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Biochemistry

Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase

(eukaryotic expression vector/genetic engineering/transcriptional signals/poxvirus)

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ABSTRACT **DNA coding for bacteriophage T7 RNA polymerase was ligated to a vaccinia virus transcriptional promoter and integrated within the vaccinia virus genome. The recombinant vaccinia virus retained infectivity and stably expressed T7 RNA polymerase in mammalian cells. Target genes were constructed by inserting DNA segments that code for β-galactosidase or chloramphenicol acetyltransferase into a plasmid with bacteriophage T7 promoter and terminator regions. When cells were infected with the recombinant vaccinia virus and transfected with plasmids containing the target genes, the latter were expressed at high levels. Chloramphenicol acetyltransferase activity was 400–600 times greater than that observed with conventional mammalian transient-expression systems regulated either by the enhancer and promoter regions of the Rous sarcoma virus long terminal repeat or by the simian virus 40 early region. The vaccinia/T7 hybrid virus forms the basis of a simple, rapid, widely applicable, and efficient mammalian expression system.**

Recombinant DNA technology has made it possible to develop molecular cloning vectors that allow expression of heterologous genes in prokaryotic and eukaryotic cells. Bacterial systems provide important advantages, such as ease of use and high expression, but impose a number of limitations for synthesis of eukaryotic proteins. In particular, correct folding, proteolytic processing, glycosylation, secretion, and subunit assembly may not occur or may occur incorrectly in bacteria. For these reasons, eukaryotic cells are preferred for expression of eukaryotic genes. We considered that a hybrid vector system that would utilize the highly efficient bacteriophage T7 RNA polymerase in a eukaryotic environment might have significant advantages. T7 RNA polymerase is a single-subunit enzyme, with high catalytic activity and strict promoter specificity (1, 2), that has already found wide application for *in vitro* synthesis of RNA and as the basis for high-level gene expression systems in *Escherichia coli* (3, 4). One potential problem with use of a prokaryotic RNA polymerase in a eukaryotic cell, however, is the requirement for mRNA to be processed, capped, methylated, and polyadenylylated. It would seem that at the very least, either the T7 RNA polymerase would have to be transported from its site of synthesis in the cytoplasm into the nucleus of the cell or else the eukaryotic RNA-modifying enzymes would have to function in the cytoplasm. The strategy explored here involves the introduction of the T7 RNA polymerase gene into a cytoplasmic virus.

Poxviruses comprise a widespread family of DNA viruses that transcribe and replicate their DNA in the cytoplasm. Vaccinia virus, the prototypal member of this family, has a large linear double-stranded DNA genome that encodes an entire transcription system including RNA polymerase, capping/methylating enzymes, and poly(A) polymerase (5). Additional advantages of vaccinia virus include its large capacity for foreign DNA (6), genome stability, and wide vertebrate host range. These characteristics have been utilized in the development of vaccinia virus as a eukaryotic expression vector (7, 8). In this communication, we describe the construction of an infectious recombinant vaccinia virus that synthesizes T7 RNA polymerase and the use of this recombinant in a simple, widely applicable, and highly efficient transient-expression system.

MATERIALS AND METHODS

Enzymes. Enzymes were supplied by the companies indicated and used in accordance with their instructions. Restriction endonucleases were from Bethesda Research Laboratories, New England BioLabs, or Boehringer Mannheim. The Klenow fragment of DNA polymerase I and T4 DNA ligase were from New England Biolabs. Calf intestinal alkaline phosphatase was obtained from Boehringer Mannheim.

Virus and Cells. Vaccinia virus (strain WR) was originally obtained from the American Type Culture Collection, replicated in HeLa cells, and purified as reported previously (9). HeLa cells were grown in Eagle's minimal essential medium supplemented with 5% horse serum. Human TK^- 143 cells (10) were grown in Eagle's medium with 10% fetal bovine serum (FBS) and 25 μg of 5-bromodeoxyuridine (BrdUrd) per ml. CV-1 monkey kidney cells were grown in Dulbecco's modified Eagle's medium containing 10% FBS.

Plasmids. pGS53 contains the vaccinia virus *P7.5* promoter, unique *Bam*HI and *Sma* I restriction sites for insertion of foreign genes, and thymidine kinase (TK) flanking sequences. It differs from the previously described pGS20 vector (11), principally in the use of pUC13 (12) plasmid instead of pBR328 (13) and TK flanking sequences derived from the Wyeth strain of vaccinia virus instead of the WR strain.

Preparation and Cloning of DNA. Recombinant plasmids were constructed and used to transform bacteria following the methods of Maniatis *et al.* (14). Plasmids were prepared by the alkaline $NaDodSO_4$ method as described by Birnboim and Doly (15) and purified by CsCl/ethidium bromide equilibrium density gradient centrifugation. Plasmids were routinely checked by agarose gel electrophoresis to ensure that the majority of DNA was in the supercoiled configuration. DNA fragments were isolated from low-melting-point agarose gels, following the Elutip-d (Schleicher & Schuell) method. DNA was extracted from purified virus as described (9).

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Abbreviations: TK, thymidine kinase; CAT, chloramphenicol acetyltransferase; *cat*, gene encoding CAT; FBS, fetal bovine serum; BrdUrd, 5-bromodeoxyuridine; kbp, kilobase pair(s).

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Isolation of Recombinant Virus. CV-1 cells were infected with 0.05 plaque-forming units of wild-type vaccinia virus per cell and transfected with calcium phosphate-precipitated plasmid as described (9). Recombinant viruses formed by homologous recombination of the foreign gene into the TK locus were selected by plaque assay on TK^- 143 cell monolayers in the presence of BrdUrd (25 μg/ml). TK^- recombinant virus plaques were distinguished from spontaneous TK^- mutant virus by DNA·DNA dot blot hybridization (9). After two consecutive plaque purifications, recombinant virus was amplified by infecting TK^- 143 cell monolayers in the presence of BrdUrd, and then large stocks were made in HeLa cells without selection.

Transient Assay Conditions. For standard assays (16), CV-1 cells were grown to 80% confluence in 25-cm^2 flasks ($\approx 2.5 \times 10^6$ cells) and infected with either purified wild-type or recombinant vaccinia virus at a multiplicity of 30 plaque-forming units per cell. The virus was allowed to adsorb for 30 min at 37°C with occasional rocking of the plate. The inoculum was then removed and 1 ml of calcium phosphate-precipitated DNA (10 μg of recombinant plasmid and 10 μg of salmon sperm DNA) was added. After 30 min at room temperature, fresh medium containing 2.5% FBS was added and the flask was incubated at 37°C. Cells were harvested at 24 hr after infection and suspended in the indicated buffer.

To compare the levels of expression obtained with the vaccinia/T7 and more conventional transient systems, care was taken to follow the conditions described by Gorman *et al.* (17). On the day prior to transfection, low passage-number (<10 passages) CV-1 cells were plated at a density of 2.5×10^6 cells per 25-cm^2 flask and were refed with fresh medium containing 10% FBS at 3 hr before transfection. A 2-min glycerol shock was performed at 3.5 hr after transfection, and cell lysates were prepared at 48 hr.

T7 RNA Polymerase Assay. Transfected or infected CV-1 cells ($\approx 2.5 \times 10^6$) were resuspended in 0.25 ml of 0.01 M Tris·HCl, pH 7.6/0.01 M NaCl/1.5 mM $MgCl_2$ and Dounce homogenized. After centrifugation, 1.5 μl of cytoplasmic supernatant was assayed for T7 RNA polymerase at 37°C in 25-μl mixtures containing 40 mM Tris·HCl (pH 8.0); 8 mM $MgCl_2$; 2 mM spermidine; 50 mM NaCl; 1 mM each ATP, CTP, and UTP; 5 μM [α-^{32}P]GTP; 30 mM dithiothreitol; 1 μg of pTFLZ-1 template; and 40 units of RNasin (Promega Biotec, Madison, WI). At various times the reactions were stopped by addition of 0.05 ml of 50 mM EDTA/0.1% $NaDodSO_4$ containing 100 μg of proteinase K per ml, followed by incubation for 60 min at 37°C. Samples were applied to DE-81 paper (Whatman) and washed thrice for 5 min in 5% Na_2HPO_4, once with water, and once with 95% ethanol. The samples were dried, and radioactivity was measured in a scintillation spectrophotometer.

β-Galactosidase Assay. Infected or transfected CV-1 cells ($\approx 2.5 \times 10^6$) were suspended in 1 ml of phosphate-buffered saline, frozen and thawed three times, and dispersed by sonication. The cellular debris was removed by centrifugation and the supernatant was assayed for β-galactosidase activity, using *o*-nitrophenyl β-D-galactopyranoside as described by Miller (18). After 30 min at 37°C, the reaction was stopped by addition of 1 M Ca_2CO_3 and the yellow color was quantitated by measuring absorbance at 420 nm. β-Galactosidase activity was recorded as nmol of *o*-nitrophenol produced in 30 min per 2.5×10^6 cells.

Chloramphenicol Acetyltransferase (CAT) Assay. Infected or transfected CV-1 cells ($\approx 2.5 \times 10^6$) were suspended in 0.2 ml of 0.25 M Tris·HCl (pH 7.5). After three freeze–thaw cycles, the lysates were dispersed by sonication, and the suspensions were assayed for enzyme activity as described by Mackett *et al.* (11).

RESULTS

Construction of a Recombinant Vaccinia Virus Containing the Bacteriophage T7 RNA Polymerase Gene. Procedures for the insertion and expression of foreign genes in vaccinia virus have been described in detail (9, 11). Vaccinia virus promoters are required to regulate transcription of the DNA which is introduced by homologous recombination into the 185-kilobase-pair (kbp) linear double-stranded DNA genome. To facilitate the use of vaccinia virus as a vector, a series of plasmids were made that contain a vaccinia virus promoter, restriction endonuclease sites for insertion of foreign DNA, and flanking vaccinia TK sequences to direct recombination into the TK locus of the genome (9, 11). For this study, we used the plasmid pGS53, which contains a promoter termed *P7.5* with early and late regulatory signals (19), to permit continuous expression of foreign genes. A 2.65-kbp DNA fragment, containing the entire T7 gene *1* coding region for T7 RNA polymerase, was excised with *Bam*HI from plasmid pAR1173 (20) and inserted into the unique *Bam*HI site of pGS53 (Fig. 1). A plasmid designated pTF7-3, with the vaccinia promoter and T7 RNA polymerase in proper orientation, was isolated from transformed *E. coli*. Plasmid pTF7-3 was used to transfect cells that were infected with vaccinia virus, and then TK^- recombinant virus plaques were selected. Correct insertion of the T7 RNA polymerase gene in the

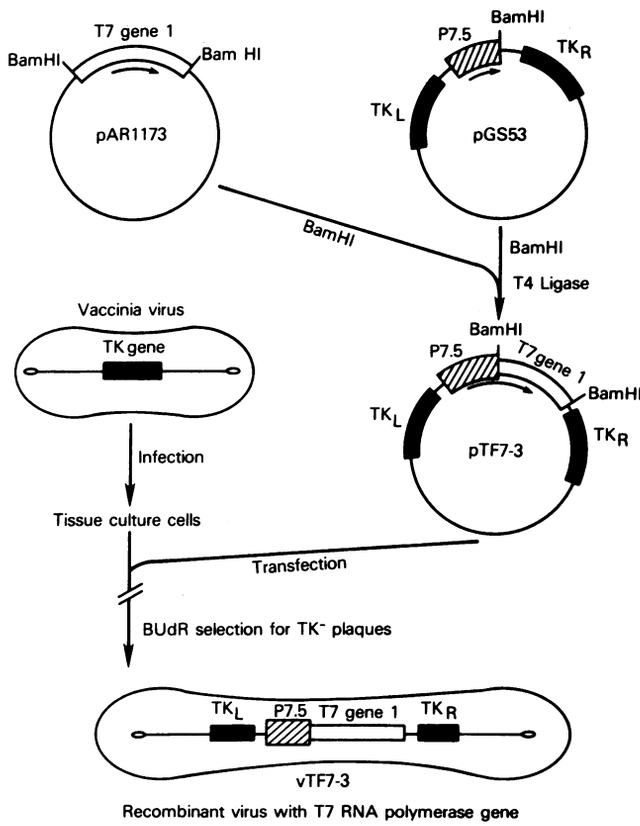

FIG. 1. Insertion of bacteriophage T7 gene *1* into the genome of vaccinia virus. A 2.65-kbp *Bam*HI fragment containing T7 gene *1* was excised from pAR1173 and inserted into the unique *Bam*HI site of pGS53 to form pTF7-3. In the latter plasmid, the coding sequence for T7 RNA polymerase is downstream of the vaccinia *P7.5* promoter and the chimeric gene is flanked by the left (TK_L) and right (TK_R) vaccinia TK gene sequences. DNA segments are not drawn to scale. CV-1 cells were infected with vaccinia virus and transfected with pTF7-3. After 48 hr, the cells were harvested and the virus was plaqued on TK^- cells in the presence of BrdUrd ("BUdR"). Virus plaques were amplified and screened by dot blot hybridization to T7 gene *1* DNA.

genome of vTF7-3 was confirmed by DNA blot hybridization. Either plasmid pTF7-3 or recombinant virus vTF7-3 was used for expression studies.

Expression of T7 RNA Polymerase in Mammalian Cells. Previous studies (16) indicated that plasmids containing genes under control of a vaccinia virus promoter are specifically transcribed in cells infected with vaccinia virus. Similarly, we wished to determine whether T7 RNA polymerase would be expressed when vaccinia virus-infected cells were transfected with the plasmid pTF7-3. T7 RNA polymerase activity in cell lysates was assayed using a DNA template containing a T7 promoter. Control experiments established that RNA polymerase activity measured with this template was not increased after vaccinia virus infection (Fig. 2). When vaccinia virus-infected cells were also transfected with pTF7-3, however, a significant increase in activity was observed (Fig. 2). This activity was not detected when a similar DNA template lacking the T7 promoter was used in the enzyme assay. Additional experiments demonstrated that T7 RNA polymerase activity was not detected when uninfected cells were transfected with pTF7-3 or when infected cells were transfected with a plasmid containing the T7 gene *1* without a vaccinia promoter (data not shown).

Next, we wished to determine whether higher levels of T7 RNA polymerase would be expressed when the T7 gene *1*, under control of a vaccinia promoter, was integrated into the vaccinia virus genome. As shown in Fig. 2, vTF7-3-infected cell extracts contained several times more T7 RNA polymerase activity than was present in cells that had been transfected with pTF7-3 in the presence of wild-type vaccinia virus. This quantitative difference between recombinant virus and transient-expression systems was consistent with previous observations (16).

Construction of Plasmids Containing Target Genes with T7 Promoters. To determine whether bacteriophage T7 RNA polymerase made under control of vaccinia virus can function in mammalian cells, we constructed plasmids containing target genes flanked by T7 promoter and termination regulatory elements. Plasmid pAR2529 (A. H. Rosenberg, J. J. Dunn, and F.W.S., unpublished work) contains the T7 gene *10* (*φ10*) promoter separated by a unique *Bam*HI site from the T7 terminator *Tφ*, which has a potential stem–loop structure followed by a run of thymidylate residues (2). As targets, we chose the *E. coli* β-galactosidase gene (*lacZ*) and the CAT gene (*cat*) derived from the Tn*9* transposon. These genes are ideal for expression systems because simple and quantitative assays are available for the enzyme products and there is no detectable background activity in mammalian cells (11, 17, 21). The *lacZ* or *cat* gene, each with an associated ATG translation initiation codon, was inserted into the unique *Bam*HI site of pAR2529 (Fig. 3). Plasmids with *lacZ* and *cat* in the correct orientation were designated pTF7LZ-1 and pTF7CAT-1, respectively.

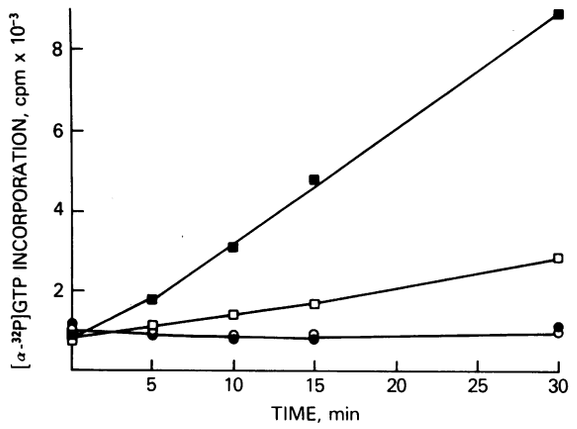

FIG. 2. Synthesis of T7 RNA polymerase. CV-1 cells were infected and transfected as described in *Materials and Methods*. After 24 hr, extracts from uninfected cells (○), cells infected with vaccinia virus (●), cells infected with vaccinia virus and transfected with pTF7-3 (□), or cells infected with vTF7-3 (■) were prepared and assayed for T7 RNA polymerase. Incorporation of [α-32P]GTP into RNA that bound to DEAE-cellulose filters was measured.

Transient Expression of β-Galactosidase. Above, we showed that cells infected with vaccinia virus and transfected with a plasmid containing the T7 gene *1* under control of a vaccinia virus promoter synthesized T7 RNA polymerase. We now asked whether vaccinia virus-infected cells would express β-galactosidase if they were transfected with plasmids containing the T7 gene *1* under control of a vaccinia promoter and the *lacZ* gene under control of a T7 promoter. Transient expression would thus depend on vaccinia virus-regulated synthesis of T7 RNA polymerase, the intracellular functioning of the T7 RNA polymerase, the production of translatable mRNA from a T7 promoter, and the synthesis of a prokaryotic enzyme. As shown in Table 1, β-galactosidase was detected in cell lysates. Omission of either vaccinia virus or the plasmid containing the T7 RNA polymerase gene prevented expression of β-galactosidase. Negative results also were obtained with either the T7 gene *1* or the *lacZ* gene was oriented oppositely with respect to the vaccinia or T7 promoter, respectively (data not shown).

In the above experiments, both the T7 gene *1* and the *lacZ* gene were transcribed from plasmids. Since more T7 RNA polymerase is made when gene *1* is integrated into vaccinia virus (Fig. 1), we anticipated that higher amounts of β-galactosidase would be produced if cells were infected with recombinant vaccinia virus vTF7-3 and then were transfected with the *lacZ* plasmid pTFLZ-1. As shown in Table 1, more than twice as much β-galactosidase was made when T7 RNA polymerase was expressed by a recombinant virus than when it was expressed from a plasmid.

We also wished to compare the transient expression of *lacZ* under control of T7 and vaccinia virus promoters. The vaccinia virus promoter used, *P7.5*, was the same as that regulating expression of T7 gene *1*. When cells were infected with vaccinia virus and transfected with the plasmid containing *lacZ* under control of the vaccinia promoter, β-galactosidase activity was about 5% of that obtained with the vaccinia/T7 transient system.

An additional comparison was made with a recombinant vaccinia virus that has the *lacZ* gene, under control of the *P7.5* promoter, inserted into the vaccinia virus genome. In this case, there was no need to transfect with a plasmid containing a target gene. Nevertheless, a mock transfection with pUC18 vector was performed in order to keep conditions equivalent. The data (Table 1) indicated that *lacZ* expression was actually about 5-fold higher with the vaccinia/T7 transient system than with the *lacZ* recombinant vaccinia virus. However, if optimal conditions for recombinant virus infection are used (i.e., transfection with calcium phosphate-precipitated DNA is not performed) then the *lacZ* recombinant expresses β-galactosidase at a level that is 2–3 times higher than with the vaccinia/T7 transient transfection system (data not shown).

Transient Expression of CAT. We considered it most important to compare the vaccinia/T7 transient expression system with more conventional ones used in mammalian cells. Since *cat* is the most common target gene used for comparison of expression levels (17, 22), experiments similar to those performed with *lacZ* were repeated (Table 1). As in the case of *lacZ*, we found that transient expression of *cat* from the T7 promoter was higher when the T7 gene *1* was integrated into vaccinia virus than when it was cotransfected on a second plasmid and that expression was much higher in

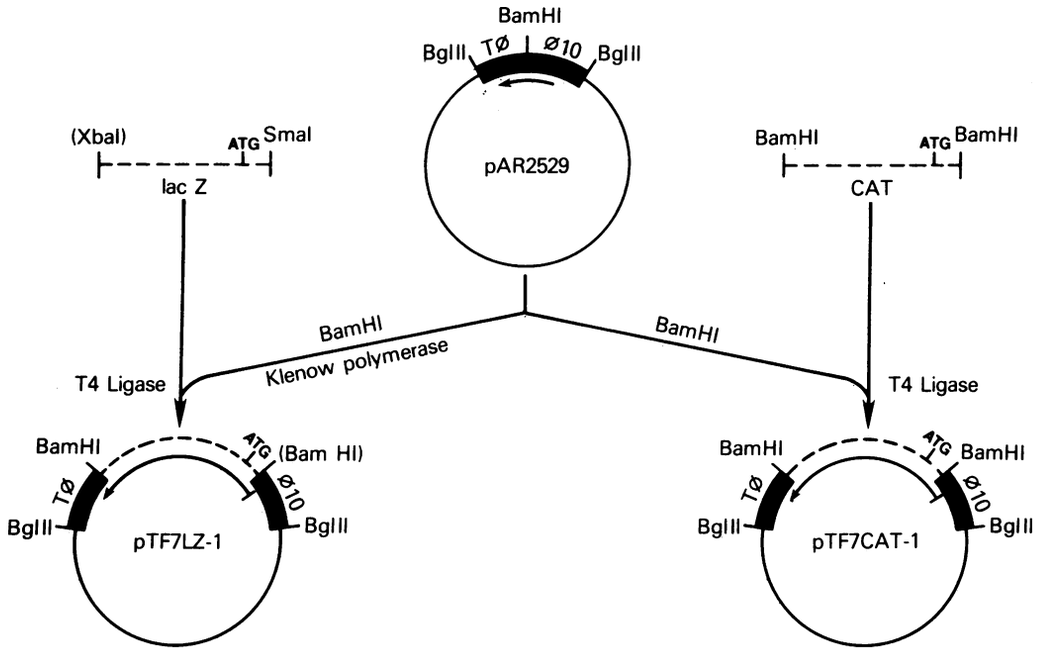

FIG. 3. Construction of plasmids containing target genes flanked by T7 promoter and terminator sequences. A 3.2-kbp DNA segment containing the *lacZ* gene with translation and termination codons was obtained by cleavage of pWS61 (provided by A. Majmdar, National Institutes of Health) with *Xba* I, filling in the staggered end with the Klenow fragment of DNA polymerase I and dNTPs, and cleaving with *Sma* I. The fragment was then blunt-end-ligated to pAR2529 that had been cleaved with *Bam*HI and treated with Klenow fragment. The resulting plasmid, pTF7LZ-1, has the β-galactosidase coding sequence flanked by the T7 *ϕ10* promoter and *Tϕ* terminator. Similarly, a 0.7-kbp *Bam*HI fragment from pGS30 (11) containing the CAT gene was ligated to *Bam*HI-cleaved pAR2529 to form pTF7CAT-1.

the vaccinia virus/T7 hybrid system than when the *cat* gene was expressed directly from the vaccinia promoter.

For further comparison, CV-1 cells were transfected with pRSVcat or pSV2cat, which contain the same *cat* DNA fragment derived from the Tn*9* transposon as pTF7CAT-1 and either the enhancer and promoter from the Rous sarcoma virus (RSV) long terminal repeat or the simian virus 40 (SV40) early region (17, 22). In order not to prejudice the results in favor of the vaccinia/T7 system, previously described experimental conditions for expression of pSV2cat and pRSVcat were employed. Thus, low-passage CV-1 cells and glycerol shock were used, and cell lysates were made at 48 hr after transfection. The extracts were diluted and tested for CAT activity. As illustrated by the autoradiogram in Fig. 4, several hundred times more CAT was made in the vaccinia/T7 system than with either pSV2cat or pRSVcat. More quantitative results, obtained by scintillation counting, indicated that 4560 nmol of chloramphenicol was acetylated per 2.5×10^6 cells using the vaccinia/T7 system, compared to

Table 1. Transient expression of β-galactosidase and CAT

Vaccinia virus	Plasmid 1		Plasmid 2		Expression	
	Promoter	Gene	Promoter	Gene	LacZ	CAT
WT	VV	T7 *1*	T7	*lacZ*	1100	
–	VV	T7 *1*	T7	*lacZ*	0	
WT			T7	*lacZ*	0	
T7 gene *1*			T7	*lacZ*	2406	
WT			VV	*lacZ*	137	
lacZ	(control plasmid)				480	
WT	VV	T7 *1*	T7	*cat*		1650
–	VV	T7 *1*	T7	*cat*		0
WT			T7	*cat*		0
T7 gene *1*			T7	*cat*		4330
WT			VV	*cat*		300
cat	(control plasmid)					1430

CV-1 cells were uninfected (–) or were infected with wild-type (WT) or recombinant (T7 gene *1*, *lacZ*, or *cat*) vaccinia virus. The recombinant viruses had the foreign gene under control of the vaccinia virus *P7.5* promoter and inserted into the TK locus. Uninfected or infected cells were transfected with one or two plasmids containing either the T7 gene *1*, *lacZ*, or the *cat* gene under control of the vaccinia virus *P7.5* promoter (VV) or the T7 *ϕ10* promoter. When cells were infected with a recombinant vaccinia virus containing either *lacZ* or *cat*, transfection was carried with a control pUC18 plasmid. Cells were harvested after 24 hr and lysates were assayed for β-galactosidase (LacZ) or CAT. Expression is given as nmol of product formed in 30 min per 2.5×10^6 cells.

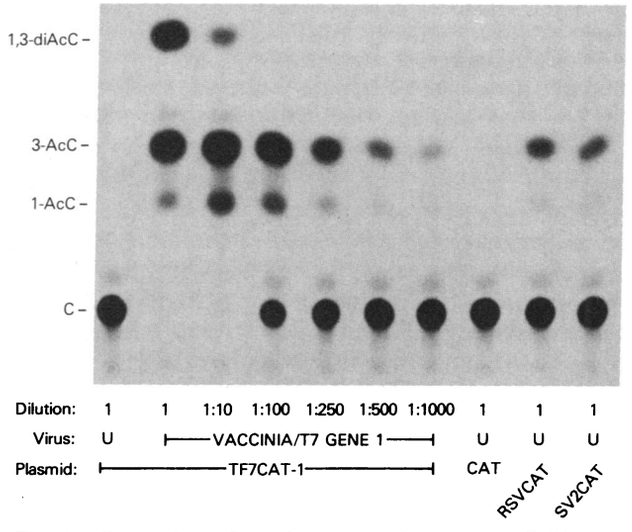

FIG. 4. Comparison of transient-expression systems. Cell lysates were prepared at 48 hr after infection with vTF7-3 (vaccinia/T7 gene *1*) and/or transfection with the indicated plasmid and assayed for CAT. (Virus "U" indicates uninfected cells.) Samples were spotted on a silica gel plate and chromatographed. An autoradiogram is shown, with the positions of chloramphenicol (C) and acetylated forms of chloramphenicol (AcC) indicated.

only 6.8 nmol with pSV2cat or 9.1 nmol with pRSVcat. This difference was even greater when glycerol shock was omitted and cells were lysed at 24 hr after transfection (data not shown).

DISCUSSION

Genetic engineering has been used extensively to express eukaryotic genes in prokaryotes and vice versa. Previously, however, this involved exchanges of coding sequences. The more radical transfer of a transcription system from a prokaryote to a eukaryote opens up new opportunities for regulating gene expression. In this study, we have integrated a functional bacteriophage RNA polymerase gene into a eukaryotic virus. Our choice of a single-subunit RNA polymerase with stringent promoter specificity and a cytoplasmic DNA virus that encodes its own RNA-modifying enzymes may have been critical to the success of the hybrid system for gene expression.

Transcription of the T7 RNA polymerase gene in vaccinia virus-infected cells is accomplished by the vaccinia RNA polymerase and is therefore dependent on the fusion of the bacteriophage gene to a vaccinia promoter. Expression of T7 RNA polymerase could be obtained either by transfecting vaccinia virus-infected cells with a plasmid containing the chimeric gene or by integrating the gene into a nonessential site within the genome of vaccinia virus. Recombinant viruses were stable, could be grown to high titer, and produced higher levels of T7 RNA polymerase than transfected plasmids.

Target genes chosen for expression by T7 RNA polymerase were inserted into a plasmid at a unique restriction site separating a T7 promoter from a T7 terminator. For these studies, the target genes (*lacZ* and *cat*) had associated translational initiation codons, but other plasmid vectors that supply the ATG and appropriate flanking nucleotides could be used for production of fusion proteins. The key step was to transfect these plasmids into cells that were infected with the vaccinia virus recombinant which expressed the T7 RNA polymerase gene. We compared the synthesis of β-galactosidase and CAT by the vaccinia/T7 hybrid system to that which occurred with a straight vaccinia transient-expression system (in which the target gene has a vaccinia promoter) and to a conventional transient-expression system (in which either the enhancer and promoter from the long terminal repeat of Rous sarcoma virus or the early region of simian virus 40 was used). The vaccinia/T7 system was 15- to 20-fold more efficient than the straight vaccinia system and 400- to 600-fold more efficient than the conventional system.

The efficiency of the vaccinia/T7 transient system, compared to that of more conventional ones, may be attributed to several factors. Since it is possible to infect tissue culture cells synchronously with vaccinia virus, all cells should have T7 RNA polymerase. Moreover, T7 RNA polymerase is a very active enzyme with a 5-fold faster elongation rate than that of *E. coli* RNA polymerase (1). Evidently the bacteriophage enzyme is able to function within the eukaryotic milieu. In addition, since the vaccinia virus RNA-modifying enzymes and, presumably, T7 RNA polymerase are localized in the cytoplasm, the transfected plasmid does not have to enter the nucleus for transcription and the mRNA produced does not have to be processed and transported back to the cytoplasm for translation. In this regard, a previous report (23) suggests that the transport of calcium phosphate-precipitated DNA into the nucleus may be a limiting factor in mammalian transient-expression systems.

Previous experience with vaccinia virus expression vectors should be directly applicable to this new system. For example, there is abundant evidence that eukaryotic proteins made in vaccinia virus-infected cells are properly processed, glycosylated, and transported to the plasma membrane (refs. 24 and 25 and refs. therein). In addition, because of the wide host range of vaccinia virus, a variety of vertebrate cells of mammalian and avian origin are suitable.

The T7 promoter is especially versatile because of its use for *in vitro* synthesis of translatable mRNA and in prokaryotic expression vectors (3, 4). Development of the vaccinia/T7 hybrid virus system makes it possible to use previous or slightly modified plasmid vectors for a third purpose: efficient expression of genes in eukaryotic cells. We have concentrated our initial efforts on developing the vaccinia/T7 system for analytical purposes because of the simplicity and potentially wide application provided by transient expression of target genes in plasmids. However, even higher levels of expression, which are more suitable for production purposes, can be achieved when both the T7 RNA polymerase gene and the target gene are carried by vaccinia virus vectors (T.R.F. and B.M., unpublished data).

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