cotranslationally glycosylated high-mannose intermediate with an apparent molecular weight of 104,000 (104K). This 104K intermediate is subsequently cleaved to generate two components, the 31,000- and 72,000-dalton high-mannose intermediates (31K and 72K, respectively). The 72K high-mannose intermediate is then further glycosylated to yield the mature gG-2 glycoprotein of 108,000 daltons (108K) which is found on the envelopes of virions and on the plasma membranes of virus-infected cells. The 31K intermediate is processed and subsequently secreted into the extracellular medium as a 34,000-dalton (34K) component (37).

The events responsible for the cleavage and processing of gG-2 are not well understood. It remains to be determined whether other HSV-2 gene products play any specific role in the processing of gG-2. To approach this problem, the synthesis of gG-2 in the absence of an HSV-2 infection was studied. Such a study was facilitated by the recent identification and sequencing of the gG-2 gene encoded within the US4 open reading frame (28). In this paper, we report the cloning and subsequent expression of the gG-2 gene in a mammalian cell line and provide evidence indicating that the proteolytic processing of gG-2 can occur in the absence of an HSV-2 infection.

* Corresponding author.

MATERIALS AND METHODS

Cell culture. NIH 3T3 cells were cultured in Dulbecco modified Eagle medium (Whittaker M.A. Bioproducts, Inc.) supplemented with 10% newborn calf serum, penicillin-streptomycin, 40 mM L-glutamine, and 0.075% sodium bicarbonate (all purchased from GIBCO Diagnostics). Cells were maintained in a maintenance medium containing 2% newborn calf serum.

Virus. HSV-2 strain 186 was used throughout this study. The virus stocks were propagated at a low multiplicity of infection in HEp-2 cells and assayed by plaque formation on Vero cells (5).

Plasmid construction. The cloning of the entire gG-2 gene into the expression vector pMSG (Pharmacia, Inc.), such that the gG-2 gene is under the control of the mouse mammary tumor virus (MMTV) promoter, is depicted in Fig. 1. Several subcloning steps were required to eliminate sequences upstream of the gG-2-coding region.

Plasmid pB-B2 contains a 4.5-kilobase (kb) *Bam*HI fragment excised from pRT1-4, a plasmid containing the *Hin*dIII L fragment of HSV-2/HG (a kind gift from A. Kessous-Elbaz, Institut du Cancer de Montreal, Department of Pathology, University of Montreal, Canada). A 1.3-kb *Sal*I fragment was subcloned from pB-B2 into pUC19 to produce pS-S1. pS-S1 was restricted with *Dde*I, made blunt ended with mung bean nuclease, and subsequently digested with *Aat*II to produce a 1.0-kb fragment which was subcloned into the *Aat*II to blunted *Pst*I sites of pUC19. The resulting plasmid, pD-A, was digested with *Hin*dIII and *Mlu*I to release a 0.9-kb fragment containing the trimmed 5′ end of the gG-2 gene. This fragment was inserted into the *Hin*dIII-*Mlu*I sites of pB-B2 to generate pD-B. A 2.4-kb fragment encoding gG-2 was excised from pD-B by cutting with *Hin*dIII, blunt-ending, and then digesting with *Xho*I. This fragment was ligated into the *Sma*I to *Xho*I sites of pMSG to yield pD-X.

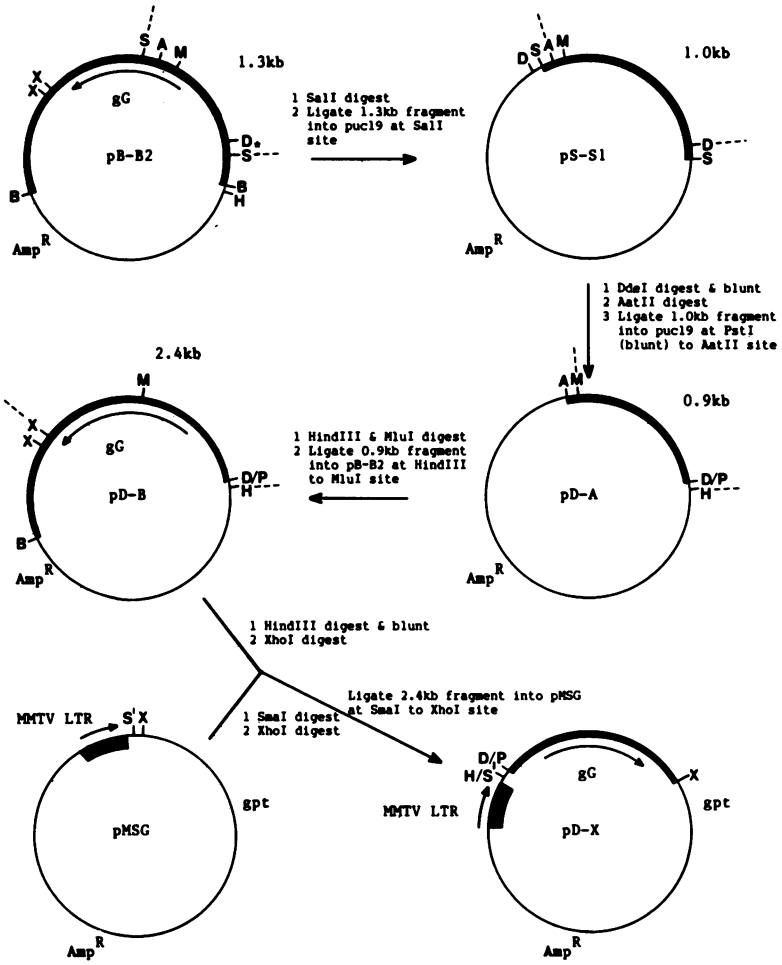

FIG. 1. Construction of plasmid pD-X for expression in NIH 3T3 cells. Details of the plasmid construction are described in Materials and Methods. Briefly, pB-B2 was generated by subcloning a 4.5-kb *Bam*HI fragment from a pBR322 construct containing the *Hin*dIII L fragment of HSV-2 DNA into pUC19. A 1.3-kb *Sal*I fragment was removed from pB-B2 and subcloned into pUC19 to generate pS-S1. pS-S1 was then digested with *Dde*I, made blunt ended, and further digested with *Aat*II to remove a 1.0-kb fragment. The 1.0-kb fragment was subcloned into pUC19, resulting in the plasmid pD-A. A 0.9-kb *Hin*dIII-*Mlu*I fragment was excised from pD-A and ligated back into pB-B2, replacing its *Hin*dIII-to-*Mlu*I fragment. From the resultant plasmid, pD-B, a 2.4-kb fragment containing gG-2 was removed by first cutting the plasmid with *Hin*dIII, blunt-ending, and *Xho*I digestion. This fragment was ligated into pMSG at the *Sma*I to *Xho*I sites to generate the plasmid pD-X. Restriction enzyme sites are abbreviated as follows: A, *Aat*II; B, *Bam*HI; D, *Dde*I; H, *Hin*dIII; M, *Mlu*I; P, *Pst*I; S, *Sal*I; S', *Sma*I; and X, *Xho*I. Not all *Sal*I and *Dde*I restriction sites are indicated.

Plasmid constructions were performed by standard recombinant DNA techniques (25). The JM83 strain of *Escherichia coli* was used for all plasmid transformations. Restriction enzymes, mung bean nuclease, and T4 DNA ligase were purchased from New England BioLabs, Inc., and employed following the specifications of the manufacturer.

Transfection and selection. Plasmids were prepared from bacteria grown in superbroth (15) and extracted by the sodium dodecyl sulfate (SDS) lysis method (14). The DNA was purified by cesium chloride equilibrium centrifugation (25). Transfection of NIH 3T3 cells was performed by the calcium phosphate precipitation method (16) as modified by Chen and Okayama (7), except *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-buffered saline was used in place of *N*,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES)-buffered saline. Each 100-mm dish was seeded with 5×10^5 NIH 3T3 cells and transfected with 30 μg of pD-X DNA. The cells were allowed to recover in growth medium for 24 h before replating for selection. The expression vector, pMSG, contains the xanthine-guanine phosphoribosyl-transferase (*gpt*) gene (29); therefore, stable transformants were selected by growth in medium supplemented with mycophenolic acid (25 μg/ml), xanthine (250 μg/ml), hypoxanthine (15 μg/ml), aminopterin (2 μg/ml) and thymidine (5 μg/ml), all purchased from Sigma Chemical Co. Surviving cell clones were picked after 2 weeks and expanded. A resulting cell line, designated pDX, was used in the studies reported here.

Protein sample preparation. Cells were harvested and solubilized in 1% SDS with boiling for 3 min. The boiled samples were diluted in 4 volumes of a solution containing 1.25% Triton X-100 in 10 mM Tris (pH 7.4)–10 mM NaCl–1 mM EDTA (2). The resultant samples were then clarified by centrifugation at 100,000 × *g* for 30 min. The supernatants served as solubilized antigens for either direct analysis on SDS-polyacrylamide gel electrophoresis (SDS-PAGE) or immunoprecipitations (10). Immune complexes were recovered by addition of *Staphylococcus aureus* cells (Pansorbin, La Jolla, Calif.). Samples were prepared from extracellular

medium by trichloroacetic acid and acetone precipitations as previously described (37).

Immunoblotting. Analysis of samples by SDS-PAGE was performed as described previously (32). Samples were electrophoresed on a 7% acrylamide gel cross-linked with *N*,*N*-methylene-bis-acrylamide. Immunoblotting was performed essentially as reported by Towbin et al. (39) with some modifications (8). As indicated in the text, the immunoblots were reacted with either anti-gG-2 or anti-pgG-2 rabbit sera (37). Reactive proteins were visualized by reaction with [^{125}I]protein A (ICN Pharmaceuticals Inc.) followed by autoradiography.

Indirect immunofluorescence. Indirect immunofluorescence was conducted as described previously (11). Cells were seeded on cover slips at an approximate density of 5×10^5 cells per 60-mm dish. Cover slips from mock-infected or HSV-2-infected NIH 3T3 cells were harvested for immunofluorescence at 12 h postinfection. Cover slips seeded with pDX cells were harvested 12 h after induction with 10^{-6} M dexamethasone (Sigma). For internal immunofluorescence, cells were fixed with acetone before being stained with the primary antibody. The anti-gG-2 serum used for both membrane and internal immunofluorescence was preabsorbed with NIH 3T3 cells and rabbit liver powder (GIBCO) (23). After reaction with the primary antiserum, all cover slips were then stained with fluorescein isothiocyanate-conjugated goat anti-rabbit gamma globulin (Cappel Laboratories). Cover slips were visualized with an Olympus (BHS) microscope by using a tungsten light source.

RESULTS

Establishment of cell lines expressing gG-2. The synthesis and processing of gG-2 in the absence of an HSV-2 infection was accomplished by introducing a cloned gG-2 gene into NIH 3T3 cells. During an HSV-2 infection, transcription of the gG-2 gene presumably requires the presence of other viral gene products (19). To study the synthesis of gG-2 in the absence of HSV-2 infection, we cloned the gG-2 gene behind the MMTV promoter on the expression vector, pMSG. The expression of gG-2 should be inducible with the glucocorticoid dexamethasone (22).

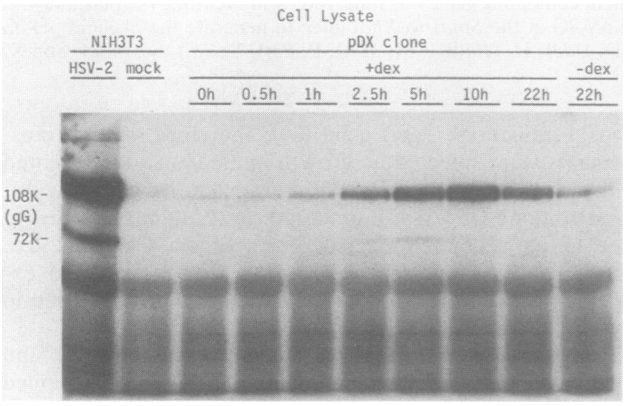

FIG. 2. Expression of gG-2 in the pDX cells after addition of dexamethasone. Parallel confluent cultures of pDX cells were incubated in the presence (+dex) or absence (−dex) of 10^{-6} M dexamethasone and harvested at the indicated times after dexamethasone addition (above lanes). Mock- or HSV-2-infected NIH 3T3 cells were harvested 22 h after infection. The harvested cell samples were solubilized and immunoprecipitated with the anti-gG-2 serum. The immunoprecipitates were analyzed by immunoblotting with the anti-gG-2 serum. The positions of the 108K and 72K proteins are indicated.

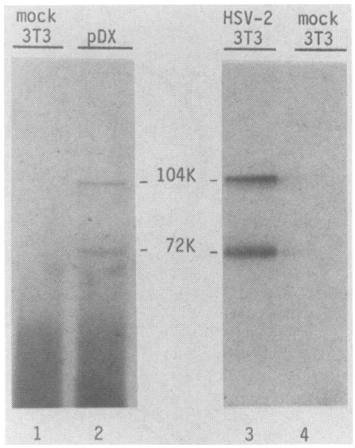

FIG. 3. Detection of the synthesis of gG-2 precursors with the anti-pgG-2 serum. NIH 3T3 cells or dexamethasone-induced pDX cells were harvested at 12 h postinduction and immunoprecipitated with anti-pgG-2 serum. The immunoprecipitated proteins were analyzed by SDS-PAGE followed by immunoblotting with anti-pgG-2 serum (lanes 1 and 2). HSV-2- or mock-infected NIH 3T3 cells were harvested at 12 h postinfection and analyzed directly by SDS-PAGE and immunoblotting with anti-pgG-2 serum (lanes 3 and 4). The positions of the 104K and 72K proteins are indicated.

The construction of the expression plasmid designated pD-X is outlined in Fig. 1 and further described in Materials and Methods. The viral sequence cloned into pD-X included the entire gG-2 coding sequence from a *Dde*I site 110 base pairs 5′ of the gG-2 initiation codon to an *Xho*I site 114 base pairs downstream from the polyadenylation signal (28). NIH 3T3 cells were transfected with pD-X DNA by the calcium phosphate precipitation technique (16), and the transformed cells were selected for *gpt* expression in medium containing 25 μg of mycophenolic acid per ml (see Materials and Methods for details). Single colonies were picked and replated in 24-well plates. A total of nine separate cell lines were established; one cell line, designated pDX, was selected for further characterization.

Inducible expression of gG-2 in pDX cells. Since the gG-2 gene within pDX cells was under the control of the MMTV promoter, gG-2 expression in this cell line was studied after induction with dexamethasone. Confluent monolayers of pDX cells were incubated in the presence or absence of 10^{-6} M dexamethasone and harvested at various times after induction. Mock- or HSV-2-infected NIH 3T3 cells were included as controls, and these cells were harvested at 22 h postinfection. At the indicated times, the various cultures were harvested and solubilized, and the gG-2 proteins were immunoprecipitated with anti-gG-2 serum. The immunoprecipitated proteins were solubilized, resolved on SDS-PAGE, and then transferred to nitrocellulose and immunoblotted with anti-gG-2 serum (Fig. 2). After the addition of dexamethasone, an increased accumulation of gG-2 was detected with time compared with noninduced pDX cells. If one compares the gG-2 components synthesized in transformed cells with those made in HSV-2-infected cells, it is apparent that after dexamethasone treatment, the 108K and the 72K precursors are detectable in the pDX cells. The amount of gG-2 (108K) and the 72K precursor detected appears to diminish at later times after induction. However, the nature of the difference in the steady-state levels of 108K versus the

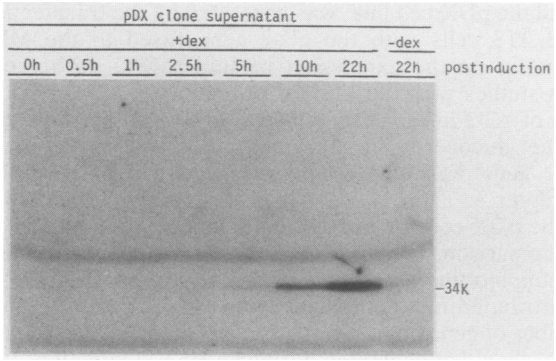

FIG. 4. Detection of the 34K secreted component in the extracellular medium of pDX cells after dexamethasone addition. The medium from pDX cells cultured in the presence or absence of dexamethasone (from Fig. 2) was harvested at the indicated times (above lanes) after addition of dexamethasone, and the proteins were precipitated with trichloroacetic acid. The recovered proteins were analyzed by immunoblotting with the anti-pgG-2 serum. The position of the 34K protein is indicated.

72K precursor is not understood and is currently under further study. The nonspecific reactivity that was present in the lower one-third of all lanes was the result of the [^{125}I]protein A reacting with the immunoglobulins originating from the immunoprecipitation step of the procedure.

The synthesis of the gG-2 precursors in the gG-2-transformed cells was further studied by using the anti-pgG-2 serum prepared against the 104K precursor (37). This anti-pgG-2 antibody was employed to facilitate the specific detection of the 104K and the 72K precursors (37). NIH 3T3 or pDX cells induced with dexamethasone for 12 h were harvested and immunoprecipitated with the anti-pgG-2 serum. The immunoprecipitated proteins were analyzed by SDS-PAGE followed by immunoblotting with anti-pgG-2 serum (Fig. 3, lanes 1 and 2). HSV-2 or mock-infected NIH 3T3 cells were harvested at 12 h postinfection and analyzed by SDS-PAGE and immunoblotting with anti-pgG-2 serum (Fig. 3, lanes 3 and 4). Both the 104K and 72K intermediates were clearly observed in the HSV-2-infected as well as the gG-2-transformed cells. These results suggest that the expression of gG-2 in the pDX cells is indeed inducible with dexamethasone and that the 104K and 72K high-mannose gG-2 precursors are generated in the transformed cells.

In HSV-2-infected cells, the synthesis of gG-2 involves a cleavage-processing event that results in the generation of the 72K component and the 34K secreted cleavage product. To verify that the 34K cleavage product was also produced in the pDX cells, the extracellular medium from transformed cell cultures incubated with dexamethasone was analyzed. At the indicated times, the cell supernatants were harvested and the proteins from the medium were precipitated, solubilized, and analyzed by SDS-PAGE followed by immunoblotting with the anti-pgG-2 serum (Fig. 4). The 34K cleavage product was clearly detectable in the extracellular medium of the pDX cells after induction with dexamethasone. The amount of the 34K secreted component appeared to accumulate with increasing time after induction. The presence of the 34K component in the culture medium of the pDX cells indicates that the cleavage-processing events for gG-2 are similar in both the transformed and HSV-2-infected cells.

Localization of gG-2 by immunofluorescence. Both internal and membrane immunofluorescence were used to study the distribution of the gG-2 antigens expressed in pDX cells. NIH 3T3 and pDX cells were seeded onto cover slips either separately or as mixed cultures. The gG-2-transformed cells were induced with dexamethasone for 12 h before harvest, while the NIH 3T3 cells, either mock infected or infected with HSV-2, were harvested at 12 h postinfection. All cover slips were then processed for internal or membrane immunofluorescence and reacted with normal rabbit serum or

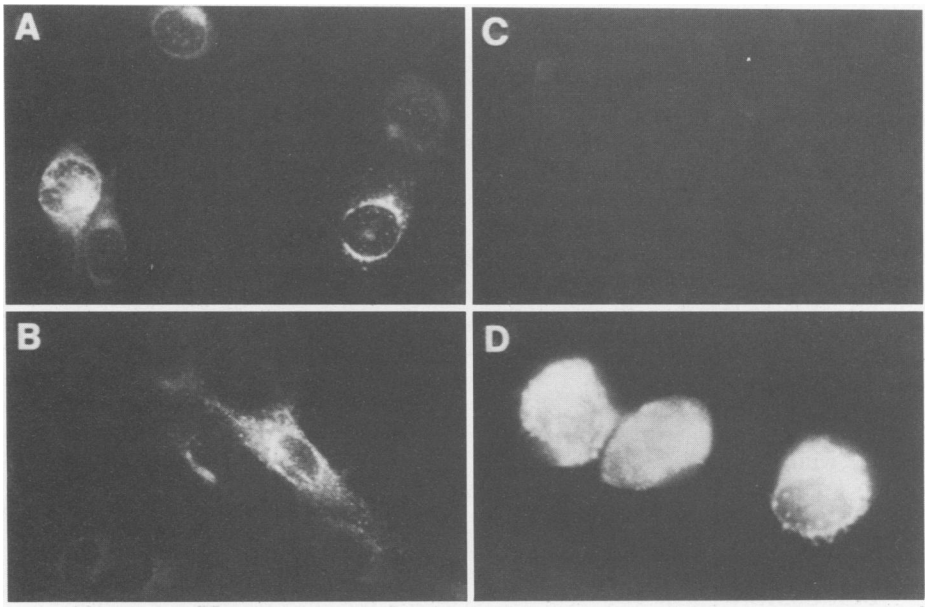

FIG. 5. Internal immunofluorescence studies of gG-2 synthesis in the pDX cells. HSV-2-infected NIH 3T3 cells (D), induced pDX cells alone (A and C), or a mixed culture of induced pDX cells and NIH 3T3 cells (B) were fixed and stained with either anti-gG-2 (A, B, and D) or normal rabbit serum (C). After being reacted with the secondary fluorescein isothiocyanate-goat anti-rabbit antibody, the cells were visualized for immunofluorescence staining.

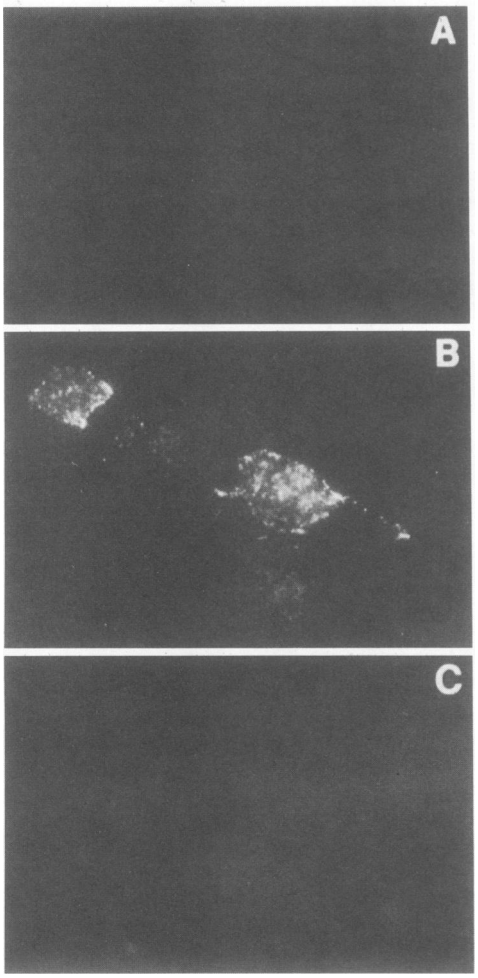

FIG. 6. Surface immunofluorescence studies of pDX cells. NIH 3T3 cells (A) or induced pDX cells (B and C) were stained with either the anti-gG-2 (A and B) or (C) normal rabbit serum and processed for membrane immunofluorescence as described in Materials and Methods.

anti-gG-2 serum. The internal immunofluorescence staining results are shown in Fig. 5. Specific perinuclear staining was observed in the gG-2-transformed cells (Fig. 5A) as well as in the HSV-2-infected NIH 3T3 cells (Fig. 5D). The intensity of the fluorescence was always greater in the virus-infected cells. In cultures containing NIH 3T3 cells and pDX cells mixed at a ratio of approximately 5:1, one observed positive cells surrounded by nonreactive cells (Fig. 5B). Cells transformed with gG-2 and reacted with normal rabbit serum (Fig. 5C) and mock-infected NIH 3T3 cells stained with anti-gG-2 serum (not shown) showed no significant staining.

The membrane immunofluorescence reactions are presented in Fig. 6. Positive staining was detected when anti-gG-2 serum was reacted with the gG-2-transformed cells (Fig. 6B). In contrast, mock-infected NIH 3T3 and pDX cells showed no reactivity with anti-gG-2 serum and normal rabbit serum, respectively (Fig. 6A and C).

DISCUSSION

In this report we describe the establishment and characterization of a NIH 3T3 cell line constitutively expressing the gG-2 gene of HSV-2. This gG-2-transformed cell line, designated the pDX cell line, was generated by the transfection of NIH 3T3 cells with the gG-2 gene fused to the MMTV promoter on the expression vector, pMSG. In agreement with studies with the MMTV promoter (6, 22), the expression of gG-2 in the pDX cells was enhanced by the addition of the glucocorticoid, dexamethasone, indicating that the gG-2 gene was indeed under the control of the MMTV promoter.

The pDX cells expressed lower levels of the mature gG-2 in comparison with HSV-2-infected cells, as determined by immunoblotting and immunofluorescence studies. This can be attributed to a combination of several factors. First, the number of gene copies in the transformed cell may be much lower than that generated during an HSV-2 infection. In one study using the pMSG expression vector, only 15 copies of the vector were present per cell (21). However, during HSV-2 infection, hundreds of copies of the gG-2 gene may be present since gG-2 is produced subsequent to the initiation of viral DNA replication. Alternatively, there may be a difference in the rate of gG-2 turnover between the pDX cells and the HSV-2-infected cells. In agreement with studies using pMSG to express a DNA-binding protein (21), accumulation of the mature gG-2 was not observed in the pDX cells at later times (22 h) after induction (Fig. 2); a constant level of mature gG-2 was maintained for up to 48 h (data not shown). This is in contrast to the increasing amounts of the 34K secreted component detected in the extracellular medium at similar times. Since the 34K secreted component and the mature gG-2 share a common precursor (37), the continued accumulation of 34K in the extracellular medium at later times suggest that synthesis of gG-2 is still occurring. The lack of a steady accumulation of the mature gG-2 therefore indicates that there may be cellular turnover of the mature gG-2 in the pDX cells. Such turnover of gG-2 during HSV-2 infection has not been described. Additionally, some as yet unidentified viral function may allow for a more efficient expression and processing of gG-2 in the infected cells than in the transformed cells. None of these possibilities can be excluded at present.

With the availability of a stable cell line expressing gG-2, the processing events for gG-2 can be further dissected. Consistent with the processing events of gG-2 already established in HSV-2-infected cells (3, 9, 37), the mature 108K gG-2, as well as the 104K and 72K intermediates, were all detected in the gG-2-transformed cells. In the presence of tunicamycin, the synthesis of the mature gG-2 was inhibited in the pDX cells (data not shown), as is the case in tunicamycin-treated HSV-2-infected cells (3, 35). Furthermore, the synthesis of gG-2 in the pDX cells also involves the cleavage-processing event that results in the secretion of the 34K component into the extracellular medium. Taken together, these results suggest that the normal processing of gG-2 is not dependent on the presence of other HSV-2 gene products. Although autoproteolytic cleavage of gG-2 precursor cannot be entirely ruled out, the cleavage event is probably mediated by a host cell-specific protease. Such host cell-specific proteolytic cleavage has been shown for the processing of the glycoproteins of several RNA viruses, including HA of orthomyxovirus, F of paramyxovirus (12), and E2 of coronavirus (20). Although cleavage of HA, F, and E2 plays a role in the activation of viral infectivity of their respective viruses (12, 13, 20), no such role is expected for gG-2 since recent studies have indicated that both gG-1 (24, 40) and gG-2 (17) are nonessential functions for viral replication in tissue culture. The gX glycoprotein of pseudorabies (a suggested homolog of gG-2 [31]) appears to resemble gG-2

in that synthesis of gX involves a cleavage-processing step as well as secretion of the cleavage product (4, 33). The gX glycoprotein is also nonessential for virus growth in tissue culture (38). The function of gG-2 remains to be determined.

These studies represent the first reported expression of gG-2 in mammalian cells in the absence of an HSV-2 infection. With the establishment of such a system, experiments can now be initiated to study the cleavage-processing events associated with gG-2 synthesis.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant RO1 CA42460 from the National Cancer Institute.

We are grateful to Jacqueline Fetherston for invaluable discussions and for critically reading the manuscript, Gordon Fowler for excellent technical assistance, and Carol Ann Ardoin for preparation of the manuscript.

LITERATURE CITED

1. **Ackermann, M., R. Longnecker, B. Roizman, and L. Pereira.** 1986. Identification, properties, and gene location of a novel glycoprotein specified by herpes simplex virus 1. Virology **150:** 207–220.
2. **Anderson, D. J., and G. Blobel.** 1983. Immunoprecipitation of proteins from cell-free translations. Methods Enzymol. **96:**111–120.
3. **Balachandran, N., and L. M. Hutt-Fletcher.** 1985. Synthesis and processing of glycoprotein gG of herpes simplex virus type 2. J. Virol. **54:**825–832.
4. **Bennett, L. M., J. G. Timmons, D. R. Thomsen, and L. E. Post.** 1986. The processing of pseudorabies virus glycoprotein gX in infected cells and in an uninfected cell line. Virology **155:**707–715.
5. **Bone, D. R., and R. J. Courtney.** 1974. A temperature-sensitive mutant of herpes simplex virus type 1 defective in the synthesis of the major capsid protein. J. Gen. Virol. **24:**17–27.
6. **Chapman, A. B., M. A. Costello, F. Lee, and G. Ringold.** 1983. Amplification and hormone-regulated expression of a mouse mammary tumor virus-Eco gpt fusion plasmid in mouse 3T6 cells. Mol. Cell. Biol. **3:**1421–1429.
7. **Chen, C., and H. Okayama.** 1987. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. **7:** 2745–2752.
8. **Compton, T., and R. J. Courtney.** 1984. Virus-specific glycoproteins associated with the nuclear fraction of herpes simplex virus type 1-infected cells. J. Virol. **49:**594–597.
9. **Dall'Olio, F., N. Malagolini, G. Campadelli-Fiume, and F. Serafini-Cessi.** 1987. Glycosylation pattern of herpes simplex virus type 2 glycoprotein G from precursor species to the mature form. Arch. Virol. **97:**237–249.
10. **Eberle, R., and R. J. Courtney.** 1980. Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex type 1. J. Virol. **35:**902–917.
11. **Flannery, V. L., R. J. Courtney, and P. A. Schaffer.** 1977. Expression of an early, nonstructural antigen of herpes simplex virus in cells transformed in vitro by herpes simplex virus. J. Virol. **21:**284–291.
12. **Frame, M. C., H. S. Marsden, and D. J. McGeoch.** 1986. Novel herpes simplex virus type 1 glycoproteins identified by antiserum against a synthetic oligopeptide from the predicted product of gene US4. Virology **67:**745–751.
13. **Frana, M. F., J. N. Behnke, L. S. Sturman, and K. V. Holmes.** 1985. Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: host-dependent differences in proteolytic cleavage and cell fusion. J. Virol. **56:**912–920.
14. **Godson, G. N., and D. Vapnek.** 1973. A simple method of preparing large amounts of ϕX174 RFI supercoiled DNA. Biochim. Biophys. Acta **299:**516–520.
15. **Gorman, C.** 1985. High efficiency gene transfer into mammalian cells, p. 143–190. *In* D. M. Glover (ed.), DNA cloning, vol. II. IRL Press, Washington, D.C.
16. **Graham, F. L., and A. J. Van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52:**456–467.
17. **Harland, J., and S. M. Brown.** 1988. Generation of a herpes simplex virus type 2 variant devoid of XbaI sites: removal of the 0.91 map coordinate site results in impaired synthesis of glycoprotein G-2. J. Gen. Virol. **69:**113–124.
18. **Hodgman, T. C., and A. C. Minson.** 1986. The herpes simplex virus type 2 equivalent of the herpes simplex virus type 1 US7 gene and its flanking sequences. Virology **153:**1–11.
19. **Honess, R. W., and B. Roizman.** 1974. Regulation of herpesvirus macromolecular synthesis. I. Cascade regulation of the synthesis of three groups of viral proteins. J. Virol. **14:**8–19.
20. **Klenk, H. D., and R. Rott.** 1980. Cotranslational and posttranslational processing of viral glycoproteins. Curr. Top. Microbiol. Immunol. **90:**19–48.
21. **Klessig, D. F., D. E. Brough, and V. Cleghon.** 1984. Introduction, stable integration, and controlled expression of a chimeric adenovirus gene whose product is toxic to the recipient human cell. Mol. Cell. Biol. **4:**1354–1362.
22. **Lee, R., R. Mulligan, P. Berg, and G. Ringold.** 1981. Glucocorticoids regulate expression of dihydrofolate reductase cDNA in mouse mammary tumor virus chimaeric plasmids. Nature (London) **294:**228–232.
23. **Lewis, J. G., L. S. Kucera, R. Eberle, and R. J. Courtney.** 1982. Detection of herpes simplex virus type 2 glycoproteins expressed in virus-transformed rat cells. J. Virol. **42:**275–282.
24. **Longnecker, R., and B. Roizman.** 1987. Clustering of genes dispensable for growth in culture in the S component of the HSV-1 genome. Science **236:**573–576.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
26. **Marsden, H. S., A. Buckmaster, J. W. Palfreyman, R. G. Hope, and A. C. Minson.** 1984. Characterization of the 92,000-dalton glycoprotein induced by herpes simplex virus type 2. J. Virol. **50:**547–554.
27. **McGeoch, D. J., A. Dolan, S. Donald, and F. J. Rixon.** 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. **181:**1–13.
28. **McGeoch, D. J., H. W. M. Moss, D. McNab, and M. C. Frame.** 1987. DNA sequence and genetic content of the HindIII L region in the short unique component of the herpes simplex virus type 2 genome: identification of the gene encoding glycoprotein G, and evolutionary comparisons. J. Gen. Virol. **68:**19–38.
29. **Mulligan, R. C., and P. Berg.** 1981. Selection for animal cells that express the Escherichia coli gene coding for xanthine-guanine phosphoribosyl transferase. Proc. Natl. Acad. Sci. USA **78:**2072–2076.
30. **Olofsson, S., M. Lundstrom, H. Marsden, S. Jeansson, and A. Vahlne.** 1986. Characterization of a herpes simplex virus type 2-specified glycoprotein with affinity for N-acetylgalactosamine-specific lectins and its identification as g92K or gG. J. Gen. Virol. **67:**737–744.
31. **Petrovskis, E. A., J. G. Timmons, and L. E. Post.** 1986. Use of λgt11 to isolate genes for two pseudorabies virus glycoproteins with homology to herpes simplex virus and varicella-zoster virus glycoproteins. J. Virol. **60:**185–193.
32. **Powell, K. L., and R. J. Courtney.** 1975. Polypeptides synthesized in herpes simplex virus type 2 infected HEp-2 cells. Virology **66:**217–228.
33. **Rea, T. J., J. G. Timmons, G. W. Long, and L. E. Post.** 1985. Mapping and sequence of the gene for the pseudorabies virus glycoprotein which accumulates in the medium of infected cells. J. Virol. **54:**21–29.
34. **Richman, D. D., A. Buckmaster, S. Bell, C. Hodgman, and A. C. Minson.** 1986. Identification of a new glycoprotein of herpes simplex virus type 1 and genetic mapping of the gene that codes for it. J. Virol. **57:**647–655.

35. **Roizman, B., B. Norrild, C. Chen, and L. Pereira.** 1984. Identification and preliminary mapping with monoclonal antibodies of a herpes simplex virus type 2 glycoprotein lacking a known type 1 counterpart. Virology **133**:242–247.
36. **Spear, P. G.** 1985. Glycoproteins specified by herpes simplex viruses, p. 315–356. *In* B. Roizman (ed.), The herpesviruses. Plenum Publishing Corp., New York.
37. **Su, H. K., R. Eberle, and R. J. Courtney.** 1987. Processing of the herpes simplex virus type 2 glycoprotein gG-2 results in secretion of a 34,000-M_r cleavage product. J. Virol. **61**:1735–1737.
38. **Thomsen, D. R., C. C. Marchioli, R. J. Yancey, and L. E. Post.** 1987. Replication and virulence of pseudorabies virus mutants lacking glycoprotein gX. J. Virol. **61**:229–232.
39. **Towbin, H., Staehlin, T., and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
40. **Weber, P. C., M. Levine, and J. C. Glorioso.** 1987. Rapid identification of nonessential genes of herpes simplex virus type 1 by Tn5 mutagenesis. Science **236**:576–579.