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The Promoter-Regulatory Region of the Major Immediate-Early Gene of Human Cytomegalovirus Responds to T-Lymphocyte Stimulation and Contains Functional Cyclic AMP-Response Elements

G. W. HUNNINGHAKE,[1] M. M. MONICK,[1] B. LIU,[2] AND M. F. STINSKI[2]*

Department of Medicine[1] and Department of Microbiology,[2] University of Iowa College of Medicine, Iowa City, Iowa 52242

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Prior studies have demonstrated that a small proportion of blood lymphocytes from patients with human cytomegalovirus (HCMV) infection express only the viral immediate-early (IE) genes (L. Einhorn and A. Ost, J. Infect. Dis. 149:207–214, 1984; G. P. A. Rice, R. D. Schrier, and M. B. A. Oldstone, Proc. Natl. Acad. Sci. USA 81:6134–6138,1984). The present studies demonstrate that the IE genes of HCMV are transcribed in Jurkat cells (T lymphocytes) only after activation of the cells with mitogens. Transcription of the IE genes is from an upstream enhancer promoter-regulatory region containing several different repeated sequence motifs. Chimeric plasmids were constructed with just a single copy or three copies of a synthetic oligonucleotide sequence of either the 16-, 18-, 19-, or 21-base-pair (bp) repeat elements upstream of the minimal wild-type promoter sequence to drive expression of the indicator gene, chloramphenicol acetyltransferase (CAT). The 18- or 19-bp motifs in the enhancer region were found to be important in mediating the effect of the mitogens. However, the CAT activity detected with the 19-bp repeat was always significantly higher than that found with the 18-bp repeat. There was an additive effect by multiple copies of the 18- or 19-bp repeat sequences on gene expression. The 19-bp repeat contains a sequence identical to that described for a cyclic AMP (cAMP) response element, and plasmids containing only this sequence and the minimal promoter sequences upstream of the CAT gene respond to agents which increase intracellular cAMP. Functional cAMP response elements are present in the wild-type promoter-regulatory region and are associated with the 19-bp repeat sequences. It is proposed that activation of lymphocytes results in expression of the IE genes of HCMV, in part via the activation of cellular *trans*-acting factors which interact with the 18- and 19-bp motifs in the HCMV IE promoter-regulatory region. The 19-bp repeat is the major contributor to the strength of this enhancer-containing promoter-regulatory region.

Human cytomegalovirus (HCMV) is an important cause of disease in immunocompromised patients. Allograft recipients have a very high incidence of infection, and many of these infections are thought to represent a reactivation of a latent viral infection (9, 15, 18, 19, 40, 46). A majority of normal individuals become infected early in life, but most of these infections remain entirely asymptomatic, unless the normal immune processes of the host are compromised (9, 15, 18, 19, 40, 46).

After infection, the genes of HCMV are expressed in three distinct phases, designated immediate-early (IE), early, and late (4, 26, 43, 50). The IE genes are transcribed soon after infection, and their expression is not dependent on prior viral protein synthesis (4, 26, 43, 50). Patients with allografts and potential antigen activation of host T lymphocytes have an unusually high incidence of infection by HCMV (9). It is of interest that only a small fraction of peripheral blood T lymphocytes from patients with HCMV infection express the IE genes (5, 34). Activation of T lymphocytes might result in IE gene expression.

If T-lymphocyte activation plays a role in expression of HCMV IE genes, it may occur via the activation of eucaryotic cell *trans*-acting factors that interact with *cis*-acting elements in the promoter-regulatory region of the major IE gene. The HCMV major IE promoter-regulatory region is known to have multiple binding sites for nuclear transcription factors (6–8, 13). This region contains 16-, 18-, 19-, and 21-base-pair (bp) repeats which may play a role in regulation of gene expression (2, 6, 44, 48). Prior studies indicate that of these repeat sequences, the 18- and 19-bp repeats are the most active in modulating the activity of the IE gene (44). It is of interest that the core of the 19-bp repeat has a sequence (TGACGTCA) identical to that reported for a cyclic AMP response element (28, 29, 33, 36, 49; for a review, see reference 35). It is known that in other genes this sequence binds a 43-kilodalton protein, called activating transcription factor (ATF), which is thought to mediate the effect of cAMP on gene transcription (11, 16, 17, 28, 29, 33, 35, 36, 49).

Our objectives in this investigation were to determine (i) whether T-lymphocyte activation results in activation of IE gene expression; (ii) which repeat elements in the major IE promoter-regulatory region are most responsive in mediating gene transcription in activated T lymphocytes; and (iii) whether a functional cAMP response element is present.

MATERIALS AND METHODS

Reagents. Phorbol myristate acetate (PMA), phytohemagglutinin (PHA), forskolin, prostaglandin E_2, and 8-(4-cholorphenylthio)-cAMP were obtained from Sigma Chemical Co., St. Louis, Mo. GeneScreen Plus and [γ-^{32}P]ATP were obtained from Du Pont, NEN Research Products, Boston, Mass. DEAE-dextran was obtained from Pharmacia, Upp-

* Corresponding author.

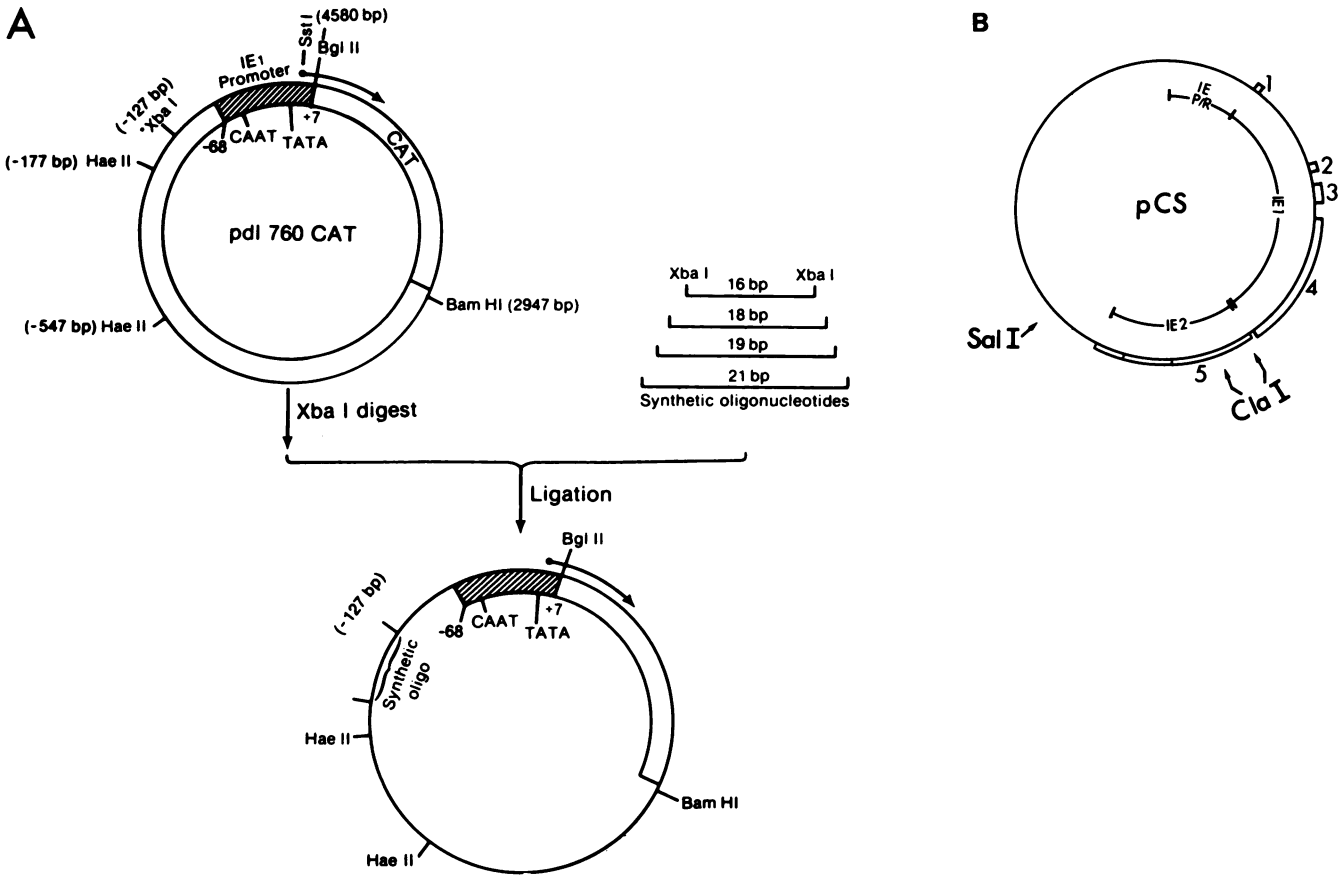


FIG. 1. Construction of plasmids with synthetic oligonucleotide repeat elements inserted upstream of the wild-type IE1 promoter and construction of a plasmid with the IE1 and IE2 genes downstream of the HCMV IE promoter-regulatory region. (A) Synthetic oligonucleotides of the 16-, 18-, 19-, and 21-bp repeats (44) with restriction endonuclease *Xba*I staggered ends were ligated at the *Xba*I site of plasmid pdl760 CAT. The resulting recombinant plasmids were designated according to the type and number of synthetic repeats inserted. (B) The IE1 and IE2 genes in plasmid pCS are designated. Numbers 1 to 5 represent the exons. Exons 1, 2, and 3 can be spliced to either exon 4 or exon 5, as described previously (42).

sala, Sweden. Heparin was purchased from Elkins-Sinn, Cherry Hill, N.J. *Escherichia coli* 23S and 16S rRNAs were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. A thin-layer chromatograph scanner (RTLC Scanner) was obtained from Radiomatic Instruments and Chemical Co., Tampa, Fla.

Tissue culture. Human fibroblast cells were cultured as previously described (43). Jurkat cells (a T-cell line) were maintained in suspension cultures in RPMI 1640 medium containing 10% endotoxin-free fetal calf serum (Hyclone Laboratories, Inc., Logan, Vt.), 4 mM L-glutamine, and 50 μg of gentamicin per ml.

Plasmid constructions. Construction of the plasmid pCAT760, which contains the wild-type promoter-regulatory region of the HCMV (Towne strain) major IE gene (see Fig. 6) upstream from the procaryotic chloramphenicol acetyltransferase (CAT) gene, has been previously described (44). A similar plasmid, pTJ278, which contains the promoter-regulatory region of the simian CMV major IE gene upstream of the CAT gene was kindly provided by G. Hayward, John Hopkins University, Baltimore, Md. DNA sequencing demonstrated that regulatory regions of HCMV and simian CMV have sets of repetitive elements which are interspersed and highly conserved but that the relative organization of these repeat motifs differs markedly. Deletion plasmids pCATdl36, pCATdl14, pCATdl4, pCATdlA231, pCATdlA23, and pCATdlNde were constructed as previously described (44). The number of 16-, 18-, 19-, and 21-bp repeats in each of these plasmid constructs has also been described previously (44). Plasmid pdl760CAT contains the minimal wild-type promoter and downstream initiation site (−68 to +7) upstream of the CAT gene. Plasmids pIE1-161, pIE1-181, pIE1-191, and pIE1-211 have a single 16-, 18-, 19-, or 21-bp synthetic repeat sequence, respectively, inserted 59 bp upstream of −68 in pdl760CAT (Fig. 1A). Plasmids pIE1-163, pIE1-183, pIE1-193, and pIE1-213 have a triplicate of the 16-, 18-, 19-, or 21-bp synthetic repeat sequence, respectively, inserted 59 bp upstream of −68 in pdl760CAT (Fig. 1A). The 59 bp between the site of insertion and −68 is vector DNA. A plasmid containing the major IE promoter-regulatory region upstream of the bona fide IE1 and IE2 genes and designated pCS(IE_1,IE_2) is diagrammed in Fig. 1B and has been described previously (14). Recombinant plasmids were prepared as described previously (44).

Transfection. All DNA concentrations were checked by comparison of the intensities of ethidium bromide-stained bands of restriction endonuclease-digested plasmid DNAs following gel electrophoresis. Jurkat cells were transfected by using 10^7 unstimulated cells and the DEAE-dextran method as described previously (30). After transfection, the cells were washed once in RPMI 1640 medium containing 1.5

units of heparin per ml and once in the same medium without heparin. After being washed, the cells were always cultured for 48 h in flat-bottom plates in 2 ml of medium containing 10% fetal calf serum. The cells remained either unstimulated or stimulated at various times during the 48-h period of culture. In most instances, the cells were stimulated 24 h after transfection with PMA (10 ng/ml) and PHA (1 μg/ml). After 48 h in culture, the cells were harvested, washed, and sonicated, and supernatants of the sonic extracts were used in the CAT assay. Human fibroblasts were transfected as described previously (14).

CAT assays. CAT assays were performed on duplicate cultures as described by Gorman et al. (10). Two or three different plasmid DNA preparations were used for each experiment. CAT activities were determined for the linear portion of the enzyme reaction. The acetylated derivatives were separated from nonacetylated chloramphenicol by ascending chromatography with a chloroform-methanol (95:5) solvent. The plates were exposed to XAR-2 film (Eastman Kodak Co., Rochester, N.Y.). The radioactivity on the plates was quantitated by using a thin-layer chromatography scanner.

Northern (RNA) blot hybridization. Whole-cell RNA was isolated by the guanidine hydrochloride-CsCl gradient procedure as described previously (45). The isolated RNA was fractionated by the method of Lehrach et al. (21) in a 1.5% denaturing agarose gel containing 2.2 M formaldehyde. *E. coli* 23S (3.3-kilobase[kb]) and 16S (1.7-kb) rRNAs were included as molecular size standards. The RNAs were transferred to GeneScreen Plus paper as suggested by the manufacturer. ^{32}P-end-labeled oligonucleotide probes were prepared and hybridized to the blots as described previously (45). Two oligonucleotide probes to the IE genes were used. One probe (5′-TCTTGGCAGAGGACTCCATCGTGTC-3′) detects a sequence in exon 2 and therefore detects both the IE1 and IE2 mRNAs described previously (42). A second probe (5′-TCATCTCCTCGGACTCACTCTCCGA-3′) detects a sequence upstream of the intron in IE2 and therefore detects only IE2 mRNA (42).

RESULTS

Effect of activation of Jurkat cells on IE gene expression. The HCMV or simian CMV major IE promoter-regulatory regions were cloned upstream of the CAT gene to test the effect of T-lymphocyte activation on IE gene expression. Therefore, the CAT gene was used to indicate the level of expression from the IE promoter as described in Materials and Methods. Low levels of CAT activity were detected in unstimulated Jurkat cells (Fig. 2A). In contrast, Jurkat cells that had been stimulated with both PHA and PMA contained significant amounts of CAT activity ($P<0.02$ for both). Both PHA and PMA activated gene expression in a dose-dependent manner (data not shown). The amounts of PMA and PHA which resulted in the greatest amount of CAT activity were 1 to 10 ng/ml and 1 μg/ml, respectively. PHA and PMA also activated gene expression in a time-dependent manner (Fig. 2B). The greatest amount of CAT activity was detected 6 to 24 h after stimulation with PHA and PMA.

To determine whether T-lymphocyte stimulation also increased the steady-state level of the bona fide HCMV IE mRNAs, we transfected Jurkat cells with plasmid pCS (IE_1,IE_2). The IE1 and IE2 mRNAs are driven by the same HCMV IE promoter-regulatory region. Both mRNAs contain exons 1, 2, and 3 from IE1 but differ in their 3′ exons as a result of an alternate splicing event as described previously

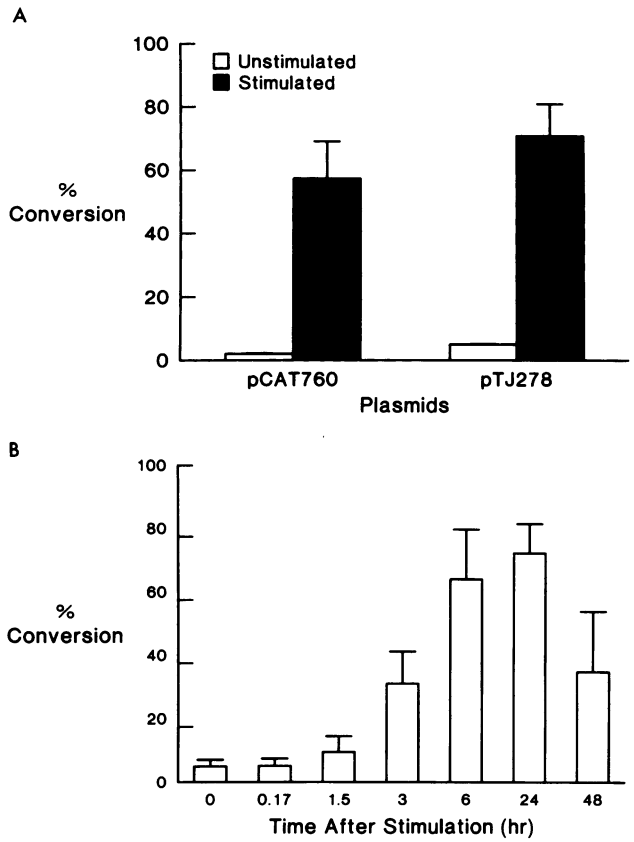


FIG. 2. Effect of activation of Jurkat cells by mitogens on the expression of IE genes of CMV. CAT activity is shown on the ordinate and is expressed as the percent conversion of [^{14}C]chloramphenicol to its acetylated derivatives. The cells remained unstimulated or were stimulated with PMA (10 ng/ml) and PHA (1 μg/ml). (A) The plasmids which were transfected into the cells are indicated on the abscissa. The bars indicate the standard error of the mean of three separate experiments. CAT gene expression is driven by the HCMV or simian CMV IE promoter-regulatory regions in pCAT760 and pTJ278, respectively. (B) Kinetic expression of CAT activity in Jurkat cells after stimulation. The time after stimulation with the mitogens is indicated on the abscissa. The bars indicate the standard error of the mean of three separate experiments. CAT gene expression is driven by the HCMV IE promoter-regulatory region in pCAT760.

(42). Little IE mRNA was detected in unstimulated cells. In contrast, activation of the Jurkat cells resulted in a significant increase in the steady-state level of IE1 and IE2 mRNAs. By using a synthetic probe with the sequence in exon 2 of IE region 1, a 2.25-kb mRNA (typical of IE2 mRNA) and a 1.90-kb mRNA (typical of IE1 mRNA) were easily detected by Northern blot analysis (Fig. 3A). By using a synthetic probe with the sequence in exon 5 of IE region 2, only the 2.25-kb IE2 mRNA was detected by Northern blot analysis (Fig. 3B). The amount of IE1 mRNA compared with IE2 mRNA in the whole-cell RNA preparation was approximately 10:1, similar to that detected during IE times in infected permissive human fibroblasts. (45).

Sequence motifs which mediate the effect of PHA and PMA. Various sequence motifs which might play a role in initiation of HCMV replication, possibly by activating IE gene expression, are present in multiple copies in the major IE promoter-regulatory region (see Fig. 6) (2, 44, 48). Specifically, there are 16-, 18-, 19-, and 21-bp repeats which may modulate gene

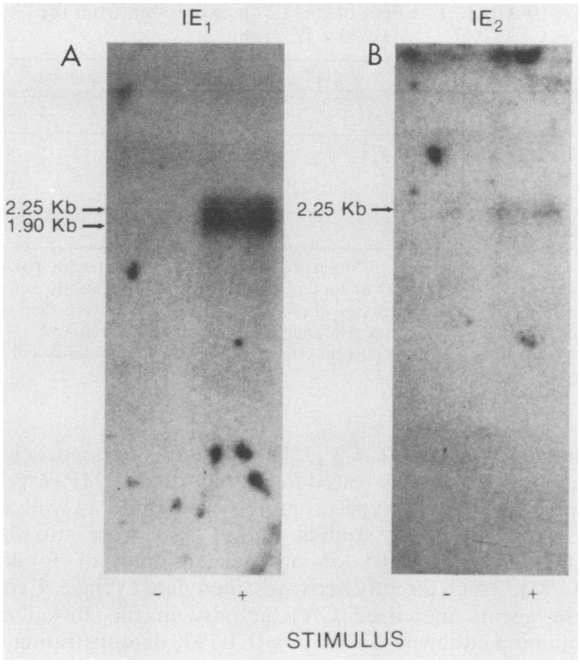


FIG. 3. Northern blot analysis of the effect of activation of Jurkat cells with mitogens on the synthesis of IE region mRNA. For these studies, the Jurkat cells were transfected with a plasmid designated pCS(IE_1,IE_2) containing the wild-type promoter-regulatory region upstream of the IE1 and IE2 genes. The cells either remained unstimulated (lanes −) or were stimulated (lanes +) with PMA (10 ng/ml) and PHA (1 μg/ml). Whole-cell RNA was isolated and was subjected to denaturing agarose gel electrophoresis and transferred to GeneScreen Plus as described in Materials and Methods. (A) Hybridization to a ^{32}P-end-labeled oligonucleotide probe which detects a sequence in exon 2 and therefore detects both IE1 and IE2 mRNAs (42). (B) Hybridization to a ^{32}P-end-labeled oligonucleotide probe which detects a sequence before the intron in IE2 and is specific for IE2 (42). The sizes of the RNAs are expressed in kilobases.

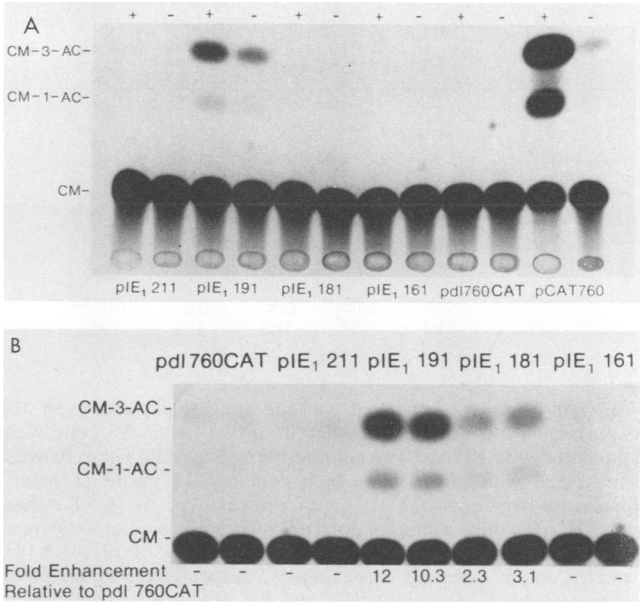


FIG. 4. Effect of various enhancer sequences in mediating expression from the major IE promoter in response to mitogens in Jurkat cells. (A) The cells either remained unstimulated (lanes −) or were stimulated (lanes +) with PMA (10 ng/ml) and PHA (1 μg/ml). The cells were transfected with pIE1-211, pIE1-191, pIE1-181, or pIE1-161, which contain a single 21-, 19-, 18-, or 16-bp synthetic oligonucleotide repeat, respectively, and the minimal wild-type promoter sequences upstream of the CAT gene; pdl760CAT, which contains only the minimal promoter sequences (−68 to +7) upstream of the CAT gene; or pCAT760, which contains the entire promoter-regulatory region of the IE genes upstream of the CAT gene. (B) All human fibroblasts were unstimulated. The assays were done in duplicate.

expression. We initially used plasmids pIE1-161, pIE1-181, pIE1-191, and pIE1-211 to determine which repeat motif sequences might mediate the effect of PHA and PMA in the Jurkat cells. CAT activity was detected only when the plasmid pIE1-191, which contains a single 19-bp repeat, was used (Fig. 4A). The CAT activity was higher in stimulated cells than in unstimulated cells. When a single 18-bp repeat was used, CAT activity was usually low or not detectable. In transfected permissive human fibroblasts, CAT activity was also highest when the plasmid pIE1-191 was used (Fig. 4B). Lower activity was also detected with pIE1-181, and no enhancement of CAT expression above the level of the minimal promoter was detected with pIE1-211 or pIE1-161 in both transfected and stimulated Jurkat cells or permissive human fibroblasts (Fig. 4A and B, respectively).

There was increased expression from the IE promoter when multiple copies of the repeat sequences were used. Using plasmids pIE1-181, pIE1-183, pIE1-191, and pIE1-193, we detected higher activity with three copies of the repeat sequences following stimulation with PHA and PMA. The activity mediated by the 19-bp repeats was greater than that mediated by the 18-bp repeats in stimulated cells (Fig. 5). In both Jurkat cells and permissive human fibroblasts, the CAT activity detected by using pIE1-183 was four- to fivefold lower than that detected by using pIE1-193. In addition, the activity detected by using pIE1-193 was five-fold higher than that detected by using pIE1-191, suggesting an additive effect of the repeat motif. No activity was detected when pIE1-163 or pIE1-213 was used (data not shown).

Deletion of the wild-type promoter-regulatory region. Since the studies described above suggested an important role for the 19-bp repeat in controlling gene expression, we further evaluated the role of the 19-bp repeat by using several deletion plasmids of the wild-type promoter-regulatory region. The deletions and the number of repeat motifs remaining are designated in Fig. 6. The 19-bp repeat between −72 and −54 is naturally altered compared with other normal 19-bp repeats as demonstrated below:

5′-C-C-C-C-A-T-T-G-A-C-G-T-C-A-A-T-G-G-G-3′
(normal 19-bp repeat)
5′-C-C-C-C-G-T-T-G-A-C-G---C-A-A T-G-G-G-3′
A
(−72) (−54)
(sequence between −72 and −54)

In pCATdl4, the dyad symmetry at the core of the 19-bp repeat (underlined) is disrupted by a missing T residue. This deletion plasmid was compared with pCAT760 and the other deletion plasmids, each of which contains at least one normal 19-bp repeat. With the exception of pCATdl4, each of the deletion plasmids resulted in significant levels of CAT activity after stimulation with PHA and PMA (Fig. 6).

Role of cAMP in IE gene expression. The core of the normal 19-bp repeat contains a sequence that is known to function as a cAMP response element in the promoter-regulatory

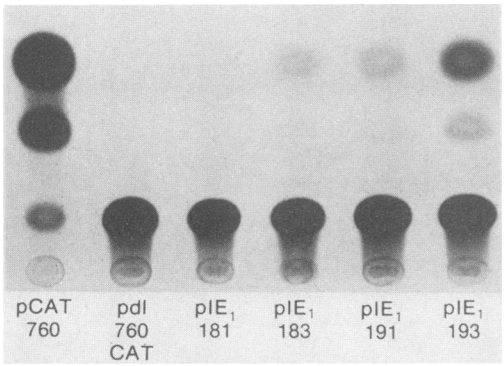


FIG. 5. Additive effect of various repeat sequences on the expression from the IE promoter in Jurkat cells. All cells were stimulated with PMA (10 ng/ml) and PHA (1 μg/ml). The cells were transfected with pCAT760, which contains the entire promoter-regulatory region of the IE genes upstream of the CAT genes; pdl760CAT, which contains only the minimal promoter sequences (−68 to +7) upstream of the CAT gene; pIE1-181 or pIE1-183, which contain one and three copies, respectively, of an 18-bp synthetic oligonucleotide repeat and the promoter sequences upstream of the CAT gene; or pIE1-191 or pIE1-193, which contain one and three copies, respectively, of a 19-bp synthetic oligonucleotide repeat and the promoter sequences upstream of the CAT gene.

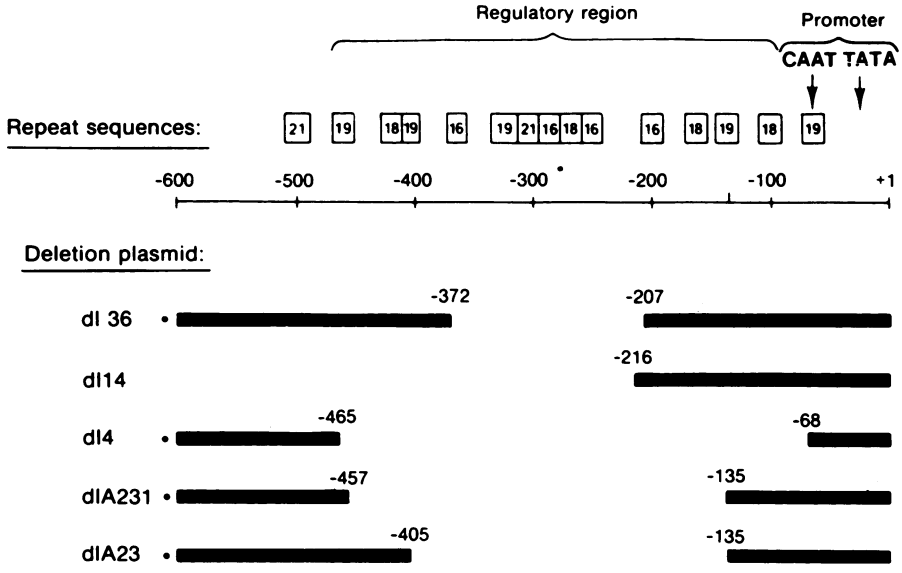
TABLE 1. Effect of cAMP on expression from the HCMV IE promoter[a]

Plasmid	% CAT activity with following stimulus[b]:		
	None	PHA + PMA	Forskolin
pCAT760	0.9	79.6	8.2
pIE1-183	0.2	0.5	0.2
pIE1-193	0.2	4.2	10.3
pCATdl4	0.2	0.5	0.6

[a] The cells either were unstimulated or were stimulated with PMA (10 ng/ml) and PHA (1 μg/ml) or forskolin (10^{-4} M), which directly activates adenylate cyclase and increases intracellular cAMP. They were then transfected with the plasmids (see Materials and Methods for details).

[b] The data represent the percent conversion of [^{14}C]chloramphenicol to its acetylated derivatives.

region (5′-TGACGTCA-3′[35]). Since this sequence is altered in pCATdl4, we tested for a functional cAMP response element in the wild-type promoter (pCAT760) as well as in pIE1-193. For these studies, Jurkat cells were stimulated with PHA and PMA or optimal amounts of forskolin (10^{-4}M), which directly activates adenylate cyclase. Each of these agents increased CAT activity in the Jurkat cells containing either pCAT760 or pIE1-193, demonstrating that



Plasmid	No. of repeats				% Conversion	
	21	19	18	16	-	+
pCAT 760	2	5	4	4	3.5	95.2
pCATdl36	1	4	3	1	8.4	96.6
pCATdl14	0	2	2	1	7.2	94.3
pCATdlA23	1	3	2	0	8.9	95.3
pCATdlA231	1	2	1	0	6.4	70.3
pCATdl4	1	0	0	0	2.9	2.5

FIG. 6. Effect of various deletions in the promoter-regulatory region of the HCMV IE gene. After transfection, the Jurkat cells either remained unstimulated (−) or were stimulated (+) with PMA (10 ng/ml) and PHA (1 μg/ml). The cells were transfected with pCAT760, which contains the entire promoter-regulatory region of the IE gene, or one of the following deletion plasmids: pCATdl36, pCATdlA23, pCATdlA231, pCATdl14, and pCATdl4. The numbers of 16-, 18-, 19-, and 21-bp repeat sequences in the plasmids are shown. With the exception of pCATdl4, all of the deletion plasmids contains at least one intact 19-bp repeat sequence as described in the text.

a cAMP response element is present in the wild-type HCMV major IE promoter-regulatory region (Table 1). CAT activity was also increased in the Jurkat cells when they were stimulated with other agents which increase intracellular cAMP, including prostaglandin E_2 and 8-(4-chlorophenylthio)-cAMP (data not shown). Since similar amounts of CAT activity were detected when pCAT760 and pIE1-193 were stimulated with forskolin, we propose that the cAMP response element in the wild-type promoter is within the 19-bp repeats and that the other repeated sequences are not necessary for the cAMP response. When the 19-bp repeat motif was defective, as described above for pCATdl4, forskolin had little or no effect (Table 1). PHA and PMA, but not forskolin, increased CAT activity when pIE1-183 was used (Table 1). Neither PHA and PMA nor forskolin increased CAT activity when pIE1-163 or pIE1-213 was used (data not shown).

DISCUSSION

These studies demonstrate that activation of Jurkat cells (T lymphocytes) with PHA and PMA increases transcription of the HCMV IE1 and IE2 genes. We propose that the 19-bp repeat is the major contributor to the strength of the CMV IE promoter-regulatory region. This repeat sequence is found in the major IE promoter-regulatory region of all animal CMVs analyzed to date, which implies that this element plays an important role in the life cycle of the virus. The IE2 gene product alone or in combination with the IE1 gene product activates, in *trans*, HCMV early promoters (3) as well as heterologous inducible promoters (14, 32, 47). Both of these mitogens increased expression from the IE promoter in a time- and dose-dependent manner. Stimulation of the cells with PHA and PMA also increased the steady-state levels of the bona fide IE1 and IE2 mRNAs when driven by the wild-type promoter-regulatory region. These studies also demonstrated that various repeat motifs in the enhancer-containing promoter-regulatory region of the IE genes were important in mediating the effect of the mitogens. This was shown by using plasmid constructs with only synthetic oligonucleotide 16-, 18-, 19-, or 21-bp repeats and the minimal promoter sequences upstream of the CAT gene. The 19-bp repeat was the element that most strongly affected CAT expression in both the permissive human fibroblasts and the stimulated Jurkat cells. When three 19-bp repeats were present, there was an additive effect in the stimulated Jurkat cells. Some CAT activity was also detected with the 18-bp repeats and was greater in activated cells. However, this activity did not approach that found with the 19-bp repeats.

Using a deletion plasmid, pCATdl4, we further demonstrated that a core sequence identical to that described for a cAMP response element in other genes (28, 29, 35, 49) must be intact to optimally increase gene expression in the stimulated Jurkat cells. As a follow-up to this observation, we found that a functional cAMP response element is present in the wild-type promoter-regulatory region and that the 19-bp repeat sequences specifically respond to cAMP. These observations are consistent with results of prior studies which demonstrated that PHA increases cAMP levels in lymphoid cells (39).

To our knowledge, this is the first description of the regulation of an HCMV gene by cAMP. However, prior studies have demonstrated that various adenovirus genes can be regulated in this manner. Leza and Hearing (24) demonstrated that a cellular transcription factor, termed ETF-A (probably identical to ATF), bound cAMP response elements in the promoter-regulatory region of the adenovirus type 5 early regions 2 and 4. In addition, E4 expression was induced in vivo by cAMP. Consistent with this observation, the HeLa cell-derived ATF binds to the adenovirus E2 and E4 promoter regions (11, 20). Green and co-workers (11, 25) found that ATF which binds to E1a-inducible genes also binds to regulatory elements in cellular cAMP-inducible promoters. On the basis of the latter studies, the authors suggested that a common cellular transcription factor, ATF, can be regulated by two different inducing agents: the adenovirus E1a protein and cAMP. In HCMV or simian CMV, cAMP might play a critical role in the stimulation of the major IE promoter-regulatory region and the subsequent expression of the IE1 and IE2 genes.

cAMP response elements are also known to be present in a number of cellular genes. These, in general, have been divided into the genes which are rapidly regulated by cAMP and those whose transcription is altered only after a prolonged exposure to cAMP (reviewed in reference 35). At least two transcription factors can mediate the effects of cAMP in genes which rapidly respond to the mediator. One factor is ATF, and it binds to the consensus sequence 5′-TGACGTCA-3′. cAMP response elements have been identified in the rat phosphoenolpyruvate carboxykinase gene (36), the alpha-subunit of the human chorionic gonadotropin gene (38), the rat somatostatin gene (29), the human vasoactive intestinal polypeptide gene (49), the rat tyrosine hydroxylase gene (27), the c-*fos* gene (38), and the human proenkephalin gene (36). The response elements in these genes closely resemble those described above in the adenovirus genes (12, 20, 24, 25, 35) and those present in the 19-bp repeat sequences of HCMV.

Prior studies have demonstrated that the 19-bp repeat sequence plays a major role in regulating gene expression in permissive human fibroblasts (44). In addition, DNase I protection studies have demonstrated that nuclear proteins present in HeLa cell extracts bind to the 19-bp repeat sequences as well as to other regions within the promoter-regulatory region (6–8). The finding that the 18-bp repeats were relatively weaker for regulating expression of the major IE gene was a surprise. This repeat motif region contains a core sequence, 5′-GGGACTTTCC-3′, identical to the immunoglobulin κ light-chain enhancer. A number of studies have demonstrated that stimulated Jurkat cells produce a factor, termed NF-*k*B1, which binds to this sequence and activates gene expression (1, 22, 31). This core sequence is also present in the promoter-regulatory region of the interleukin-2 receptor gene and the human immunodeficiency virus long terminal repeat (1, 25, 37). The observation that the 18-bp repeat by itself was not as active in increasing expression from the IE promoter is consistent with the results of studies by Ghazal et al., who found less binding of nuclear proteins to the 18-bp repeats than to the 19-bp repeats (8). However, it is likely that this sequence also plays a major role in the activation of the HCMV major IE promoter-regulatory region by acting in a synergistic manner with the 19-bp repeat sequences and with other sequences in the promoter-regulatory region. Therefore, it will be of interest to evaluate this hypothesis by using plasmids which contain both 18 and 19 synthetic oligonucleotide sequences in addition to those that were used in the present study.

The biological significance of the results of the present study is, at present, not totally appreciated. The observations suggest that a prerequisite for replication of HCMV may be the presence of adequate amounts of cellular cAMP-

dependent proteins which have the capacity to activate, in *trans*, the HCMV major IE promoter-regulatory region. These cellular proteins could be present in adequate amounts without stimulation of the cell (i.e., fibroblasts) or they could be generated after activation of the cell (i.e., Jurkat cells). Alternatively, the viral tegument proteins might trigger the generation of these factors after the process of attachment to and entry into the cell. There is evidence that a virion-associated component of HCMV acts, in *trans*, to stimulate transcription from the major IE promoter-regulatory region (41, 44). Infection of T lymphocytes in vivo occurs, but it is very inefficient. Stimulation of these infected T lymphocytes might activate HCMV IE gene expression. The effects of HCMV IE gene products on T-lymphocyte functions will require further investigation.

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