Cell type-specific induction of the major immediate early enhancer of human cytomegalovirus by cyclic AMP

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The major immediate early enhancer of human cytomegalovirus (HCMV) is known to exert a strong constitutive transcription stimulation in a broad spectrum of cells. This basal activity can be augmented considerably by elevated levels of intracellular cAMP in a cell type-specific manner. Cyclic AMP induction was observed in several lymphoid cell lines and in HeLa cells. One of the functionally important enhancer sequence modules, the 19 bp repeat element, mediates this effect as a cAMP-responsive element (CRE). It acts more efficiently than the corresponding sequence from the human chorionic gonadotropin gene. It is suggested that protein kinase C is involved in the pathway which leads to the activation of CRE-containing genes in lymphoid cells. Gel retardation assays indicated that similar, but not identical complexes are formed between nuclear protein extracts and the CREs of HCMV and the gonadotropin gene.

Introduction

Transcriptional enhancer sequences of various viral and cellular genes have been classified into two types, constitutive and inducible (Serfling et al., 1985; for a review see Hatzopoulos et al., 1988). Constitutive enhancers, such as the simian virus 40 enhancer, may be active in a broad spectrum of cell types (Picard, 1985), or show a pronounced tissue specificity, for example, the lymphotrophic papovavirus enhancer which is active only in certain cells of the haematopoietic system (Mosthaf et al., 1985). Inducible enhancers stimulate transcription when activated by a variety of agents. For instance, enhancers of interferon genes are induced by viral infection (Lengyel, 1982), and the mouse mammary tumour virus enhancer responds to glucocorticoids (Yamamoto, 1985). A few constitutive enhancers can also be induced, for example, the Moloney murine sarcoma virus enhancer (Defranco & Yamamoto, 1986; Miksicek et al., 1986). The major enhancer of human cytomegalovirus (HCMV) has been shown to exert a strong constitutive activity in a wide variety of cell types, including *Xenopus laevis* (Boshart et al., 1985) and *Drosophila melanogaster* cells (Sinclair, 1987). This enhancer sequence is located between nucleotides −524 and −65 upstream of the transcription start site of the immediate early gene 1 (IE1) of HCMV. It is proposed that the enhancer controls the expression of important regulatory proteins of the virus which are involved in the switch from restricted to extensive transcription of the genome (for a review see Mach et al., 1989). Four groups of repeat elements (of 17, 18, 19 and 21 bp) appear to act as targets for cellular trans-acting proteins and to contribute to the complex modular structure of the enhancer (Ghazal et al., 1987). The palindromic repeat element of 19 bp is essential for the constitutive activity of the enhancer in the cell lines HeLa and CV-1 (Fickenscher et al., 1989; Ghazal et al., 1988). Computer searches detected a sequence homology between the 8 bp core palindrome of the 19 bp motif and a transcription regulatory element which is contained within various hormone and enzyme genes and the proto-oncogene c-fos (Fickenscher et al., 1989; for a review see Roesler et al. 1988). This element was shown to be capable of acting as a cAMP-responsive element (CRE). However the mere presence of this 8 bp sequence in a promoter regulatory region is not sufficient to confer cAMP inducibility to the corresponding gene. The surrounding sequences may exhibit a restrictive influence, as shown for the rat glucagon or bovine parathyroid hormone gene (Deutsch et al., 1988b). In this paper we show that the major enhancer of HCMV can be regulated by cAMP treatment in a cell type-specific manner in addition to its constitutive activity. The 19 bp motif acts as a CRE in a broad spectrum of cell lines. It shows protein binding characteristics similar, but not identical, to the CRE of the gonadotropin gene.
Methods

Plasmid cloning. Cloning reactions were performed according to standard procedures. Construction of plasmid pRR55 which contains HCMV IE enhancer/promoter sequences between positions $-671$ and $+52$ of the IE1 transcription start fused to the chloramphenicol acetyltransferase (CAT) gene (Gorman et al., 1982) has been described (Fickenscher et al., 1989). Plasmid pRR56/5 was constructed by shortening of the HCMV sequence of pRR55 with nuclease Bal31 (Sihavy et al., 1984). Recessed ends were filled in with the Klenow fragment, and were religated in the presence of excess HindIII-SmaI fragment, and were religated in the presence of excess HindIII linker DNA. The resulting CAT expression plasmid contained the HCMV IE1 promoter sequences between positions $-65$ and $+52$ of the IE1 cap site. Plasmid puPCAT (Fig. 1) was created by inserting the HindIII-SmaI fragment of pRR56/5 (which contains the IE1 promoter sequences and the CAT gene) into the PsI site of the Bluescribe vector (Vector Cloning Systems) after the recessed ends had been filled in. This vector was used for cloning and polymerization of oligonucleotides in analogy to the OVEC system (Westin et al., 1987). Oligonucleotides were synthesized, using a Cyclone DNA Synthesizer (Biosearch), with SacI/Sacl sticky ends and a XhoI recognition sequence overlapping the SacI site. Oligonucleotides were ligated into SacI/SalI-digested puPCAT, and the DNA sequence of the cloned oligonucleotides was determined according to standard procedures. As cleavage with SalI and XhoI forms compatible ends, cloned oligonucleotides could be polymerized by cleavage of the appropriate vector with SalI/HindIII and XhoI/HindIII, isolation of the corresponding fragments and ligation (Fig. 1).

Cell culture, plasmid transfections and treatment of cells by 8-bromo-cAMP, forskolin and staurosporin. HeLa cells, cœropithecus kidney cells (CV-1) and human foreskin fibroblasts (HFF) were grown as described previously (Fickenscher et al., 1989, Jahn et al., 1984). BJA-B (Klein et al., 1974), T cell-derived Jurkat (Schneider et al., 1977) and Molt-4 cell lines (Minowada et al., 1972) were cultured in RPMI 1640 medium [Gibco/Bethesda Research Laboratories (BRL)] supplemented with $10\%$ foetal calf serum (FCS). PC-12 cells (Biocca et al., 1983) were obtained from the American Type Culture Collection and maintained as suspension cultures in RPMI 1640 medium supplemented with $5\%$ FCS and $10\%$ horse serum. L-Glutamine and gentamicin were added to all cultures.

Plasmid transfections for transient expression assays were done by the DEAE-dextran procedure according to Queen & Baltimore (1983). HFF cells were transfected by the calcium phosphate precipitation method as described (Fickenscher et al., 1989). For stimulation experiments, cells were transfected in a batch and subsequently plated onto separate dishes. Forskolin (10 μM; Sigma) or 1 mM 8-bromo-cAMP (Sigma) were added to one of two parallel dishes 16 to 24 h before CAT activities were determined.

For the establishment of permanent lymphoid cell lines, 2 × 10⁶ cells were cotransfected with the plasmid of interest and pSV2neo at a molar ratio of 5 : 1. Transfection was done by electroporation (Döffinger et al., 1988). About 48 h after transfection, cells were plated in 24-well culture dishes (Nunc) at 2 × 10⁴ cells per well; G418 (Gibco/BRL) was added at a concentration of 800 μg/ml, and G418-resistant cells were selected. Four weeks later, G418-resistant clones were pooled.

Staurosporin, an inhibitor of protein kinase C (PKC), was obtained from Boehringer Mannheim and was used according to the manufacturer's instructions.

CAT assays and RNase protection analysis. CAT assays were performed as described (Fickenscher et al., 1989). Total cellular RNA of transfected cells was isolated according to Chomczynski & Sacchi (1987). RNA was analysed for CAT-specific transcripts with a T7 RNA polymerase-generated RNA probe. The probe was prepared using plasmid pRR61 containing a 1 kb HindIII-EcoRI fragment of pRR55 downstream of the T7 promoter of the Bluescribe vector. Total cellular RNA (40 μg) was hybridized with the probe overnight at 37 °C in 10 μl of hybridization buffer containing 50% formamide, 40 mM-PIPES pH 6.4, 400 mM-NaCl and 1 mM-EDTA. Digestion of ssRNA was performed for 1 h at 37 °C with 6 μg/ml RNase A and 12 units/ml of RNase T1 (Boehringer Mannheim) in RNase buffer containing 300 mM-NaCl, 5 mM-EDTA and 10 mM-Tris-HCl pH 7.5. After recovery of DNA/RNA mixtures by ethanol precipitation, samples were analysed on 6% (w/v) denaturing polyacrylamide gels.

Preparation of nuclear extracts and gel mobility shift assays. Nuclear extracts of several cell types were prepared as described (Fickenscher et al., 1989). For band shift assays, oligonucleotides were labelled by filling in recessed ends with the Klenow fragment (Fickenscher et al., 1989). About 5 fmol of end-labelled DNA (10000 c.p.m.) was incubated with 5 μg of nuclear protein for 15 min at room temperature in a buffer containing 1 μg poly(dI)-poly(dC) (Pharmacia), 10 mM-HEPES pH 8.0, 1 mM-mercaptoethanol, 5 mM-MgCl₂, 50 mM-KCl, 1 mM-DTT and 9% glycerol (v/v) in a final volume of 15 μl. Samples were electrophoresed on a 6% (w/v) polyacrylamide gel (29:1) gel in 0.25 mM-Tris–borate–EDTA buffer. For competition experiments, a 50-fold molar excess of unlabelled double-stranded oligonucleotide was incubated with nuclear extracts for 10 min on ice, prior to the addition of the labelled probe.

Results

Levels of induction by cAMP of HCMV enhancer activity vary for different cell lines

The first experiments addressed the question of whether cAMP treatment of cells could regulate the activity of the
Induction of HCMV IE enhancer by cAMP

A plasmid, designated pRR55, containing the entire HCMV enhancer/promoter sequence between nucleotides -671 and +52 of the IE1 transcription start inserted in front of the CAT gene (Fickenscher et al., 1989), was transfected into several cell lines. The cells were transfected in a batch and subsequently plated onto separate dishes. To minimize error from transfection, cAMP stimulation experiments were done with pairs of sister cell cultures. Cells were treated for 16 to 24 h with 8-bromo-cAMP and CAT activities were determined after 48 h. The HCMV enhancer was stimulated significantly in several cell lines (Table 1). No major difference in results was observed if forskolin (10 \mu M), a potent inducer of adenylate cyclase activity (Seamon et al., 1981), was used instead of 8-bromo-cAMP (1 mM). Highest induction levels obtained were for the T lymphoma cell line Jurkat. Molt-4, another T cell line, showed significant induction also. Upregulation of the HCMV enhancer by cAMP was also observed in BJA-B cells, an Epstein–Barr virus genome-negative B cell line, as well as in HeLa cells. When testing CV-1, a monkey kidney cell line, or PC-12, a rat pheochromocytoma cell line generally known to support cAMP regulation, only a minor rise in CAT activities was observed. Also, in repeated experiments, enhancer-driven CAT expression could not be stimulated in primary HFFs.

In all cell lines tested, the HCMV enhancer exerted a considerable constitutive activity which varied in the range of one order of magnitude. The enhancer stimulated basal transcription from the HCMV IE1 promoter by a factor of about 20 in Jurkat cells, whereas transcription enhancement was more than 250-fold in HeLa cells. The promoter plasmid pRR56/5, containing IE1 promoter sequences between -65 and +52, was tested in all cell lines that were found to be highly inducible for the enhancer. No significant cAMP induction was observed for the IE1 promoter itself, indicating that enhancer sequences upstream of the promoter are necessary for cAMP regulation (Table 1).

The 19 bp repeat motif can act as a response element for cAMP induction in a broad spectrum of cells

To test whether the 19 bp enhancer motif is a CRE, a vector was constructed which allows the insertion and polymerization of synthetic oligonucleotides upstream of the IE1 promoter (Fig. 1). The oligonucleotide sequences, representing enhancer motifs (genuine or mutated) and a known CRE element, and the nomenclature of the resulting plasmids, are listed in Table 2. The CAT plasmids were transiently expressed in Jurkat cells in the presence or absence of forskolin (Fig. 2). Insertion of one single 19 bp motif immediately upstream of the IE1 promoter resulted in 12.5-fold increase in CAT activity by cAMP. Cyclic AMP regulation was not observed when using plasmid p19(-1)PCAT. In this plasmid, the 19 bp motif had been mutated by deletion of a single nucleotide from the palindromic core, thus turning the CRE consensus into a putative target sequence for the transcription factor AP-1 (Angel et al.,)

### Table 1. Increase of CAT activity after induction of cAMP in various cell lines transiently transfected with different enhancer/promoter/CAT constructs

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Plasmid pRR55</th>
<th>Plasmid pRR56/5</th>
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<tbody>
<tr>
<td>BJA-B</td>
<td>4.6*</td>
<td>1.0</td>
</tr>
<tr>
<td>Jurkat</td>
<td>6.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Molt-4</td>
<td>3.5</td>
<td>1.1</td>
</tr>
<tr>
<td>HeLa</td>
<td>5.2</td>
<td>1.1</td>
</tr>
<tr>
<td>CV-1</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>HFF</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>PC-12</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

* CAT activity increased by 4.6-fold in BJA-B cells transfected with pRR55, etc. The numbers tabulated here represent the averages of at least two experiments performed for each line.

### Table 2. Sequence of oligonucleotides inserted in pUC19CAT and nomenclature of resulting plasmids

<table>
<thead>
<tr>
<th>Sequence of oligonucleotides</th>
<th>1 copy</th>
<th>2 copies</th>
<th>4 copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 bp motif</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19(-1) motif</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRE-go</td>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 bp motif</td>
<td>G</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Nomenclature of plasmid**

<table>
<thead>
<tr>
<th>Nomenclature of plasmid</th>
<th>1 copy</th>
<th>2 copies</th>
<th>4 copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>p19PCAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p2-19PCAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p4-19PCAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p17PCAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p2-17PCAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p4-17PCAT</td>
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</tbody>
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HCMV enhancer served as negative controls and CAT activities were not induced by cAMP for these plasmids. In contrast to the high cAMP responsiveness found with the intact 19 bp motif, there was only a minor transcriptional stimulation of the reporter gene containing the CRE of the human chorionic gonadotropin gene α subunit (CRE-go) (Silver et al., 1987) linked to the HCMV promoter. This sequence conferred little inducibility by cAMP in Jurkat cells (Fig. 2), even as a dimer.

To exclude transfection artefacts in cAMP induction, CAT fusion genes containing the HCMV or RSV enhancer/promoter sequences were stably integrated into the genome of Jurkat and BJA-B cells. Cellular RNA, isolated from untreated cells or from cells cultured for 6 h in the presence of forskolin, was analysed for CAT-specific transcripts by quantitative RNase protection (Fig. 3). These experiments showed that the addition of forskolin to both BJA-B and Jurkat cells, carrying the intact 19 bp palindromic fragment in front of the IE1 promoter, resulted in increased amounts of correctly initiated transcripts. CAT enzyme levels, which were analysed in parallel, correlated with these results (data not shown). The amount of transcripts originating from pRSVCAT or pRRS6/5 appeared unaffected by forskolin treatment.

As it has been shown that different CREs can differ significantly in conferring cAMP inducibility to promoter sequences (Deutsch et al., 1988b), we compared the induction levels of plasmids p19PCAT and pCREPCAT. The latter contains CRE-go in front of the HCMV IE1 promoter. The plasmids were transiently expressed in PC-12 cells, which have been used extensively to investigate cAMP regulation (Montminy et al., 1986; Delegeane et al., 1987). In repeated experiments, we observed significantly higher induction levels with p19PCAT than with pCREPCAT (Table 3). When using the plasmids puPCAT containing only the IE1 promoter or pRSVCAT, no significant induction was found in PC-12 cells. One 19 bp element conferred significant cAMP responsiveness in PC-12 cells, but the entire enhancer was not found to be inducible.

Table 3. Increase in CAT activities after induction of cAMP in PC-12 cells transiently transfected with different CAT fusion genes

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>PC-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>p19PCAT</td>
<td>24.2 (2.4)*</td>
</tr>
<tr>
<td>pCREPCAT</td>
<td>5.7 (1.1)</td>
</tr>
<tr>
<td>puPCAT</td>
<td>1.8 (0.3)</td>
</tr>
<tr>
<td>pRSVCAT</td>
<td>1.4 (0.5)</td>
</tr>
</tbody>
</table>

* CAT activity increased by 24.2-fold in PC-12 cells transfected with p19PCAT, etc. The numbers tabulated represent the average of at least three experiments performed for each plasmid. The standard deviations are given in parentheses.
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Fig. 3. RNase protection analysis of RNA from stably transfected BJA-B and Jurkat cells in the presence or absence of forskolin. BJA-B and Jurkat cells were stably transfected with various CAT fusion genes. Cells were cultured with or without forskolin and RNA was isolated after 6 h. Total cellular RNA was analysed for CAT-specific transcripts. Solid squares (●) indicate treatment of cells with forskolin. Lanes 1 to 4, BJA-B cells stably transfected with pRR55 (entire enhancer); lanes 1 and 2 represent a short exposure of lanes 3 and 4; lanes 5 and 6, BJA-B cells stably transfected with pRSVCAT; lanes 7 and 8, BJA-B cells stably transfected with pRR56/5 (IE1 promoter); lanes 9 and 10, BJA-B cells stably transfected with p2-19PCAT; lanes 11 and 12, BJA-B cells stably transfected with p2-(19-1)PCAT; lanes 13 and 14, Jurkat cells stably transfected with pRR55; lanes 15 and 16, Jurkat cells stably transfected with p2-19PCAT; lanes 17 and 18, Jurkat cells stably transfected with pRSVCAT. For nomenclature and detailed description of plasmids see Table 2.

Forskolin induction in Jurkat cells can be inhibited by staurosporin

It has been proposed that PKC is involved in the signal transmission pathway leading to the activation of CRE-containing genes (Yamamoto et al., 1988; Gonzalez et al., 1989; Deutsch et al., 1988a). Therefore we studied the participation of this kinase in the cAMP induction of HCMV enhancer-mediated transcription in Jurkat cells by using staurosporin. The latter is a microbial alkaloid with antifungal properties (Tamaoki et al., 1986), which has been shown to inhibit PKC efficiently by binding to the catalytic domain. It also induces the translocation of PKC to membranes where subsequent protein phosphorylation is inhibited in a concentration-dependent manner with an IC50 value of 2.7 nM (Wolf & Baggioni, 1988). Jurkat cells bearing the CAT plasmid pRR55 stably integrated were incubated for 6 h in the presence of increasing concentrations of staurosporin. Forskolin was added 16 h before CAT activities were measured (Fig. 4). The response of enhancer-driven CAT transcription to forskolin was diminished by 50% at 3 nM staurosporin, when a half maximum block of PKC activities should be expected. Increasing concentrations of staurosporin diminished further the forskolin response. The same kinetics were seen, when O-tetradecanoylphorbol 13-acetate (TPA) was used to stimulate enhancer activities or when CAT RNA was analysed (data not shown). These experiments suggested that PKC is involved in the signal transmission pathway which leads to cAMP-mediated stimulation of enhancer activities in Jurkat cells.

A similar set of proteins bind to the HCMV 19 bp palindrome and the CRE-go

Binding characteristics of the HCMV 19 bp sequence were compared with CRE-go by gel mobility shift assays.
Fig. 4. Inhibition by staurosporin of forskolin induction of enhancer-mediated transcription in Jurkat cells. Jurkat cells stably transfected with plasmid pRR55 (entire enhancer) were incubated in the presence of increasing concentrations of staurosporin. Forskolin was added 6 h later and CAT activities were determined after 22 h. Forskolin induction of enhancer-driven CAT expression was blocked by 50% at 3 nM-staurosporine, whereas the basal level of CAT expression without induction was not affected significantly, even at higher staurosporin concentrations. With forskolin (■); without forskolin (□).

Nuclear extracts were prepared from HeLa cells, allowed to react with 32P-labelled oligonucleotides, and subjected to PAGE on 6% (w/v) non-denaturing gels (Fig. 5). Both the 19 bp motif and CRE-go gave rise to one major complex, migrating at an identical position for both probes (Fig. 5). The specificity of binding was determined by competition with unlabelled 19 bp or CRE-go oligonucleotides, respectively. Complexing with the labelled 19 bp motif was inhibited efficiently both by the unlabelled 19 bp motif and by the CRE-go at a 50-fold molar excess. When the 21 bp repeat element of the HCMV enhancer was used at the same molar excess, no competition was achieved. The CRE-go oligonucleotide used as the probe for the gel shift assay behaved identically (Fig. 5). An oligonucleotide corresponding to the AP-1-binding sequence of the collagenase gene (Angel et al., 1987) was also able to compete for factors binding to the CRE elements. However, competition was incomplete, indicating a significantly lower affinity of this sequence. Identical competition results were obtained when nuclear extracts from Jurkat cells were allowed to bind to the 19 bp probe (data not shown). In summary, both the CRE-go and the 19 bp motif of HCMV appear to bind a very similar set of proteins, with minor differences in low abundance, faster migrating complexes.

Fig. 5. Gel shift patterns obtained with nuclear extracts from HeLa cells were compared for the 19 bp motif of the HCMV IE1 enhancer (19) and the CRE-go (CRE). One major complex migrating at identical positions for both probes could be observed (▲). Competition experiments with unlabelled oligonucleotides corresponding to the 19 bp motif, the CRE-go and the 21 bp repeat motif of the HCMV enhancer (21) showed the specificity of binding and the relationship of proteins complexing with the 19 bp motif and CRE-go. Low abundance complexes which differ for both probes are indicated (▲). Absence of competitor denoted by (−).

Discussion

The major enhancer of HCMV which controls the expression of a group of regulatory IE proteins (Akrigg et al., 1985; Boshart et al., 1985; Thomsen et al., 1984) exerts a high constitutive activity in a broad spectrum of vertebrate and non-vertebrate cells (Boshart et al., 1985; Foerking & Hofstetter, 1986; Sinclair, 1987). In this study we show that the HCMV IE enhancer can be induced by cAMP in several cell lines in addition to its constitutive activity. Thus, it appears that a cellular signal transmission pathway regulates an important control element of viral gene expression. The degree of regulation varies between different cell types. The epithelial cell line HeLa and cell lines of the haematopoietic system permitted high induction levels; lymphoid cells may be relevant for the natural pathogenicity of the virus (Schrier et al., 1985). Regulation of HCMV enhancer activity was observed neither in the rat...
(1989) report this element to be almost silent without induction. However in the latter study the activity of the HCMV enhancer was not quantified relative to the IE1 promoter or to another enhancer.

A monomer of the 19 bp element forms a strong response element for cAMP regulation in Jurkat cells, whereas basal transcription of the promoter is hardly affected. The transcription-enhancing activity exerted by a multimer of this motif after stimulation of Jurkat cells by cAMP exceeds values observed for the entire HCMV enhancer after induction. In PC-12 cells a single 19 bp element in front of the HCMV IE1 promoter forms a strong CRE, several times stronger than CRE-go, whereas the entire enhancer showed no response. As a large nucleoprotein complex forms on the enhancer (Ghazal et al., 1987), steric hindrance might be one reason for the lack of response; further experiments are required to test this hypothesis.

Proteins that bind to CRE sequences of various genes have been purified to apparent homogeneity by several groups (Hurst & Jones, 1987; Montminy & Bilezikjian, 1987; Cortes et al., 1988). One of the proteins, termed CREB, which was isolated from PC-12 cells, has been shown to be similar or identical to the HeLa factor ATF which binds to adenovirus promoter sequences (Hurst & Jones, 1987; Hardy & Shenk, 1988; Montminy & Bilezikjian, 1987; Lin & Green, 1988). Hai et al. (1988) proposed the existence of a family of transcription factors that are related by DNA-binding specificity and by antigenic cross-reactivity; it includes multiple forms of ATF and of the transcription factor AP-1. By using the gel mobility shift technique, we found a band shift pattern for the HCMV 19 bp repeat motif similar to the well characterized CRE of the α-subunit of the human chorionic gonadotrophin gene (Silver et al., 1987; DeLegeane et al., 1987; Deutsch et al., 1988b). Cross-competition studies have shown the relationship of factors binding to both sequences. The significance of some low abundance complexes that differ for CRE-go and the 19 bp motif remains to be determined. An oligonucleotide corresponding to the AP-1 recognition sequence of the collagenase gene (Angel et al., 1987) was also capable of competing for factors binding to the CRE-go and the 19 bp motif; however its binding affinities seemed to be low. This relationship has also been reported in earlier studies (Hurst & Jones, 1987; Fickenscher et al., 1989; Maekawa et al., 1989). In transient expression assays, a single base pair mutation of the 19 bp motif that turns its inner core into an AP-1 consensus sequence abolished both inducibility by cAMP and constitutive activation of basal transcription. Thus, in spite of the observed cross-reactivity of AP-1 and CRE sequences in cell-free systems (Hurst & Jones, 1987; Fickenscher et al., 1989; Maekawa et al., 1989; Gonzalez et al., 1989; Hyman et al., 1988), the in vivo experiments show that distinct functions are exerted by these related sequences (Deutsch et al., 1988a).

It has been shown that CREs can be modified by phosphorylation by protein kinases A and C (Montminy & Bilezikjian, 1987; Yamamoto et al., 1988; Gonzalez et al., 1989). By using staurosporin, known to be a potent inhibitor of PKC (Wolf & Baggioni, 1988), we were able to block the forskolin response of enhancer-mediated transcription in Jurkat cells. This may suggest the involvement of PKC in the signal transmission pathway leading to activation of CRE-containing genes in this cell line. However participation of protein kinase A cannot be excluded. It has been reported for lymphoid B cells that elevated levels of intracellular cAMP activate the translocation of PKC from the cytosol to the nucleus (Cambier et al., 1987). It may be a plausible hypothesis that a similar mechanism is responsible for activation of CRE-containing genes in the T cell line Jurkat. In summary, we have shown that an important cellular signal transmission pathway acts upon a transcriptional control element of viral IE gene expression in a cell type-specific manner. This mechanism may be relevant in promoting the reactivation of HCMV from its latent state.

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References


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