Overproduction of the protein product of a nonselected foreign gene carried by an adenovirus vector

(thymidine kinase/expression vector/hybrid virus/helper function/simian virus 40 tumor antigen)

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ABSTRACT We have constructed a recombinant adenovirus that carries the herpes simplex virus type I gene for thymidine kinase (EC 2.7.1.21) and expresses thymidine kinase under control of adenovirus major late promoter. A DNA fragment carrying thymidine kinase coding sequences but lacking the thymidine kinase promoter was sandwiched between a piece of adenoviral DNA and simian virus 40 early DNA on a plasmid. The aligned fragment was then inserted into the adenoviral genome, replacing internal adenoviral DNA. Hybrid viruses carrying the thymidine kinase gene were obtained by selecting for viruses that express simian virus 40 tumor antigen (T antigen) in monkey cells. The thymidine kinase gene was positioned in the third segment of the adenovirus tripartite leader downstream from the major late promoter by *in vivo* DNA recombination between the duplicated adenoviral sequences present in the plasmid insert and the viral vector. Levels of thymidine kinase activity in human or monkey cells infected with this hybrid virus were several times higher than in cells infected with herpes simplex virus. Infected cells produced thymidine kinase protein at very high levels, similar to those found for adenovirus late major capsid proteins. The thymidine kinase protein represented 10% of the newly synthesized protein in late infected cells and accumulated to represent 1% of total cell protein under optimal conditions. This vector system offers a procedure by which a variety of gene products that are biologically active and properly modified can be produced at high levels in mammalian cells.

Several systems have been developed to express cloned genes of mammalian origin (1–3). The expression of these genes in mammalian cells produced sufficient mRNA, protein products, or both for analytical purposes—i.e., to detect the gene products and to investigate mechanisms of gene regulation. On the other hand, cloned genes were sometimes expressed at high levels when introduced into bacteria and yeast (4). Although the products synthesized are useful— e.g., to raise antisera—they do not always correspond to their mammalian counterparts. Such products would not be expected to have the posttranscriptional and posttranslational modifications seen in a higher eukaryote. Therefore, if a cloned gene could be expressed at high levels in mammalian cells, the abundant products would be a useful source for isolation and purification to elucidate their biochemical properties. We have been developing an adenovirus vector system to maximize the expression of foreign genes in mammalian cells and have succeeded in expressing simian virus 40 (SV40) tumor antigen (T antigen) at high levels (5–7). The T-antigen gene was a selected marker in these systems. In this report, we have developed this system further to apply it to any nonselected foreign gene.

Adenovirus has a double-stranded linear DNA genome of 36 kilobase pairs (kb) (8). In the late stage of infection, host protein synthesis is suppressed, and more than 90% of the proteins synthesized are encoded by the adenovirus genome. Nearly all of the late viral mRNAs are initiated at the strong major late promoter at 16.5 map units, and they share a tripartite leader sequence at their 5′ ends (8). We previously inserted the coding sequence of the SV40 T-antigen gene at various positions downstream from the major late promoter in adenovirus (7). Although the T-antigen mRNA was always synthesized efficiently in these constructions, T-antigen protein was synthesized at the highest levels in the hybrid virus AdSVR284, in which the T-antigen mRNA contained almost the entire tripartite leader sequence at its 5′ end. This suggests that to obtain high levels of expression, a nonselected foreign gene should be inserted at the same position as the SV40 T-antigen gene in AdSVR284—that is, in the middle of the third segment of the tripartite leader sequence. A foreign gene can be inserted at a predetermined position in adenovirus by a combination of *in vivo* and *in vitro* DNA recombination (6). Hybrid adenoviruses expressing SV40 T antigen can be selected for by their growth in monkey cells because T antigen provides a helper function (9), which overcomes the block to adenovirus growth in simian cells. If the foreign gene to be inserted into the adenovirus genome is first linked to a functional SV40 T-antigen gene, the hybrid virus carrying the foreign gene can be obtained by selecting for the viruses that express SV40 T-antigen helper function.

In this communication, we report experiments introducing the thymidine kinase (TK; ATP:thymidine 5′-phosphotransferase, EC 2.7.1.21) gene of herpes simplex virus (HSV) type I into this system. The *TK* gene was chosen as a nonselected foreign gene because its biological activity and the level of mRNA and protein produced could be measured easily. Enzymatically active TK protein is synthesized in hybrid-virus-infected cells at very high levels that are similar to those found for adenovirus late major capsid proteins.

MATERIALS AND METHODS

Construction of the Hybrid Adenovirus. The procedures for plasmid constructions and the conditions of cell culture were previously described (7, 10, 11). The procedures used to construct hybrid adenoviruses were essentially the same as described (5–7). Briefly, DNAs of the plasmids, pMY591 or pMY599, and the adenovirus 2/5 recombinant, 1 × 51, were digested with *Bam*HI and ligated with T4 DNA ligase. The ligation mixture was mixed with adenoviral 1 × 51 DNA, which served as a helper, and used to transfect 293 cells by the calcium phosphate coprecipitation method (12). After incubation at 37°C for 3 days, cells were collected and

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Abbreviations: SV40, simian virus 40; T antigen, tumor antigen; HSV, herpes simplex virus; TK, thymidine kinase; kb, kilobase pair(s); moi, multiplicity of infection; hyb, proportion of hybrid virus in a virus stock.

disrupted to produce virus lysates. The resulting hybrid viruses containing SV40 DNA were amplified by two cycles of infection in CV-1 monkey cells with adenovirus 1 × 51 as a helper, followed by plaque purification on CV-1 cells. Three to five plaques were chosen from each independent transfection experiment and again amplified twice in CV-1 cells along with helper viruses.

DNA and RNA Analysis. Virus DNA was isolated from infected HeLa cells by a modified Hirt procedure (5, 13). Cytoplasmic RNA was isolated by lysis of infected HeLa cells with Nonidet P-40 followed by phenol extraction (7, 11), and polyadenylylated RNA was selected by oligo(dT)-cellulose column chromatography (14). Southern analysis of DNA was carried out by transfer of restriction enzyme fragments to nitrocellulose filters (15) and hybridization with ^{32}P-labeled DNA fragments made by nick-translation (11, 16–18). RNA was analyzed by electrophoresis in agarose gels containing formaldehyde (19) and transfer of the RNA to nitrocellulose filters for hybridization (20).

Protein Analysis. Infected cells were labeled for 1 hr with 100 μCi (1 Ci = 37 GBq) of [^{35}S]methionine in methionine-depleted medium (GIBCO). Cell extracts were obtained by lysis of the cells with 1% Nonidet P-40/50 mM Tris·HCl, pH 7.4, followed by centrifugation (Eppendorf; 12,000 × g) for 1 min. A portion of the extract was incubated for 1 hr at 4°C with goat antiserum raised against the *ompF-TK-lacZ* "tribrid" protein (36) (kindly supplied by G. Weinstock) with gentle shaking in NET/GEL buffer (0.05% Nonidet P-40/50 mM Tris·HCl, pH 7.4/150 mM NaCl/5 mM EDTA/0.25% gelatin/0.02% sodium azide) (21). Immunoreactive material was precipitated with staphylococcal protein A-Sepharose CL-4B (Pharmacia) precoated with rabbit IgG directed against goat IgG (Dako, Santa Barbara, CA), then boiled in Laemmli sample buffer and fractionated by NaDodSO$_4$/polyacrylamide gel electrophoresis (22). A ^{14}C-labeled protein mixture (Amersham) was used for molecular weight standards. TK activity in cell lysates prepared by sonication was measured as described (23). Protein concentrations were measured by the Bio-Rad protein assay, using bovine serum albumin as a standard.

RESULTS

Construction of a Hybrid Virus Carrying the HSV *TK* Gene. To place the HSV *TK* gene in the middle of the third segment of tripartite leader in the adenovirus genome, we first made bacterial plasmid pMY591, in which the *TK* gene is sandwiched between a piece of adenoviral DNA and SV40 early DNA (Fig. 1). The adenoviral segment in pMY591 contains a part of the third segment of the tripartite leader and serves as an element necessary for *in vivo* recombination. The SV40 early region in pMY591 contains not only the entire T-antigen coding sequence but also the early promoter and replication origin. The SV40 segment serves as an element coding for the helper function that allows the hybrid adenovirus to grow in monkey cells. The *TK* segment in pMY591 contains the entire continuous 1128-nucleotide coding sequence (25). In addition, the *TK* segment contains 57 nucleotides of the 5' untranslated leader sequence but does not contain the *TK* promoter and the proximal 50 nucleotides of the 5' untranslated leader (26). The *TK* fragment also contains the polyadenylylation signal, which appears 60–90 nucleotides downstream from the termination codon. The entire adenovirus-TK-SV40 fragment in pMY591 was excised with *Bam*HI and ligated to *Bam*HI-digested adenovirus 1 × 51 DNA, which has two *Bam*HI sites, at 29 and 59.5 map units. The ligation products were transfected into 293 cells along with 1 × 51 helper DNA, and hybrid viruses expressing SV40 T antigen were selected on CV-1 monkey cells as described in *Materials and Methods*. The genome structure of several
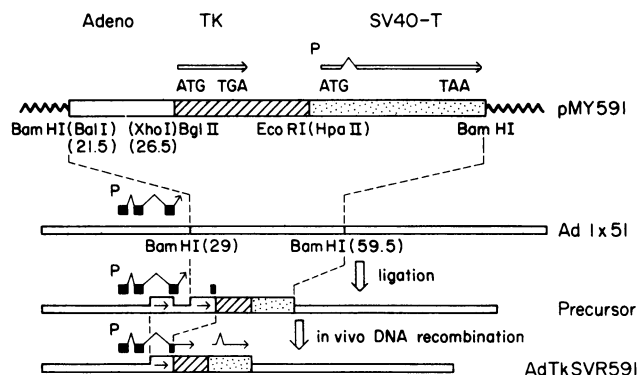

Fig. 1. Construction of a hybrid adenovirus that expresses the HSV *TK* gene. The *TK* gene was first sandwiched between adenovirus and SV40 DNAs to form pMY591 (top line). The adenovirus segment (empty bar) in pMY591 originates from a *Bal* I (21.5 map units)/*Xho* I (26.5 map units) fragment and carries most of the third segment of the tripartite leader at its distal end (24). The SV40 segment (stippled bar) is derived from a *Hpa* II (nucleotide 346)/*Bam*HI [nucleotide 2533, SV number (8)] fragment and carries the SV40 early region, which includes the entire T-antigen coding sequence, the early promoter (*P*), enhancer, replication origin, and polyadenylylation signal. The 2.4-kb *Bgl* II/*Eco*RI fragment (hatched bar) carries the HSV TK-encoding sequence but lacks its promoter. The three fragments were linked to each other with a short polylinker sequence or after conversion of the ends with linkers. The entire adenovirus-TK-SV40 fragment was inserted into the *Bam*HI site of pBR322 (wavy line). The direction of *TK* and SV40 early transcription, which is shown by arrows, is the same as that of adenoviral late mRNAs. The second line is a map of the adenovirus vector 1 × 51 showing the major late promoter (*P*), three segments of the tripartite leader (■), and its two *Bam*HI sites (29 and 59.5 map units). The insert in pMY591 was excised with *Bam*HI and ligated with *Bam*HI-digested adenovirus 1 × 51 DNA to form the proposed precursor to AdTkSVR591. The duplication of adenoviral DNA from map position 21.5 to 26.5 is emphasized by the open boxes with arrows. Intramolecular or intermolecular recombination between the duplicated regions of adenoviral DNA would result in the formation of AdTkSVR591 as shown at the bottom of the figure. The *TK* gene is positioned in the middle of the third segment of the tripartite leader.

candidate viruses was analyzed by restriction enzyme digestion and Southern blot hybridization of viral DNA using *TK* and T-antigen sequences as probes (data not shown). A hybrid virus, designated AdTkSVR591, had the expected DNA structure as shown at the bottom of Fig. 1. The hybrid virus had lost a portion of adenoviral DNA (26.5–59.5 map units, 11.9 kb) and gained 2.4- and 3.1-kb inserts of TK and SV40 DNAs, respectively, resulting in a total genome length of 29.6 kb (82% of full-length adenovirus). Although several hybrid viruses with rearranged DNA structures were detected in the lysate obtained after the initial transfection, the hybrid viruses were stable after they were plaque purified.

For control experiments, a hybrid virus (AdSVR599), which contained the same SV40 early region present in AdTkSVR591 but not the *TK* gene, was constructed in a similar way (data not shown). AdSVR599 had a similar structure to AdSVR284, the high producer virus of T antigen (7), except that AdSVR599 has the SV40 origin and promoter located between the adenoviral third leader segment and the SV40 T-antigen-encoding sequences, while SV40 T-antigen-encoding sequences were directly linked to the adenoviral third leader segment in AdSVR284.

Properties of the Hybrid Virus Carrying the *TK* Gene. The hybrid virus, AdTkSVR591, is defective because it lacks a large segment of the adenoviral genome essential for viral growth. Thus stocks of AdTkSVR591 contain a mixture of hybrid and helper viruses. The proportion of the hybrid virus in a lysate was estimated by comparing the number of plaques that appeared on HeLa and CV-1 cells. As expected for a

mixture of defective and helper viruses, recombinant virus populations displayed two-hit kinetics in a plaque assay on CV-1 cells. The proportion of hybrid virus was low (about 1%) in the original viral populations. After two further cycles of plaque purification and growth on CV-1 cells, infected cell lysates were analyzed for the proportion of the hybrid virus they contained and for the level of TK activity (see below). One lysate (AdTkSVR591-3ABC) was found to contain the hybrid virus in the 25–35% range. The proportion of hybrid virus in the population did not increase further after additional cycles of plaque purification and growth. The genome of AdTkSVR591-3ABC was analyzed by digestion with several restriction enzymes. No differences were detected between AdTkSVR591-3ABC and the original AdTkSVR591 genome. Possible mechanisms by which the amount of the hybrid virus in the AdTkSVR591 stocks increased are discussed below. AdTkSVR591-3ABC was used for most of the following experiments.

Expression of TK mRNA and Protein. HeLa or CV-1 cells were infected with the hybrid virus AdTkSVR591 and TK mRNA and protein were analyzed in a variety of ways. The *TK* gene was expressed at late times after infection, but the time of optimal expression varied depending on the host cell and multiplicity of infection (moi) used (see below). The TK mRNA and protein used for experiments shown in Figs. 2 and 3 were isolated at times that gave maximal expression. The conditions used, including the moi and incubation period, are indicated in each figure legend. In the case of defective hybrid viruses, both moi of helper virus and the estimated proportion of the hybrid virus (hyb) in the population are indicated.

An abundant polyadenylylated TK mRNA species of 1550 nucleotides could be detected in the cytoplasm of AdTk-SVR591-infected cells by blotting analysis (Fig. 2). The size of the TK mRNA is consistent with a transcript that starts at the adenovirus major late promoter, has normally processed leader sequences, and ends just downstream from the *TK* polyadenylylation signal.

To detect TK protein, infected cells were labeled with [^{35}S]methionine for 1 hr, and extracts were subjected to immunoprecipitation with TK antisera. A protein of apparent M_r 47,000 was precipitated from both AdTkSVR591- and HSV-infected cells (Fig. 3, lanes D and E). No TK protein could be detected in cells infected with wild-type adenovirus or AdSVR599. The TK antisera used could not precipitate all the TK protein in the samples (36) and thus the amount of TK protein in the immunoprecipitates did not reflect the total amount present in the extracts. Addition of antisera to the supernatant of immunoprecipitated samples brought down almost the same amount of TK protein as did the first challenge (data not shown). In addition to the major product, two minor protein species could be detected as products of AdTkSVR591. One minor product (M_r 41,000) is clearly detected both by immunoprecipitation with TK antisera (Fig. 3, lane D) and in the total cell extract (Fig. 3, lane I). The other minor product (M_r 39,000) could be detected by immunoprecipitation but only after longer exposure of the gel shown in Fig. 3. The ratio of these three products was deduced as 100:9:1 by densitometry. The minor species were probably not TK degradation products because they showed the same half life as the major TK product (4–6.5 hr) in pulse–chase experiments (data not shown). The origins of these minor products are discussed below. Variable amounts of a minor species (M_r 45,000) were sometimes detected in CV-1 cells infected with AdTkSVR591 and HSV (Fig. 3, lanes D and E). This species was never seen in infected HeLa cells.

The TK protein in AdTkSVR591-infected cells was produced at sufficient levels to be detectable without immunoprecipitation. A prominent band representing TK protein of apparent M_r 47,000 can be easily detected among total labeled
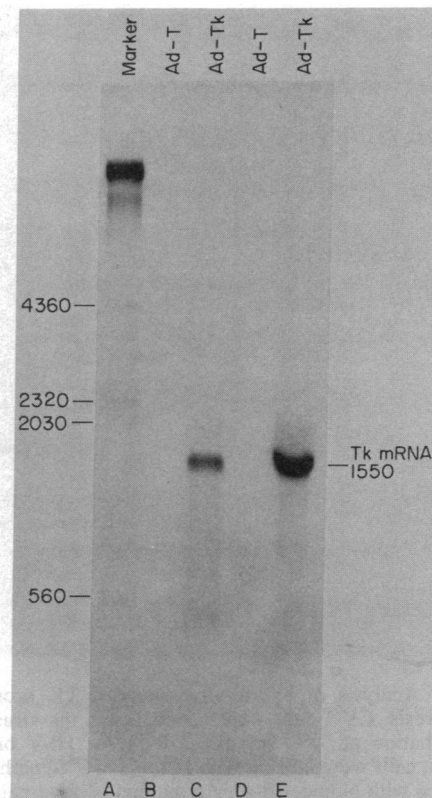

FIG. 2. Sizing of TK RNAs by blot hybridization. Cytoplasmic RNA was isolated from HeLa cells infected with AdSVR599 (moi = 40, hyb = 9%) or AdTkSVR591 (moi = 40, hyb = 25%) at 24 hr after infection. One-half microgram of poly(A)$^+$ and 80 μg of total cytoplasmic RNA were denatured by treatment with formamide and formaldehyde and were fractionated on a 1.2% agarose gel as described (19). The RNA was transferred to nitrocellulose filters and transcripts containing *TK* sequences were identified by hybridization with ^{32}P-labeled *TK* DNA. The lanes are as follows: A, DNA fragments of *Hind*III-digested λ phage for size markers; B, poly(A)$^+$ RNA from AdSVR599-infected cells; C, poly(A)$^+$ RNA from Ad-TkSVR591-infected cells; D, total cytoplasmic RNA from AdSVR-599-infected cells; E, total cytoplasmic RNA from AdTkSVR591-infected cells. The size of the TK mRNA is in agreement with the size expected for a transcript comprising the sum of the first, second, and part of the third tripartite leader (41, 72, and 56 nucleotides), linker and upstream *TK* sequences (18 and 57 nucleotides), the TK-encoding sequence (1128 nucleotides), and the 3' noncoding sequence, including the poly(A) tail.

proteins of AdTkSVR591-infected cells by NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 3, lane I). Analysis of the autoradiograms by densitometry showed that the TK protein was the third most abundantly labeled protein in AdTkSVR591-infected CV-1 cells after the major capsid proteins, hexon (M_r 120,000) and fiber (M_r 65,000). The TK protein represents about 8–10% of the newly synthesized proteins present in the extract. In infected HeLa cells, it was the fourth most abundantly labeled protein, constituting about 3–4% of the radioactivity in the extract (data not shown). The Coomassie-stained gel shown in lane K of Fig. 3 demonstrates that the TK protein produced by AdTkSVR-591 accumulated to the level of about 1% of the total cellular proteins.

Enzymatic Activity of TK in AdTkSVR591-Infected Cells. Permissive human or monkey cells were infected with several viruses, and the TK activity in cell extracts was determined by measuring the extent of conversion of [^{3}H]thymidine to [^{3}H]dTMP. When almost confluent plates of CV-1 cells were infected with wild-type adenovirus or AdSVR599, the TK
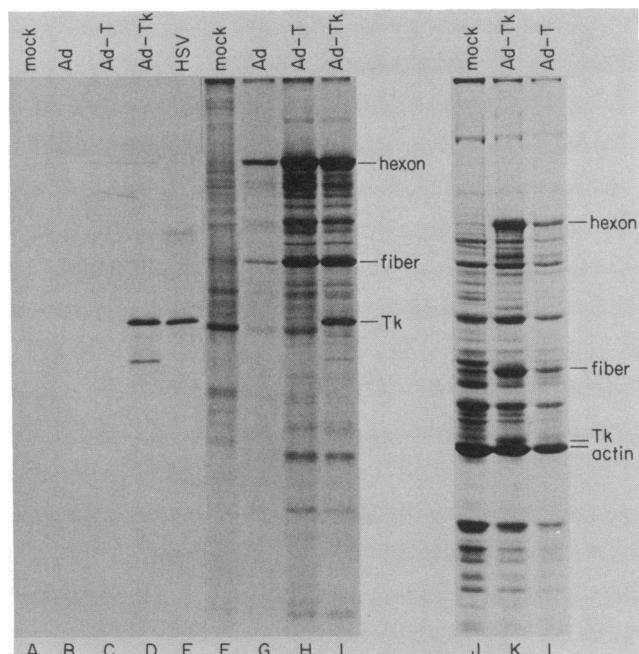
FIG. 3. Analysis of hybrid-virus-encoded TK protein by gel electrophoresis. CV-1 cells were infected with the virus indicated. After incubation at 37°C for either 8 hr for HSV or 20 hr for adenovirus, cells were labeled with 100 μCi of [^{35}S]methionine for 1 hr and lysed with Nonidet P-40. An amount of supernatant fraction equivalent to 2×10^5 cells was incubated with 2 μl of anti-TK antiserum at 4°C, and immunocomplexes were precipitated with an excess of protein A-Sepharose precoated with rabbit IgG directed against goat IgG. The precipitate was boiled in sample buffer and analyzed by electrophoresis on a 10% acrylamide gel (lanes A–E). An amount of the same extract equivalent to 2×10^4 cells was also analyzed without immunoprecipitation by electrophoresis on the same gel (lanes F–I). Extracts were also prepared from nonlabeled cells at 26 hr after infection. An amount equivalent to 2×10^5 cells (containing about 70 μg of protein) was analyzed on an 8% acrylamide gel (lanes J–L). Infections were as follows: A, F, and J, mock; B and G, adenovirus 1×51 (moi = 50); C, H, and L, AdSVR599 (moi = 200, hyb = 10%); D, I, and K, AdTkSVR591 (moi = 200, hyb = 30%); E, HSV (moi = 10).
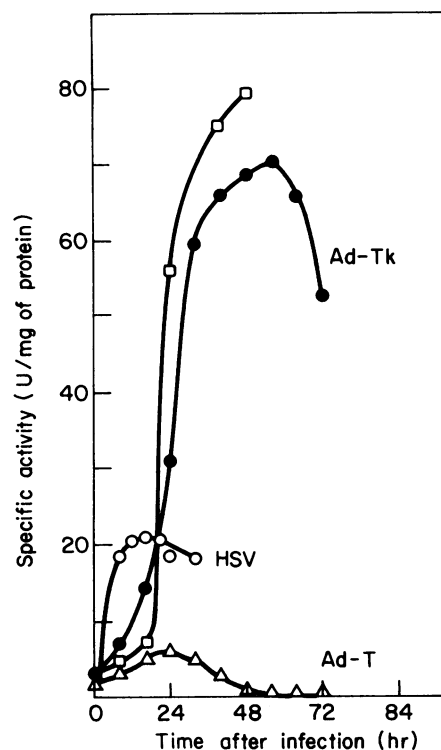

FIG. 4. Time course of TK activity expressed in AdTkSVR591-infected cells. Almost confluent plates of CV-1 cells were infected with the virus indicated and incubated at 37°C. The cells were collected at 4- or 8-hr intervals until about 50% of the cells detached from the plates. TK activity in the cell lysates was measured as described (23). A unit of TK activity is defined as the conversion of 1 nmol of thymidine per hr. The specific activity is defined as units (U) of TK activity per mg of protein. The amount of protein obtained from a plate first increased and then decreased as cells started to detach from the surface of the dish. Therefore, the total TK activity reached a maximum at an earlier time than the specific activity. $\triangle$, AdSVR599 (moi = 40, hyb = 3%); $\square$, AdTkSVR591 (moi = 200, hyb = 30%); $\bullet$, AdTkSVR591 (moi = 40, hyb = 8%); $\circ$, HSV (moi = 3).

activity increased 2- to 3-fold after 24 hr. This represents the activity of the endogenous cellular *TK* gene(s) because it is sensitive to the presence of 50 μM dTTP in the reaction (27). When CV-1 cells were infected with AdTkSVR591, the TK activity increased with the kinetics shown in Fig. 4. The activity found at 0 and 8 hr after infection was sensitive to dTTP, while that found at 24 hr and later was insensitive to dTTP (data not shown). The TK activity of HSV is known to be insensitive to dTTP (27). Therefore, the increased TK activity at late times in AdTkSVR591-infected cells could be accounted for by the expression of the inserted HSV *TK* gene.

CV-1 cells infected at a wide range of multiplicities of the hybrid virus (0.5–60) present in different AdTkSVR591 lysates, including AdTkSVR591-3ABC, showed virtually the same maximal specific TK activities (50–81 units/mg of protein). The TK activity in infected cells may reach the saturation level at an effective moi of 1. CV-1 cells infected with AdTkSVR591 showed 3- to 4-fold more TK activity than HSV-infected cells when the activities were compared at the respective optimal times and at the same low moi (1).

The hybrid virus AdTkSVR591 expressed TK activity in a variety of human and monkey cells. The TK activity in infected HeLa cells was similar to that found in CV-1 cells. The levels of TK activity expressed in infected human 143Tk$^-$ cells were lower than in CV-1 cells. However, the

activity was clearly detected because the cells have no endogenous cellular TK activity.

DISCUSSION

We have demonstrated that a recombinant adenovirus carrying the HSV *TK* gene in the middle of the third segment of the tripartite leader produces large quantities of enzymatically active TK protein. The amount of TK protein produced in infected cells is comparable to the level of adenoviral late major capsid proteins. The TK protein represented 10% of newly synthesized protein in infected cell extracts, and accumulated to a level of 1% of the total cell protein under optimal conditions.

Several factors are responsible for this high level of expression: (*i*) The copy number of the *TK* gene was high because it was efficiently introduced into cells by infection with the hybrid adenovirus and was replicated along with the adenoviral genome. (*ii*) Transcription of the *TK* gene is under the control of the efficient adenoviral major late promoter. (*iii*) Translation of the TK mRNA was efficient because it contained almost the entire adenoviral tripartite leader at its 5′ end. It has been demonstrated previously that the adenoviral tripartite leader enhances the efficiency with which a mRNA is translated at late times after infection (7, 28).

The first AUG codon found in the hybrid TK mRNA that contains most of the tripartite leader and untranslated TK leader sequences at its 5' end is the authentic *TK* initiation codon. Although the sequences surrounding the *TK* initiation codon (C-G-C-G-T-*A-U-G*-G) differ from the most common class of consensus sequences found by Kozak (C-C-^A_G-C-C-*A-U-G*-G) (29), the *TK* AUG initiation codon may serve as an efficient translational initiation signal in AdTkSVR591-infected cells. Preston and his colleagues have analyzed TK proteins by *in vitro* translation of hybrid-selected TK mRNA (30) and two-dimensional gel electrophoresis of proteins produced after HSV infection (31). They reported the appearance of M_r 39,000 and 38,000 minor TK products that arose by initiation at the second and third in-frame AUG codons in the TK mRNA. Two minor AdTkSVR591-encoded proteins (M_r 41,000 and 39,000; see *Results*) that we have detected probably result from initiation of *TK* translation at these downstream AUG codons. Use of the recombinant adenovirus may help in further clarifying the nature of the minor proteins, because they are also overproduced.

A direct selection for SV40 T-antigen helper function was used to isolate a hybrid virus carrying the *TK* gene. The SV40 early region inserted into the hybrid virus contained its own promoter as well as the SV40 replication origin. T antigen binds to the SV40 early promoter region, where it operates as an autorepressor (32, 33). Thus we would expect small amounts of T antigen to be synthesized by hybrid adenoviruses carrying the entire SV40 early region. This is, in fact, the case for the original isolate of AdTkSVR591, which contained a low proportion of the hybrid virus (1%). In contrast, one lysate (AdTkSVR591-3ABC) obtained after further plaque purification and growth in monkey cells contained a high proportion of the hybrid virus (35%) and produced more T antigen. However, unlike the TK protein, T antigen produced in CV-1 cells infected with AdTkSVR591-3ABC cannot be detected without immunoprecipitation. It remains to be determined whether a mutation in the genome of AdTkSVR591-ABC has occurred and is responsible for either the high levels of T-antigen expression or the high proportion of hybrid virus in the population. The SV40 replication origin in the hybrid virus is probably inert even in the presence of T antigen. For example, in coinfections of SV40 and adenovirus in monkey cells, the adenovirus was able to grow because of the helper function provided by SV40, while SV40 could not replicate. Adenoviral gene products probably inhibit host cell proteins needed for SV40 DNA replication (34, 35).

We have also introduced the gene for the α subunit of human chorionic gonadotropin or its corresponding cDNA into this adenovirus vector system. Cells infected with these viruses produced the hormone at levels equivalent to the TK protein synthesized in AdTkSVR591-infected cells. The hormone synthesized was glycosylated and secreted into the culture medium (unpublished data). Polyoma virus small, middle, and large T antigens have also been overproduced by using this system (37). We believe this adenovirus system is applicable to the overproduction of a wide variety of eukaryotic gene products, which will be properly modified and biologically active.

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