
Transcriptional enhancer within the human placental lactogen and growth hormone multigene cluster

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ABSTRACT

Human placental lactogen (hPL) and human growth hormone (hGH) are members of a multigene family that share amino acid sequence homology and similarity in gene structure and nucleotide sequence, but differ in both function and expression. To determine the sequence requirements for tissue specific expression recombinant plasmids containing the members of the hPL-hGH multigene family and flanking regions were analyzed by both transient and stable transfection assays.

We have identified a transcriptional enhancer in a 1.0 kb region located 2.0 kb downstream of the hPL₃ structural gene. This enhancer sequence is not strictly cell-type specific since it functions in cell lines of both placental (JEG-3) and pituitary (18-54,SF) origin. However, its efficiency is several fold higher in placental cells than in pituitary cells.

INTRODUCTION

Human placental lactogen (hPL) and human growth hormone (hGH) are polypeptide hormones which are closely related in structure (85% homology in amino acid sequence), but they differ in both function and expression (1). hGH is involved in the regulation of growth and metabolism during development and hPL stimulates mammary gland development and lactogenesis. hPL is expressed in increasing amounts during pregnancy, accounting for >5% of the total poly(A⁺)RNA in the placenta during the third trimester of pregnancy (2), whereas hGH is expressed in the anterior lobe of the pituitary.

The hPL/hGH gene cluster is contained within a 60 kilobase region of DNA on chromosome 17 at region q22-24 (3). There are two hGH genes interspersed with three hPL genes, all in the same transcriptional orientation (4). A detailed restriction map of this cluster is well defined (4,5), and each of the genes have been cloned into plasmid vectors (6) and sequenced (7,8). Two of the three hPL genes (hPL₃ and hPL₄) are transcribed in term placenta (6). Furthermore, the third hPL gene (hPL₁), not known to be transcriptionally active in vivo, is capable of directing transcriptional initiation in vitro (9).

The mechanisms whereby the members of the hPL-hGH multigene family are differentially expressed in a tissue-specific manner are not understood. Tissue specific transcriptional enhancers have been detected in the murine κ and heavy chain immuno-

globulin genes (10-12) and may play a role in the tissue-specific differential expression of these genes. Viral enhancers have also been shown to exhibit host cell specificity in their activity (13). Other striking properties of enhancers include their abilities to function on heterologous promoters regardless of orientation and over long distances (several thousand bp). Although several of these properties have not been demonstrated for all putative enhancers, distal 5' upstream sequences from a number of genes exert a profound effect on the level of transcription and exhibit tissue-specific activity. These control regions, candidates for enhancer-like activity, have been defined for the several eukaryotic genes including: *Drosophila* glue protein *Sgs4* gene (14), silkworm fibroin gene (15), mammalian insulin and chymotrypsin genes (16), and the rat albumin gene (17).

The elucidation of the mechanisms for the control of gene expression of the members of the hPL-hGH multigene family is complicated by the high sequence homology among their structural genes (7,8). In the present work, several cell lines transformed by pSV2gpt or pSER recombinants containing the structural genes encoding hPL or hGH have been isolated and shown to express the introduced genes. DNA fragments containing the hPL and hGH structural genes and their flanking regions have been assayed for enhancer function. Transfection assays using plasmid constructs containing a 3.9 kb 3' flanking sequence of the hPL₃ gene indicates the presence of a transcriptional enhancer within this region. The hPL₃ gene enhancer functions in an orientation independent manner and in transient transfection assays was able to markedly elevate transcription directed from a heterologous promoter. The enhancer containing sequence has been localized to an AccI-SacI generated 1.0 kb DNA fragment. It is not strictly tissue-specific but shows a marked preference for placental cells.

MATERIALS AND METHODS

Plasmid Constructions

DNA fragments were isolated from recombinant plasmids that contain the genes for hPL₁, hPL₃, hPL₄, or hGH (5,6). hPL₁ is contained within a 3.5 kb EcoRI - Xba I fragment, whereas the hPL₃, hPL₄, and hGH genes are carried within 2.9 kb and 2.6 kb EcoRI generated fragments, respectively (5). The hPL₃, hPL₄, and hGH genes were isolated (18) and cloned directly into the unique EcoRI site of pSV2gpt (19), pSER (12) or pSER2 in the two possible orientations. Cloned inserts are designated (+) or (-) to indicate their orientation relative to the transcriptional orientation of the gpt gene. The hGH gene cloned in all the constructs (Fig. 1) refers exclusively to hGH₁ and not the variant gene, hGH₂ (4,5). The EcoRI-XbaI fragment that contains the hPL₁ gene was cloned into the same site following EcoRI linker addition (20). hGH-hPL flanking regions were cloned in the same site after EcoRI digestion of recombinant phage λH19, λH20, and λH22 previously selected from a human genomic DNA library (5). Another cloning

vector, pSER2, was constructed from pSV2gpt by PvuII - SphI double digestion followed by a T_4 -DNA polymerase-catalyzed fill-in and blunt-end ligation of filled-in hPL₃ using T_4 - DNA ligase (20). This clone reconstructed an EcoRI site immediately adjacent the 5' end of the gpt gene. Plasmid pSV1CATfrag1(+) was constructed by standard methods (20) after partial EcoRI digestion of the enhancer-deleted pSV1CAT vector (21).

Sau3A generated fragments of fragment 1 were cloned by standard methods (20) after digestion of pSV1CAT with BamHI. Several clones including pSV1CATfrag4, pSV1CATfrag5, pSV1CAT_{hGH}, pSV1CAT_{AS} and pSV1CAT_{EA} were constructed by blunt-end ligation of the T_4 -DNA polymerase-catalyzed filled-in fragments into the filled-in BamHI site of pSV1CAT. Plasmids pC3GEB and pC3hPL₃EB were constructed by ligation of the 5' flanking EcoRI-BamHI fragments of hGH and hPL₃ into the pCAT3M vector (22). The pCAT3M vector was first digested with XbaI restriction endonuclease and the ends filled-in by incubation with T_4 -DNA polymerase and free nucleotides. The EcoRI-BamHI fragments were also filled-in by the same procedure and were blunt-end ligated (20) into the filled-in XbaI site of pCAT3M. Clones with the EcoRI-BamHI fragment in the positive orientation relative to the chloramphenicol acetyltransferase gene were selected. The recombinant clones were routinely screened using minilytate procedures (23), and clones of interest were subjected to large scale plasmid preparations (24).

Cell Culture and Transfections

The JEG-3 cell line is derived from a human choriocarcinoma and produces chorionic gonadotropin and progesterone (25). The 18-54,SF cell line is derived from a pituitary tumor and produces prolactin (26). Cells were routinely grown as a monolayer in minimal essential medium supplemented with 10% fetal calf serum (Gibco). Cloned DNA was introduced into JEG-3 cells grown in 24 well dishes using the DNA-CaPO₄ coprecipitate method (27,28). After incubation of JEG-3 cells in the presence of medium plus DNA-CaPO₄ coprecipitate (28) for 4 hours at 37°C, the medium was removed and replaced with standard medium. For stable transformation the medium was removed after 24 h incubation and replaced every other day with selection medium (21). Transfection assays were quantitated by the number of foci of transformants observed after 21 days in selection medium containing mycophenolic acid, xanthine, and hypoxanthine (21). Stably transformed cell lines were obtained by pooling individual transformants and growth in selection medium. For chloramphenicol acetyl-transferase (CAT) assays, subconfluent cultures of cells were transfected with 20 µg/75 cm² flask of supercoiled plasmid DNA by the CaPO₄ coprecipitate technique (27,28). CAT assays were performed as described by Gorman et al. (21).

Northern Blot Analysis

Poly(A)⁺RNA was isolated from stably transformed JEG-3 cell lines essentially as described by Frazier et al. (29). RNA was glyoxalated (30) and fractionated on 1.2%

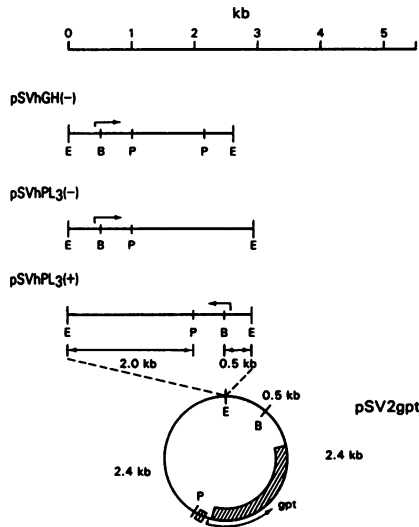


Figure 1. Restriction map of recombinant clones containing hPL and hGH inserts in pSV2gpt. The 2.9 kb EcoRI fragments containing hPL₃ or hPL₄ and the 2.6 kb EcoRI fragment containing hGH (hGH₁ described in Fig. 4) cloned into pER322 were purified and subcloned into the EcoRI site of pSV2gpt or pSER. pSER has had the SV40 enhancer sequences deleted and in the process has lost its SphI and PvuII sites. A second vector, designated pSER2, has a second EcoRI site in place of the lost PvuII site and hPL₃ and hGH were cloned in each orientation into this site (not shown). All clones are designated (+) or (-) to indicate their transcriptional orientation relative to that of gpt. The sites of initiation and direction of transcription are indicated by arrows. The SV40 enhancer region is represented by boxes. Abbreviations used include: E, EcoRI; P, PvuII; B, BamHI; and gpt, xanthine-guanine phosphoribosyl-transferase.

agarose gels in recirculated NaPO₄ buffer, pH 7.0. The RNA was transferred to nitrocellulose as described previously (30). Human 28S and 18S and Escherichia coli 23S and 16S ribosomal subunits served as RNA size markers. Filters were air-dried, baked at 80°C for 2 h under vacuum, and prehybridized in plastic bags at 42°C for 18 h in 5xSSC, 50% formamide, 0.1% SDS, 0.02% ficoll, 0.02% polyvinylpyrrolidone and 0.25 mg/ml of heat denatured sheared calf thymus DNA. The prehybridization solution was replaced by the hybridization solution (50% formamide, 5 x SSC, 0.02% ficoll, 0.02% polyvinylpyrrolidone, 0.1% SDS, 12.5% dextran sulfate, 0.02 N HCl, 0.25 mg/ml heat denatured sheared calf thymus DNA plus 2x10⁷ cpm of heat denatured ³²P-labeled probe). Lambda DNA digested with HindIII served as DNA size markers. Hybridization was carried out at 42°C for 16 h in plastic bags. The filter was washed at room temperature in 2 x SSC,

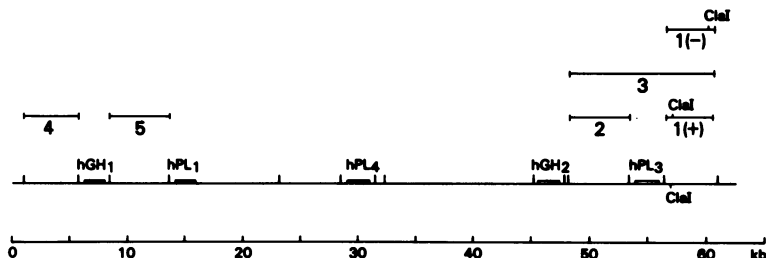


Figure 2. Restriction map of recombinant clones containing hPL and hGH flanking regions cloned into pSER. The vertical bars represent EcoRI restriction endonuclease sites. The map and gene nomenclature is taken from maps previously published (3,4). The direction of transcription is from left to right with the structural gene regions indicated by the open blocks. Cloned intergenic and flanking regions are indicated by numbered fragments (e.g. frag1 through 9). The EcoRI fragments, designated (+) or (-) depending on their orientation relative to the gpt gene, were cloned into the EcoRI site of pSER. Fragment 1 has an artificial EcoRI site produced upon cloning into λ Charon 4A.

0.1% SDS with 4 changes of 10 min each followed by 4 washes at 42°C in 0.1 x SSC, 0.1% SDS at 30 min each. The filter was exposed to X-ray films for 1-3 days in the presence of intensifying screens.

RESULTS

Subcloning of hPL and hGH Structural Genes in pSV2gpt and pSER; Transfection of JEG-3 Cells

EcoRI fragments encoding the entire hPL₃, hPL₄, and hGH structural genes were cloned into the EcoRI site of the vector pSV2gpt or pSER in both possible orientations designated (+) or (-) relative to the direction of transcription of the gpt gene (Fig. 1). The EcoRI - XbaI fragment that contains the hPL₁ gene was cloned into the EcoRI site of pSV2gpt following EcoRI linker addition. Another pSER cloning vector was constructed, designated pSER2, which has an EcoRI site in place of the deleted PvuII - SphI fragment containing the SV40 enhancer region. hPL₃ and hGH structural genes were cloned in place of the deleted SV40 enhancer such that in one of the orientations (designated (-)) the 5' region is adjacent to the gpt promoter. The DNA constructs illustrated in Fig. 1 were successfully introduced into JEG-3 cells, and stably transformed cell lines were obtained. These cell lines were derived from pools of 50-100 or 4-8 individual transfectants in the case of pSV2gpt and pSER, respectively.

Detection of Enhancer Activity Within a Flanking Region

To screen the intergenic regions of the hPL-hGH multigene cluster for enhancer activity, EcoRI fragments flanking the hGH and hPL₃ genes were cloned into the EcoRI

Table I. Relative transformation frequencies of pSV2gpt and derivative plasmids in JEG-3 cells.

Plasmids	Relative Frequency
pSV2gpt	1.0
pSER	<0.03
pSERhPL ₃ (+)	0.02
pSERhPL ₄ (+)	0.02
pSERhGH ₁ (+)	0.02
pSER2hPL ₃ (-)	0.02
pSER2hGH ₁ (-)	0.02
pSERfrag1(+)	0.30
pSERfrag1(-)	0.29
pSER2frag1(+)	1.3
pSER2frag1(-)	2.3
pSERfrag2	0.02
pSERfrag3	0.10
pSERfrag4	0.01
pSERfrag5	0.01
pSERfrag6	0.02
pSERfrag7	0.01
pSERfrag8	0.02
pSERfrag9	0.01

The transformation frequencies were normalized to the frequency obtained with pSV2gpt (1.1×10^{-4} for JEG-3 cells). All transfections were done in duplicate and in the case of pSV2gpt, pSER, pSERfrag1(+), pSERfrag1(-), pSER2frag1(+), and pSER2frag1(-), the frequencies listed were deduced from at least three different experiments using two different plasmid preparations.

site of pSER (Fig. 2). When pSER was used to transfect JEG-3 cells, the transformation frequency (relative to that of the plasmid pSV2gpt) was reduced by a factor of more than 30 (Table I). The DNA fragments shown in Figs. 1 and 2 were tested for their ability to substitute for the SV40 enhancer. These fragments include the structural genes for hGH, hPL₃ and hPL₄ as well as selected flanking regions (Fig. 2). As shown in Table I, the hPL and hGH structural genes did not contain DNA sequences capable of substituting for the deleted SV40 enhancer region. However, the structural gene sequences were at least 2 kb from the gpt promoter. Consequently, hPL₃ and hGH structural genes cloned into an EcoRI site close to the gpt promoter of pSER2 were also tested (Table I, pSER2hPL₃(-) and pSER2hGH₃(-). It has been reported previously (31) that the EcoRI - BamHI 5' fragment of hGH contains DNA sequences responsible for glucocorticoid induction. Recently, Moore et al. (32) detected a glucocorticoid receptor binding region within the first intron of hGH. By analogy with the enhancer activity in the MMTV LTR (34) it was

considered that the 5' end of the hGH and hPL genes were the best candidates for enhancer activity. However, even in the orientation (designated (-)) when the 5' end was nearest the gpt promoter this sequence failed to exhibit enhancer activity (Table I). Large EcoRI digested flanking regions cloned into pSER were also tested (Fig. 2 and Table I). The pSERfrag1(+) clone containing a 3.9 kb 3' flanking region of the hPL₃ gene exhibited an almost 10-fold higher transformation frequency compared to the pSER vector (Table I). In this construct the enhancer containing fragment 1 is cloned into the 3' region relative to the direction of the gpt gene transcription. Another construct, pSER2frag1(+), which has fragment 1 cloned into an EcoRI site 5' to the SV40 promoter exhibited an even higher relative transformation frequency (Table I). It is concluded that fragment 1, a 3' flanking region of hPL₃, contains an active enhancer sequence which can work in a position independent manner. Furthermore, the enhancer can function in an orientation independent manner as indicated by the elevated relative transformation frequencies of pSERfrag1(-) and pSER2frag1(-) (Table I). The low relative transformation frequencies of pSER clones containing fragments 6 through 9 indicate that there is no enhancer associated with the hPL₄ gene (Table I). The enhancer containing fragment 1, cloned into pSER in the opposite orientation and designated pSERfrag1(-) was also tested for enhancer activity (Table I). In the pSER2frag1(+) or (-) constructs, the enhancer is positioned 2 kb closer to the SV40 promoter than in the pSERfrag1(+) or (-) constructs and this may contribute to the observed higher relative transformation frequencies (2.3 and 1.3 versus 0.3 and 0.29, respectively).

The pSERfrag2 clone containing a 5.2 kb 5' flanking region of the hPL₃ gene did not exhibit an increased relative transformation frequency. Clones containing 3' and 5' flanking regions of hGH, designated pSERfrag5 and pSERfrag4 respectively, also failed to have an effect on relative transformation frequency. It should be noted that pSERfrag5 contains a flanking region which could be assigned as 5' to the hPL₁ gene (Fig. 2). The pSERfrag3 clone containing the 5' fragment 2, the 2.9 kb hPL₃ structural gene and the 3' fragment 1 showed an intermediate frequency (Table I).

The Enhancer can Directly Activate Transcription of a Heterologous Promoter

Relative transformation frequency assays have proved to be a valid test of enhancer activity (12,34,35). A more direct assay of the effect of enhancers on transcription using the E.coli CAT gene was developed by Gorman *et al.* (21). Using this transient assay (Fig. 3), the 3.9 kb fragment 1 markedly activates transcription of the SV40 early promoter in the pSV1CATfrag1(+) construct when introduced into JEG-3 cells. The time of incubation was chosen such that the amount of chloramphenicol conversion is in the linear range under the experimental conditions (data not shown).

The hPL₃ Enhancer is the Only Enhancer Detected in the hGH/hPL Cluster

Flanking regions throughout the hGH/hPL multigene cluster were examined for the

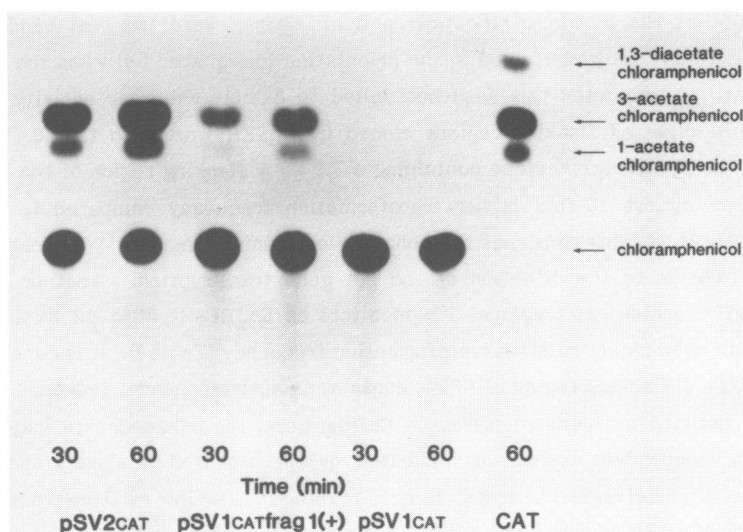


Figure 3. Assay of CAT activity in JEG-3 cells transfected with pSV2CAT and derivative plasmids. Cell lysates were assayed for CAT activity (21) 44 h after transfection with pSV2CAT, pSV1CAT, pSV1CATfrag1(+), pSV1CATfrag1EA, pSV1CATfrag1AS or pOTCO. Purified CAT enzyme served as a control and the radioactive products detected by autoradiography are indicated by arrows. Plasmid pSV2CAT possesses the SV40 enhancer and early promoter whereas the pSV1CAT is an enhancer-deleted variant (21). The pSV1CATfrag1(+) has the enhancer containing fragment 1 (Fig. 2) cloned in an *EcoRI* site 3' to the CAT gene. The elevated CAT activity observed with pSV1CATfrag1(+) transfection was observed in five independent experiments using two different plasmid preparations. pOTCO contains a thymidine kinase promoter in a CAT vector.

presence of enhancer sequences (Table I). Other than the hPL₃ enhancer (fragment1), no other enhancer containing sequences were found using this assay. Since the stable transformation assays were performed using JEG-3 cells as recipients it was possible that any enhancers present were acting in a cell-type specific manner. Consequently, we examined the hGH₁ section of the cluster for a hGH enhancer elements, using 18-54,SF cells as recipients in CAT assays. Fragments 4 and 5 as well as the hGH₁ structural gene were cloned into the *Bam*HI site of pSV1CAT. Each construct was introduced into both JEG-3 and 18-54,SF cells and CAT assays were performed. No construct exhibited higher activity than that observed with pSV1CAT (data not shown) and we conclude that there is no enhancer associated with the hGH₁ region of the cluster that is detectable under the conditions used.

In several of the constructs assayed in Table I, the structural hPL or hGH genes contained promoter regions spanning 500 bp upstream from the transcriptional start. It

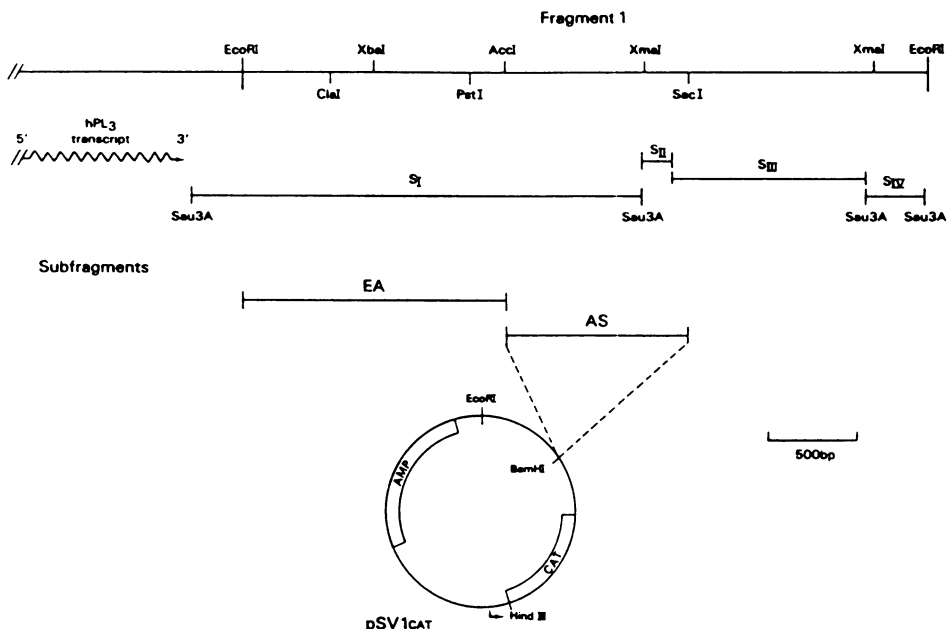


Figure 4. Subfragments of the enhancer containing hPL₃ 3' flanking regions cloned into pSV1CAT. Four Sau3A generated fragments, designated S_I through S_{IV}, were cloned into the BamHI site of pSV1CAT. Two fragments, EA and AS, were generated by AccI-SacI digestion of purified fragment1. The ability of the cloned fragments to substitute for the deleted SV40 enhancer was assessed by CAT assay.

was considered that the presence of two promoters in the pSER construct (SV40 and hPL or hGH) may interfere with the enhancer assay. The 500 bp EcoRI-BamHI fragments that contain the hGH and hPL promoters (9) were cloned upstream of the promoter-less pCAT3M vector (pC3GEB and pC3hPL₃EB, respectively). These two constructs were introduced into both JEG-3 and 18-54,SF cells and CAT assays were performed. Neither of these constructs exhibited CAT activity above that observed for pSV1CAT (data not shown). It is concluded that the 500 bp EcoRI-BamHI promoter containing segment does not contain an enhancer and furthermore the promoters did not exhibit cell-type preference as assessed by CAT activity (data not shown).

Localization of the Enhancer within the hPL₃ Flanking Region

Fragment 1 was cloned into the EcoRI site of pSV1CAT and CAT assays demonstrated that fragment 1 contains an enhancer that can act directly to increase transcriptional activity from a heterologous promoter (Figs. 3 and 4). The enhancer containing fragment 1 was purified and digested with a series of endonucleases and a

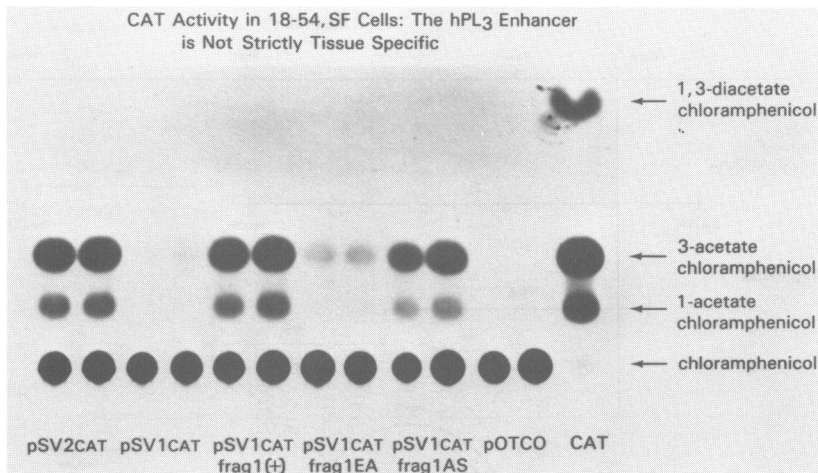


Figure 5. CAT activity in transfected pituitary 18-54,SF cells: localization of the hPL₃ enhancer to an AccI-SacI restriction fragment (frag 1AS). Cell lysates were assayed for CAT activity 44 h after transfection with the plasmid constructs listed. Purified CAT enzyme served as a control and the radiolabeled products detected by autoradiography (24 h) are indicated by arrows.

number of subfragments were cloned into pSV1CAT (Fig. 4). CAT assays demonstrated that Sau3A fragments, designated S_I through S_{IV}, did not contain sequences capable of acting as an enhancer (data not shown). The same assay system demonstrated that the enhancer is located within an AccI-SacI generated fragment of approximately 1.0 kb (Fig. 3, 4; pSV1CATAS). Presumably, the S_I, S_{II} and S_{III} fragments contain only part of the active enhancer sequence and this is why they failed to exhibit enhancer activity (data not shown).

The hPL₃ Enhancer is not Strictly Cell-Type Specific

The hPL₃ enhancer is not strictly tissue specific since it functions in human-cell lines of placental (JEG-3) and pituitary (18-54,SF) origin (Fig. 3 and 5). Nonetheless, the enhancer in pSV1CATAS functions more efficiently, relative to the SV40 enhancer activity, in JEG-3 cells than in 18-54,SF cells. Enhancer efficiency was estimated from densitometric scans of the products of the CAT assay. In placental cells the hPL enhancer fragment 1 has twice the activity of the SV40 enhancer while the subcloned fragment 1AS has ~9 times the activity of the SV40 enhancer. In pituitary cells fragment 1 has about the same enhancer activity as SV40, while the subfragment AS has about one-half the activity of the SV40 enhancer. These experiments show that the hPL₃ enhancer is severalfold more active than the SV40 enhancer in placental cells but much less active in pituitary cells. Since the EA fragment (Fig. 4) is a subfragment of S_I, its failure to exhibit enhancer activity (Fig. 3 and 5) is expected.

DISCUSSION

In the present work, a highly efficient enhancer of the hPL₃ gene has been identified. The hPL₃ enhancer has all three of the major distinguishing characteristics of a transcriptional enhancer: function is independent of orientation and position relative to the transcriptional start and it can act on a heterologous promoter to directly stimulate transcriptional activity (Table I; Fig. 3 and 5). The hPL₃ enhancer has been localized to a 1 kb region ~2 kb downstream from the hPL₃ gene. The enhancer fragment enhances CAT expression in human placental cells (JEG-3) ~9 times more than the SV40 enhancer but only one-half as efficiently as the SV40 enhancer in pituitary cells (Fig. 3 and 5). Thus, this highly efficient enhancer shows ~20 fold tissue preference for placental cells relative to pituitary cells, but not strict tissue specificity. The ability of this sequence to dramatically stimulate transcription clarifies the prominent role the hPL₃ gene plays in placental lactogen synthesis (~1g/day in term placenta).

The location of the enhancer adjacent to the hPL₃ gene makes a compelling argument that its biological role is to enhance hPL₃ gene transcription. Barsh *et al* (4) screened the hPL/hGH multigene family for sequences related to hPL. They found only the five hPL and hGH genes with no homology within 16 kb 3' of the hPL₃ gene. Thus, there is no evidence for a transcribed gene within 6 kb of the enhancer except for the hPL₃ gene.

It is not surprising that hPL₃ has a strong enhancer. Five to ten percent of the total mRNA in term placenta is hPL mRNA (6) and all of it is transcribed from two genes, hPL₃ and hPL₄. About 10-20% of it is transcribed from hPL₄ and 80-90% from hPL₃ (6). In vitro transcription experiments in a HeLa cell extract showed similar promoter efficiencies for the two genes. Perhaps Nature was clever to insert an enhancer 3' of the outside gene (hPL₃) of the cluster rather than 5' of the gene, because of the possibility of its influencing hGH transcription (hGH₂ and hPL₃ are separated by 6 kb).

The control of hPL expression is poorly understood. In contrast, the other members of the multigene family, hGH and prolactin, have been extensively studied. Two groups (36,37) have used experiments with different gene-fusion constructs to suggest that transcription of the hGH gene is stimulated in the presence of dexamethasone. However, there is disagreement on the regions responsible for this induction, with some authors (31) implicating the first intron sequence and others (37) the region within 500 bp 5' to the hGH transcriptional start site. Experiments described in the present work failed to detect an enhancer within the hGH or hPL₄ structural genes or their 5' and 3' flanking regions. These experiments were performed in the absence of added hormones and consequently, enhancer sequences that are hormone inducible would not be readily detected. Hence, the hPL₃ enhancer described here remains the only enhancer so far

detected in the hGH/hPL multigene cluster. It would seem that in the evolution of this multigene family the regulation of the hPL genes has been radically altered from that of the hGH genes. This is interesting when it is considered that the hGH and hPL genes are thought to have evolved from a duplicated ancestral gene (1) and recently (~10 million years ago) underwent a gene conversion event leading to the high homology found in the multigene complex (4,38). During evolution of this multigene family an enhancer from elsewhere in the genome may have been translocated to its present position in the 3' flanking region of the cluster. Such an event would explain the high expression of hPL₃ in vivo, and would explain why the other hPL and hGH genes or its flanking regions do not possess a counterpart to the hPL₃ enhancer. In several cell lines, translocations or retroviral insertions have been shown to lead to transcriptional activation of adjacent genes. This mechanism of gene activation has been reported to play a role in c-myc expression in Manca cells (39), interleukin-3 expression in WEHI-3B cells due to retroviral insertion 5' upstream of the gene (40), and interleukin-2 production in MLA144 cells due to retroviral insertion 3' to the gene (41).

There is considerable evidence that enhancers are regions of DNA where site-specific DNA binding proteins interact (42). Specific interactions have been described between nuclear proteins and the polyoma virus (43) and immunoglobulin heavy chain (44) enhancers. The search for trans-acting factors that interact specifically with the hPL₃ enhancer in placental tissues is in progress.

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