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Luman, a New Member of the CREB/ATF Family, Binds to Herpes Simplex Virus VP16-Associated Host Cellular Factor

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The human host cell factor (HCF) is expressed in a variety of adult and fetal tissues, and its gene is conserved in animals as diverse as mammals and insects. However, its only known function is to stabilize the herpes simplex virus virion transactivator VP16 in a complex with the cellular POU domain protein Oct-1 and *cis*-acting regulatory elements in promoters of immediate-early viral genes. To identify a cellular function for HCF, we used the yeast two-hybrid system to identify a cellular ligand for HCF. This protein, Luman, appears to be a cyclic AMP response element (CRE)-binding protein/activating transcription factor 1 protein of the basic leucine zipper superfamily. It binds CREs in vitro and activates CRE-containing promoters when transfected into COS7 cells. This activation of transcription was synergistically enhanced by the presence of CCAAT/enhancer-binding protein elements and inhibited by AP-1 elements in the promoter. In addition to a basic DNA binding domain, Luman possesses an unusually long leucine zipper and an acidic amino-terminal activation domain. These features in Luman are also present in what appear to be homologs in the mouse, *Drosophila melanogaster*, and *Caenorhabditis elegans*. Luman and VP16 appear to have similar mechanisms for binding HCF, as in vitro each competitively inhibited the binding of the other to HCF. In transfected cells, however, while VP16 strongly inhibited the ability of GAL-Luman to activate transcription from a GAL4 upstream activation sequence-containing promoter, Luman was unable to inhibit the activity of GAL-VP16. Luman appears to be a ubiquitous transcription factor, and its mRNA was detected in all human adult and fetal tissues examined. The possible role of HCF in regulating the function of this ubiquitous transcription factor is discussed.

The induction of transcription of the immediate-early (IE or α) genes of herpes simplex virus (HSV) by the virion protein VP16, also known as Vmw65 or α-TIF, is one of the better-known models for the combinatorial control of gene expression by selective protein-protein interactions (reviewed in references 38 and 52). Unlike most other transcription activators, VP16 does not bind directly to DNA but is recruited to IE gene promoters by its association with the POU domain protein Oct-1 (28, 33, 34, 39, 51). However, this association is unstable and requires another cellular protein, host cell factor (HCF; also called C1, VCAF, or CFF), to be functional (25, 26, 50, 59). When HSV infects permissive cells, VP16 is released from the virion into the cell and forms a complex with HCF. The interaction of VP16 with HCF precedes recognition of Oct-1 bound to the *cis*-regulatory target of VP16 activation, the TAATGARAT motif (R is a purine), found in HSV IE promoters (4, 28, 34, 39, 41, 51).

The human HCF, purified from HeLa cells, is a family of polypeptides ranging in molecular mass from 100 to 300 kDa (29, 56). These polypeptides originate from a single large protein by posttranslational processing (15, 56, 57). The HCF protein has six copies of a 26-amino-acid (aa) repeat located near its center (27, 56). These repeats are sites for proteolytic cleavage which results in a series of polypeptides of various lengths (15, 57). Almost all the amino-terminal and carboxy-terminal polypeptides that result are held together by noncovalent interactions (56). The interaction between processed HCF fragments is strong, such that the two terminal regions will remain associated even in the presence of 3 M urea (29). The functional relevance of the association between the amino and carboxyl termini of HCF is underscored by the observation that a fusion protein comprising the termini associates with VP16 and participates in complex formation with TAATGA RAT sequences (29a). The gene encoding HCF, designated *HCFC1*, consists of 26 exons spread over a region of 26 kb in chromosome Xq28 (15, 57).

The *HCFC1* gene is expressed in many human adult and fetal tissues and in many mammalian cell types (55, 57). Since HCF is also evolutionarily conserved in animals as diverse as mammals and insects (26), it likely has important cellular functions. Yet the only known biological function of HCF is in the stabilization of HSV VP16-induced complex. The higher levels of HCF protein in embryonic tissues than in adult tissues suggest that HCF may be involved in cell proliferation and differentiation. Recent data on a temperature-sensitive HCF mutant hamster cell line with a temperature-sensitive lesion in HCF support this hypothesis (19). At the nonpermissive temperature, the cells were arrested at the G_0/G_1 decision point. Thus, it is suggested that HSV may use HCF levels to monitor the proliferative status of the infected cell to determine whether to follow a lytic or latent pathway (56). However, the function of HCF in uninfected cells and its mechanism of action are unclear.

To elucidate the normal functions of HCF, we chose to identify cellular ligands for the protein by using the yeast in-

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TABLE 1. Oligonucleotides used in this study

No.	Purpose	Used in construct:	Sequence
1	PCR	pCRLuman	5′-TGAAGATACCCCACCAAACC
2	PCR	pCRLuman	5′-TCCTCATCTGTCAGTACTAGCC
3	PCR	pCRLuman	5′-GATGAGGAGAAGAGTCTATTGG
4	PCR	pCRLuman	5′-CACGACGTTGTAAAACGACG
5	PCR	pCRLuman	5′-CGGAATTCCAGTTGTCCCAAATGGAGC
6	Linker	pcLumanΔ38	5′-GATCCGAATG
7	Linker	pcLumanΔ38	5′-AATTCATTCG
8	Linker	pcLuman	5′-GATCCTCTACCATGGACTACAAAGACGATGACGACAAGC
9	Linker	pcLuman	5′-AATTGCTTGTCGTCATCGTCTTTGTAGTCCATGGTAGAG
10	Linker	pGEXLuman	5′-GATCCAATGGAGAATTCCTGCAGGATATCGGGCCCT
11	Linker	pGEXLuman	5′-CTAGAGGGCCCGATATCCTGCAGGAATTCTCCATTG
12	EMSA	(CRE)[a]	5′-AGCTGCCGGTGACGTCATCGCAT
13	EMSA	(CRE)	5′-CTAGATGCGATGACGTCACCGGC
14	EMSA	(C/EBP)	5′-AGCTGGTATTGCGTAATTGATAT
15	EMSA	(C/EBP)	5′-CTAGATATCAATTACGCAATACC
16	EMSA	(AP-1)	5′-AGCTACCGGTGACTCAATGGCT
17	EMSA	(AP-1)	5′-CTAGAGCCATTGAGTCACCGGT

[a] Parentheses denote oligonucleotides not used for constructing plasmids.

teraction trap/two-hybrid system (10, 20). In the present study, a cDNA clone (designated *Luman*, named after a legendary hero in ancient China) was isolated from a HeLa cDNA library. Both sequence analysis and in vitro/in vivo assays demonstrated that Luman is a transcription factor that belongs to the cyclic AMP response element (CRE)-binding protein (CREB)/activating transcription factor (ATF) gene family. Northern blot analysis showed that Luman is ubiquitously expressed in all human adult and fetal tissues examined. Its DNA binding domain is remarkably well conserved among proteins in nematodes and insects. These results suggest that Luman is an important regulator of certain fundamental events in the cell. The phenomenon that both the viral and cellular transactivators, VP16 and Luman, bind to HCF implies a unique mechanism for the regulation of the function of transcriptional factors and gene activation.

MATERIALS AND METHODS

Materials. All restriction endonucleases, modifying enzymes, *Taq* DNA polymerase, and oligonucleotide primers were purchased from Canadian Life Technologies. Amino acids were purchased from Sigma Chemical Company. All other reagents were also obtained from Canadian Life Technologies unless otherwise stated.

Screening a two-hybrid cDNA library. Bait plasmid pGBThcfNC was constructed by removing an *Eco*RI-*Xho*I fragment from plasmid SL2 (S. LaBossiere and P. O'Hare, Marie Curie Institute, Surrey, United Kingdom), which contains fused sequences coding for the amino-terminal (aa 2 to 490) and carboxyl-terminal (aa 1495 to 2035) regions of HCF (hcfNC). This fragment was then inserted between *Eco*RI-*Sal*I sites of pGBT9 vector (Clontech). The control plasmid pGBTGST had an *Eco*RI-*Bam*HI fragment containing the glutathione *S*-transferase (GST) gene subcloned into pGBT9 vector digested by the same enzymes. Screening of a human HeLa cDNA library (MatchMaker cDNA library; Clontech), which is fused to GAL4 activation domain (GAL4 AD), using the yeast two-hybrid system was performed according to the manufacturer's instructions. *Saccharomyces cerevisiae* HF7c [*MAT***a** *ura3-52 his3-200 lys2-801 ade2-101 trp1-901 leu2-3,112 gal4-542 gal80-538 LYS2::GAL1-HIS3 URA3::* (*GAL4* 17-mers)$_3$-*CYC1-lacZ*] was sequentially transformed with pGBThcfNC and the HeLa MatchMaker cDNA library by using the lithium acetate method (24). Transformants were spread on a synthetic minimal dropout medium (0.67% [wt/vol] yeast nitrogen base, 2% [wt/vol] glucose, 2% [wt/vol] agar, appropriate auxotrophic supplements lacking leucine, tryptophan, and histidine) supplemented with 25 mM 3-amino-1,2,4-triazole to suppress background growth. HIS3-positive colonies were picked up and restreaked on the same selective medium to make master plates. β-Galactosidase colony lift assays were carried out on these plates to confirm the phenotype of the clones.

The cDNA library plasmids from yeast transformants were isolated by a phenotype rescue strategy as follows. Crude preparations of plasmid DNA from yeast cells were introduced into *Escherichia coli* HB101 by electroporation (2.5 kV, 25 μF, and 200 ohms in a 0.2-cm cuvette) and plated on M9 minimum medium supplemented with all amino acids except leucine. Since HB101 carries a *leuB* mutation, it can be rescued only by cDNA harboring plasmids containing a *LEU2* marker. The cDNA inserts in these plasmids were confirmed by restriction analysis. Subsequently, these purified plasmids were reintroduced into yeast strain HF7c along with the original bait plasmid pGBThcfNC or control plasmids pGBTGST and pGBT9. Transformants were spread on the selective medium and tested for β-galactosidase activity. An estimated 5×10^6 independent colonies were screened. Two positive clones were selected for further analysis, one of which, clone pHL1A, is discussed here.

DNA sequencing. Plasmid DNA was purified by using a Wizard miniprep kit (Promega). Double-stranded cDNA insert was sequenced by using Sequenase 2.0 as instructed by the manufacturer (Amersham).

Cloning the full-length cDNA by overlapping PCR. To clone the full-length cDNA, three separate PCRs were performed. The DNA sequences of the primers used in the reactions are shown in Table 1. The first PCR was designed to isolate the 5′ region of the cDNA from the cDNA library. Primer 1 was targeted to the 3′ end of the GAL4 AD region of the cDNA library vector pGADGH, and primer 2 was targeted to the 5′ portion of the cDNA insert in pHL1A. The PCR products were electrophoresed through a 0.8% agarose gel. The largest bands were isolated, reamplified, and cloned into pCRII vector (Invitrogen), using the T-A cloning strategy. Six independently isolated clones were purified and sequenced. Two of the clones had identical inserts whose 3′ ends matched the sequence of pHL1A. In another PCR, primer 3 was designed to have an 8-bp overlap with primer 2 in the opposite orientation; primer 4 targeted a sequence 3′ of the multiple cloning site of pGADGH. Two separate PCRs were carried out: primers 1 and 2 were used to amplify the insert in pCR1A, and primers 3 and 4 were used to amplify the insert in pHL1A. The PCR products were purified from an agarose gel, mixed, and reamplified by using primers 5 and 4. Primer 5 was to introduce an *Eco*RI site at the predicted start codon of the cDNA. The subsequent PCR product was cloned into pCRII. The DNA sequence was verified by DNA sequencing (Fig. 1). The cDNA was designated *Luman*, and the PCR clone was named pCRLuman.

The amplification conditions were the same for all the PCRs. The 50-μl standard reaction mixture consisted of 100 ng of cDNA library DNA, 50 ng of plasmid DNA, or 2 μl of gel-purified DNA, 0.25 mM each dATP, dTTP, dGTP, and dCTP, 0.5 μM primers, 1.5 mM $MgCl_2$, 75 mM KCl, and 2.5 U of *Taq* DNA polymerase. The PCR program began with one cycle of 3-min denaturation at 94°C, 2-min annealing at 55°C, and 3-min extension at 72°C, followed by 30 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C and then a final 8-min extension at 72°C.

Plasmids. Plasmids pM1 and pM2, for constructing GAL4 fusion proteins, and pG5EC, a plasmid containing the coding sequences of the gene for chloramphenicol acetyltransferase (CAT) linked to five GAL4 binding motifs in its promoter region, were obtained from Ivan Sadowski, University of British Columbia (45). Plasmid pGEX-KG, for constructing GST fusion proteins, was a gift from Gerry Weinmaster (University of California, Los Angeles). Reporter plasmids p-109C3, p-109A3, p-68D3, and p-68CRE, which contain different basic leucine zipper (bZIP) protein binding sites in the promoter region of *CAT* gene, were obtained from William Roesler (University of Saskatchewan) (42–44). The numbers in the plasmid names indicate the locations of each element in relation to the beginning of the transcripts of CAT.

To construct pGALLuman, an *Eco*RI-*Xho*I fragment of pCRLuman, containing the entire predicted coding sequence of Luman, was cloned between *Eco*RI-*Sal*I sites of pM1. The *Eco*RI-*Xho*I fragment from pHL1A was cloned into pM2

by the same strategy. Since this *Eco*RI-*Xho*I insert (pHL1A) lacks the first 38 aa after the presumed start codon of Luman, it was named pGALLumanΔ38.

In the construction of pcLumanΔ38, a pair of oligonucleotides (6 and 7 in Table 1) were annealed and cloned into pcDNA3 (Clontech) between *Bam*HI and *Eco*RI to introduce an ATG start codon. The *Eco*RI-*Xho*I fragment from pHL1A was subsequently cloned between the same sites of pcDNA3. Oligonucleotides 8 and 9 (Table 1) code for a FLAG tag (Kodak) and have *Bam*HI and *Eco*RI cohesive ends when annealed. This linker and an *Eco*RI-*Xho*I fragment from pCRLuman containing the entire Luman cDNA were simultaneously cloned between *Bam*HI-*Xho*I sites of pcDNA3 by a three-way cloning strategy. The resulted plasmid was called pcLuman. Plasmid pchcfNC was constructed by removing an *Eco*RI-*Xho*I fragment from plasmid SL2, which contains hcfNC as mentioned above, into *Eco*RI-*Xho*I sites of pcDNA3.

To construct pGEXLuman, an oligonucleotide linker (oligonucleotides 10 and 11 in Table 1) was cloned into *Bam*HI-*Xba*I of pGEX-KG to realign the reading frame at the *Eco*RI site. The *Eco*RI-*Xho*I fragment of pCRLuman, containing the entire predicted coding sequence of Luman, was cloned into the same sites of the new pGEX vector. The constructions of plasmids pcVP16, pGALVP16, and pGEXVP16 were described elsewhere (35, 36).

Cell culture, transfections, and CAT assays. The growth conditions of COS7 cells have been described previously (35, 36). COS7 cells were transfected by using Lipofectamine (Canadian Life Technologies) in six-well plates according to the manufacturer's instructions. In each transfection, a total of 2 μg of DNA (pUC plasmid DNA was used to make up the total amount when necessary) and 8 μl of Lipofectamine were used. The cells were harvested after 48 h of transfection and assayed for CAT expression by using a CAT enzyme-linked immunosorbent assay kit (Boehringer Mannheim) according to the manual provided by the manufacturer.

Expression and purification of GST fusion proteins, GST pull-down assay, and in vitro translation. The fusion proteins GST-Luman and GST-VP16 were produced in *E. coli* BL21(DE3) (Novagen) and purified (35, 36). A rabbit reticulocyte in vitro transcription-translation system (TnT; Promega) was used to produce ^{35}S-labeled and unlabeled Luman, LumanΔ38, VP16, hcfNC, and human bone alkaline phosphatase according to the manufacturer's protocol.

Electrophoretic mobility shift assay (EMSA). Oligonucleotides representing binding sites for CRE, CCAAT/enhancer-binding protein (C/EBP), and AP-1 (oligonucleotides 12 and 13, 14 and 15, and 16 and 17, respectively, in Table 1) were annealed and labeled with [α-^{32}P]dCTP and the Klenow fragment of *E. coli* DNA polymerase and used as probes. Details of the procedure were as described elsewhere (35, 36) except that purified GST fusion proteins were used instead of cell extracts.

RNA analysis. MTN Northern blots (Clontech) containing poly(A)$^+$ RNA from adult and fetal human tissues were hybridized with the full-length Luman cDNA, hcfNC, and actin according to the recommendations of the manufacturer. The Luman and hcfNC probes were prepared by *Eco*RI-*Xho*I excision from plasmids pCRLuman and SL2 and were labeled by the random labeling method (13).

Nucleotide sequence accession number. The sequence reported here has been deposited with GenBank under accession no. AF009368.

RESULTS

Cloning of a human HCF-binding protein, Luman cDNA. To identify cellular ligands for HCF, we screened a HeLa cDNA and GAL4 AD fusion library by using pGALhcfNC as bait. This plasmid expresses hcfNC linked with the GAL4 DNA binding domain (GAL4 BD). The hcfNC protein should be a working version of native HCF because the terminal regions of HCF associate with each other under natural conditions (57). In addition, hcfNC, which lacks the internal HCF repeats, efficiently promotes complex formation in vitro between VP16, the POU domain of Oct-1, and oligonucleotides containing a TAATGARAT motif. The association between hcfNC and VP16 can also be demonstrated in yeast and mammalian two-hybrid systems (29a). The HeLa cDNA library was chosen because (i) VP16 transactivates transcription in HeLa cells and (ii) HCF is present in abundance in HeLa cells and was initially purified and characterized from this cell line (27, 56). Since pGALhcfNC itself can activate transcription, albeit weakly, 3-amino-1,2,4-triazole was used to suppress the background growth on His-lacking SD selective medium of HF7c yeast cells containing this plasmid. From the 5×10^6 colonies screened, two cDNA clones that interacted strongly with HCF were identified. The study of one of these clones, pHL1A, is presented here.

pHL1A contained a 1,282-bp cDNA insert. Preliminary sequence analysis of pHL1A revealed that it lacked an AUG codon in a translation initiation context. To obtain cDNA clones containing the entire coding region, we designed an overlapping PCR strategy to amplify the missing 5′ region. Of the six clones obtained, two had the same insert and also overlapped cDNA sequence in pHL1A. Thus, an additional 124 bp was recovered and linked to the cDNA sequences in pHL1A. The resulting entire cDNA was designated *Luman.*

The complete nucleotide sequence contains an open codon reading frame of 1,113 bp. This region would specify a protein of 371 aa with a predicted molecular mass of 41 kDa (Fig. 1).

Luman is a novel member of the CREB/ATF family. A search of GenBank with the amino acid sequence of Luman revealed a homolog (70% similarity), LZIP, in mice (8). LZIP can efficiently bind to CRE sites and to AP-1 sites to a lesser degree, and it belongs to the bZIP superfamily of transcription factors. Proteins of the bZIP superfamily bind to DNA as dimers and are characterized by their unique structure: all members have a highly positively charged basic domain that contacts DNA. An adjacent region, the leucine zipper, contains repeating leucine residues, separated by 6 aa. The leucine zipper mediates dimerization with molecules of the same protein or other proteins that have similar motifs (reviewed in references 2 and 30). The members of the bZIP superfamily can be divided into three families on the basis of their ability to cross-dimerize (reviewed in reference 30): (i) the C/EBPs, (ii) the FOS/JUN group of transcription factors, and (iii) the CREB/ATF family, comprising the ATFs, the original CREBs, and the CRE modulators (14, 30, 49). As shown in Fig. 2B, Luman has a typical bZIP region (aa 155 to 220), with the 5-aa spacer between the basic domain and leucine zipper perfectly preserved. The bZIP region of Luman is strikingly similar to that of ATF-A and ATF6, representative members of the human CREB/ATF family. The basic region of Luman is also related to members of FOS-JUN and C/EBP families, although to a lesser extent. A unique feature of Luman sequence is that it has an unusually long stretch of leucine repeats. Apart from substitutions of a tyrosine, two serines, and an isoleucine, the leucine repeats extend 13 times (Fig. 1). Most other members of the bZIP family have zippers with four to five leucine repeats. The tyrosine and isoleucine substitutions probably do not affect dimerization, as other bZIP proteins harboring such replacements can still dimerize (22, 53). Secondary structure predictions by different algorithmic methods also indicate the formation of an α helix in this region (references 12, 17, 18, and 31 and data not shown).

Examination of the amino acid composition of Luman suggests the presence of other structural domains in the protein. The amino terminus of Luman is highly negatively charged. Of the 52 aa in the amino-terminal portion of the protein, 15 are aspartic acid or glutamic acid residues (29%). There is only one positively charged arginine. This 52-aa segment is reminiscent of the acidic activation domains of transcription factors; indeed, it has nearly 50% similarity to a region of the *Bombyx mori* nuclear polyhedrosis virus transcription activator IE-1 (23). The carboxyl terminus of Luman is highly rich in proline (20%), although a search of GenBank with this domain did not yield any significant matches. Two serine-rich regions were found at aa 56 to 106 (20%) and aa 221 to 254 (26%). These may represent possible phosphorylation sites.

Luman is a transcription factor with an amino-terminal activation domain. Since all members of bZIP family are transcription activators and/or repressors, we decided to determine whether Luman could function as a transcription activator. The Luman coding sequences were cloned as fusions with the

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1                                                                    gTAGttgtcccaa    13
14   ATGGAGCTGGAATTGGATGCTGGTGACCAAGACCTGCTGGCCTTCCTGCTAGAGGAAAGTGGAGAT    79
1    M  E  L  E  L  D  A  G  D  Q  D  L  L  A  F  L  L  E  E  S  G  D     22
80   TTGGGGACGGCACCCGATGAGGCCGTGAGGGCCCCACTGGACTGGGCGCTGCCGCTTTCTGAGGTA   145
23   L  G  T  A  P  D  E  A  V  R  A  P  L  D  W  A  L  P  L  S  E  V     44
146  CCGAGCGACTGGGAAGTAGATGATTTGCTGTGCTCCCTGCTGAGTCCCCCAGCGTCGTTGAACATT   211
45   P  S  D  W  E  V  D  D  L  L  C  S  L  L  S  P  P  A  S  L  N  I     66
212  CTCAGCTCCTCCAACCCCTGCCTTGTCCACCATGACCACACCTACTCCCTCCCACGGGAAACTGTC   277
67   L  S  S  S  N  P  C  L  V  H  H  D  H  T  Y  S  L  P  R  E  T  V     88
278  TCTATGGATCTAGAGAGTGAGAGCTGTAGAAAAGAGGGGACCCAGATGACTCCACAGCATATGGAG   343
89   S  M  D  L  E  S  E  S  C  R  K  E  G  T  Q  M  T  P  Q  H  M  E    110
344  GAGCTGGCAGAGCAGGAGATTGCTAGGCTAGTACTGACAGATGAGGAGAAGAGTCTATTGGAGAAG   409
111  E  L  A  E  Q  E  I  A  R  L  V  L  T  D  E  E  K  S  L  L  E  K    132
410  GAGGGGCTTATTCTGCCTGAGACACTTCCTCTCACTAAGACAGAGGAACAAATTCTGAAACGTGTG   475
133  E  G  L  I  L  P  E  T  L  P  L  T  K  T  E  E  Q  I  L  K  R  V    154
476  CGGAGGAAGATTCGAAATAAAAGATCTGCTCAAGAGAGCCGCAGGAAAAAGAAGGTGTATGTTGGG   541
155  R  R  K  I  R  N  K  R  S  A  Q  E  S  R  R  K  K  K  V  Y  V  G    176
542  GGTTTAGAGAGCAGGGTCTTGAAATACACAGCCCAGAATATGGAGCTTCAGAACAAAGTACAGCTT   607
177  G  L  E  S  R  V  L  K  Y  T  A  Q  N  M  E  L  Q  N  K  V  Q  L    198
608  CTGGAGGAACAGAATTTGTCCCTTCTAGATCAACTGAGGAAACTCCAGGCCATGGTGATTGAGATA   673
199  L  E  E  Q  N  L  S  L  L  D  Q  L  R  K  L  Q  A  M  V  I  E  I    220
674  TCAAACAAAACCAGCAGCAGCAGCACCTGTATCTTGGTCCTACTAGTCTCCTTCTGCCTCCTCCTT   739
221  S  N  K  T  S  S  S  S  T  C  I  L  V  L  L  V  S  F  C  L  L  L    242
740  GTACCTGCTATGTACTCCTCTGACACAAGGGGGAGCCTGCCAGCTGAGCATGGAGTGTTGTCCCGC   805
243  V  P  A  M  Y  S  S  D  T  R  G  S  L  P  A  E  H  G  V  L  S  R    264
806  CAGCTTCGTGCCCTCCCCAGTGAGGACCCTTACCAGCTGGAGCTGCCTGCCCTGCAGTCAGAAGTG   871
265  Q  L  R  A  L  P  S  E  D  P  Y  Q  L  E  L  P  A  L  Q  S  E  V    286
872  CCGAAAGACAGCACACACCAGTGGTTGGACGGCTCAGACTGTGTACTCCAGGCCCCTGGCAACACT   937
287  P  K  D  S  T  H  Q  W  L  D  G  S  D  C  V  L  Q  A  P  G  N  T    308
938  TCCTGCCTGCTGCATTACATGCCTCAGGCTCCCAGTGCAGAGCCTCCCCTGGAGTGGCCATTCCCT  1003
309  S  C  L  L  H  Y  M [P  Q  A  P  S  A  E  P  P  L  E  W  P  F  P    330
1004 GACCTCTCTTCAGAGCCTCTCTGCCGAGGTCCCATCCTCCCCCTGCAGGCAAATCTCACAAGGAAG  1069
331  D  L  S  S  E  P  L  C  R  G  P  I  L  P  L  Q  A  N  L  T  R  K    352
1070 GGAGGATGGCTTCCTACTGGTAGCCCCTCTGTCATTTTGCAGGACAGATACTCAGGCtagatatga  1135
353  G  G  W  L  P  T  G  S  P  S  V  I  L  Q  D  R  Y  S  G] *    *    371
1136 ggatatgtgggggtctcagcaggagcctgggggctccccatctgtgtccaaataaaagcggtg     1201
1202 ggcaagggctggccgcagctcctgtgccctgtcaggacgactgagggctcaaacacaccacactta  1267
1268 atggctttctgggtcttttatttgtacccatgtgtctgtcacaccatgaatgtacctggggaaatc  1333
1334 aactgacctccctgaacatttcacgcagtcagggacaggtgaggaaagaAATAAAtaagtgattct  1399
1400 aatgctg                                                             1406
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FIG. 1. The cDNA sequence of Luman. The sequence contains a coding region of 1,113 bp, encoding a protein of 371 amino acids with a predicted molecular mass of 41 kDa. The protein contains a bZIP region and belongs to the bZIP superfamily. It also has a highly acidic region at the amino terminus (dotted line) and a proline-rich region at its carboxyl terminus (in brackets). It has an unusually long leucine zipper with a tyrosine substitution at the second and an isoleucine substitution at the seventh leucine heptad positions (boldface and underlined). Apart from two serine substitutions in the heptad repeats, the leucine zipper extends through 13 repeats (boldface). The putative polyadenylation site is in boldface and underlined.

GAL4 BD. This plasmid was designated pGALLuman. The initial Luman clone, which is missing the first 38 aa, was also cloned as a GAL4 BD fusion, resulting in pGALLumanΔ38 (Fig. 3A). The reporter plasmid pG5EC has five GAL4-upstream activation sequence (UAS) sites in the *CAT* promoter region. Plasmids expressing the GAL4 fusion proteins and pG5EC were introduced into COS7 cells. The cloning vector pM1 and pGALVP16 were used as controls.

The results showed that Luman is a strong transcription activator whose activity is comparable to that of VP16 (Fig.

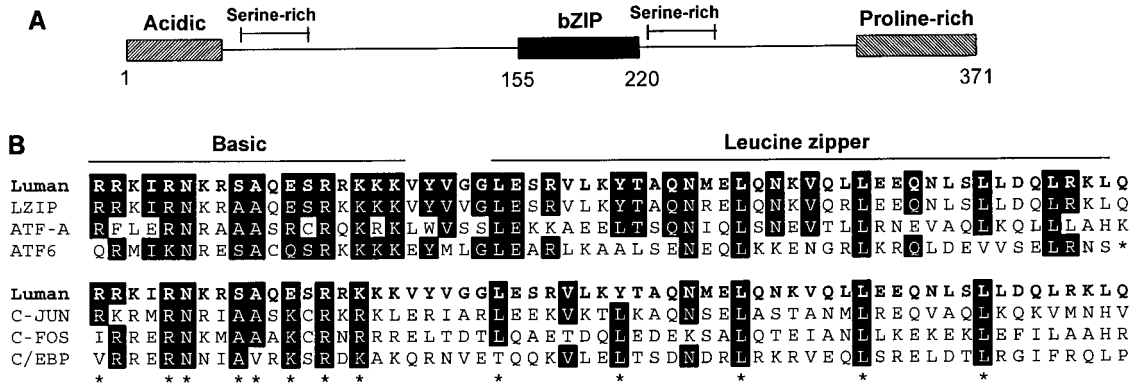

FIG. 2. Putative domains of Luman and its similarity to members of the CREB/ATF family. (A) Schematic diagram showing the putative domains of Luman. (B) Protein sequence alignment of bZIP region of Luman with members of the CREB/ATF family and representatives of the bZIP superfamily. Amino acids in shaded boxes are either identical sequences or consensus sequences. Residues that align with the consensus are indicated by asterisks.

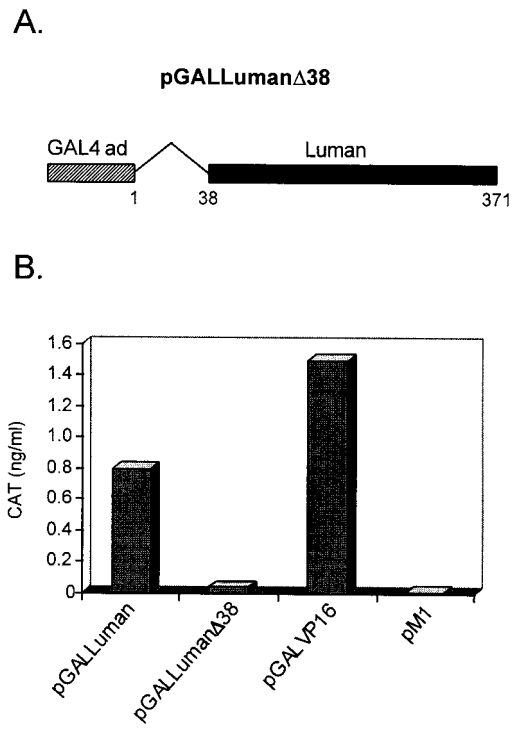

FIG. 3. Luman is a transcription factor with an amino-terminal activation domain. (A) Structure of pGALLumanΔ38, showing the 38-aa deletion. (B) Plasmids pGALLuman, pGALLumanΔ38, and pGALVP16 were introduced into COS7 cells with the reporter plasmid pG5EC.

3B). A deletion of the first 38 aa of Luman (pGALLumanΔ38) resulted in a sharp decrease in its ability to activate transcription. This result suggests that Luman has an activation domain at its amino terminus. This region has a strong overall negative charge, as have the members of the class of activation domains represented by VP16.

Binding of Luman to CRE and C/EBP sites. Sequence analyses (see above) suggest that Luman is a member of the CREB/ATF family of proteins. This family of proteins preferentially bind to CRE sites with a core sequence, 5′-CGTCA. They usually do not bind to C/EBP or AP-1 sites or bind with much less efficiency. We therefore assessed the ability of Luman to bind these sequences. We used EMSA to characterize the binding specificity in vitro. The 17- or 18-mer (excluding flanking restriction sites) consensus sequences of CRE, C/EBP, and AP-1 sites were compiled from the TRANSFAC binding site distribution matrix database, TFMATRIX (58) (Table 1). Double-stranded oligonucleotides labeled with α-^{32}P were annealed and incubated with the test proteins. Initially intact Luman protein was produced in the TnT rabbit reticulocyte expression system or in pcLuman-transfected COS7 cells in the form of nuclear extract. However, endogenous CRE-binding proteins resulted in very high background binding to CRE (data not shown), and thus a bacterial expression system was chosen. A GST-Luman fusion protein was produced in bacteria and purified by using glutathione-Sepharose beads. The purified GST-Luman fusion protein was incubated with labeled oligonucleotides and analyzed by standard nondenaturing polyacrylamide gel electrophoresis (PAGE). The GST protein, produced in the same system, was used as a control. Our results (Fig. 4) showed that GST-Luman could form a complex with the CRE; it could also bind the C/EBP

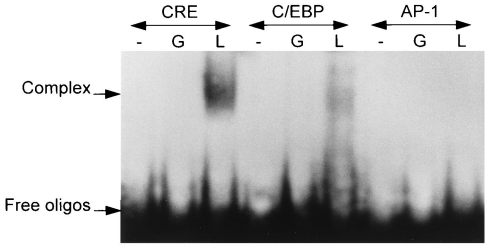

FIG. 4. Luman binds to CRE and C/EBP sites in vitro. Double-stranded oligonucleotides (Table 1) representing CRE, AP-1, and C/EBP were labeled with ^{32}P, annealed, and incubated with GST-Luman (L) recombinant fusion protein produced in bacteria and purified by using glutathione-agarose beads, GST protein produced in the same system (G), or bovine serum albumin (−). Complexes were analyzed by standard nondenaturing PAGE.

element but with a significantly lower affinity. GST-Luman did not form a detectable complex with oligonucleotides representing the AP-1 element. The binding of GST-Luman to radiolabeled CRE could be specifically inhibited by unlabeled CRE. In addition, the affinity of the association, as determined by densitometric analysis of the autoradiographs, was approximately sixfold higher than for GST-Luman binding to C/EBP (data not shown). Indeed, when a high-ionic-strength buffer (0.25 M Tris, 0.5 M glycine) was used to separate the complex on a polyacrylamide gel, the GST-Luman-C/EBP complex was disrupted (data not shown). None of the oligonucleotides formed complexes with GST. Therefore, we conclude that, like other CREB/ATF proteins, Luman is able to preferentially complex with CREs and that this association, at least in vitro, does not require the involvement of other cellular factors.

Luman can activate transcription through CRE and C/EBP elements. To determine if the Luman protein could activate promoters containing bZIP protein response elements, we used CAT reporter plasmids with a basal promoter containing various combinations of CRE (5′-CCCTTACGTCAGAGGA) and AP-1 (5′-TCAAAGTTTAGTCAA) sequences from the phosphoenolpyruvate carboxykinase promoter and the C/EBP sequence (5′-TGATTTTGTAATGGGG) from the mouse albumin promoter (16, 43, 44). Plasmid designations as well as the numbers and locations of the response elements are shown in Fig. 5. For these experiments we used COS7 cells, in which these plasmids exhibit only basal levels of CAT activity.

As shown in Fig. 5, Luman could activate transcription from the promoter p-68CRE, which contains three CREs, and to a lesser extent from p-68D3, which has three C/EBP motifs. Interestingly, the combination of C/EBP and CRE sites in p-109C3 had a synergistic effect on transcription activation. In contrast, the presence of AP-1 sites in combination with CRE had a marked inhibitory effect. These results are consistent with the in vitro EMSA data and demonstrate that the Luman protein is a CRE- and C/EBP element-binding transcription factor. Although other explanations for the synergistic effect of CRE and C/EBP elements cannot be ruled out, the results suggest that simultaneous binding of Luman to both sites, or interactions between Luman at the CRE and other factors bound to C/EBP (or AP-1) sites, may influence activation by Luman.

Luman interacts with HCF in vivo and in vitro. The Luman cDNA was isolated by detecting the positive interaction with HCF in yeast cells by the two-hybrid system. To establish that this interaction can also occur in mammalian cells, we used a mammalian two-hybrid system in which transcriptional activation by Luman would depend on tethering to a GAL4 UAS-containing promoter via the hcfNC-GAL4 BD fusion protein.

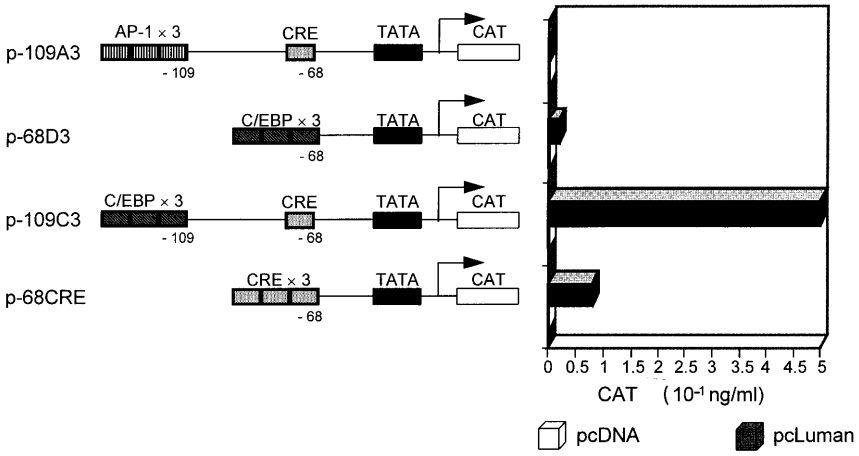

FIG. 5. Luman activates transcription through CRE and C/EBP elements. Plasmid pcLuman was introduced into COS7 cells with the promoter reporter constructs shown diagrammatically on the left.

Since Luman itself has an activation domain, pcLuman (without fusion to GAL4 AD) was cotransfected into COS7 cells with pGALhcfNC, as well as the CAT reporter plasmid pG5EC. Controls included transfections in which the original blank vector, pM, was substituted for pGALhcfNC. The results (Fig. 6) showed that Luman could interact with hcfNC and activate transcription on the GAL4 promoter. Luman or GAL-hcfNC alone had a negligible effect.

Since it appears that both Luman and VP16 can bind to HCF, we decided to investigate whether the two transcription factors had similar mechanisms for associating with HCF. We examined the ability of GST-linked Luman or VP16 to associate with radiolabeled hcfNC and determined if a twofold excess of unlabeled proteins could inhibit this interaction. The GST fusion proteins were purified from *E. coli*, while all other proteins were made in the TnT rabbit reticulocyte transcription-translation system. The hcfNC protein was labeled with ^{35}S. Figure 7A shows that glutathione-Sepharose beads linked to GST-Luman, but not to GST alone, could pull down ^{35}S-hcfNC. The addition of unlabeled VP16 or Luman could inhibit this association, while a control protein, bone alkaline phosphatase, could not. In a parallel experiment (Fig. 7B), the association of GST-VP16 with ^{35}S-hcfNC was also inhibited by either unlabeled VP16 or Luman but not by alkaline phosphatase. Although unlabeled VP16 and Luman reduced binding of ^{35}S-hcfNC to the GST-linked proteins to near background levels, the binding could not be completely eliminated by the amounts of unlabeled competitors used. These results indicate that Luman and VP16 may have similar mechanisms for binding HCF, since each could inhibit the binding of the other. Our other GST pull-down assays showed that Luman did not bind to VP16 or indirectly bind to VP16 through binding of HCF (data not shown). Thus, the tertiary complex, VP16-HCF-Luman does not form, at least not in vitro.

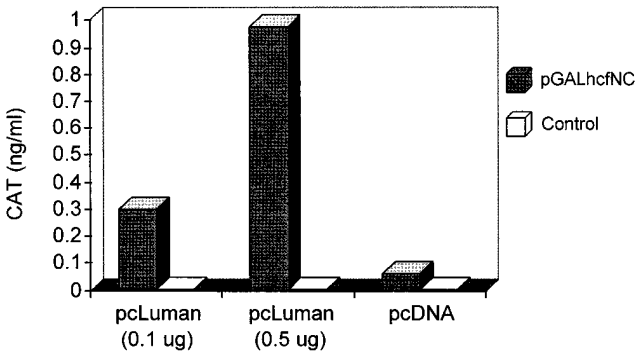

FIG. 6. Luman interacts with HCF in vivo. A modified mammalian two-hybrid system was used to test the interaction between HCF and Luman. Since Luman has an activation domain, GAL AD-Luman fusion protein is not needed. Plasmid pGALhcfNC was introduced into COS7 cells along with pcLuman and reporter plasmid pG5EC.

VP16 inhibits Luman's transactivation activity in vivo. Our in vitro experiments suggested that the presence of either VP16 or Luman could mutually interfere with their association with HCF. VP16 requires HCF for initiating the viral lytic cycle in HSV-infected cells, and we presume that Luman requires HCF for its function as well. Mutual inhibition may therefore have implications for both viral and cellular expression in virus-infected cells that contain functionally active Luman. To determine if interference occurs in cells as well as in vitro, we designed a competition CAT assay. Plasmid pGALLuman (0.1 μg) was cotransfected into COS7 cells with the reporter plasmid pG5EC (0.5 μg). Plasmids expressing the competitors pcVP16 (0.01 to 0.1 μg), pcLuman (0.1 to 0.5 μg), and pcLumanΔ38 (0.1 to 0.5 μg) were also added. We found (Fig. 8) that an equal amount (0.1 μg) of pcVP16 could almost completely inhibit the ability of GAL-Luman to transactivate. Luman and LumanΔ38, its mutant lacking the amino-terminal activation domain, could also inhibit the activity of GAL-Luman, but to a lesser degree, and much higher concentrations of competitor plasmids were required for significant inhibition. In contrast to the potent ability of VP16 to inhibit activation by GAL-Luman, neither Luman nor VP16 inhibited activation by GAL-VP16. Also, in other transient expression assays, exogenously supplied Luman was unable to inhibit the ability of VP16 to activate a TAATGARAT-containing promoter (results not shown). These results suggest that while VP16 is a strong transcription inhibitor of Luman, Luman has little effect on the transcription activity of VP16. To rule out the possibility that the VP16-mediated inhibition of activation by GAL-Luman was through sequestering, by the strong VP16 activation domain, of a limiting component of the transcriptional apparatus (a phenomenon known as squelching), we examined the effect of VP16 mutants in which the activation domain had been deleted or debilitated. Both mutants were as efficient as wild-

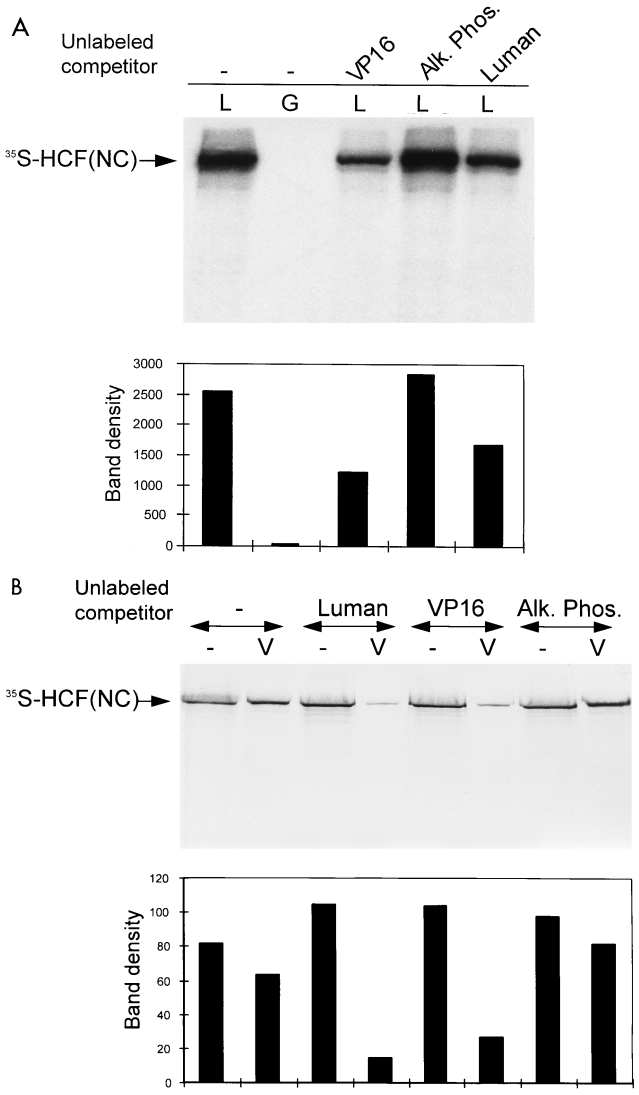

FIG. 7. Luman interacts with HCF in vitro. All proteins except GST-Luman and GST-VP16 fusion proteins were produced in a rabbit reticulocyte in vitro transcription-translation system. The hcfNC protein was labeled with ^{35}S. The band intensity was analyzed by densitometric analysis. (A) Glutathione-Sepharose beads linked to GST-Luman fusion protein (L) or GST alone (G) were used to precipitate ^{35}S-hcfNC, with (twofold excess) or without the addition of unlabeled competitor protein VP16, Luman, or the control protein bone alkaline phosphatase (Alk. Phos.). (B) Equal aliquots of ^{35}S-hcfNC were incubated with a twofold excess of unlabeled competing protein Luman, VP16, or alkaline phosphatase or with no additional protein (−) for 1 h on ice. One-tenth of the reaction mixture was removed and analyzed as input (−). Glutathione-Sepharose beads linked to GST-VP16 (V) were added to the remainder. After mixing at 4°C for 1 h, the beads were washed extensively and analyzed by sodium dodecyl sulfate-PAGE. Input samples (1/10) were electrophoresed beside each sample.

type VP16 at inhibiting activation by GAL-Luman (results not shown). This result suggests that the inhibition was exerted by the binding of other limiting components, possibly HCF.

Luman is a ubiquitous transcription factor. To determine the tissue expression patterns of Luman and to compare them to those of HCF, poly(A) RNAs from eight different adult tissues (heart, brain, placenta, lung liver, skeletal muscle, kidney, and pancreas) (Fig. 9, left three panels) and four fetal tissues (brain, lung, liver, and kidney) (Fig. 9, right three panels) were hybridized with ^{32}P-labeled full-length Luman as well

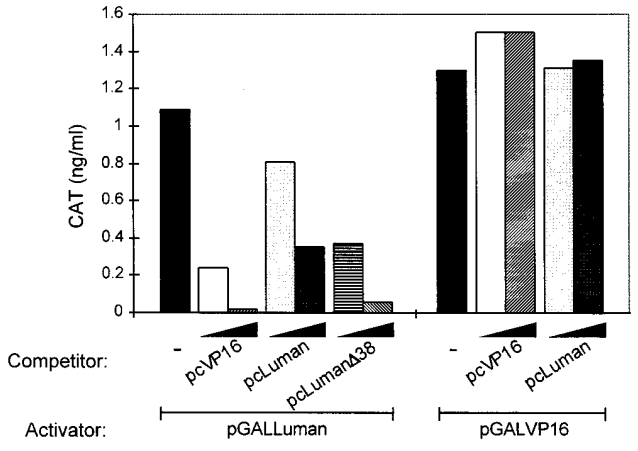

FIG. 8. VP16 inhibits the ability of Luman to activate transcription. As in other cotransfection assays, pGALLuman (0.1 μg) or pGALVP16 was introduced into COS7 with the reporter pG5EC (0.5 μg) and various amounts of plasmids expressing the competing proteins: pcVP16 (0.01 to 0.1 μg), pcLuman (0.1 to 0.5 μg), and pcLumanΔ38 (0.1 to 0.5 μg).

as with ^{32}P-labeled hcfNC. After analysis, the blots were stripped of probe and rehybridized to ^{32}P-labeled Luman and ^{32}P-labeled actin separately. The results of the three successive hybridizations are depicted as separate panels in Fig. 9. In all the tissues examined, the Luman probe revealed a strong signal at approximately 1.4 kb and a weaker band at 4.0 kb. On the basis of the size of Luman cDNA (1.4 kb), the 1.4-kb band is probably the transcript of Luman. The 4-kb band could be the transcript of a gene with significant homology to regions of Luman (e.g., a well-conserved bZIP region) or an alternative spliced RNA from a precursor shared with Luman. The results show that Luman mRNA is expressed to comparable levels in most adult and fetal tissues. Contrary to a previous report (57), our data show that HCF is also universally expressed in all tissues at the mRNA level. Because we do not have immunological probes for Luman or HCF, we could not determine if the proteins were as widely distributed in the tissues.

DISCUSSION

The protein HCF is strongly conserved among animals as diverse as mammals, insects, and helminths and is expressed in various adult and embryonic tissues. This suggests that it is fundamental to the biology of these organisms. Yet its only known function is to stabilize the HSV IE gene transactivator VP16 during productive infections of host cells. To elucidate the normal functions of HCF, we have identified a previously undescribed human protein, Luman, which interacts with HCF.

Our structure-function analyses demonstrate that Luman is a transcription factor of the bZIP superfamily and is most closely related to members of the CREB/ATF family (reviewed in references 2 and 30). Like other bZIP proteins, CREB/ATF transcription factors have a modular structure consisting of basic regions that bind DNA, which for CREB/ATF proteins are the CREs. They also possess leucine zippers for dimerization and activation domains for affecting transcription. The DNA binding domain of Luman is more closely related to those of CREB/ATF proteins than to those of other bZIP proteins. Like the CREB/ATF proteins, Luman binds preferentially to CRE in vitro and activates promoters containing CRE elements in transfected cells.

A homology search of GenBank revealed three proteins with

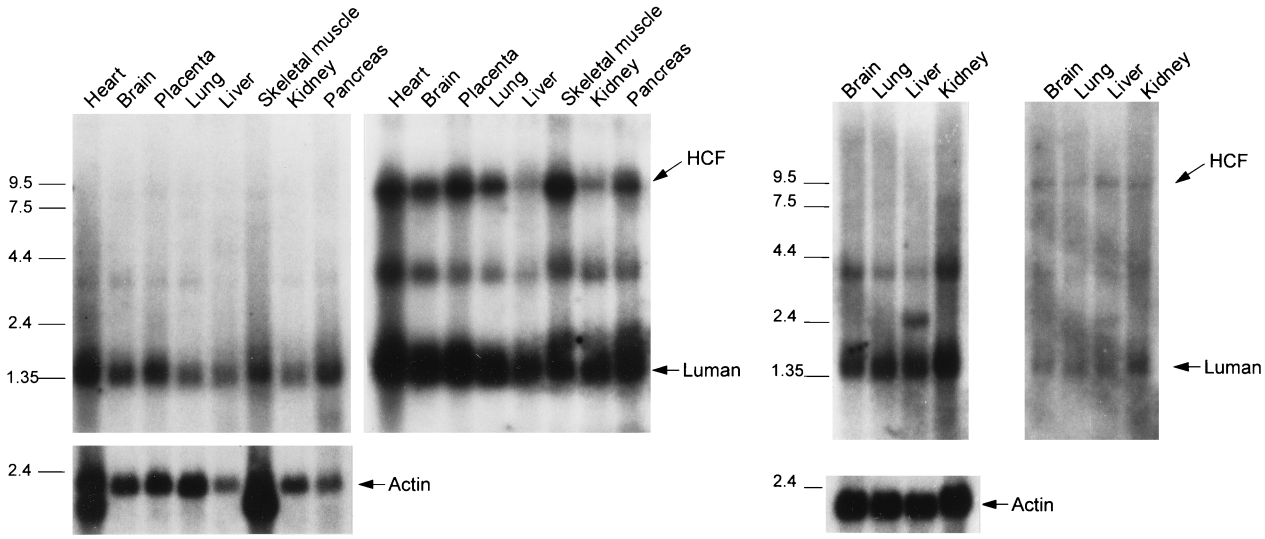

FIG. 9. Universal expression of Luman mRNA in human tissues. Molecular size standards (in kilobase pairs) and tissues of origin are indicated. The ^{32}P-labeled probe of the full-length Luman was hybridized to human adult (left two upper panels) and fetal (right two upper panels) tissues, with (right of each pair of panels) or without (left of each pair of panels) radiolabeled hcfNC probe. The same blots were also hybridized with the actin probe (bottom), used as a loading reference.

substantial homology to Luman. The protein LZIP (8) is most closely related to Luman and is probably its mouse homolog. For two other proteins, a *Drosophila melanogaster* transcription factor called BBF-2 (1) or dCREB-A (47) and the *Caenorhabditis elegans* GenBank entry g726428, amino acid sequence homology is restricted to a 53-aa stretch including the basic domain and the sequence immediately preceding it (Fig. 10A). Like Luman, all of these proteins possess unusually long leucine zippers in which tyrosine replaces leucine in one of the heptads. All four proteins also have extremely acidic amino termini. The acidic amino terminus of BBF-2/dCREB-A functions as an activation domain on GAL4 UAS-containing promoters if fused to GAL4 BD (47). Our results suggest that the acidic amino terminus region of Luman may also constitute an activation domain since deletion of this region drastically reduced the ability of Luman to transactivate (Fig. 3). In EMSA, Luman (Fig. 4), LZIP (1), and BBF-2/dCREB-A (8) all preferentially bind CRE. Luman, in addition, bound C/EBP elements but not AP-1 sequences, while LZIP binds AP-1 but not C/EBP (8). This discrepancy may be because oligonucleotides used for assessing the AP-1 binding of LZIP contained an AGTCA motif. This sequence is similar to the core CRE motif, CGTCA. Furthermore, BBF-2/dCREB-A has been shown to bind the AGTCA motif (1). The AP-1 oligonucleotides used to assess BBF-2/dCREB-A or Luman binding (Fig. 4 and Table 1) did not have such a CRE-like sequence. Failure to detect LZIP and BBF-2/dCREB-A binding to C/EBP was probably due to the use of high-ionic-strength buffer in those assays. Our assays, done in a low-ionic-strength buffer, showed some binding to C/EBP elements, albeit at a reduced level. Based on these observations, we suggest that Luman, L-ZIP, BBF-2/dCREB-A, and possibly g726428 belong to a subgroup of CREB/ATF proteins that may have similar properties and biological roles. At present the roles of LZIP, Luman, and the g726428 protein are not known. BBF-2/dCREB-A is expressed in a several adult and embryonic tissues and may be involved in regulating the alcohol dehydrogenase gene in yolk and fat body (1). Recent evidence suggests that during embryogenesis, it functions in signaling cascades that decide the patterning of cuticular structures (3).

We detected Luman because of its ability to bind to HCF. While this has not been demonstrated for LZIP and BBF-2/dCREB-A, all three proteins possess a region (Fig. 10B) that bears a strong similarity to a segment of HSV VP16 that is believed to contact HCF (21). This region is also highly conserved in homologs of VP16 from other herpesviruses. A mutagenic analysis of this region in Luman to determine if it is involved in HCF binding is in progress. If the functions of these proteins can indeed be modulated by HCF, this would represent a unique mechanism for regulating transcription factors and, consequently, gene expression.

HCF is initially detected by its association in the VP16-induced transactivation complex (27, 56). It is required by VP16 for the formation of DNA–Oct-1–VP16–HCF quater-

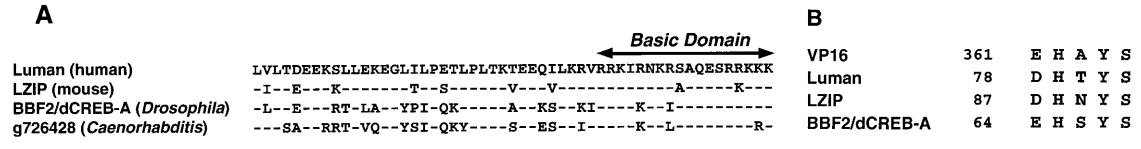

FIG. 10. Alignment of the amino acid sequence of portions of Luman with corresponding regions from related proteins. (A) Basic DNA binding domain and the amino acids preceding it. A search of GenBank using the deduced Luman amino acid sequence as a query revealed this extremely conserved region among Luman, mouse LZIP, *Drosophila* BBF-2/dCREB-A, and a predicted protein, g726428, from *C. elegans*. In this 53-aa region, Luman has similarities of 92% with LZIP, 81% with BBF-2, and 79% with g726428. Allowing for conservative changes, the 18-aa basic domain is nearly perfectly conserved, except for one nonconservative difference. (B) Putative HCF binding region. Alignment of segments of Luman, LZIP, and BBF-2/dCREB-A with the portion of VP16 believed to interact with HCF (21, 46). The location of the first residue of each domain in the amino acid sequence of the protein is indicated. A peptide representing this domain in VP16 inhibits complex formation by VP16 (21, 46), and changes in any of the residues (EHAY) drastically affect the ability of the peptide to prevent HCF-VP16 interaction (46).

nary complex, possibly as a means of neutralizing the effects of the highly acidic activation domain of VP16. Wilson et al. have suggested (56) that the activity of HCF may vary with changes in cellular metabolism, and thus the virus might use it to gauge the physiological state of the cell. In this model, the presence of functional HCF in a metabolically receptive host cell would allow for the formation of the VP16 transcriptional complex and hence a commitment to the lytic program of gene expression. In a metabolically unreceptive cell, in the absence of HCF, latency of the viral genome or an abortive infection would follow. Our EMSA results (Fig. 4) suggest that Luman does not require HCF for complex formation with CRE (although at this stage we cannot rule out a role for HCF in the stabilization of the complex). However, it is possible that HCF, instead of, or in addition to, promoting complex formation, plays a more active role in transcription activation. The Tax protein of human T-cell leukemia virus 1 (HTLV-1) may provide a model for how HCF might affect transcription. Tax is the viral gene product responsible for the autoregulation of HTLV-1 promoter. It does not bind to target DNA directly (9, 48). Nonetheless, Tax can stabilize the binding of CREB and other CRE-binding proteins to the CREs in the HTLV-1 promoter (6, 40, 54). Bodor et al. (7) have reported that Tax stimulates CREB-mediated transcription of the HTLV-1 long terminal repeat by a mechanism in addition to the stabilization of DNA binding. When the DNA binding bZIP domain of CREB was substituted by GAL4 BD, so that Tax was not required for DNA binding, a single amino acid substitution of serine-133, a protein kinase A phosphorylation site that is also essential for the activation function of CREB, disrupted both Tax and protein kinase A-mediated transcription response. The authors (7) suggest that Tax may enhance interactions of CREB with CBP, a cyclic AMP-responsive protein that couples enhancer-binding proteins to the basal RNA polymerase complex (5, 11, 32).

The results of the GST pull-down assays using GST-Luman and GST-VP16 (Fig. 7) show that the two proteins bound HCF equally well and that either protein can inhibit the binding of the other to HCF. One explanation for these data is that VP16 and Luman interact with HCF in similar manners. This explanation would have implications for viral as well as host gene expression in host cells that contain Luman. It implies that the relatively small amount of VP16 introduced into cells with the infecting virion would be unsuccessful at competing with the potentially larger amount of Luman in cells expressing the gene. The opposite appears (Fig. 8) to happen. While minute amounts of plasmid expressing VP16 drastically inhibited the ability of GAL-Luman to transactivate a promoter with a GAL4 UAS, neither VP16 nor Luman inhibited activation by GAL-VP16. Luman was also not able to affect VP16-mediated activation of a TAATGARAT-containing promoter. This finding suggests either that in cells VP16 has a much higher affinity for HCF than Luman, a property not apparent in GST pull-down experiments, or that the inhibition by VP16 in cells is by a mechanism that does not involve HCF. It is interesting that in transfected cells even VP16 was unable to inhibit activation by GAL-VP16. Nevertheless, the results, if they reflect what happens in infected cells, imply that VP16, in addition to activating transcription of viral IE promoters, may also inhibit the expression of cellular genes that are activated by Luman.

What role does Luman play in cell biology? Our results suggest that Luman mRNA is present in many adult and fetal tissues (Fig. 9). Although we do not know if the Luman protein is as widely distributed (translational regulation mechanisms as for HCF [57] or human D-site binding protein [DBP] [37] may exist), our findings suggest that Luman may be a ubiquitous transcription factor. Like other members of the CREB/ATF family, it may play an important role in regulating the transcription of a wide variety of genes that have CREs in their promoters. The possibility of the involvement of Luman, despite being ubiquitous, in selective gene activation in response to diverse stimuli also exists. The specificity of Luman binding to its CRE, or its effect, may potentially be regulated at several different levels: by HCF binding, by leucine zipper-mediated dimerization with other proteins, and by posttranslational modification such as phosphorylation. Binding of coactivators/repressors to CRE-attached Luman can also be potentially affected by sequences flanking the CRE. Recently we showed (36) that selective activation by Oct-1, another ubiquitous transcription factor, is modulated in this manner.

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