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# Chicken Ovalbumin Gene Fused to a Herpes Simplex Virus α Promoter and Linked to a Thymidine Kinase Gene Is Regulated Like a Viral Gene

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We are describing a system for the introduction, selection, and expression of eucaryotic genes in higher eucaryotic cells. The carrier consisted of the herpes simplex virus 1 (HSV-1) *tk* gene covalently linked to an HSV-1 α promoter directed away from the *tk* gene. In this study we fused to the α promoter the 5′ transcribed noncoding sequences and the coding sequences of the chicken oviduct ovalbumin gene. Cells converted to the $TK^+$ phenotype with this chimeric fragment produced an ovalbumin precursor which was processed and secreted into the extracellular fluid. The ovalbumin gene utilized the HSV-1 α promoter and was regulated as a viral gene inasmuch as inversion of the genomic DNA relative to the α promoter resulted in no ovalbumin synthesis, and production of ovalbumin was enhanced after superinfection with HSV-1. Synthesis of ovalbumin was not detected when cDNA was linked to the HSV-1 α promoter. The carrier system described in this study is suitable for introduction, selection, and expression of eucaryotic genes whose natural promoter is either weak or requires the presence of regulatory elements which may be absent from undifferentiated cells in culture.

In an earlier paper, this laboratory described a system for the identification of promoter-linked regulatory gene sequences using the herpes simplex virus 1 (HSV-1) thymidine kinase gene (*tk*) as the test gene (15). That system was tested with HSV-1 genes. Specifically, HSV genes form three groups, designated α, β, and γ, whose expression is coordinately regulated and sequentially ordered in a cascade fashion (5, 6). The natural HSV *tk* gene is regulated as a β gene and in infected cells requires an α gene product for its optimal expression. By fusing the coding sequences and a portion of the 5′ transcribed noncoding sequences of the *tk* gene with a DNA fragment coding for the 5′ terminus of the mRNA and the upstream sequences of the α protein no. 4 gene, the *tk* gene is converted to an α gene (15). The advantage of this system is that the chimeric gene can be used to convert cells from $TK^-$ to $TK^+$ (designated $Vtk^+$ cells) and the readily selectable $Vtk^+$ cells can then be analyzed for regulation of the *tk* gene. That study revealed that the sequences determining whether genes are regulated as either α or β are upstream from the 5′ terminus of the coding sequences of the gene and that the expression of α genes is enhanced by non-α viral gene products.

In this paper we describe a system suitable for the introduction, selection, and expression of eucaryotic genes whose natural promoter either is weak or requires the presence of regulatory elements which may be absent from undifferentiated cells in culture. The system consists of two elements. The first is the HSV-1 *tk* gene, which functions as a selectable marker to discriminate between cells that have received the gene under study from those that have not. The second element, covalently linked to the *tk* gene, is the α protein no. 4 promoter region identified previously (11, 15). The α protein no. 4 promoter is then linked to the 5′ transcribed noncoding sequences and the coding sequences of the gene under study. We present evidence that the system allows the synthesis of ovalbumin from ovalbumin genomic DNA and that the expression of this gene is under viral control.

The system we describe differs significantly from that described by Wigler et al. (22), in which cells are transfected with a mixture of DNA fragments carrying the HSV-1 *tk* gene and the gene under study. In our system the gene under study was covalently linked to the selectable marker and regulated by a promoter incorporated into the system. After this paper was submitted for publication, Subramani et al. (18) reported on the expression of mouse dihydrofolate reductase gene under the direction of signals supplied by simian virus 40.

Ovalbumin expression in mammalian cells has

been detected either in the form of transcripts (1) or by very sensitive immunological techniques in the form of a protein which does not comigrate in polyacrylamide gels with hen oviduct albumin (9). In this study we demonstrate by direct immune precipitation both a precursor ovalbumin molecule in cell lysates and a processed form secreted into the extracellular medium which comigrated with oviduct ovalbumin on polyacrylamide gels.

## MATERIALS AND METHODS

**Cells and viruses.** The Ltk$^-$ cell line (7) was the generous gift of S. Kit and was maintained in Dulbecco modified Eagle medium with 10% calf serum. TK$^-$ cells converted to TK$^+$ phenotype (LVtk$^+$) cells were grown in a modified HAT medium consisting of $1.6 \times 10^{-5}$ M thymidine, $10^{-5}$ M hypoxanthine, and $4.4 \times 10^{-7}$ M methotrexate (15).

The *ts*502Δ305 virus produces a temperature-sensitive α protein no. 4, and only α genes are expressed at the nonpermissive temperature (39°C); the virus also contains a deletion within the *tk* gene. The construction of the virus has been described elsewhere (15).

**Plasmid construction.** The cloning of HSV-1 (F) *Bam*HI fragments in pBR322 and the authenticity of the cloned DNA were reported by Post et al. (14). pRB103 and pRB114 are pBR322-derived plasmids containing *Bam*HI-Q and BamHI-N, respectively. The construction of a *tk* gene under the control of the α-regulated promoter of the α protein no. 4 gene has been described elsewhere (15).

The ovalbumin plasmids pOV12 and pOV230 were generously provided by B. W. O'Malley, Baylor College of Medicine, Houston, Tex. The construction and properties of these plasmids have been described elsewhere (9, 12). The *Taq*I fragments from these plasmids containing the ovalbumin gene sequences were cloned into the *Bam*HI site of pBR322 by treatment with T4 DNA polymerase (Pabst Laboratories, Milwaukee, Wis.) before ligation (19).

Methods and references for DNA cloning and plasmid preparation have been described in a previous publication (14).

**Conversion of Ltk$^-$ cells to Ltk$^+$ phenotype.** Ltk$^-$ cells were transfected with approximately 0.1 μg of intact circular plasmid DNAs by the calcium phosphate method described by Wigler et al. (22). This resulted in 50 to 100 foci growing in HAT medium. These foci were passaged without cloning.

**Preparation of radiolabeled cellular antigens and antigens accumulated in the growth medium.** The cell lines were grown in monolayer cultures in 75-cm$^2$ flasks (except as stated in the text) and were either mock infected or infected in mixture 199 supplemented with 1% (vol/vol) fetal calf serum at a multiplicity of infection of 5 PFU of HSV-1 *ts*502Δ305 per cell. The cells were incubated at 39°C from the beginning of the adsorption period. At 3 h postinfection the cells were washed with medium free of methionine and fetal calf serum and overlaid with 5 ml of serum-free medium containing 1/20 of the normal concentration of methionine but supplemented with 50 μCi of [$^{35}$S]methionine per ml of medium ([$^{35}$S]methionine, 900 mCi/mmol; New England Nuclear Corp.). The radioactive labeling of the cells was continued until 8 h postinfection, when the medium was removed and the cell monolayer was washed in phosphate-buffered saline (PBS) containing 10 mM $Na_2PO_4$, 1.5 mM $KH_2PO_4$, 0.14 M NaCl, 3 mM KCl, 0.5 mM $MgCl_2$, and 1 mM $CaCl_2$. The cells were then scraped into 4 ml of PBS and pelleted by low-speed centrifugation. The cell pellet was suspended in 0.6 ml of PBS-A (PBS without $MgCl_2$ and $CaCl_2$) containing 1% (vol/vol) Nonidet P-40, 1% (wt/vol) deoxycholate, and $10^{-5}$ M of protease inhibitors TPCK (L-1-tosylamide-2-phenylethyl chloromethyl ketone) and TLCK (*N*-α-*p*-tosyl-L-lysine chloromethyl ketone) (Sigma Chemical Co., St. Louis, Mo.), disrupted by ultrasonic treatment, and centrifuged for 1 h at 25,000 rpm and 4°C in an SW50.1 rotor. The supernatant fluid was the source of the cell lysate antigen.

To the 5 ml of labeling medium from each cell culture were added 0.1% (vol/vol) Nonidet P-40, 0.1% (wt/vol) deoxycholate, 1 μg of ovalbumin, and $10^{-6}$ M of protease inhibitors TPCK and TPLK. The medium was dialyzed against 10-fold-diluted PBS-A to remove the excess of free [$^{35}$S]methionine and concentrated to 0.5 ml by lyophilization. This 10-fold-concentrated medium was tested for the presence of secreted [$^{35}$S]methionine-labeled ovalbumin as described below.

**Antibodies.** Preimmune rabbit serum and immune rabbit serum made to chicken ovalbumin were obtained from Cappel Laboratories (Cochranville, Pa.). The immune serum (2 ml) was absorbed two times on Ltk$^-$ cell monolayers grown in 150-cm$^2$ cultures before use in immunoprecipitation studies. Sodium azide (0.1%; wt/vol) was added to the sera before immune precipitation.

**Radioactively labeled ovalbumin.** [$^{14}$C]methyl-labeled chicken ovalbumin (0.020 mCi/mg, 0.002 mCi/0.2 ml; New England Nuclear Corp., Cambridge, Mass.) was used as an immunological standard. A total of 20 μl of $^{14}$C-labeled ovalbumin was immunoprecipitated in the presence of 0.1 μg of unlabeled carrier ovalbumin (Sigma) with 25 μl of the absorbed immune serum and suspended in disruption mixture. The precipitated equivalent of 10 μl of [$^{14}$C]ovalbumin standard was loaded on each gel.

**Immunoprecipitation of cellular extracts.** A total of 50 μl of the cell lysates was incubated either with 50 μl of preimmune rabbit serum or with 50 μl of immune rabbit serum made to chicken ovalbumin and absorbed with Ltk$^-$ cells. After 1 h of incubation at 4°C, 5 mg of protein A-Sepharose (Sigma) was added to each sample. After incubation for 1 h at 4°C, the immunocomplexes bound to the protein A-Sepharose were washed four times in PBS-A with 1% (vol/vol) Nonidet P-40, 0.5% (wt/vol) deoxycholate and 0.1% (wt/vol) sodium dodecyl sulfate and one time in 0.1 M NaCl buffered with 0.05 M Tris-hydrochloride, pH 7.4. The concentration of serum used in these experiments was based on preliminary titrations with increasing serum concentrations.

**Immunoprecipitation of the 10-fold-concentrated tissue culture medium.** A total of 200 μl of the medium was incubated either with 50 μl of preimmune rabbit serum or with 50 μl of the absorbed immune serum. The immunoprecipitation was done as described above.

**Polyacrylamide gel electrophoresis.** The procedures for the solubilization of cell lysates or of immune

precipitates, polyacrylamide gel electrophoresis, fixation, staining, and autoradiography of the electrophoretically separated polypeptides were as described by Morse et al. (13).

## RESULTS

**Construction of HSV Pα vectors.** The objective was to construct a vector which would include a selectable marker and an HSV α promoter (Pα) that would direct transcription of DNA inserted into a unique restriction enzyme cleavage site. Table 1 lists the plasmids used in these constructions. The α-regulated promoter of the α protein no. 4 gene has been located very precisely (11; and S. Mackem and B. Roizman, manuscript in preparation) at the left end of *Bam*HI-N, and the *Bam*HI cleavage site at the end of *Bam*HI-N has been used to join the α protein no. 4 promoter to the *tk* gene (15). The first step in the construction was to subclone a piece of *Bam*HI-N containing the α protein no. 4 promoter such that the *Bam*HI site at the left end of *Bam*HI-N was the only *Bam*HI site in the subcloned plasmid. This was accomplished by cutting pRB114 with *Bam*HI plus *Pvu*II, religating, and screening for the plasmid (pRB403) containing the α protein no. 4 promoter within the *Pvu*II-*Bam*HI fragment of *Bam*HI-N (Fig. 1a).

The selectable marker was introduced into plasmid pRB403 by inserting a *Pvu*II fragment containing a functional *tk* gene into the single *Pvu*II cleavage site of pRB403. Two vectors were constructed. pRB334 was made by inserting the *Pvu*II fragment from pRB103, which contains a natural HSV *tk* gene with its β-regulated promoter. pRB335 was made by inserting the *Pvu*II fragment from pRB316, which contains a *tk* gene under the control of the α-regulated promoter of the α protein no. 4 gene (15). Both of these vectors contain a functional *tk* gene and a unique *Bam*HI site immediately downstream from an α promoter (Fig. 1a).

**Construction of Pα-ovalbumin plasmids.** To insert the ovalbumin genes under control of Pα of pRB334 or pRB335, it was desirable to have

TABLE 1. Plasmids used in these experiments

| Designation | Vector | Construction |
|---|---|---|
| HSV plasmids | | |
| pRB103 | pBR322 | HSV-1 (F) *Bam*HI-Q cloned into *Bam*HI site (14) |
| pRB114 | pBR322 | HSV-1 (F) *Bam*HI-N cloned into *Bam*HI site (14) |
| pRB316 | pBR322 | *Bam*HI-N inserted into *Bgl*II site of pRB103 with α protein no. 4 promoter proximal to the *tk* gene (14) |
| pRB403 | pBR322 | *Pvu*II-*Bam*HI fragment containing the α protein no. 4 promoter from *Bam*HI-N cloned between *Pvu*II and *Bam*HI sites of pBR322 |
| pRB334 | pRB403 | *Pvu*II fragment carrying the *tk* gene from pRB103 inserted into the *Pvu*II site of pRB403 |
| pRB335 | pRB403 | *Pvu*II fragment carrying the *tk* gene from pRB316 inserted into the *Pvu*II site of pRB403 |
| Ovalbumin plasmids | | |
| pOV12 | pBR322 | Entire ovalbumin gene from chicken genome inserted into *Hin*dIII site of pBR322 (9) |
| pOV230 | pMB9 | Full-length cDNA from ovalbumin message inserted with polydeoxyadenylic acid-polydeoxythymidylic acid tails (12) |
| pRB7 | pBR322 | *Taq*I fragment containing entire ovalbumin coding sequences from pOV230 inserted into *Bam*HI site |
| pRB8 | pBR322 | *Taq*I fragment containing entire ovalbumin coding sequences plus introns from pOV12 inserted into *Bam*HI site |
| Pα-ovalbumin plasmids | | |
| pRB351 | pRB334 | *Bam*HI fragments from pRB7 inserted into *Bam*HI site of pRB334; orientation of α protein no. 4 promoter and ovalbumin sequences same |
| pRB352 | pRB334 | *Bam*HI fragment from pRB8 inserted into the *Bam*HI site of pRB334; orientation of α protein no. 4 promoter and ovalbumin sequences same |
| pRB353 | pRB335 | *Bam*HI fragment from pRB8 inserted into *Bam*HI site of pRB335; orientation of α protein no. 4 promoter and ovalbumin sequences same |
| pRB354 | pRB335 | *Bam*HI fragment from pRB8 inserted into *Bam*HI site of pRB335; orientation of α protein no. 4 promoter and ovalbumin sequences opposite |
| pRB355 | pRB334 | *Bam*HI fragment from pRB7 inserted into *Bam*HI site of pRB334; orientation of α protein no. 4 promoter and ovalbumin sequences opposite |

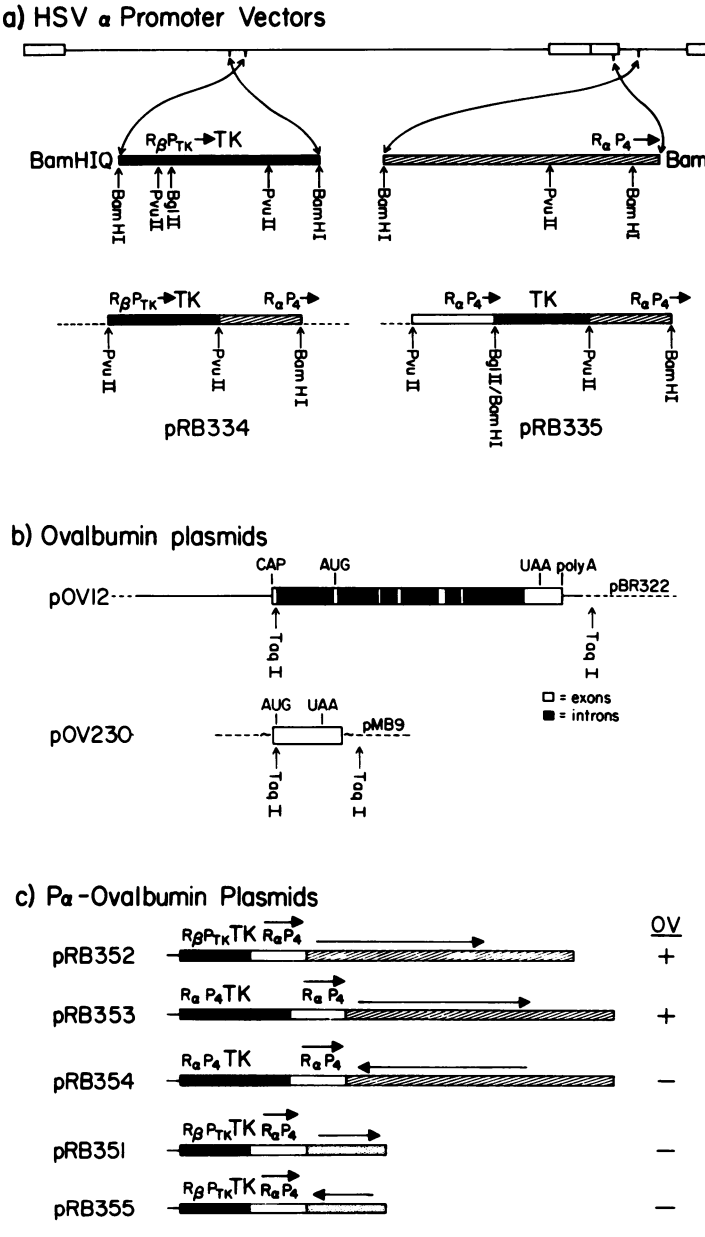


FIG. 1. Construction of Pα-ovalbumin plasmids. (a) Vectors. The top line shows a schematic representation of the HSV genome (16). The expanded diagrams of HSV-1 *Bam*HI fragment Q, containing the *tk* gene, and of *Bam*HI fragment N, containing the α promoter of the α protein no. 4 gene, are inverted from their arrangement in the prototype isomer of HSV-1 DNA. HSV-1 *Bam*HI-Q and *Bam*HI-N DNAs were used to construct the two vectors shown in the third line. pRB334 contains a *tk* gene with its natural β-regulated promoter ($R_\beta P_{tk}$) and the α-regulated promoter of the α protein no. 4 gene ($R_\alpha P_4$) at the only *Bam*HI site in the plasmid; pRB335 contains a *tk* gene under the control of the α-regulated promoter of the α protein no. 4 gene (15) and another copy of the α protein no. 4 gene promoter at the *Bam*HI site. (b) Ovalbumin plasmids obtained from B. W. O'Malley. pOV12 contains the entire chromosomal ovalbumin gene (9), whereas pOV230 is a cloned nearly full-length cDNA of ovalbumin mRNA (12). (c) Pα-ovalbumin plasmids. All were constructed by inserting the ovalbumin genes from either pOV12 or pOV230 into the *Bam*HI site of either pRB334 or pRB335. Filled bar represents a *tk* gene, the open bar represents the α-controlled promoter of the α protein no. 4 gene, the cross-hatched bar represents the genomic ovalbumin gene, and the stippled bar represents ovalbumin cDNA. The + or − designations refer to the detection of ovalbumin.

the genes cloned as *Bam*HI fragments. The ovalbumin cDNA clone (pOV230) has the entire ovalbumin coding sequences within a *Taq*I fragment (2) which extends from within the leader sequence of the ovalbumin mRNA to sequences within the vector. In the genomic DNA, the *Taq*I site in the leader sequence is in the first exon (17), and there are no more *Taq*I sites in the ovalbumin gene. Thus the entire genomic ovalbumin gene was carried on one *Taq*I fragment of approximately 7.5 kilobases (Fig. 1b). An especially desirable feature of these two fragments is that the *Taq*I site is downstream from the transcriptional initiation site and upstream from the translational initiation site. This feature made the *Taq*I fragments ideal for ligation to the Pα fragment which ends between its natural transcriptional and translational initiation sites. These *Taq*I fragments were converted to *Bam*HI fragments by cloning into the *Bam*HI site of pBR322 after the treatment of fragment and vector with T4 DNA polymerase (19). These *Bam*HI fragments were then cloned into pRB334 and pRB335 to give the plasmids listed in Table 1 and diagrammed in Fig. 1c. For analysis of the expression of the chimeric ovalbumin plasmids, $Ltk^-$ cells were converted to $TK^+$ phenotype, as described above with the plasmids depicted in Fig. 1 and described in Table 1. The $LVtk^+$ lines were designated according to the number of the plasmid used for conversion (L351, L352, L353, L354, L355).

**Production of ovalbumin.** $^{14}C$-labeled ovalbumin obtained from New England Nuclear formed three major and a minor band on electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, and all of these bands were present in the autoradiograms of solubilized and electrophoretically separated precipitates of the labeled ovalbumin with antiovalbumin serum. Analysis of the cell lines with antiovalbumin antibody by immune precipitation showed the presence of an ovalbumin-reactive antigen in L352 and L353 in which Pα was upstream from and in the same orientation as the ovalbumin genomic DNA (Fig. 1 and 2). The antigen was not made in any of the other cell lines derived in this study, including L354, in which the genomic DNA is in the opposite orientation to the HSV Pα (Fig. 2). The antigen precipitated by the antiovalbumin antibody migrated slightly faster than the second, major ovalbumin band in the standard. Evidence that this antigen is related to ovalbumin is based on two observations. First, it was absent in mock precipitates obtained by mixing the preimmune serum with cell lysates (data not shown). Second, the radioactive antigen in the precipitates was competed out with unlabeled ovalbumin, but not with equivalent amounts of unlabeled RNase (Fig. 2).

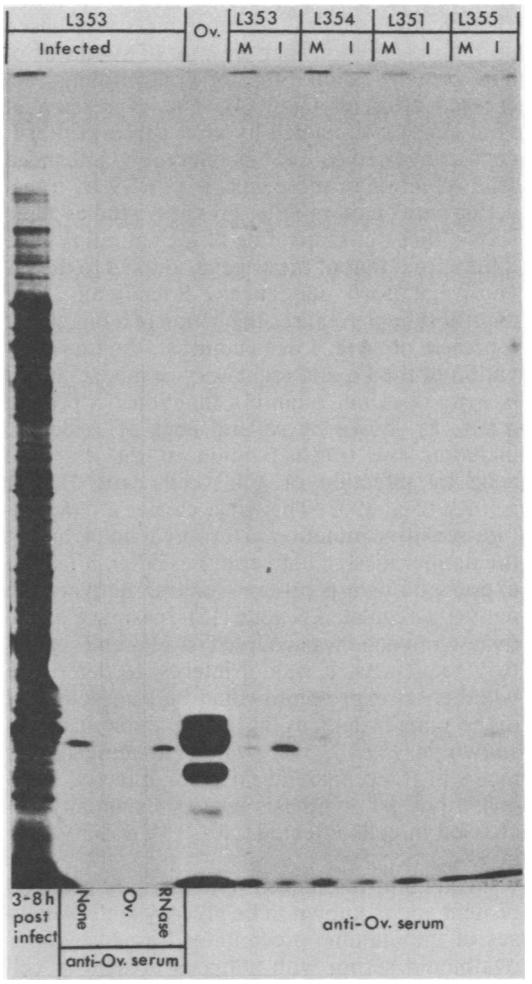


FIG. 2. Detection and identification of the antigen reactive with antiovalbumin serum produced at 39°C in infected or mock-infected cell lines L353, L354, L351, and L355. The figure shows autoradiograms of labeled proteins electrophoretically separated in sodium dodecyl sulfate-9.25% polyacrylamide gels. The left lane shows the electrophoretic profiles of the proteins labeled with [$^{35}S$]methionine from 3 to 8 h postinfection of L353 cells with HSV-1 *ts*502Δ305. The procedure for the preparation of the cell lysate is described in the text. The next three lanes show the electrophoretic mobility of the precipitate obtained by mixing the cell lysate shown in the left lane with antiovalbumin serum in the presence of no additive (none), in the presence of 50 μg of unlabeled ovalbumin (Ov), and in the presence of 50 μg of RNase obtained from Sigma. The other lanes in this autoradiogram show the electrophoretic mobility of the precipitates formed with [$^{14}C$]ovalbumin obtained from New England Nuclear Corp. and lysates of cell lines L353, L354, L351, and L355 labeled from 3 to 8 h postinfection (I) or mock infected (M).

A major criterion for discrimination between HSV and the cellular proteins used in the initial identification of viral gene products was based on the observation that the rate of synthesis of host proteins, unlike that of viral proteins, decreased after infection (4). The expression of viral genes exemplified by viral *tk* retained in L cells converted to $TK^+$ phenotype is enhanced after infection of these cells with HSV by transacting viral factors (10). Previous studies have shown that transactivation of the natural *tk* gene differs from that of the *tk* gene coupled to the Pα and regulatory sequences. Specifically, the natural *tk* gene requires the action of a functional α protein no. 4 (8, 15). In contrast, the transactivation of the Pα and regulatory sequence-linked *tk* gene does not require a functional α protein no. 4, as shown by several lines of evidence, including the transactivation of the chimeric gene by infection of $Vtk^+$ cells with HSV-1 *ts*502Δ305 at 39°C. This virus carries a temperature-sensitive mutation in α protein no. 4, and at the nonpermissive temperature (39°C), it fails to express its own β proteins or transactivate the native, β-regulated *tk* gene (15). Inasmuch as the ovalbumin gene was coupled to a Pα and regulatory sequences, it was of interest to determine whether its expression would be transactivated in the same fashion as that of the chimeric *tk*. As shown in Fig. 2, the ovalbumin antigen was made in trace amounts in mock-infected L353 cells, but its synthesis was substantially increased in cells infected with HSV-1 *ts*502Δ305 at 39°C.

Ovalbumin in chicken oviducts is a secreted protein and is known to be glycosylated. Analyses of the immune precipitates formed by antiovalbumin serum with antigens present in cell lysates and in extracellular media showed that the extracellular media of L352 (Fig. 3) and L353 cells (data not shown) contained an antigen which reacted with antiovalbumin immune serum but not with normal, preimmune rabbit serum. This antigen was not present in the extracellular medium of any of the other cell lines. The precipitated antigens comigrated with the major ovalbumin band formed by the standard $^{14}C$-labeled commercial ovalbumin preparation.

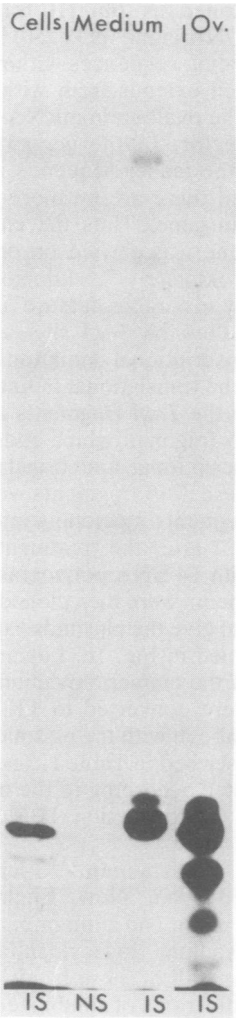


FIG. 3. Autoradiographic images of electrophoretically separated [$^{35}S$]methionine-labeled antigens precipitated with antiovalbumin serum from L352-infected cell lysates and from extracellular medium. The cells grown in 25-$cm^2$ Corning flasks were infected with HSV-1 *ts*502Δ305 at 39°C and labeled from 3 to 8 h postinfection with one third of the amounts of medium described in the text. The medium was lyophilized and dissolved in 200 μl of water. A 100-μl amount of concentrated medium was mixed either with 50 μl of preimmune rabbit serum (NS) or with 50 μl of antiovalbumin serum (IS). The immune complexes were precipitated as described in the text. One half of the solubilized immune precipitate was subjected to electrophoresis on a sodium dodecyl sulfate-9.25% polyacrylamide gel. The gel was processed for fluorography. The fluorogram was developed after 1 day of exposure. Identical results were obtained with cell line L353 (data not shown). Overexposed fluorograms failed to show secretion of ovalbumin antigens from infected or mock-infected L354, L351, and L355 cell lines (data not shown).

## DISCUSSION

This paper describes a selection procedure that allows the introduction and expression of eucaryotic genes in eucaryotic cells under the control of a promoter that can be regulated. Specifically, we constructed a DNA fragment consisting of the *tk* gene and a Pα available for fusion with the coding sequences of any eucaryotic gene. The purpose of the *tk* gene is to allow for selection of the cells that had incorporated

the chimeric DNA fragment. The function of the Pα is to replace the natural promoter, which may require special conditions for its expression. An additional advantage of the Pα is that the expression of the gene linked to it can be enhanced (15). In this paper we demonstrate that the chicken oviduct ovalbumin gene linked to the Pα is fully expressed and that the product is made and secreted into the extracellular medium, as predicted from its known function in the hen oviduct. We also demonstrate that the expression of the gene is under viral control inasmuch as the ovalbumin is enhanced after superinfection, as would be predicted from studies published previously (15) and from the observation that when the ovalbumin gene is inverted relative to the Pα, no expression was obtained. The significance of the findings are as follows.

(i) The observation that the ovalbumin gene when linked to the HSV-1 Pα is expressed but is regulated as a viral gene reinforces the conclusions presented in the earlier paper that regulatory signals are contained upstream from the 5′ terminus of the coding sequences of the gene. Implicit in this conclusion is that genes with very weak promoters or which require highly specialized regulatory signals for their expression may be made to be expressed by the simple procedure of substituting the natural promoter regulatory region with a stronger promoter with known regulatory features.

(ii) Our results with the cDNA fused with the Pα in both direct and inverted orientations are consistent with other attempts to express in higher eucaryotic cells cDNA of a gene that is naturally spliced (3, 20).

(iii) As noted above, we have described a useful system for introducing test genes to identify and study their products, which in some instances may not be expressed in the absence of factors which would normally regulate their expression.

The system we have described consists of two components. First, a covalently linked *tk* gene allows for efficient and unambiguous selection of cells which carry the gene to be studied. The second component is the α protein no. 4 promoter, which can readily be fused to other structural genes.

The α protein no. 4 promoter cut at the *Bam*HI cleavage site has now been fused to two very different genes: the HSV *tk* gene (15), which has no introns, and the chicken ovalbumin gene, which has seven introns. In both cases synthesis of the gene product was readily detected after the fusion of the α protein no. 4 promoter and the gene at the most convenient restriction enzyme cleavage site. It seems likely that the HSV α protein no. 4 promoter is a general tool that can be used to obtain efficient expression of spliced and unspliced genes in mammalian cells.

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## LITERATURE CITED

1. **Breatnach, R., N. Mantei, and P. Chambon.** 1980. Correct splicing of a chicken ovalbumin gene transcript in mouse L cells. Proc. Natl. Acad. Sci. U.S.A. **77**:740–744.
2. **Fraser, T. H., and B. J. Bruce.** 1978. Chicken ovalbumin is synthesized and secreted by *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **75**:5936–5940.
3. **Hamer, D. H., and P. Leder.** 1979. Splicing and the formation of stable RNA. Cell **18**:1299–1302.
4. **Honess, R. W., and B. Roizman.** 1973. Proteins specified by herpes simplex virus. XI. Identification and relative molar rates of synthesis of structural and nonstructural herpes virus polypeptides in the infected cell. J. Virol. **12**:1347–1365.
5. **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.
6. **Honess, R. W., and B. Roizman.** 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. Proc. Natl. Acad. Sci. U.S.A. **72**:1276–1280.
7. **Kit, S., D. R. Dubbs, L. J. Piekarski, and T. C. Hsu.** 1963. Deletion of thymidine kinase activity from L cells resistant to bromodeoxyuridine. Exp. Cell Res. **31**:297–312.
8. **Kit, S., D. L. Dubbs, and P. A. Schaffer.** 1978. Thymidine kinase activity of biochemically transformed mouse cells after superinfection by thymidine kinase-negative, temperature-sensitive, herpes simplex virus mutants. Virology **85**:456–463.
9. **Lai, E. C., S. L. C. Woo, M. E. Bordelon-Riser, T. H. Fraser, and B. W. O'Malley.** 1980. Ovalbumin is synthesized in mouse cells transformed with the natural chicken ovalbumin gene. Proc. Natl. Acad. Sci. U.S.A. **77**:244–248.
10. **Leiden, J. M., R. Buttyan, and P. G. Spear.** 1976. Herpes simplex virus gene expression in transformed cells. I. Regulation of the viral thymidine kinase gene in transformed L cells by products of superinfecting virus. J. Virol. **20**:413–420.
11. **Mackem, S., and B. Roizman.** 1980. Regulation of herpesvirus macromolecular synthesis: transcription-initiation sites and domains of α genes. Proc. Natl. Acad. Sci. U.S.A. **77**:7122–7126.
12. **McReynolds, L. A., J. F. Cattaral, and B. W. O'Malley.** 1977. The ovalbumin gene: cloning of a complete ds-cDNA in a bacterial plasmid. Gene **2**:217–231.
13. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. J. Virol. **26**:389–410.
14. **Post, L. E., A. J. Conley, E. S. Mocarski, and B. Roizman.** 1980. Cloning of reiterated and non-reiterated herpes simplex virus 1 sequences as BamHI fragments. Proc. Natl. Acad. Sci. U.S.A. **77**:4201–4205.

15. **Post, L. E., S. Mackem, and B. Roizman.** 1981. Regulation of α genes of herpes simplex virus: expression of chimeric genes produced by fusion of thymidine kinase with α gene promoters. Cell **24**:555–565.
16. **Roizman, B.** 1979. The organization of the herpes simplex virus genomes. Annu. Rev. Genet. **13**:25–57.
17. **Roop, D. R., M. Tsai, and B. W. O'Malley.** 1980. Definition of the 5′ and 3′ ends of transcripts of the ovalbumin gene. Cell **19**:63–68.
18. **Subramani, S., R. Mulligan, and P. Berg.** 1981. Expression of the mouse dihydrofolate reductase complementary deoxyribonucleic acid in simian virus 40 vectors. Mol. Cell. Biol. **1**:854–864.
19. **Wartell, R. M., and W. S. Reznikoff.** 1980. Cloning DNA restriction endonuclease fragments with protruding single-stranded ends. Gene **9**:307–314.
20. **Wickens, M. P., S. Woo, B. W. O'Malley, and J. B. Gurdon.** 1980. Expression of a chicken chromosomal ovalbumin gene injected into frog oocyte nuclei. Nature (London) **285**:628–634.
21. **Wigler, M., S. Silverstein, L. Lee, A. Pellicer, Y. Cheng, and R. Axel.** 1977. Transfer of purified herpes virus thymidine kinase gene to cultured mouse cells. Cell **11**:223–232.
22. **Wigler, M., R. Sweet, G. K. Sim, B. Wold, A. Pellicer, E. Lacy, T. Maniatis, S. Silverstein, and R. Axel.** 1979. Transformation of mammalian cells with genes from prokaryotes and eukaryotes. Cell **16**:777–785.