JOURNAL OF VIROLOGY, Dec. 1985, p. 867–878 Vol. 56, No. 3
0022-538X/85/120867-12$02.00/0
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Differential Activation of Hybrid Genes Containing Herpes Simplex Virus Immediate-Early or Delayed-Early Promoters After Superinfection of Stable DNA-Transfected Cell Lines

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Received 13 May 1985/Accepted 19 August 1985

We compared the levels of gene expression obtained after herpes simplex virus (HSV) superinfection of cell lines containing integrated human β-interferon (IFN) or chloramphenicol acetyltransferase (CAT) genes under the control of HSV immediate-early (IE) or delayed-early class promoters. DNA-transfected mouse Ltk$^+$ cell lines harboring coselected IE175-IFN or thymidine kinase (TK)-IFN hybrid genes gave only low basal expression of human IFN. However, infection of both cell types with HSV type 1 or HSV type 2 produced abundant synthesis of IFN-specific RNA and biologically active IFN protein product. The IE175-IFN cell lines consistently gave 20- to 150-fold increases in IFN titers, and several TK-IFN cell lines yielded 100- to 500-fold induction. In the IE175-IFN cells, expression of IFN RNA also increased up to 200-fold and was detectable within 30 to 60 min after virus infection. Qualitatively similar results were obtained with hybrid G418-resistant Ltk$^-$ or Vero cell lines containing coselected IE175-CAT and TK-CAT constructs, except that there was relatively high basal expression of IE175-CAT. All three sets of IE cell lines (but not the delayed-early cell lines) responded to virus infection both in the presence of cycloheximide and with mutants defective in IE gene expression, demonstrating specific *trans*-activation by the pre-IE virion factor. In contrast, activation in the TK hybrid cell types required viral gene expression and the presence of a functional IE175 gene product. Up to 30-fold amplification in the copy number of the resident IFN or CAT DNA sequences also occurred within 20 h after HSV infection in IE175 hybrid cells but not in TK hybrid cells. Amplification was abolished either by treatment with phosphonacetate or by superinfection with a *ts* mutant unable to synthesize viral DNA, demonstrating specific HSV activation of the viral DNA replication origin (ori_S) present in the IE hybrid constructs.

The immediate-early (IE), delayed-early (DE), and late classes of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) genes are expressed in a defined temporal sequence after infection and respond to different forms of viral inducing factors. Individual IE and DE promoters possess characteristic class-specific features. For example, the promoter-regulatory region of the DE thymidine kinase (TK) gene is relatively simple, occupying less then 109 base pairs (bp) (8, 27). During infection, transcription from the TK promoter is totally dependent on the presence of an active IE175 (ICP4) gene product (23, 37, 50), although this requirement can be bypassed in appropriate selected TK$^+$ cell lines (22, 29, 42) and in microinjected *Xenopus laevis* oocytes (26, 27). In contrast, the 5′ promoter and regulatory region of the IE175 gene is 330 bp in size and consists of numerous short G+C-rich stretches together with three TAATGARAT elements with enhancer-like features (6, 21, 24). This region also contains an A+T-rich viral DNA replication origin (ori_S) (31, 47). Five HSV IE genes are transcribed after virus infection in the absence of de novo protein synthesis (5, 17, 19), and at least two genes (IE175 and IE110) are expressed abundantly after DNA transfection or microinjection of the isolated intact genes (6, 28, 34). Expression of IE genes is also stimulated by a pre-IE factor carried in virions (2, 25, 36).

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A number of researchers have been attempting to reconstruct these *trans*-activation processes with isolated genes and promoters by DNA transfection and cotransfection procedures. Short-term transfection systems have proved useful for identifying both *cis*-acting elements and *trans*-acting proteins involved in HSV gene regulation mechanisms (6, 9, 21, 33, 34). However, because of the potential deficiencies of transient assays, we also wished to carry out similar studies with isolated herpesvirus promoters stably inserted in the cellular genome. We anticipated that these studies would be particularly relevant and informative for herpesviruses, the genomes of which presumably persist in a repressed nucleosome-associated state during latency (44). Therefore, we extended previous studies of the type described by Reyes et al. (40), with a cell line containing a TK-interferon (IFN) hybrid gene, and by Herz and Roizman (15), with a cell line containing a TK-ovalbumin hybrid gene. We were particularly interested in the following points: (i) asking whether the very high efficiency induction of TK-IFN after HSV superinfection in the LH_2p192-8 cell line resulted from the coselection procedure used to establish this cell line; (ii) comparing the efficiency and mechanism of transcriptional activation in cell lines containing hybrid genes with IE promoters (IE175-IFN and IE175-chloramphenicol acetyltransferase [CAT]) relative to cell lines containing hybrid genes with DE promoters (TK-IFN and TK-CAT); (iii) studying the *trans*-activation processes at the RNA level as well as assaying protein gene products; (iv) examining the specificity and magnitude of these responses, particularly with regard to previous suggestions of nonspecific activation

of DNA-transfected genes by both adenovirus Ela and pseudorabies virus IE gene products (9, 18, 20); (v) beginning to utilize the potential of these systems for studying features of herpesvirus gene regulation associated with DNA replication and host shutoff functions.

The results described in this report demonstrate that strong class-specific HSV regulatory processes occurring at both the RNA and DNA levels can be reconstructed efficiently and reproducibly with foreign hybrid genes introduced into permanent coselected cell lines and suggest that experiments and reagents of this type will prove to be invaluable for defining the mechanisms and control of herpesvirus gene regulation.

(Preliminary accounts of portions of this work were first presented at the 1982 Cold Spring Harbor Herpesvirus Meeting and the 1984 Seattle Herpesvirus Meeting.)

MATERIALS AND METHODS

Cells and viruses. Mouse Ltk$^-$ cells, African green monkey kidney (Vero) cells, human diploid fibroblasts (HF and GM2504), and cloned Ltk$^+$ cell lines containing transfected plasmid DNA were grown in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (FCS). Maintenance medium for Ltk$^+$ cell lines contained in addition selective HAT medium (15 μg of hypoxanthine per ml, 1 μg of aminopterin per ml, and 5 μg of thymidine per ml). HSV-1 (MPcl-20), HSV-1(KOS)tsB2, HSV-1(HFEM-STH2)tsB7, and HSV-2 (333cl-6) virus stocks were prepared, and titers were determined as described previously (28, 40). Human Namalwa lymphoblast cells were grown in RPMI 1640 medium with 20% FCS and were induced by treatment with 50 U of α-IFN per ml for 1 h follwed by infection with 300 hemaglutinizing units of Sendai virus per ml for 20 h.

Plasmid DNA. pHFβ contains a 560-bp human IFN cDNA originally selected with a probe provided by W. Fiers (38, 40). The TK-IFN plasmids pGR191 and pGR192 have been described by Reyes et al. (40), and the TK-CAT and IE175-CAT plasmids (pPOH3 and pPOH2) have been described by O'Hare and Hayward (33). The hybrid IE175-IFN genes were constructed by fusing the 560-bp human IFN cDNA coding region from pGR191 to the nontranslated leader sequence of the IE175 gene. pGH12 contains a 1.9-kilobase (kb) *Hin*dIII-*Bam*HI subfragment from the *Bam*HI M fragment of HSV-1(MP) DNA (map coordinates 0.864 to 0.896) in pBR322. The *Bam*HI site in pGH12 lies at position +30 after the RNA initiation site from the IE175 promoter-regulatory region. The 560-bp IFN-cDNA fragment bounded by *Bgl*II sites was inserted into the *Bam*HI site in pGH12 such that the first AUG codon in the fused mRNA would be that for the IFN signal peptide. The IE175-IFN hybrid construction in plasmid pGR238 retains the IFN UGA termination signal but has no defined poly(A) addition site. Plasmid pGR239 contains the IFN insert in the opposite orientation relative to that in pGR238. All plasmids were grown in *Escherichia coli* HB101, and DNA preparations were purified in cesium chloride-ethidium bromide density gradients followed by ethanol precipitation and extensive dialysis against 0.01 M Tris–0.001 M EDTA (pH 8.0).

Microinjection, DNA transfection, and coselection. Microinjection procedures in *X. laevis* oocytes followed those described by McKnight and Gavis (26) and Reyes et al. (40).

DNA transfection was carried out by the calcium phosphate precipitation procedure of Graham and Van der Eb (13) essentially as described previously (30, 42). Transfection mixtures for 60-mm dishes of Ltk$^-$ cells contained 1 μg of the supercoiled plasmid to be coselected, together with 100 ng of pGR18 DNA containing the intact HSV-2 TK gene (41) and 5 μg of isogenic Ltk$^-$ as carrier. Selection for G418 resistance in Ltk$^-$ or Vero cells was carried out in 40-mm culture wells with 5 μg of coselected DNA plus 0.5 μg of pSV$_2$neo DNA (45) without carrier. The transfected cells were shocked with glycerol at 4 h and subjected to butyrate enhancement (5 mM sodium butyrate in Dulbecco modified Eagle medium–10% FCS for 16 h). At 48 h, freshly trypsinized cells were placed in 100-mm culture dishes in medium containing 800 μg of G418 per ml and grown to confluence. The cells were further split 1:10 and placed in medium containing 1 mg of G418 per ml. Resistant colonies were grown to mass culture in the absence of selection.

Assays for IFN or CAT expression before and after superinfection. Confluent Ltk$^+$ cell lines at 8×10^6 cells per 100 mm plate either received a fresh medium change for basal expression assays or were infected with HSV-1 at a multiplicity of infection of 3 to 5 PFU per cell. After adsorption of virus for 90 min at 37°C in 4 ml of medium without FCS, the cells were overlaid with 2 ml of Dulbecco modified Eagle medium supplemented with 10% FCS and incubated further at 37°C. In some experiments phosphonoacetic acid (PAA; 300 μg/ml; Richmond Organics) was included in the medium to inhibit viral DNA replication. At the times indicated (given as hours after the addition of virus) the medium was collected, and the IFN titer was determined on either GM2504 cells (human fibroblasts trisomic for chromosome 21) or Vero cells (African green monkey kidney cells deficient in IFN production). The sensitivity of IFN detection was usually 30 to 120 U/ml in Vero cells and 1 to 2 U/ml in GM2504 cells.

G418-resistent Ltk$^-$ and Vero cell lines were grown and infected as described above. Where appropriate, cycloheximide (50 μg/ml) or actinomycin D (2 μg/ml) were added to the medium. Cycloheximide reversal was accomplished by three washes with phosphate-buffered saline followed by incubation in fresh medium. For CAT assays, cells were harvested by trypsinization, followed by the addition of Dulbecco modified Eagle medium containing 10% FCS, and then washed in phosphate-buffered saline. Extracts were prepared by freeze-thawing in 0.25 M Tris hydrochloride (pH 7.6). CAT enzyme activity assays were performed with 0.05 μCi of [^{14}C]chloramphenicol (40 to 50 μCi/mmol; New England Nuclear Corp., Boston, Mass.) as described by Gorman et al. (12) and O'Hare and Hayward (33).

Preparation of cellular DNA and RNA. High-molecular-weight DNA for both Southern gel blot and DNA dot blot hybridization analyses was prepared from detergent-salt lysates of cells, followed by treatments with proteinase K, ribonuclease, phenol, and chloroform and by ethanol precipitation (42, 43). Total cellular RNA for RNA dot blot hybridization analysis was prepared from guanidine thiocyanate lysates of cell monolayers in 3 ml of buffer containing 0.04 M sodium acetate (pH 5.0), 0.01 M EDTA, and 0.1% sarcosyl per 100-mm dish (3). The DNA in the lysate was sheared by vortexing, and then 1 g of CsCl was added, and the lysate was layered over a 1-ml cushion of 5.7 M CsCl in 0.1 M EDTA (pH 5.0) and centrifuged for 12 h at 40,000 rpm in a Beckman SW60 rotor. Pelleted RNA was suspended and precipitated with ethanol. Alternatively, to separate into cytoplasmic and nuclear RNA fractions, the cells were lysed in 10 mM NaCl–3 mM magnesium acetate–20 mM Tris hydrochloride (pH 7.4) buffer containing

0.5% Nonidet P-40 and 10 mM vanadyl ribonucleoside complex (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). After 15 min at 0°C, the nuclei were pelleted and then washed twice and treated with 2 μg of pancreatic DNase per ml for 15 min at 20°C. Both nuclear and cytoplasmic samples were then incubated for 1 h at 42°C with 0.5% sodium dodecyl sulfate and 100 μg of proteinase K per ml, followed by extraction with phenol-chloroform and precipitation with ethanol.

DNA and RNA blot hybridization. For dot blot analysis, twofold dilutions of either DNA or RNA were applied to nitrocellulose sheets using a 96-well filtration manifold (Bethesda Research Laboratories). Cell DNA was treated with 0.5 M NaOH for 10 min on ice, neutralized with an equal volume of 4 M ammonium acetate, and applied to nitrocellulose in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.7]). RNA precipitated with ethanol was evaporated to dryness and suspended in 10 μl of formamide containing 8% formaldehyde and 20 mM (MOPS; pH 7.0), 5 mM sodium acetate, and 1 mM EDTA. The RNA sample was heated at 65°C for 15 min and diluted with 200 μl of 10× SSC containing 3% formaldehyde and applied to the nitrocellulose. Southern blot analysis of DNA samples was carried out as described previously (40, 42). Prehybridization and washing procedures were those described previously by Thomas (48), and hybridizations were carried out at 37°C in 50% formamide for 48 to 72 h in 5× SSC with a nick-translated DNA probe (10^8 counts per min per μg) prepared from an isolated 920-bp *Eco*RI to *Bgl*II fragment of human IFN genomic DNA from plasmid pIF_R (51).

RESULTS

Rearranged structure of the integrated TK-IFN DNA in LH_2p192-8 cells. The construction of a hybrid TK-IFN gene inserted into a TK vector plasmid, together with assays for IFN expression in microinjected oocytes and permanent DNA-transfected Ltk^+ cell lines, have been described previously by Reyes et al. (40). In addition to an intact HSV-2 TK gene, plasmid pGR192 contains human IFN cDNA bounded by *Bgl*II linkers and inserted at position +50 within the 5'-untranslated leader sequence behind the DE HSV-1 TK promoter. This hybrid gene construct yields a potentially bicistronic mRNA, including the complete coding region and 3' poly(A) sites from the HSV-1 TK gene. We were particularly interested in the structure of the integrated TK-IFN hybrid DNA in the Ltk^+ cell line established by linked coselection (LH_2p192-8), because of the unusually high level of *trans*-activation obtained after virus superinfection (up to 500-fold; see below). In comparison, stimulation of the intact HSV TK gene in Ltk^+ cell lines selected directly for basal TK expression in HAT medium is never more than 5- to 15-fold (8, 22, 29, 52). Analysis of several sets of Southern blot hybridization experiments using an isolated IFN cDNA probe (Fig. 1a), as well as HSV-1 TK- and HSV-2 TK-specific probes (data not shown), revealed that LH_2p192-8 cells contain three separate, integrated portions of the input plasmid DNA. An overall summary and interpretation of the results is presented in Fig. 1b. Two integration sites contained rearranged single copies of the IFN sequences, and the third site occurred within sequences that had been duplicated two- to threefold after integration or ligation (14, 42). All three copies retained intact the 2,200-bp *Pvu*II, 1,750-bp *Bst*EII, and 3,000-bp *Eco*RI fragments from pGR192, indicating the presence of a complete HSV-1 TK promoter-IFN hybrid gene, including the adjacent HSV-1 TK coding region and the 3' termination signals. The right-hand boundary of both single-copy inserts mapped between the *Eco*RI and *Sal*I sites, but the amplified insert retained pBR322 sequences beyond the *Sal*I site. On the left-hand side the data indicate that one of the three inserts lacks HSV-2 TK gene sequences beyond the first *Kpn*I site and that neither of the other two reached as far as the *Bst*EII site which lies 500 bp beyond the HSV-2 poly(A) site. Although the data did not enable us to determine unambiguously whether either of the two larger inserts contained a complete HSV-2 TK gene, these results suggest that this particular cell line may have been selected for expression of its HSV-1 TK gene lying downstream on the bicistronic hybrid TK-IFN mRNA rather than from the intact HSV-2 TK gene.

Fusion of IFN cDNA to the promoter region for the IE175 gene. To place the same human IFN-β cDNA coding sequence behind the HSV-1 IE175 promoter, a *Bam*HI-*Hin*dIII fragment from HSV-1(MP) *Bam*HI-M was subcloned into pBR322 to give the pGH12 plasmid (33). This DNA fragment insert contains all 700 bp between the 5' ends of two divergent IE genes and includes also the complete ori_S DNA replication origin (31, 47). The 560-bp *Bgl*II IFN cDNA subfragment from pGR191 (40) was placed in both orientations at the *Bam*HI site 30 bp downstream from the mRNA start site for the IE175 gene. Plasmid pGR238 contains the IFN-coding region in the sense orientation relative to that of the IE175 promoter, and the first AUG initiator codon in the insert would be that for the IFN-β signal peptide region, whereas pGR239 contains the IFN cDNA insert in the antisense orientation. The presence of several additional *Bam*HI sites within the 4.3-kb IE175 gene precluded construction of a hybrid IE175-IFN gene retaining the intact 3' poly(A) region.

Constitutive synthesis of IE175-IFN in microinjected oocytes. To examine the validity of the IE175-IFN constructions, the IFN hybrid plasmids were microinjected into the nuclei of *X. laevis* oocytes, and the yields of biologically active IFN produced were assayed after 48 h of incubation. The IFN titers detected from either homogenized oocytes or released into the supernatant medium were initially assayed by the standard cytopathic dilution method on vesicular stomatitis virus-infected GM2504 cells. However, because previous attempts to demonstrate poly(rI):poly(rC) induction in DNA-transfected mouse L cells (35) appear to have been complicated by carry-over of poly(rI):poly(rC) into the very sensitive GM2504 assay cells (J. Mosca, unpublished data), we also assayed IFN titers on the less sensitive, noninducible Vero cells. Most of the activity was usually found in the supernatant medium. Both assay systems gave essentially equivalent results at the higher IFN titers, but GM2504 cells were necessary to establish the lower limits for relatively inactive constructs (Table 1). The results of several representative experiments revealed that the IE175-IFN hybrid gene in pGR238 gave approximately 15-fold higher total yields of IFN than that of the TK-IFN hybrid in pGR192. As expected, the IE175-IFN construction with the IFN cDNA in the antisense orientation (pGR239) was essentially inactive in the assay (less than 2% of the level produced by pGR238). Considering that the IE175-IFN construction lacks a poly(A) site, these results presumably underestimate the comparative strength of the HSV IE promoter relative to the HSV TK promoter, although on the other hand the polyadenylation process is known to be relatively inefficient in oocytes.

IFN expression in DNA-transfected cell lines. Our primary objective initially was to examine the efficiency of establishing stable DNA-transfected cell lines containing hybrid

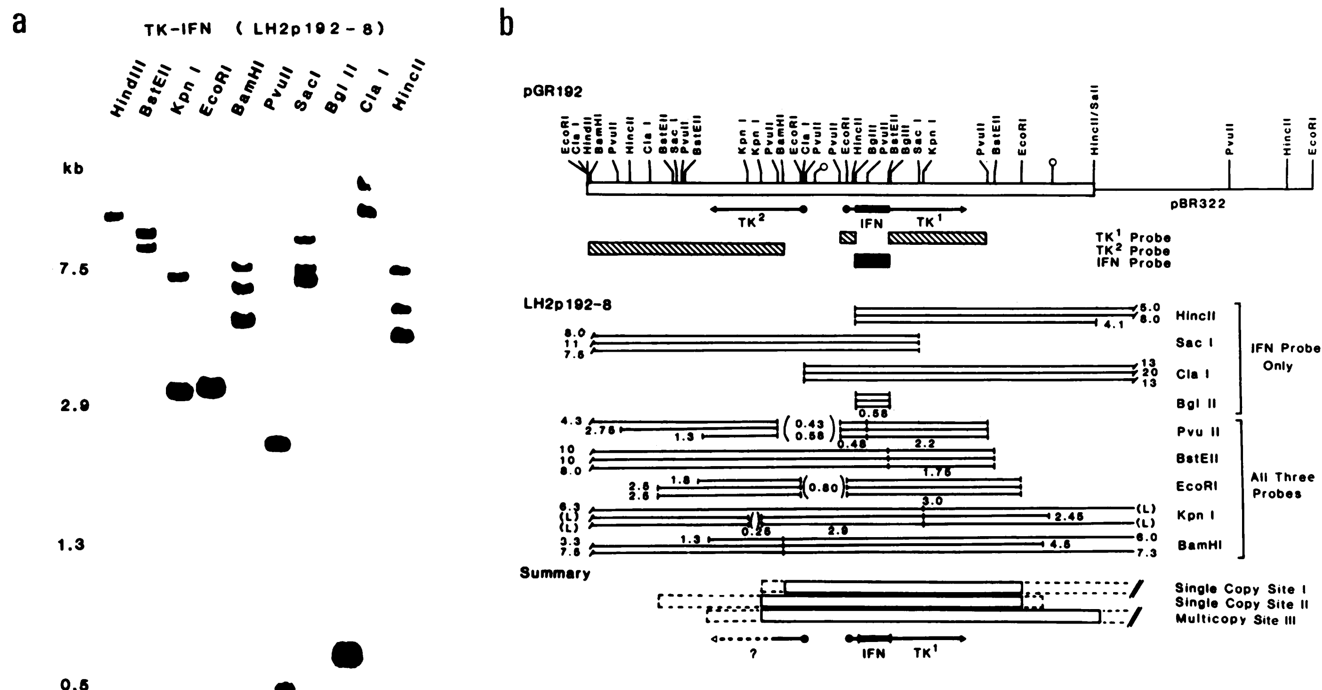

FIG. 1. Mapping by Southern blot hybridization of stably integrated TK-IFN DNA sequences in the highly inducible cell line that was established by linked TK coselection. (a) Samples of LH_2p192-8 cell DNA (15 μg) were cleaved with various restriction enzymes, followed by agarose gel electrophoresis and transfer to a nitrocellulose filter sheet. The figure shows an autoradiograph obtained after hybridization with a ^{32}P-labeled 560-bp IFN-specific DNA probe. (b) Diagram summarizing the structure of the three integrated TK-IFN hybrid gene fragments in LH_2p192-8 cells deduced from the results of two sets of Southern blot DNA hybridization analyses. The data include a comparison of the cleavage maps of each of the three integrated fragments relative to that of the input pGR192 plasmid DNA from an experiment in which blots were prepared in triplicate and hybridized separately to each of three different probes: a 2.0-kb *Pvu*II fragment from pHSV106 containing the HSV-1 TK gene, a 3.4-kb *Bam*HI fragment from pGR18 containing the HSV-2 TK gene, and the 560-bp *Bgl*II fragment containing the IFN coding region from pGR191. Numbers in the figure are in kilobases. Hatched bars denote the locations of the TK probe sequences, and solid bars represent the IFN probe sequences. Arrows indicate the positions and orientations of the HSV-2 TK and hybrid HSV-1 TK-IFN mRNA transcripts.

genes that respond to specific *trans*-activating factors. The construction of the TK-IFN (LH_2p192) series of cell lines by linked TK coselection after DNA transfection of mouse Ltk^- cells (40) yielded only 1 of 16 tested Ltk^+ colonies that possessed an intact inducible TK-IFN hybrid gene, and as we have shown above, even in this cell line only fragments of the input plasmid DNA were retained. Therefore, we used unlinked coselection with an HSV TK plasmid (pGR18) to attempt to increase the efficiency of retaining intact input DNA and to obtain cell lines with higher copy numbers of transfected DNA. A relatively large number of Ltk^+ colonies from unlinked cotransfection experiments with pGR238

TABLE 1. IFN activity expressed from hybrid IE175-IFN genes after microinjection into *X. laevis* oocytes

Hybrid gene[a]	Plasmid DNA	Interferon titer (U/ml) in[b]:			
		Expt 1		Expt 2	
		GM2504	Vero	GM2504	Vero
None	pBR322	<40	<250	<40	<500
TK-IFN(+)	pGR192	320	<500	120	<500
TK-IFN(−)	pGR191	<40			
IE175-IFN(+)	pGR238	5,120	3,000	1,680	1,000
IE175-IFN(−)	pGR239	80			
IFN-βcDNA	pHFβ	<40	<250		

[a] +, Sense orientation; −, antisense orientation.

[b] Combined values from the supernatant and homogenized oocytes.

and from both linked and unlinked cotransfection with pGR192 DNA were isolated and assayed for basal IFN titers produced in the absence of infection. Data for several representative stable cell lines of each type are given in Table 2. Basal expression was defined as the level of IFN accumulated in the supernatant of uninfected cells over a 16- to 20-h period after a fresh medium change. After the first several passages, most of the IE175-IFN and TK-IFN lines yielded significant but low basal IFN synthesis (60 to 240 U/ml) compared with background levels of less than 10 U/ml in the parent Ltk^- cells or in LH_2p18 cells that received the HSV TK gene only and in cell lines cotransfected with plasmids containing IFN cDNA (data not shown).

***trans*-Activation of IFN expression in mouse cell lines after HSV infection.** The IFN titers that accumulated in the medium from a number of independent clones of DNA-transfected mouse Ltk^+ cell lines over a 20-h period after superinfection with HSV-1 are also given in Table 2, together with the ratio of virus-induced IFN to basal IFN expression. Activation of IFN expression in the $LH_2$192-8 cell line was frequently measured to be between 250- and 500-fold in the present experiments, and 100-fold viral-mediated induction was also observed with six of eight additional TK-IFN cell clones constructed by either linked or unlinked coselection (LH_2p192-18 to LH_2p192-24). Furthermore, HSV-1 infection efficiently induced IFN production (30- to 190-fold) in approximately 40% of all independently derived cloned cell lines tested that had received the

coselected IE175-IFN hybrid gene (LH_2p238 cells). The high proportion of Ltk^+ colonies that yielded inducible cell lines implies that the ability to respond to factors introduced by superinfecting virus almost certainly resides within the input hybrid gene sequences and is relatively independent of the site of integration within the cellular genome. The properties of continuous low level basal IFN expression, together with the ability to be induced by HSV infection, have remained stable during passaging of the cultures for nearly 3 years in those cell lines studied in detail (e.g., LH_2p192-8 and LH_2p238-38; Table 2).

Superinfection with the heterologous HSV-2 virus instead of HSV-1 also stimulated IFN production in both cell types. An examination of the effect of the multiplicity of infection (MOI) on induction with each virus subtype on the TK-IFN and IE175-IFN cell lines revealed that the IFN yields were strongly dependent on input PFU/cell ratios (Table 3). Maximum yields at 16 h with HSV-1 occurred at an MOI of 1 to 3 with decreased yeilds at higher MOIs. Using HSV-1 at an MOI of 5, no additional active IFN protein accumulated after the 14-h time point in IE175-IFN cells, whereas the bulk of the IFN activity accumulated only after 14 h in the TK-IFN cells.

Examination of *trans*-activation at the RNA level. The use of human IFN sequences in mouse cells gave us the opportunity to examine the synthesis of IFN-specific RNA from the transfected hybrid genes in the absence of any background homology to either mouse DNA sequences or any incoming viral genes. Human IFN-specific RNA was barely detectable by dot blot analyses of the total RNA from uninfected TK-IFN (LH_2p192-8) cells but was induced at least 8- to 16-fold at both 5 and 24 h after HSV-1 infection (Fig. 2a). Similar analyses showed 8- to 24-fold RNA stimulation in the unlinked coselected TK-IFN cell lines (LH_2p192-23 and LH_2p192-24; data not shown). IFN-specific RNA was detectable in total cell RNA fractions from several different uninfected IE175-IFN (LH_2p238) cell lines, and both cytoplasmic and nuclear RNA was induced between 32- and 128-fold by 20 h after HSV-1 infection (Fig. 2b). In these cells the IFN RNA was predominantly retained in the nucleus, but a surprisingly large amount was also found in the cytoplasm, despite the lack of a poly(A) site in the hybrid gene construction. Results of other experiments with S1 nuclease analysis or Northern RNA blot hybridization (data not shown) confirmed that in both the TK-IFN and IE175-IFN cell lines a substantial portion of induced hybrid RNA was initiated at or close to the authentic mRNA start sites for these promoters. A cumulative time course of total IFN RNA levels present in infected IE175-IFN cells at early stages after infection is shown in Fig. 2c. The amount of IFN RNA was increased by six- to eightfold within 1 h, and twofold stimulation was detectable even within 30 min after the addition of the virus inoculum. In this experiment, the steady-state RNA levels were 30- to 40-fold above basal levels by 9 h, although other experiments have demonstrated maximal activation of RNA expression of up to 200-fold from the same cell line as early as 5 h after infection.

Control HSV-1 infections of diploid HF cells did not yield any detectable IFN RNA or biological activity (data not shown), and similarly, HSV infection of the parent Ltk^- cells did not induce any complicating mouse IFN titers or significant amounts of RNA complementary to the human IFN DNA probe. The amount of IFN RNA induced in the TK-IFN cells was comparable to that from Namalwa lymphoblast cells after Sendai virus induction, which yielded 10,000 U of IFN per ml in this experiment (Fig. 2a). This result and other positive controls with Newcastle disease virus induction in HF cells (data not shown) revealed that

TABLE 2. Basal and inducible levels of human IFN expressed in stable mouse cell lines receiving hybrid genes

Input hybrid IFN DNA gene	Cell line	IFN titer (U/ml)		Induction ratio
		Basal (20 h)	HSV-1 infected (20 h)	
None	Ltk^-	<60	<60	
	HF	<60	<60	
Intact TK	LH_2p18-1	<60	<60	
TK-IFN	LH_2p192-8(p6)	60	15,360	250×
	192-8(p10)	<100	25,000	>250×
	192-8(p22)	120	61,500	500×
	192-8(p38)	120	32,000	250×
	192-18[a]	<60	1,920	>32×
	192-19[a]	60	8,000	132×
	192-21[b]	60	7,680	125×
	192-22[c]	120	16,000	132×
	192-23[c]	150	16,000	100×
	192-24[c]	240	24,000	100×
IE175-IFN	LH_2p238-6	<200	6,400	>32×
	238-27	8	1,000	120×
	238-37	200	12,800	64×
	238-38(p6)	50	3,200	64×
	238-38(p12)	60	3,840	64×
	238-38(p32)	40	7,680	190×
	238-47	120	7,680	65×
	238-59	100	2,000	20×
	238-63	100	12,800	128×
	238-64	100	2,000	20×

[a] Linked coselection with pGR192 alone.
[b] Linked coselection with pGR192 with TK^2 inactivated.
[c] Unlinked coselection with pGR192 in which TK^2 was inactivated plus pGR18.

TABLE 3. Both HSV-1 and HSV-2 activate IE175-IFN and TK-IFN expression in stable cell lines

Virus	Time (h)	MOI (PFU/cell)	IFN titer (U/ml)	
			IE175-IFN (LH_2p238-38)	TK-IFN (LH_2p192-8)
Expt. 1				
Mock	6	0	<60	<60
	14	0	<60	<60
	24	0	60	60
HSV-1	6	5	7,680	1,920
	14	5	30,720	3,840
	24	5	30,720	61,440
HSV-2	6	5	960	240
	14	5	3,840	3,840
	24	5	15,360	3,840
Expt. 2				
HSV-1	16	0	<125	<125
	16	0.1	<125	500
	16	0.3	1,000	4,000
	16	1	4,000	16,000
	16	3	8,000	16,000
	16	9	8,000	8,000
HSV-2	16	0	<30	<30
	16	0.1	<30	<30
	16	0.3	480	240
	16	1	960	960
	16	3	3,840	1,920
	16	9	15,360	7,680

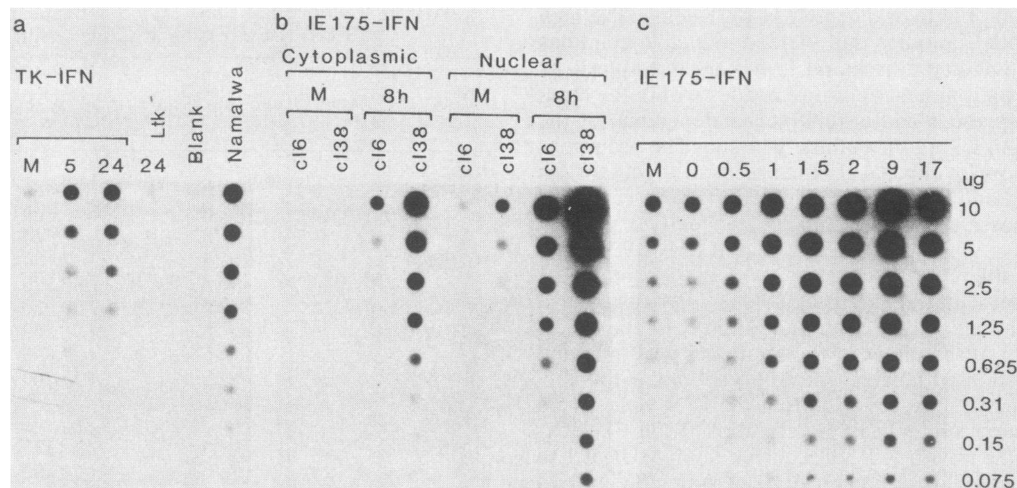

FIG. 2. Detection of human IFN-specific RNA expression in transfected Ltk$^+$ cell lines after HSV superinfection. (a) Response to HSV-1 infection at 5 and 24 h in TK-IFN (LH_2p192-8) and Ltk$^-$ cells. Each vertical series of spots represents twofold dilutions from 10 μg of total cell RNA which was extracted and purified by the guanidine thiocyanate-cesium chloride procedure. The RNA was applied to the filters and hybridized to an IFN DNA probe as described in the text. The negative control samples (M) represent mock infection after 24 h. The positive control sample contains 10 μg of RNA from the Namalwa human B lymphoblast line after Sendai virus infection. (b) Response to HSV-1 infection in two independent IE175-IFN cell lines (LH_2p238-6 and LH_2p238-38). Serial twofold dilutions were prepared from 10 μg of cytoplasmic or nuclear RNA extracted at 5 h after infection or mock infection (M). (c) Detailed analysis of the rapid response to HSV-1 infection in IE175-IFN (LH_2p238-38) cells at the indicated hours after addition of virus. The mock-infected sample (M) was incubated for 24 h in fresh medium.

the overall efficiency of translation of HSV-induced TK-IFN mRNA and of transport and secretion of the resulting IFN protein was almost as great in the LH_2p192-8 cell line as that of natural IFN mRNA produced in human cells in response to RNA virus infection. By comparison, the IE175-IFN cells produced high levels of RNA (especially in the nucleus) after HSV infection, but the ratio of active protein product to total homologous IFN-specific mRNA was relatively poor.

Different responses of IE175-IFN and TK-IFN cell lines to infection with HSV *ts* mutants. To demonstrate that the two different hybrid IFN constructs were responding to different viral activation mechanisms, we examined the patterns of IFN RNA induction after superinfection with the HSV-1 *ts*B_2 mutant which produces a nonfunctional IE175 gene product at nonpermissive temperatures and fails to synthesize viral DE mRNA (7). We have shown previously that synthesis of biologically active IFN from the LH_2p192-8 cell lines is temperature sensitive after *ts*B2 infection (40). Induction of TK-IFN RNA in these cells also proved to be temperature sensitive (Fig. 3). However, neither the increased IFN RNA nor protein production was affected by carrying out the infection at nonpermissive temperatures in the IE175-IFN cell line. These results indicate that the IE175-IFN cells are probably responding to the pre-IE virion transcription factor, whereas the TK-IFN cells require viral gene expression and a functional IE175 gene product.

Stable retention of IE175-IFN DNA sequences. Southern DNA blot analysis of cleaved cell DNA from a number of different IE175-IFN cell lines revealed that each inducible clone tested retained multiple copies of all DNA bands representative of the input plasmid DNA sequences (in contrast to the TK-IFN cell line described earlier). The patterns from 10 different IE175-IFN clones were very similar to one another and varied mostly in total copy number, ranging from approximately 1 copy per cell up to 30 copies per cell. Furthermore, the cell lines giving the highest spontaneous and inducible IFN levels tended to be those with the highest input DNA copy numbers. Additional studies with zero-cut and single-cut restriction enzyme digests showed that the input IE175-IFN DNA sequences were retained in a high-molecular-weight integrated form rather than in a monomer-sized unintegrated plasmid form (data not shown).

Amplification of IFN DNA copy number in IE175-IFN cell lines. The pGR238 plasmid used for constructing the IE175-IFN cell lines retained the HSV-1 ori_s DNA replication signal, and therefore we wondered whether DNA amplification might also be occurring after superinfection. Analysis of the IFN-specific DNA sequences by Southern DNA hybridization experiments revealed that the total amount of IFN gene DNA in IE175-IFN cells increased from approximately

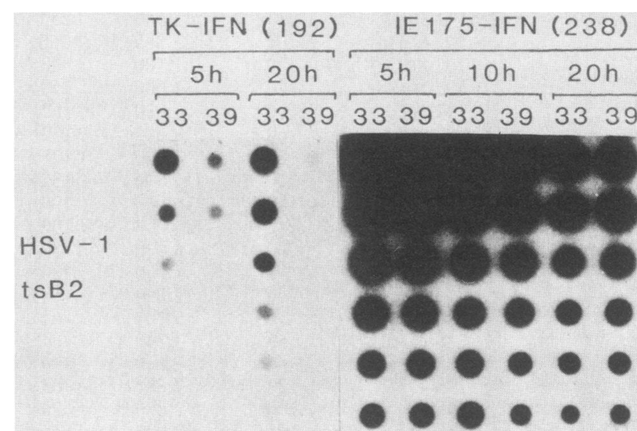

FIG. 3. Lack of requirement for a functional IE175 protein product during superinfection induction of IE175-IFN RNA. The autoradiograph shows an RNA dot blot hybridization assay for total IFN-specific RNA present in the LH_2p192-8(TK-IFN) and LH_2p238-38(IE175-IFN) cell lines at 5 and 20 h after infection with HSV-1(KOS)*ts*B2 at permissive (33.5°C [33]) and nonpermissive (39.5°C [39]) temperatures.

10 copies per haploid genome equivalent to between 80 and 160 copies by 20 h after HSV-1 infection (Fig. 4a). The major resident IFN-specific DNA species present both before and after amplification comigraged with the 3.6-kb *Pst*I fragment from the input pGR238 plasmid DNA. In contrast, no increase in the copy number of the IFN DNA sequences in IE175-IFN cells occurred at either 5 or 20 h in the presence of PAA, a specific inhibitor of HSV DNA polymerase.

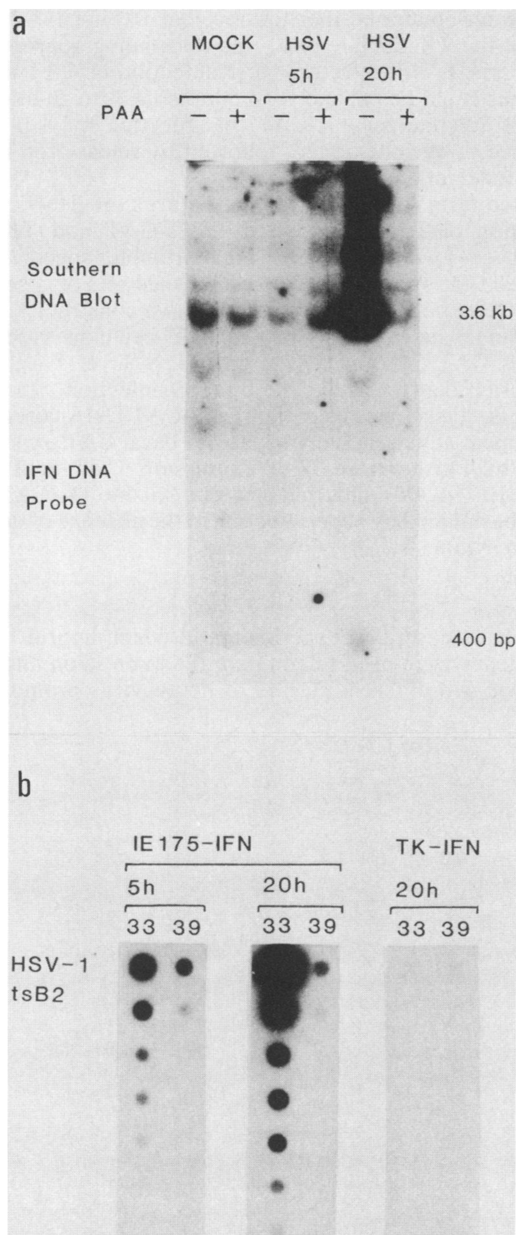

FIG. 4. Amplification of the resident IFN-specific DNA sequences in the IE175-IFN cell line after HSV infection. (a) Southern DNA blot hybridization analysis with 10-μg samples of LH_2p238-38 cell DNA extracted at 5 or 20 h from HSV-1-infected or mock-infected cells in the presence and absence of the HSV DNA polymerase inhibitor PAA. The DNA samples were cleaved with *Eco*RI-*Pst*I and hybridized after blotting with the 560-bp [^{32}P]IFN DNA probe. (b) Comparison by DNA dot blot analysis of amplification of IFN DNA sequences in LH_2p238-38 and LH_2p192-8 cells at 5 and 20 h after infection with HSV-1(KOS)*ts*B2 at permissive (33.5°C [33]) and nonpermissive (39.5°C [39]) temperatures.

Confirmation that the DNA amplification events in IE175-IFN cells required viral gene expression was obtained by repeating the experiment with a temperature-sensitive virus that is conditional in its ability to synthesize the DE-class virus-specific enzymes needed for replication at the viral DNA origin sequences. Superinfection with *ts*B2 virus produced amplification at permissive temperatures but failed to alter the IFN DNA content in IE175-IFN cells at nonpermissive temperatures (Fig. 4b). The ratio of DNA amplification at 33 over 39°C in this experiment was 3-fold at 5 h and 25-fold at 20 h. Very similar results were obtained with an IE175-CAT-containing Vero cell line (see below), which also gave 20- to 30-fold amplification of the CAT DNA sequences after HSV-1 infection (data not shown). In contrast, infection of cells containing the TK-IFN construct, which lacks a viral DNA replication origin, did not lead to any amplification of the IFN DNA sequences even at the permissive temperature.

Effects of PAA on levels of IFN RNA and protein yields. Transcription of IE genes might normally be expected to have been shut down by the time DNA replication occurs; however, a relatively unexpected feature of IE175-IFN *trans*-activation was the extended RNA synthesis (to at least 9 h after infection at 37°C Fig. 2c), as well as the great abundance of IFN RNA produced. To investigate whether the increased template copy number may affect the levels of IE175-IFN activation observed, we carried out additional studies which showed that the presence of PAA failed to reduce more than twofold the amount of IFN RNA present at 5 or 20 h after infection of IE175-IFN cells (Fig. 5). Therefore, either the actively replicating IE175-IFN gene was not a template for transcription at these late times or normal shut off and degradation of IE175-IFN transcripts in the absence of PAA counterbalanced any positive transcriptional effects of amplification of the DNA template. The use of PAA reproducibly lowered the yields of biologically active IFN from HSV-1-infected IE175-IFN cells approximately eightfold in these experiments, but this effect also occurred with TK-IFN cells (data not shown).

Evidence for a viral shutoff mechanism acting on IE175-IFN expression. Although an active IE175 gene product is needed throughout the HSV replication cycle (50), both its transcription and translation are believed to be subject to specific, but as yet undefined, viral shutoff mechanisms. Evidence supporting a virus-induced negative regulation mechanism acting on IE175-IFN transcripts in the DNA-transfected cell lines came from two sets of related observations. First, infection with wild-type virus at 39°C resulted in rapid induction of IE175-IFN RNA compared with that at 33°C within the first 5 h, followed by a return to basal levels of IFN RNA by 20 h (Fig. 6). Almost no IE175-IFN RNA remained after 20 h at 39°C in this experiment, despite the extensive synthesis at earlier times. Similar results were obtained with the TK-IFN cell line (data not shown). The degradation of the RNA appeared to be a relatively late event because by 20 h at 33°C the steady-state RNA levels were still at a maximum. Second, two different classes of *ts* mutants unable to synthesize DE viral gene products at 39°C each gave greatly increased (or stabilized) levels of IE175-IFN RNA at 20 h compared with infection with wild-type virus at 39°C. The *ts*B2 mutant, which produces an inactive IE175 gene product at 39°C, might be expected to continue to synthesize IE gene products even at late times after infection (50), but *ts*B7 is an uncoating mutant in which the

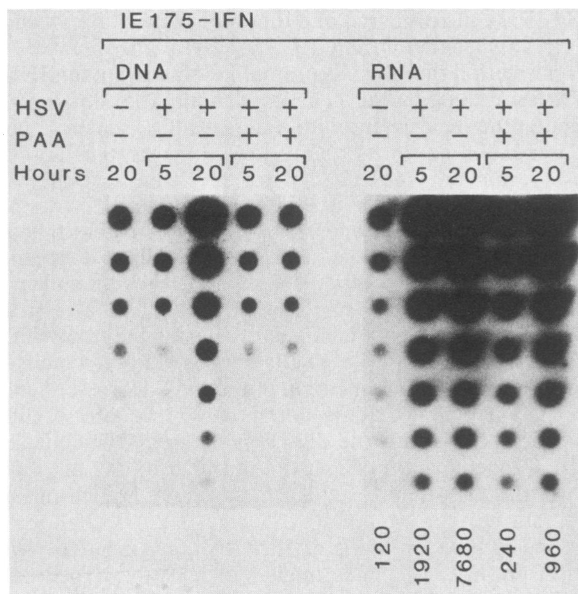

FIG. 5. Lack of effect of PAA-blocked DNA amplification on steady-state RNA levels after induction. IFN-specific DNA or RNA sequences were detected in serial twofold dilutions from 10-μg samples of total infected cell DNA or RNA after dot blot hybridization analysis. The diagram shows comparisons of LH_2p238-38 cells at 5 and 20 h after HSV-1(MP) infection (+) or mock infection (−) and in the presence (+) or absense (−) of PAA. The RNA samples were derived from parallel cultures infected at the same time as those used for the DNA analysis. Measurements of IFN titers (units per milliliter) from the same cultures are given below each column in the RNA blots.

input virion DNA does not reach the nucleus at 39°C (1). Therefore, in the absence of progression of the lytic cycle, either the RNA-destablizing factors are not produced or positive transcriptional stimulation by the pre-IE virion factor occurs continuously.

Specific activation of hybrid CAT genes in coselected cell lines. O'Hare and Hayward (33) have described plasmids containing hybrid CAT genes that are under the transcriptional control of the HSV-1 IE175 and TK promoter regions and have presented evidence for differential *trans*-activation of CAT expression from these two constructs by superinfecting virus after short-term DNA transfection into Vero cells. To determine whether these hybrid CAT genes, like the IFN hybrids, would respond to specific viral regulatory signals in stable long-term transfected cells, we have also established Vero cell lines containing these two plasmid DNAs by unlinked coselection with pSV2neo DNA (45). G418-resistant cell lines were cloned and tested for both basal and virus-induced levels of CAT activity (Fig. 7a). Of 12 tested clones from the culture that received TK-CAT, 2 were inducible at least 20-fold at 8 h after HSV infection, but neither clone gave significant basal expression. In contrast, 6 of 10 IE175-CAT cell clones gave strong basal CAT expression, and the only one examined further could be induced at least eightfold by HSV superinfection. Note that, because of the high constitutive expression in the IE175-CAT cells before infection, the actual level of induction after infection was difficult to evaluate. The specificity of the *trans*-activation was examined by carrying out the infection in the presence of cycloheximide for 4 h to prevent de novo viral protein synthesis followed by reversal for 4 h in the presence of actinomycin D. Under this protocol, the only viral proteins synthesized would be IE gene products, the mRNA of which is transcribed in the presence of cycloheximide. Similarly, increased CAT protein synthesis can be directed only by CAT mRNA produced during the cycloheximide block. The TK-CAT Vero cell line failed to be induced under these conditions, whereas cycloheximide treatment did not prevent the stimulation in CAT expression observed after superinfection of the IE175-CAT Vero cell line (Fig. 7a). This result confirmed that the resident IE175-CAT hybrid, but not the TK-CAT hybrid, was responding appropriately to the pre-IE virion factor. Viral induction of CAT in both cell lines could be enhanced an additional 3- to 10-fold (to a total of 200-fold for TK-CAT) by infection for 4 h in the presence of cycloheximide, followed by release for 16 h in the absence of actinomycin D.

To confirm that the major features of HSV *trans*-activation of DNA-transfected IE175-CAT and TK-CAT hybrid genes were reproducible and promoter specific rather than cell type specific, we also established sets of coselected mouse Ltk^- cells that received these two constructs. Again, 1 of 10 independent G418-resistant Ltk^- cell clones receiving the TK-CAT plasmid DNA gave low level basal CAT expression that was inducible by HSV infection, whereas 8 of 10 Ltk^- cell clones from the IE175-CAT DNA coselection experiment gave relatively high level basal CAT expression (Fig. 7b). Furthermore, CAT expression in all eight of the Ltk^- IE175-CAT lines, but not that in the TK-CAT line, could be induced by superinfection in the absence of de novo protein synthesis.

DISCUSSION

We have described experiments in which hybrid IFN or CAT genes were used to compare the expression and regulation of two different classes of herpesvirus promoters in

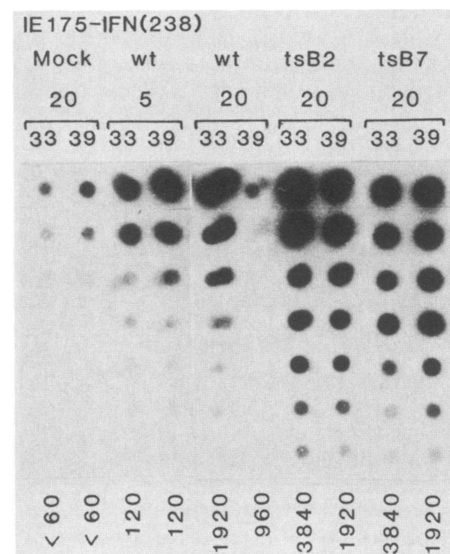

FIG. 6. Failure to shutoff IE175-IFN RNA induction in the absence of a functional IE175 gene product. RNA dot blot hybridization analysis of IFN-specific total cell RNA levels in LH_2p238-38 cells at various times (5 and 20 h) after infection with HSV-1(MP), HSV-1(KOS)*ts*B2, or HSV-1(HFEM-STH2)*ts*B7 at permissive (33.5°C [33]) and nonpermissive (39.5°C [39]) temperatures. Parallel measurements of IFN titers (units per milliliter) from the same culture samples are given below each column. wt, Wild type HSV-1(MP).

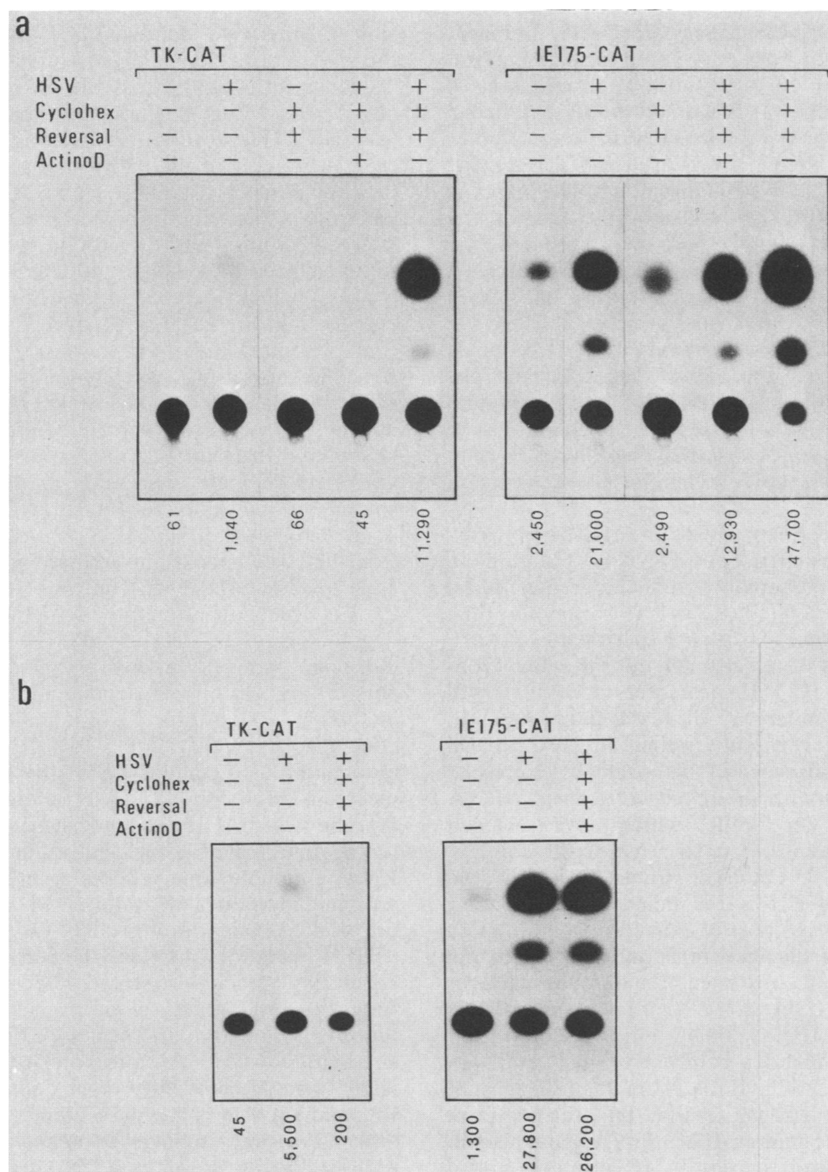

FIG. 7. Specificity of TK-CAT and IE175-CAT induction in stable DNA-transfected cell lines after various superinfection protocols. (a) Comparison of basal and induced CAT activity in G418-resistent Vero cells containing TK-CAT (VNpPOH3-6) or IE175-CAT (VNpPOH2-1). Infection with HSV-1(MP) at 36°C in the absence of cycloheximide (Cyclohex) was carried out for 8 h. Cycloheximide treatment was maintained from 0 to 4 h after infection and reversal in the presence of actinomycin D (ActinoD) was performed for an additional 4 h. Samples from cycloheximide reversal carried out in the absence of actinomycin were harvested at 16 h. (b) Similar comparison as in panel a above of basal and induced CAT activity in G418-resistant Ltk^- cell lines containing TK-CAT (LNpPOH3-5) or IE175-CAT (LNpPOH2-10). Measurements of radioactivity (counts per minute) present as [^{14}C]-2 acetylchloramphenicol are given below each lane.

microinjected oocytes and in stable DNA-transfected cell lines. Expression of the hybrid IFN or CAT genes was inducible by viral superinfection in at least 20 to 50% of permanent coselected Ltk^+ or Vero cell lines tested. This high efficiency, together with the appropriateness of the phenotype of all cell lines examined in detail, implies that specific signals contained within the input DNA are directly responsive to viral *trans*-activating factors, with minimal influence from the local genomic environment.

Activation of IE175 hybrid mRNA and protein synthesis occurred in the absence of any viral IE gene expression and in the absence of a functional IE175 protein. Therefore, we conclude that the IE175-IFN and IE175-CAT hybrid genes (even in an integrated, chromatin-associated form) are efficiently recognized and activated in *trans* by the virion-associated pre-IE transcription factor within 30 to 60 min of the addition of virus to the cell. The question of the specificity of DE activation remains to be resolved. The TK-IFN and TK-CAT hybrid genes obviously were not responding to the pre-IE virion factor, but the possibility that any transfected gene might respond to HSV IE gene products remains feasible (9). On the other hand, as we will describe elsewhere, expression of cytomegalovirus IE94-IFN hybrid genes in DNA-transfected Ltk^+ cell lines is also stimulated by HSV superinfection, but by a different mechanism from either the virion factor or IE175K-dependent

induction responses described here (J. Mosca, K. T. Jeang, P. M. Pitha, and G. S. Hayward, submitted for publication).

The 500-fold induction of IFN titers after infection of LH_2p192-8 (TK-IFN) cells was 40- to 100-fold greater than that observed in a number of previous superinfection studies in which the intact viral TK gene was used and TK enzyme levels were assayed (8, 22, 29, 52). Our initial DNA mapping analysis raised the interesting possibility that in the LH_2p192-8 cells the active TK gene product expressed may be encoded by a bicistronic IFN-HSV-1 TK hybrid mRNA rather than by the HSV-2 TK gene. However, since three other TK-IFN cell lines tested that were established by unlinked coselection also gave relatively high IFN yields after infection, we attribute the higher induction ratio primarily to coselection of the target TK-IFN hybrid gene and suggest that a fully inducible DE phenotype is unlikely to be displayed by viral TK assay genes that have already been subjected to selection for relatively high basal expression in HAT medium.

The higher IFN titers obtained with the IE175-IFN plasmid relative to those with the TK-IFN plasmid in microinjected oocytes presumably reflects greater basal transcription from the isolated HSV-1 IE promoter than from an HSV DE promoter. In other experiments, in vitro runoff transcription analysis also yielded considerably stronger signals from several HSV IE promoters compared with those from HSV DE promoters (K.-T. Jeang and P. O'Hare, unpublished data). The relatively strong levels of basal IE175-CAT expression observed in the coselected Vero cell lines are also consistent with the results of transient DNA transfection assays in Vero cells, which have revealed similar differences between the constitutive expression levels of TK-CAT and IE175-CAT constructs (33). Only the Ltk^+ cell lines receiving IE175-IFN failed to show higher basal expression than their TK-IFN counterparts. Although this could be related to the lack of a poly(A) site in the pGR238 construct, we also observed that an even stronger promoter (that from the IE94 gene of cytomegalovirus) fails to drive significant basal IFN production in coselected Ltk^+ cell lines, which is in distinct contrast to the results in oocytes and with IE94-CAT hybrids in Ltk^+ and Vero cell lines. We have argued elsewhere that this results from intrinsic instability of the human IFN mRNA sequences in mammalian cells and that activation by HSV infection also involves an mRNA stabilization mechanism (Mosca et al., submitted).

In contrast to an 8- to 16-fold increase in TK-IFN RNA levels after viral infection, the IE175-IFN cell lines gave up to 200-fold activation of IFN RNA levels. At present we do not know whether this represents a real difference in the level of inducibility of these two promoters or whether the TK-IFN induction was underestimated because of extremely low basal expression below the background detection levels. Nevertheless, the efficiency of transport and translation of the IE175-IFN RNA was far below that of the TK-IFN RNA. Presumably, this difference primarily reflects the lack of a known poly(A) site in the IE175-IFN construction, and we anticipate that much higher IFN protein induction titers would be obtained from similar constructs containing the IE175 promoter together with an efficient 3′ poly(A) signal region. This prediction is partially supported by the higher constitutive and induced levels of CAT expression observed in both the Ltk^- and Vero cell lines receiving IE175-CAT relative to those receiving TK-CAT genes. In these latter two constructs, both the IE and DE promoters drive expression of otherwise identical CAT coding regions containing the simian virus 40 3′ polyadenylation site.

Transcription and translation of most cellular genes usually ceases within the first few hours after HSV infection (11). HSV IE transcription and translation also are thought to be negatively regulated by viral gene products, although a functional and presumably stable IE175 protein is required continuously to support both DE and late viral gene expression (50). Shutoff of host protein synthesis has been shown to take place in two stages (10, 16, 32). An early, very rapid process, which included disaggregation of polysomes, occurs even in the absence of viral RNA synthesis and therefore, presumably involves a virion factor. This is followed by a second, slower process that does require viral gene expression. Recently, Read and Frenkel (39), using virus mutants deficient in the virion factor-mediated host shutoff process, observed overproduction of IE gene products and postulated that viral IE gene expression may also be susceptible to shutoff by this same mechanism. In this regard, expression of the resident IE175-IFN hybrid gene in our DNA-transfected cell lines showed three major features: (i) steady-state levels of IE175-IFN mRNA continued to accumulate until at least 9-h after infection; (ii) complete shutoff and degradation of IE175-IFN and TK-IFN mRNA expression did occur, but only at relatively late times after infection; and (iii) this shutoff was dependent on viral gene expression, including the synthesis of a functional IE175 protein. We can conclude, first, that IE175-IFN RNA apparently escapes recognition as a cellular mRNA by the pre-IE virion factor mechanism for shutoff of host protein synthesis and, second, that the hybrid IE175-IFN construct is probably not subject to the anticipated early shutoff mechanisms for viral IE RNA, although some process resulting from later stages of viral infection does ultimately lead to cessation of transcription and degradation of the RNA.

HSV infection provides helper functions in *trans* for generating tandemly repeated defective DNA from free viral fragments containing ori_L or ori_S sequences (4, 46, 49), and the precise location and structure of one of these initiation sites (ori_S) within the promoter-regulatory region of the IE175 gene has recently been defined (47). Mocarski and Roizman (30) also have presented preliminary evidence that an HSV ori_S region linked to a coselected viral TK gene and viral packaging signals in a DNA-transfected cell line could undergo virus-activated DNA amplification. Although overall cellular DNA synthesis is shut down rapidly after HSV infection (10), our results show, first, that amplification of transfected IE175-IFN and IE175-CAT DNA sequences is a reproducible phenomenon related specifically to the presence of the adjacent integrated ori_S sequences and, second, that the amplification requires viral gene expression and a functional viral DNA polymerase. Details of the replication mechanism involved in selective amplification of DNA sequences associated with an integrated ori_S region are unknown, but cell lines of this type should provide useful new approaches for studying both HSV DNA replication events and the effects of active DNA replication on the expression of adjacent viral genes, especially those of the late DNA replication-dependent class.

The use of reconstructed model systems of this type, involving hybrid IFN or hybrid CAT cell lines, that are subject to apparently correct and specific HSV gene regulation, should aid in further detailed characterization of both the viral factors and transcriptional or posttranscriptional mechanisms involved in these processes and may help to

explain how infected cells discriminate between viral and cellular mRNA species.

ACKNOWLEDGMENTS

These studies were supported by Public Health Service grants ROI CA28473 and ROI CA22130 to G.S.H. from the National Cancer Institute and by an American Cancer Society of Maryland grant MV-208 to P.M.P. J.D.M. was a Postdoctoral Fellow funded by a Pharmacology/Oncology Training Grant at Johns Hopkins Medical Institutions (Anti-Cancer Drug Development 5T32 CA09243). G.R.R. was a predoctoral trainee in the Johns Hopkins University Medical Scientist Training Program (NIH GM07309). G.S.H. is a Faculty Research Awardee of the American Cancer Society (FRA-247).

We thank Elizabeth Gavis for assistance with the oocyte microinjection experiments, Peter O'Hare for donating the hybrid CAT plasmids, Dolores Ciufo and Merrill Kellum for excellent technical assistance, and Nancy Standish for typing the manuscript.

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