Host cell factor 1 (HCF-1) is a cellular transcriptional coactivator which coordinates the assembly of enhancer complex through direct interactions with viral and cellular trans-activators such as VP16, Oct-1, LZZIP, and GA-binding protein. These interactions are mediated by the β-propeller domain comprising the first 380 residues of HCF-1 with six kelch repeats. Here we describe the identification and characterization of a novel HCF-like kelch repeat protein, designated HCLP-1. HCLP-1 is a ubiquitously expressed nuclear protein which is composed almost entirely of a six-bladed β-propeller. HCLP-1 selectively interacts with LZZIP but not with VP16. The physical interaction between HCLP-1 and LZZIP leads to the repression of the LZZIP-dependent transcription. The HCLP-1-binding domain of LZZIP maps to residues 109–315, which contain the bZIP DNA-binding motif. Electrophoretic mobility shift assay demonstrates that HCLP-1 indeed interferes with the binding of LZZIP to its DNA target. Thus, HCLP-1 serves a transcriptional co-repressor function mediated through its inhibitory interaction with the LZZIP transcription factor. Our findings suggest a new mechanism for transcriptional regulation by HCF-like proteins.

Transcriptional regulation in eukaryotes involves a complicated interplay between activators, repressors, basal transcription factors, and chromatin (1). To coordinate the orderly assembly of multicomponent enhancer complexes onto DNA targets, cells have evolved specialized groups of regulators that interact with multiple transcription factors. Host cell factor 1 (HCF-1) represents one of these multipartner transcriptional regulators and it functions as a coordinator in the assembly of enhanceosome on viral and cellular DNAs (2–5).

HCF-1 was first identified through its association with herpes simplex virus transactivator VP16, a key regulator of lytic infection (6, 7). The formation of the VP16-HCF-1 complex facilitates the nuclear accumulation of VP16 (8) and the recruitment of the POU-homeodomain transcription factor Oct-1 (9), leading to activation of viral immediate early genes. In addition to VP16 and Oct-1, HCF-1 also directly interacts with GA-binding protein, another cellular transcription factor critically involved in the regulation of immediate early gene expression (4). One notable mechanism for the regulation of HCF-1 activity is through subcellular localization. Thus, HCF-1 is abundantly found in sensory neurons and nuclear translocation leads to reactivation of the virus from latency (10).

HCF-1 is expressed as a 230-kDa precursor, which is autocatalytically cleaved into an array of tightly associated polypeptides ranging from 50 to 150 kDa (4, 6, 11–14). The interaction of HCF-1 with VP16 is mediated by the first 380 residues of HCF-1 (2, 15). This N-terminal domain, which consists of six kelch repeats, sufficiently stabilizes VP16-induced complex with Oct-1 and activates transcription (8, 15). In addition, this domain has an essential role in cell proliferation (16). The kelch repeats represent an evolutionarily conserved module for protein-protein interaction and they form a tertiary structure called β-propeller, which has been found in many different polypeptide contexts (17). In HCF-1, gross alterations in the six-bladed β-propeller structure disrupt both the VP16-binding and the cell-cycle progression activities (9, 15, 16).

One additional cellular target of the β-propeller domain of HCF-1 is a bZIP transcription factor known as LZZIP or Luman (18, 19). LZZIP is the human ortholog of the fruit fly BBF2/dCREB-A protein, which has been shown to activate Drosophila fat body- and mammalian liver-specific transcription (20, 21). The subportion of LZZIP that contains its bZIP domain is highly homologous to counterparts in other members of the bZIP family of transcription factors including CREB, CREM, and ATF6 (18, 19, 22). LZZIP binds to a canonical cAMP-responsive element (CRE) and activates CRE-dependent transcription (19, 22–24). While the cellular targets of LZZIP are poorly understood, LZZIP has been implicated in cell proliferation and it acts as a binding partner and transforming cofactor of the hepatitis C virus core protein, a viral protein with oncogenic potential (24). HCF-1 recognizes a tetrapeptide HCF-binding motif (HBM) shared by LZZIP, VP16, and another HCF-1 partner termed Zhangfei (18, 23, 25). However, VP16 and LZZIP show drastically different sensitivities to individual HCF-1 point mutants, suggesting that other structural motifs in the β-propeller may also contribute to the specificity of the binding (9). HCF-1 functions as a coactivator in the context of LZZIP, and the physical interaction with HCF-1 is necessary for coor-
Inhibition of LZIP-mediated Transcription by HCLP1

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—Plasmid pBDHCLP1 expressing Gal4 DNA-binding domain (BD)-HCLP-1 was constructed by in-frame insertion into plasmid pAS2-1 (CLONTECH) of a cDNA (GenBank™ AF113131) encoding the full-length 406 amino acids of HCLP-1. Gal4BD-HCLPN and Gal4BD-HCLPC express truncated Gal4BD-HCLP-1 corresponding to amino acids 1–331 and 298–406, respectively. pADLZIP was obtained by cloning the complete LZIP coding sequence into Gal4 activation domain (AD) plasmid pGADGH (CLONTECH). pADCREB, pADATF4, pADATF6, pADeFOS, pADDeJUN, and pADCEBP contain the complete coding regions of human CREB, ATF4, ATF6, c-Fos, c-Jun, and CEBP-α, respectively. Our findings suggest that HCLP-1 is a specific transcriptional corepressor for LZIP.

**RESULTS**

**Identification of HCLP-1 as a Novel HCF-like Protein**—To mine for novel human kelch repeat proteins related to HCF, we searched the current nucleotide data bases via the tblastn server (www.ncbi.nlm.nih.gov) for expressed sequence tags homologous to the coding sequences of the β-propeller domain of HCF-1 (30). Further analysis of the HCF-1-related sequences identified from this search revealed that a group of human expressed sequence tags including AA483867, AA167356, and AW992905 encode a single non-HCF-1 and non-HCF-2 kelch repeat protein. A fetal brain cDNA library was then screened for clones that match the above ESTs and a full-length human cDNA for a novel HCF-like protein designated HCLP-1 was obtained.

HCLP-1 is a 406-amino acid protein composed almost entirely of six internally repeated sequences of 45–71 residues (Fig. 1A). These kelch repeats are expected to form four-stranded, anti-parallel β-sheets that fold into propeller-like barrel structures (17). Thus, the whole HCLP-1 molecule will form a six-bladed β-propeller. A kelch repeat is characterized by a pair of glycines immediately preceded by four hydrophobic residues and followed by a tryptophan (17). In HCLP-1, phenylalanine is found at position −1 relative to the glycine pair in five of the six repeats, and valine or leucine is present at position −2. However, the third and the sixth kelch repeats of HCLP-1 are relatively long. HCLP-1 shares significant homology with the HCF-1 and HCF-2 kelch domains (Fig. 1B).

To determine the expression patterns of HCLP-1 mRNA, we analyzed poly(A)^+ RNA from various human tissues and cancer cell lines for the presence of transcripts that hybridize to an HCLP-1 cDNA probe. HCLP-1-specific transcripts of ~2 kilobases and/or 1.8 kilobases in size were detected in all tested human tissues and cancer cell lines (Fig. 2). Thus, HCLP-1 is a novel and ubiquitously expressed β-propeller protein related to HCF-1 and HCF-2.

**HCLP-1 Associates with LZIP but Not with VP16**—HCLP-1 has been shown to associate with VP16 (6, 7) and LZIP (18, 19). These interactions are mediated by the β-propeller domain of HCF-1 and a tetrapeptide HBM motif present in both VP16 and LZIP (18, 23). Studies with point mutants suggest that VP16 might recognize HCF-1 and HCF-2 through similar mechanisms, albeit the interaction with HCF-2 is weaker (26). Because HCLP-1 forms a structurally related β-propeller, we asked whether HCLP-1 would also interact with LZIP and/or VP16.

We addressed this issue by using a Gal4-based yeast two-hybrid assay (Fig. 3A). In this experiment, we used two irrelevant pairs of proteins (columns 1 and 6) as positive controls. Western blot analysis was performed to verify the interaction. The Tax oncoprotein of human T-cell leukemia virus type 1 can self-associate (30). Therefore, Tax fused to Gal4BD interacts potently with Tax fused to Gal4AD (Fig. 3A, column 1). Likewise, Tax binds to a cellular partner called Int-6 (34), albeit with lower affinity (column 6). Next we verified that the β-propeller domain of HCF-1 is able to interact with both LZIP and VP16 (columns 2 and 3). In contrast,
HCLP-1 can associate only with LZIP (compare column 4 to column 7), but not with VP16 (compare column 5 to column 7). Hence, HCLP-1 can discriminate between LZIP and VP16.

To define the specificity of the interaction between HCLP-1 and LZIP, we tested whether HCLP-1 could distinguish LZIP from other cellular bZIP transcription factors. Seven bZIP proteins fused to Gal4AD were queried in parallel for binding to Gal4BD-HCLP-1 (Fig. 3A, columns 4 and 8–13). Notably, HCLP-1 interacts with LZIP only but not with any of the other six bZIP factors, namely CREB, ATF4, ATF6, c-Jun, c-Fos, and CEBPα. Thus, HCLP-1 is a specific partner of LZIP.

The observed interaction between HCLP-1 and LZIP in yeast cells does not exclude that it could be mediated through another yeast protein. To challenge this possibility, we performed in vitro pull-down assays with GST-HCLP-1 and His-tagged TRX-LZIP purified from *E. coli*. In agreement with a direct physical contact between HCLP-1 and LZIP, Fig. 3B verified that TRX-LZIP bound to GST-HCLP-1 (lanes 3 and 5; also compare lane 3 to lane 2), but not to GST alone (lanes 1 and 4).

In addition to the in vivo yeast two-hybrid analysis and in vitro protein affinity binding assays, confocal laser-scanning immunofluorescence microscopy was also performed to further probe the interaction between HCLP-1 and LZIP within human cells. We generated anti-HCLP-1 serum (α-HCN) directed against bacterially produced HCLP-1 protein containing the N-terminal 331 residues. The specificity of this antiserum was verified by immunofluorescent staining. HeLa cells were probed with α-HCN, or preimmune serum, or α-HCN neutralized with excess amount of purified His-tagged HCLP-1 protein (His-HCLP-1). HCLP-1-specific staining was found in the nucleus of interphase HeLa cells probed with α-HCN (Fig. 3C, panel 1), but not in cells stained with preimmune (data not shown) or neutralized (Fig. 3C, panel 3) sera. Because both α-HCN and the anti-LZIP antibody α-ZN were from rabbits, a direct co-localization of HCLP-1 and LZIP cannot be demonstrated with indirect immunofluorescence microscopy. However, when considered together with the previously documented (24) nuclear localization of LZIP (Fig. 3C, panel 2), our data indicate that endogenous HCLP-1 and LZIP proteins reside in the same intranuclear compartment within human cells, consistent with an intracellular protein-protein contact between the two entities.

We carried out additional yeast two-hybrid interactive assays to define the functional domains of LZIP and HCLP-1 (Fig. 4). Various truncated mutants of LZIP were constructed and their HCLP-1 binding activities were individually assessed. We
observed that the mutant LZIP-M4 induced LacZ reporter expression as potently as the wild type LZIP (Fig. 4A). It is noteworthy that this mutant with amino acids 109–315 contains an intact bZIP region. By contrast, neither the LZIP-M2 mutant comprising amino acids 1–109 nor the LZIP-M5 mutant comprising amino acids 235–371 can interact with HCLP-1. One interpretation for these data is that the bZIP region (amino acids 152–220) is required for interaction with HCLP-1. We noted that mutants LZIP-M3 and LZIP-M4, both lacking the HBM motif, still interact with HCLP-1. These results implicate that the HBM region, which mediates the association between HCF1 and LZIP, might be dispensable for the interaction between HCLP-1 and LZIP.

On the other hand, we were unable to narrow down a minimal LZIP-binding domain in HCLP-1. Neither the N-terminal (amino acids 1–331) nor the C-terminal (amino acids 298–406) portion of HCLP-1 is sufficient to bind LZIP (Fig. 4B). Thus, the entire β-propeller structure of HCLP-1 might be necessary for interaction with LZIP.

HCLP-1 Inhibits LZIP-dependent Transcription in Mammalian Cells—Previously, we and others have shown that LZIP is a nuclear CRE-activating factor (19, 24). The finding that HCLP-1 interacts with LZIP predicts a functional impact of the former on the transcriptional activity of the latter. To explore this, we expressed epitope-tagged HCLP-1 protein in HeLa cells and assessed the effects of HCLP-1 on Gal4BD-LZIP activation of luciferase expression driven by Gal4-binding enhancer elements. The expression of HCLP-1 tagged with a V5 epitope was verified by Western blot analysis, and a 50-kDa species recognized by the anti-V5 antibody was specifically detected from the pcHCLP-1-transfected cells (Fig. 5A, compare lane 2 with lane 1). When we increased the expression of HCLP-1, the Gal4BD-LZIP-dependent transcription of luciferase reporter decreased progressively (Fig. 5B; GAL4-LZIP, +). By sharp contrast, the reporter expression dependent on a Gal4BD version of VP16, which does not interact with HCLP-1 (see Fig. 3A for reference), was totally unaffected by HCLP-1.
A monoclonal anti-V5 antibody (Invitrogen) was used at 1:1000 dilution. 

Fig. 5. HCLP-1 protein inhibits LZIP-dependent transcription. A, expression of HCLP-1 in HeLa cells. Cells were mock transfected (lane 1) or transfected with pHCLP-1 (0.1 μg; lane 2). Western blotting was performed to verify expression of the V5-tagged HCLP-1. The mouse monoclonal anti-V5 antibody (Invitrogen) was used at 1:1000 dilution. B, effects of HCLP-1 expression on Gal4BD-LZIP transactivation. HeLa cells were transiently transfected with pGalLUC (0.1 μg) plus either the Gal4-VP16-expressing plasmid pGalVP16 (0.1 μg, □) or the Gal4-LZIP-expressing plasmid pGalLZ (0.1 μg, ■) plus the indicated amounts of pHCLP-1. C, effects of HCLP-1 expression on LZIP activation of CRE. HeLa cells were transiently transfected with pCRELUC (0.1 μg) plus the indicated amounts of pHCLP-1 plus 0.1 μg of empty vector (0.1 μg, ◇) or expression plasmids for the indicated proteins (LZIP, □, LZM4-VP16, ◆). All values represent the means of three independent transfections, and error bars indicate S.D. from the mean.

(Fig. 5B; GAL4-VP16, ◇). These results correlate the protein-protein interaction with an inhibitory effect on transcription.

To further characterize this inhibitory effect, the HCLP-1-expressing plasmid (pHCLP-1) paired with either an LZIP-expressing plasmid or an empty vector was transfected into HeLa cells, and the activation of CRE-dependent reporter expression was assayed. Again, we observed that increased expression of HCLP-1 reproducibly repressed cell-endogenous (Fig. 5C; ■) and LZIP-stimulated (Fig. 5C; □) CRE-dependent luciferase activity. One interpretation of this experiment is that HCLP-1 protein physically binds endogenous LZIP in HeLa cells in a manner similar to its interaction with exogenously overexpressed LZIP, leading to repression of transcription. To assess whether the HCLP-1-binding domain of LZIP can confer HCLP-1 responsiveness to transcription factors which do not bind HCLP-1, we constructed a LZM4-VP16 chimera, which contains the HCLP-1-binding domain (amino acid 109–315) of LZIP and the activation domain of VP16. Interestingly, LZM4-VP16 also stimulated CRE-dependent activity and again was repressed by HCLP-1 in a dose-dependent manner (Fig. 5C, ◆). All of this supports the notion that the interaction between HCLP-1 and LZIP leads to the inhibition of LZIP-mediated transcription.

Above, we showed a direct interaction of HCLP-1 with LZIP (Fig. 4) and the functional consequence of this interaction (Fig. 5). One salient point of our findings is that the HCLP-1-binding domain in LZIP contains an intact bZIP DNA-binding region. This prompts us to ask whether HCLP-1 would inhibit the DNA binding activity of LZIP. We and others have shown that LZIP binds canonical CRE sites in vitro and in vivo (19, 24). We therefore assessed the influence of HCLP-1 on LZIP binding to CREs in an electrophoretic mobility shift assay (Fig. 6). Various combinations of recombinant proteins produced from E. coli were incubated with 32P-labeled CRE oligonucleotides and the protein-DNA complex was analyzed on a nondenaturing PAGE gel. An LZIP-specific retarded band (Fig. 6; highlighted by an arrow) was observed when TRX-LZIP was mixed with labeled probe containing CRE sites (Fig. 6, lane 2). When TRX was added, this band was not seen (Fig. 6, lane 1). The addition of a 50-fold excess of unlabeled CRE oligonucleotides depleted the activity to bind with the labeled probe (Fig. 6, lane 3), lending further support to the specificity of the band. This LZIP-specific signal diminished progressively when increased amounts of GST-HCLP-1 were added to the reaction (Fig. 6, lanes 7–9). The diminution was caused by HCLP-1 but not by GST, since the binding activity was unaffected by the addition of GST (Fig. 6, lanes 4–6). Notably, GST-HCLP-1 alone was unable to bind the CRE oligonucleotides (Fig. 6, lane 10). Thus, HCLP-1 inhibits LZIP-mediated transcription through the modulation of its DNA binding activity.

DISCUSSION

Here we report on the identification and characterization of human HCLP-1, a novel and ubiquitously expressed β-propeller protein related to HCF-1 and HCF-2 (Figs. 1 and 2). The direct interaction of HCLP-1 with the cellular transcription factor LZIP was demonstrated in two independent assays for
HCF-1 is a unique transcriptional coactivator which functions as a coordinator in the assembly of multicomponent enhancosomes onto DNA targets (2–5). HCF-1 has no histone acetyltransferase activity and a major mechanism for its regulation of transcription is through protein-protein interaction with viral and cellular transactivators. Thus, HCF-1 associates with VP16, Oct-1, LZIP, and GABP. In one perspective, the kelch β-propeller domain of HCF-1 serves as a specialized platform for interactions with multiple partners. It is of great interest to understand better the modular structure and the molecular mechanism through which the kelch domain recognizes the target transcription factors. In this regard, the comparison with other HCF-like proteins provides novel opportunities to study the structure and functions of HCF-1. The findings that a closely related HCF-2 interacts poorly with VP16 and hardly with LZIP (26) strongly suggest that the recognition of targets by the β-propeller has specificity.

In this context, it is not surprising that the HCF-like HCLP-1 protein identified in this study interacts selectively with LZIP but not with VP16. Although LZIP and VP16 share a common tetrapeptide HBM recognized by HCF-1, they exhibit dramatically different sensitivities to HCF-1 point mutants in the β-propeller domain (9), implicating that HCF-1 binds VP16 and LZIP through different mechanisms. Our findings that HCLP-1 interaction with LZIP is independent of the HBM (Fig. 4) revealed that the β-propeller domain of HCLP-1 can recognize a distinct motif in LZIP. Further analysis will shed light on the identity of this recognition motif.

HCLP-1 is smaller in size and the entire molecule consists of a six-bladed β-propeller (Fig. 1). Structurally, HCLP-1 is most similar to a newly identified and naturally occurring 50-kDa fragment of HCF-1, which has been found in the cytoplasm of primary G0 cells as a proteolytic product of the full-length protein (14). This HCF-β60 fragment contains the intact β-propeller domain and retains the ability to interact with VP16. HCF-β60 has been suggested to mediate the suppression of viral immediate early gene expression through sequestration of VP16 in the cytoplasm (14). This resembles the action of the hepatitis C virus core protein, which retains LZIP in the cytoplasm (24). We note that endogenous HCLP-1 protein localizes to the nucleus of interphase HeLa cells (Fig. 3C). However, we do not rule out the possibility that HCLP-1 could be a cytoplasmic protein during particular phases of the cell cycle. Taking the similarity between HCLP-1 and HCF-β60 into account, we postulate that HCLP-1 might also inhibit LZIP activity by sequestering it in the cytoplasm under certain circumstances. On the other hand, the HCLP-1 modulation of the LZIP-dependent DNA binding activity demonstrated in this study (Fig. 6) provides novel mechanistic insights into how HCF-like β-propeller proteins regulate transcription. It is of great interest to see whether similar forms of HCF-like proteins such as HCF-β60 might act through a similar mechanism when targeted to the nucleus.

Both HCF-1 and LZIP have been implicated in cell proliferation. A proline-to-serine point mutant at position 134 in the β-propeller domain of HCF-1 leads to G0/G1 cell cycle arrest (15, 16). In contrast, loss of LZIP function induced by the hepatitis C virus core protein correlates with oncogenic transformation (24). We have previously proposed a model in which LZIP serves a tumor suppressor function in the development of hepatocellular carcinoma (24). Intriguingly, HCLP-1 has recently been identified independently as a specific tumor antigen for hepatocellular carcinoma (GenBank™ AF244137). This raises the possibility that HCLP-1 may have a role in hepatocarcinogenesis by targeting the LZIP tumor suppressor. Further investigations are required to test this hypothesis.
Inhibition of LZIP-mediated Transcription through Direct Interaction with a Novel Host Cell Factor-like Protein
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