Functional Interactions between Sp1 or Sp3 and the Helicase-like Transcription Factor Mediate Basal Expression from the Human Plasminogen Activator Inhibitor-1 Gene*

(Received for publication, March 23, 1999)

Hao Ding‡§, Abderraﬁ M. Benotmane‡, Guntram Suske¶, Désiré Collen‡, and Alexandra Belayew‡

From the ‡Center for Molecular and Vascular Biology, University of Leuven, 3000 Leuven, Belgium and the ¶Institut für Molekularbiologie und Tumorforschung, Philipps-Universität, 35037 Marburg, Germany

Basal expression of the human plasminogen activator inhibitor-1 (PAI-1) is mediated by a promoter element named B box that binds the helicase-like transcription factor (HLTF), homologous to SNF/SWI proteins. Electrophoretic mobility shift assays performed on a set of B box point mutants demonstrated two HLTF sites flanking and partially overlapping with a GT box binding Sp1 and Sp3. Mutations affecting either the Sp1/Sp3 or the two HLTF sites inhibited by 6- and 2.5-fold, respectively, transient expression in HeLa cells of a reporter gene fused to the PAI-1 promoter. In Sp1/Sp3-devoid insect cells, co-expression of PAI-1–lacZ with Sp1 or Sp3 led to a 14–26-fold induction while HLTF had no effect. Simultaneous presence of Sp1 or Sp3 and the short HLTF form (initiating at Met-123) provided an additional 2–3-fold synergistic activation suppressed by mutations that prevented HLTF binding. Moreover, a DNA-independent interaction between HLTFMet123 and Sp1/Sp3 was demonstrated by co-immunoprecipitation from HeLa cell extracts and glutathione S-transferase pull-down experiments. The interaction domains were mapped to the carboxyl-terminal region of each protein; deletion of the last 85 amino acids of HLTFMet123 abolished the synergy with Sp1. This is the first demonstration of a functional interaction between proteins of the Sp1 and SNF/SWI families.

Plasminogen activator inhibitor-1 (PAI-1), a member of the serine protease inhibitor (serpine) family, plays a key role in the regulation of fibrinolysis by binding to and rapidly inactivating both tissue-type- and urokinase-type plasminogen activators (reviewed in Ref. 1). Increased plasma levels of PAI-1 have been shown to be associated with venous thrombosis and to predispose to arterial thrombosis (reviewed in Ref. 2). Transgenic mice deficient for PAI-1 show increased endotoxin-induced venous thrombosis and enhanced neointima formation upon vessel wall injury, whereas mice overexpressing PAI-1 suffer from spontaneous venous occlusions (3, 4). Components of the fibrinolytic system are also involved in extracellular matrix degradation required for invasion and metastasis of neoplastic cells. PAI-1 is thought to play two independent roles in such processes by protecting the tumor stroma from urokinase-type plasminogen activator-mediated degradation and by favoring detachment of vitronectin-bound cells (5, 6). The poor prognosis of high PAI-1 levels in many cancer patients might also stem from its recently demonstrated role in tumor vascularization (7).

PAI-1 gene expression was shown to be stimulated by a variety of cytokines, growth factors, hormones, phorbol esters, and endotoxins (8). Some cis-elements involved in gene regulations have been identified in the human PAI-1 promoter, such as those involved in the induction by transforming growth factor-β (9, 10), glucocorticoids (11, 12), phorbol ester (13, 14), p53 (15), and glucose (16).

We have previously cloned and characterized a novel transcription factor involved in basal expression of the human PAI-1 gene in HeLa cells (17). The protein, named helicase-like transcription factor (HLTF) has a specific DNA-binding domain, a RING finger domain, and the seven conserved DNA helicase domains; it is homologous to proteins of the SNF/SWI family that play a role in chromatin remodeling and facilitate trans-factor interaction with nucleosomes (reviewed in Refs. 18–21). Two HLTF proteins differing in translation initiation site were observed, only the smaller of which, HLTFMet123, is transcriptionally active. The same protein was independently isolated by other groups because it interacted with other DNA targets (the human immunodeficiency virus long terminal repeat and the simian virus enhancer, the myosin light chain locus enhancer, the rabbit uteroglobin promoter, the tumor necrosis factor response element), and shown to display DNA-dependent ATPase activity (22–25). HLTF activates the PAI-1 promoter via specific interaction with the B box that was initially identified as a phorbol ester-responsive element (13), but was later shown only to be involved in basal expression (17). The B box is highly similar to the GT box (also called CACCC motif), which has been shown to bind Sp1 as well as other recently identified members of the expanding Sp family of transcription factors (26–28). Sp1 is ubiquitously expressed and is essential for early embryonic development (29); it can activate transcription of a large number of regulated and constitutively expressed genes, whether the promoter comprises a TATAA box or not (30). Sp1, Sp3, and Sp4 contain a similar DNA-binding domain, with three zinc fingers and two glutamine- and serine/threonine-rich trans-activation domains (28, 31). Sp3 contains an additional inhibitory domain, leading to either activation or repression depending on the promoter and cellular context (31–37).

In the present work, we investigated the interplay between...
Functional Interaction between Sp1/Sp3 and HLTF

EXPERIMENTAL PROCEDURES

Materials—The pGL-3 Basic vectors, luciferin, and reporter-lysis buffer were from Promega (Leiden, The Netherlands); the GalactoLight kit was from Tropix (Bedford, MA). The Protein A-Sepharose (Pharmacia Biotech, Uppsala, Sweden), culture media, fetal calf serum, media additions, and Lipopectamine transfection reagent were from Life Technologies, Inc. (Ghent, Belgium). The Qiagen plasmid extraction kits were from Westergaard (Seraing, Belgium). Oligonucleotides were from Eurogentec (Seraing, Belgium). Monoclonal antibodies against Sp1 and Sp3 were from Santa Cruz Biotechnology (Santa Cruz, CA); the polyclonal antiserum against Sp1 or Sp3 have been described elsewhere (31), as has the antiserum against murine placminogen (38). The fusion protein between glutathione S-transferase and the HLTF DNA-binding domain (GST-MLTFDBD), previously named GST-6D3, and mAb2F6, a monoclonal antibody directed against 6D3, have been described (17). The HeLa and Drosophila SL2 cells were provided by Dr. C. Backendorf (University of Leiden, Leiden, The Netherlands) and Dr. W. Wahl (University of Geneva, Geneva, Switzerland), respectively. The pPacUbSp1 and pPacUbSp3 expression vectors and GST-Sp3 mutants have been described elsewhere (34), the GST-Sp1 mutants were kindly provided by Dr. E. Wintersberger (University of Vienna, Vienna, Austria), and the pPacSp1N619(ΔD) and the J44-LacZ vectors were provided by Dr. Tjian (University of California, Berkeley, CA) and Dr. Spear (University of Kentucky, Lexington, KY), respectively.

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts from HeLa cells were prepared as described (17). Double-stranded oligonucleotides (sequence of top strands given in Fig. 2A) were end-labeled with [γ-32P]ATP and T4 polynucleotide kinase. EMSA was performed with 6 μg of HeLa cell nuclear extract that were incubated with 20,000 cpm of 32P-labeled probe in a 20-μl volume containing 15 mM Hepes (pH 7.9), 100 mM (or other concentrations as indicated) NaCl, 10% glycerol, 1 mM dithiothreitol, and 1 μg of poly(dI·dC)·poly(dI·dC). After 15 min on ice, temperature, electrophoresis was performed at 4 °C on a 4% polyacrylamide gel in 45 mM Tris borate, pH 8.5, 1 mM EDTA, which was prerun at 4 °C for 30 min. When needed, 1 μl of antibody (corresponding to 100 ng of proteins) was added to the binding assay mixtures and incubated for 30 min on ice before addition of labeled probes.

Plasmid Constructions—The 336-bp fragment (coordinates –318 to +18 bp) of the human PAI-1 promoter was obtained by PCR amplification with appropriate primers and cloning into the pGL3-1 Basic plasmid yielding PAIL-1uc. For transfection experiment in Drosophila SL2 cells, the test DNA fragments were subcloned into the BamHI and HindIII sites of the pJ4-A Luc vector (39) replacing the original alkaline phosphatase promoter to yield PAIL-1uc. The promoter chosen since, in contrast to pGL3-G2 or pGL3-3 Basic or pGL3-RS, it presented no induction upon Sp1 or Sp3 transient co-expression. The 336-bp fragment (coordinates -336 to 0) of a 50:50 slurry of the glutathione-Sepharose-bound GST fusion protein (1 μg) were incubated with either 10 μg of reticulocyte lysate translation mix containing 35S-labeled GST fusion protein (wild type or mutant) or 200 μg of HeLa nuclear extract in 200 μl of binding buffer at 4 °C for 1 h. The binding buffer was 20 mM HEPES, pH 7.9, 1 mM MgCl2, 40 mM KCl, 0.1 mM EDTA, 0.1% Nonidet P-40, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 100 μg/ml ethidium bromide. The beads were washed four times with binding buffer and then boiled in 50 μl of SDS-sample buffer followed by analysis on 8% PAGE. Coomassie Blue staining demonstrated the presence of similar amounts of GST fusion proteins. The HLTF proteins were visualized either by autoradiography (35S label) or Western blotting using 10 μg/ml mAb2F6 and the ECL detection system.

Protein/Protein Interactions—35S-Labeled HLTF proteins were obtained by in vitro transcription/translation in the TNT reticulocyte lysate of pGEM2Z constructs into which the cDNA was under control of the T7 promoter. The GST-Sp1, GST-Sp3, and mutant proteins were produced in Escherichia coli from pGEX vectors and purified by adsorption onto glutathione-Sepharose according to the manufacturer's instructions. Transfections were done in triplicate and repeated three to five times with different preparations of the same plasmid.

Statistical Analysis—Student's t test for paired values was used to evaluate luciferase and pβ-galactosidase transient expression.
FIG. 1. HLTF, Sp1, and Sp3 are the B box binding proteins. A. EMSAs were performed with 6 µg of HeLa nuclear extract and 20,000 cpm of 32P-labeled B box oligonucleotide. The binding buffer contained 150 (lane 1), 125 (lane 2), 100 (lane 3), 75 (lane 4), and 50 mM NaCl (lane 5). The oligonucleotide sequence is given in Fig. 2A. The protein-DNA complexes (C1, C2, C3a, and C3b) and free DNA (F) are indicated. B. EMSA was performed as above with 100 mM NaCl in the absence (lane 1) or presence of 100 ng of antibodies directed against Sp3 (lane 3), Sp1 (lane 4), both Sp1 and Sp3 (lane 2), GST (lane 5), or HLTF (lane 6).

RESULTS

HLTF, Sp1, and Sp3 Bind to the B Box of PAI-1 Promoter—In a previous study, EMSAs performed with a 32P-labeled B box oligonucleotide (coordinates −86 to −60 bp in the human PAI-1 promoter; Ref. 13) and HeLa nuclear extracts had shown a major shifted band corresponding to several protein/DNA complexes (17). In order to identify the proteins involved in these complexes, EMSA conditions have now been modified allowing resolution of four distinct complexes (C1, C2, and the C3a/C3b doublet in Fig. 1). Protein/DNA interactions were strongly affected by the salt concentration of the binding buffer. At 150 mM NaCl, a strong C3a/C3b doublet and a weak C1 complex were observed (lane 1) while decreasing the salt concentration led to progressive decline of the doublet intensity and simultaneous appearance of a strong C1 and a weak C2 complex (lanes 2–5). Addition of antibodies directed against Sp3 to the EMSA reaction caused a supershift of complex C2 (Fig. 1B, lane 3). A partial supershift of complex C1 was caused by Sp1 antibodies (lane 4), and further addition of Sp3 antibodies suppressed most of the remaining complex (lane 5). None of the antibodies used suppressed complex C3b. In aggregate, these data indicate that HLTF (complex C3a), Sp1 (complex C1), and Sp3 (complexes C1 and C2) can bind to the B box.

Delineation of the HLTF and Sp1 Binding Sites in the B Box—A degenerate consensus binding site, 5′-T(A/G)[C/T]/G(A/T)[G(A/T)][T/C/A]-3′ was identified in a target definition assay performed according to Ref. 45 with the GT box/DNA-binding domain produced in bacteria as a GST fusion protein (GST-HLTF-DBD; Ref. 17 and data not shown). The B box of the PAI-1 promoter contains a tandem repeat of sequences (5′-GTGGTG-3′ and 5′-GGGGAA-3′) that present one or two mismatches (bold) with this target. These repeats flank the GT box (5′-GGGGTG-3′) that partially overlaps with the upstream putative HLTF site (Fig. 2A, upper line).

A panel of B box oligonucleotide mutants affecting G nucleotides either in the putative HLTF binding sites or in the GT box (Fig. 2A) were synthesized. They were evaluated in a competition assay for their ability to suppress, at a 50-fold molar excess, complexes formed in the radiolabeled wild type B box in EMSA performed as above (Fig. 1B). Mutant 1 bearing mutations at bp −72, −73, −75, and −76 had previously been shown to have strongly reduced affinity for HLTF (17); consistently, it could suppress neither HLTF- nor Sp1/Sp3-containing complexes (Fig. 2B). Mutant 3 with a single mutation at bp −76 could not interact with Sp1/Sp3 either but was able to bind HLTF since it suppressed its complex (C3a). Mutants 4, 5 (data not shown), 6, 9, and 10 could still bind Sp1/Sp3 since they prevented formation of their complexes (C1 and C2) with the
FIG. 3. Transcriptional activity of the B box mutants in HeLa cells. A, B box mutations introduced into the (Bbox)x2-TATA-luc reporter vector. Left panel, schematic presentation of the vectors and the mutations (X) in the GT box (empty box) or the two HLTF sites (gray boxes). Right panel, 2.5 × 10^5 cells were transiently co-transfected with 10 ng of CMV β internal control and 2 μg of pGL-3 vector containing either no promoter (Luc), a minimal adenovirus major late promoter (TATA-luc) or this promoter fused to two copies of the B box, either wild type or mutated as stated in Fig. 2. Luciferase and β-galactosidase activities were measured 16 h after transfection. The data are average ± S.D. of luciferase activities normalized to β-galactosidase activities from three different experiments performed in triplicates. B, B box mutations introduced into the PAI-1-Luc reporter vector. Left panel, schematic presentation of the vectors as above. Right panel, 2.5 × 10^5 cells were transiently co-transfected with 10 ng of CMV β internal control and 2 μg of pGL-3 vector containing either no promoter (Luc), the 318-bp PAI-1 promoter (PAI-1-Luc), or PAI-1 promoter mutated in the B box (PAI-1 Mut3-Luc; PAI-1 Mut10-Luc). Luciferase and β-galactosidase activities were measured as stated in panel A.

PAI-1 Promoter Activation by Sp1 and Sp3—In order to further study the interplay between Sp1/Sp3 and HLTF on the B box, Drosophila SL2 cells, which lack endogenous Sp1-like activity, were used for transient expression experiments. The 318-bp PAI-1 promoter fused to the lacZ reporter gene was activated in a dose-dependent manner by co-expression with either Sp1 or Sp3, reaching maximal 26.0 ± 0.3- and 14.3 ± 0.2-fold stimulation in the presence of 500 ng of pPacUbxSp1 or pPacUbxSp3 expression vector, respectively. This effect was mediated by the GT box, since only minor stimulations (1.3 ± 0.1-fold and 1.4 ± 0.1-fold with 100 ng of Sp1 or Sp3 expression vector, respectively) were observed with the PAI-1-Mut3-LacZ into which the Sp1/Sp3 binding site was mutated, as compared with the activations reached with the same amount (1 μg) of wild type PAI-1-LacZ (6.2 ± 0.2-fold and 5.9 ± 0.2-fold with 100 ng of Sp1 or Sp3 expression vector, respectively).

In conclusion, Sp1 and Sp3 can stimulate the PAI-1 promoter provided their binding site is intact in the B box.

Transcriptional Synergy between HLTF and Sp1 or Sp3—The use of alternative translation initiation starts in the HLTF mRNAs was previously shown to yield, both in vitro and in vivo, two protein variants (HLTFMet1 and HLTFMet123), of which only the shorter activated basal expression of the PAI-1 promoter in HeLa cells (17). Introduction of expression vectors for either HLTFMet1 or HLTFMet123 in SL2 cells did not affect transient expression of PAI-1-LacZ (Fig. 4, A and B, lanes 3 and 4). Similar expression of HLTFMet1 and HLTFMet123 in these experiments was demonstrated by Western blotting, using whole cell extracts from transfected or control SL2 cells (data not shown). Co-expression of HLTFMet123 enhanced by an additional 2-fold the Sp1-mediated activation of PAI-1-LacZ (Fig. 4A, lane 6); similarly, an additional 3-fold enhancement

Transcriptional Activity of the B Box Mutants—Two copies of either the B box oligonucleotide or its mutants were fused to a luciferase reporter gene with a minimal adenovirus major late promoter (TATA-luc) and the resulting (Bbox)x2-TATA-luc reporter vectors were introduced into HeLa cells. Transient luciferase expression was not affected by mutations in either of the two HLTF binding sites (Fig. 3A, Mut6 and Mut9); however, the combined mutations (Mut10) led to a 2-fold reduced luc activity as compared with the wild type B box. Mutations in the Sp1/Sp3 binding site (Mut1 or Mut3) reduced transient luciferase activity to the level of the control TATA-luc construct.

The mutations suppressing only either Sp1/Sp3 (Mut3) or HLTF (Mut10) binding were introduced into the 318-bp PAI-1 promoter fused to the luc gene and the constructs evaluated by transient expression in HeLa cells. The single G→T mutation in the Sp1/Sp3 site (Fig. 3B, PAI-1 Mut3-Luc) led to a 6-fold reduced luciferase activity as compared with the wild type promoter (PAI-1-Luc) while the combined mutations affecting both HLTF sites (PAI-1 Mut10-Luc) resulted in a 2.5-fold inhibition. These data demonstrate a positive correlation between binding of HLTF and Sp1/Sp3 to these sites and transcriptional activity.
Functional Interaction between Sp1/Sp3 and HLTF

**Physical Interaction in Vitro between HLTF and Sp1/Sp3**—We then investigated whether direct HLTF-Sp1 or HLTF-Sp3 interactions could be involved in the observed transcriptional synergies. GST pull-down experiments were performed in the presence of ethidium bromide, which eliminates any possible interference by, or dependence on, DNA in the protein-protein interaction (62). 35S-Labeled HLTFMet123 was synthesized by in vitro translation in a reticulocyte lysate and incubated with GST-Sp1 immobilized onto glutathione-Sepharose. The bound material was analyzed by SDS-PAGE, followed by autoradiography. The molecular size markers are indicated, and HLTF is shown by an arrow.

To map the HLTF domain required for interaction with Sp1 and Sp3, a series of HLTF cDNA deletion mutants was prepared (Fig. 6A), affecting the regions coding for different helix-case domains (ΔH), the RING finger domain, the first or second half of the DNA-binding domain (ΔNDBD and ΔCDBD), and a putative nuclear location signal (ΔNLS). The encoded proteins were 35S-labeled by transcription/translation in a reticulocyte lysate and analyzed by SDS-PAGE and autoradiography (Fig. 6B). Alternative translation initiation at Met-123 (vertical arrow in panel A) yielded an additional product in all lanes except lane 3, this mutant (ΔCDBD/H I) having lost the alternative initiator codon. GST pull-down experiments were performed...
into which the same amount of each protein was incubated with GST-Sp1ZD (Fig. 6C) or GST-Sp3ZD (Fig. 6D) immobilized on glutathione-Sepharose. Only deletion of 85 amino acids from the carboxyl terminus (mutant DHI) greatly reduced the ability of HLTF to interact with Sp1 (Fig. 6C, lane 7) or Sp3 (Fig. 6D, lane 7).

In aggregate, these data demonstrate direct protein/protein interactions between HLTF and Sp1 or Sp3 that are mediated by the carboxyl-terminal region of each partner.

**Physical Interaction in Vivo between HLTF and Sp1/Sp3**—HLTF synthesized in vivo could also specifically interact with Sp1 and Sp3, as shown when glutathione-Sepharose-bound GST-Sp1ZD or GST-Sp3ZD were incubated with HeLa nuclear extracts and the adsorbed material analyzed by SDS-PAGE and Western blotting with the mAb2F6 monoclonal antibody against HLTF (Fig. 7A, lanes 1 and 3 versus the GST control in lane 2). This antibody only recognizes HLTFMet1. However, both forms of HLTF (HLTFMet1 and HLTFMet123) were visualized with a polyclonal antibody against HLTF in a similar experiment (data not shown).

To determine whether HLTF and Sp1/Sp3 could form a complex in vivo, HeLa nuclear extracts were immunoprecipitated with antibodies against HLTF, and the immune complexes were analyzed by SDS-PAGE and Western blotting demonstrating the presence of Sp1 or Sp3 (Fig. 7, B and C, lanes 1) as well as HLTF (data not shown). The additional proteins observed were also present when the control antibodies against murine plasminogen were used, which did not immunoprecipitate Sp1 or Sp3 (Fig. 7, A and B, lanes 2).

**The Carboxyl-terminal Domain of HLTF Is Required for Transcriptional Synergy with Sp1 or Sp3**—In order to investigate a transcriptional role for the HLTF domain involved in the protein/protein interactions with Sp1/Sp3, transient activation by Sp1 or Sp3 of the PAI-1-lacZ reporter vector in insect SL2 cells was challenged by co-expression of an HLTFMet123 mutant with deletion of the carboxyl terminus (DHI; Fig. 8); no synergy could be observed in either case, as compared with the control conditions, where the presence of full-size HLTFMet123 yielded a 2–3-fold activation of Sp1- or Sp3-mediated enhancement.

**DISCUSSION**

In the present study, we demonstrate that the cis-element (B box) that mediates basal expression of the PAI-1 promoter in HeLa cells comprises a GT box interacting with Sp1 or Sp3 flanked by two HLTF-binding sites. This element mediates transcriptional activation by Sp1 and Sp3, and additional synergy with HLTF, which requires DNA binding and protein/protein interaction mediated by the carboxyl-terminal region of either protein. Although the PAI-1 promoter was shown to harbor another Sp1-binding site (bp –45 to –40; Ref. 14 and 16) besides the one studied here (B box, bp –82 to –65), our data indicate that basal expression in HeLa cells was mostly mediated by the B box site (Fig. 3B).

**Protein Binding to the B Box**—Binding sites for Sp1/Sp3 and HLTF were found on a very short DNA region, raising the question of how the binding sites are recognized by the three proteins. This is particularly intriguing given the short distance between the Sp1 and Sp3 binding sites, which might allow for direct interaction between the two proteins. Further studies will be needed to elucidate the molecular basis of this interaction and its role in transcriptional regulation.
question of whether these large proteins could be present simultaneously on the B box. The deoxyribonuclease I footprinting technique cannot provide an answer to this question since the region protected in vitro by purified Sp1 extends from bp −83 to −66 in the PAI-1 promoter (14), thus including the two HLTF sites. Classical EMSA conditions use excess oligonucleotide and only allow observation of B box complexes involving individual proteins: Sp1, Sp3, or HLTF (Fig. 1). When a much reduced amount of B box was saturated with the recombinant HLTF DNA-binding domain (GST-HLTF-DBD) in EMSA, a supershift was observed upon addition of either Sp1 or Sp3, indicating that the two proteins could bind simultaneously to the oligonucleotide (data not shown). Although the full-size HLTF (116 kDa) is larger than GST-HLTF-DBD (48 kDa), indirect evidence that it binds in the presence of Sp1 is provided by the observation that the Sp1/or Sp3/HLTF synergy is abolished by mutation of the two HLTF binding sites (Fig. 4, lane 12).

Our mutagenesis analysis delineated a minimal GT box from bp −77 to −73, a location partly overlapping with the 5′ HLTF site but at a 5–6-bp distance from the 3′ HLTF site (Fig. 2A). This spacing suggests that HLTF bound to the 3′ site would be on the opposite side of the DNA double helix as compared with bound Sp1, an arrangement reducing steric hindrance. More space could even probably be available since the DNA might be locally unwound (with 11.2 bp/turn instead of 10) by the Sp1 zinc fingers as shown in vitro (46).

The two HLTF-binding sites of the B box are located 10 bp apart, i.e. on the same side of the DNA double helix, probably precluding simultaneous binding of such large molecules. HLTF might have equivalent affinities for its two binding sites since no significant difference in binding competition of GST-HLTF-DBD with oligonucleotide mutants affecting either one site or the other was observed neither in EMSA (Fig. 2, mutants 6, 8, and 9) nor in a more sensitive method allowing quantitation of DNA/protein interaction at equilibrium (enzyme-linked DNA/protein interaction assay, ELDIA; Ref. 47) (data not shown). Evaluation of the different mutants by transient expression experiments in HeLa cells demonstrated no significant activity difference upon disruption of any single HLTF binding sites (Fig. 3A). In aggregate, these data suggest that binding of a single HLTF molecule in the B box is sufficient to mediate the synergy with Sp1 or Sp3.

In addition, in several published EMSAs where Sp1/Sp3 binding to a GT box was investigated, additional uncharacterized faster migrating complexes were found (e.g. Refs. 48–52), which our data suggest might result from HLTF binding. Because the HLTF proteins are found in most adult tissues (17, 23, 24), synergies similar to the ones described here might

FIG. 7. Interaction in vivo of Sp1 or Sp3 and HLTF. A, HeLa nuclear extracts were incubated with GST (lane 2) or GST fused to the ZD domains of either Sp1 (lane 1) or Sp3 (lane 3) bound to glutathione-Sepharose. Adsorbed proteins were analyzed by SDS-PAGE, followed by Western blotting revealed by mAb2F6 directed against HLTFMet1. The molecular size markers and HLTF are indicated by arrows. B, HeLa nuclear extracts were immunoprecipitated with an antiserum directed against either the HLTF DNA-binding domain (Drosophila A lanes 3, against Sp3. Molecular size markers and Sp3 are indicated by arrows. Western blotting revealed by mAb2F6 directed against HLTFMet1. The molecular size markers and HLTF are indicated by arrows. B, lane 1 nuclear extracts were immunoprecipitated with an antiserum directed against either the HLTF DNA-binding domain (Drosophila A lanes 3, against Sp3. Molecular size markers and Sp3 are indicated by arrows. Western blotting revealed by mAb2F6 directed against HLTFMet1. The molecular size markers and HLTF are indicated by arrows. B, lane 1 nuclear extracts were immunoprecipitated with an antiserum directed against either the HLTF DNA-binding domain (Drosophila A lanes 3, against Sp3. Molecular size markers and Sp3 are indicated by arrows. Western blotting revealed by mAb2F6 directed against HLTFMet1. The molecular size markers and HLTF are indicated by arrows. B, lane 1 nuclear extracts were immunoprecipitated with an antiserum directed against either the HLTF DNA-binding domain (Drosophila A lanes 3, against Sp3. Molecular size markers and Sp3 are indicated by arrows. Western blotting revealed by mAb2F6 directed against HLTFMet1. The molecular size markers and HLTF are indicated by arrows. B, lane 1 nuclear extracts were immunoprecipitated with an antiserum directed against either the HLTF DNA-binding domain (Drosophila A lanes 3, against Sp3. Molecular size markers and Sp3 are indicated by arrows. Western blotting revealed by mAb2F6 directed against HLTFMet1. The molecular size markers and HLTF are indicated by arrows. B, lane 1 nuclear extracts were immu...
Functional Interaction between Sp1/Sp3 and HLTF

occur on several other promoters and might have remained unnoticed till now.

Role of Protein/Protein Interactions—Protein/protein interactions between HLTF and Sp1/Sp3 might further stabilize the complex formed by these factors and DNA. The Sp1 domain involved in the interaction with HLTF was delineated in the present study to the ZD region. Transient co-expression experiments performed with Sp1 lacking domain D demonstrated a synergy with HLTF similar to that observed with full-size Sp1, suggesting that the zinc finger domain was involved (data not shown). Further studies will require point mutagenesis of Sp1 since deletion of the zinc finger domain abrogates its DNA binding required for activation of the B box. Several cellular transcription factors such as YY1 (53), the p65 subunit of NF-κB (54), and GATA-1 (55) have been shown to functionally interact with the zinc finger domain of Sp1. Similarly, the carboxy-terminal region of Sp1 (Z and D domains) was found to mediate functional interaction with the cell cycle-regulated E2F transcription factor (41, 56); a more refined mapping will be required to determine if the same domain is involved in HLTF and E2F interaction. In these examples for E2F/Sp1 interaction, the mouse thymidine kinase promoter carrying the HLTF and E2F interaction. In these examples for E2F/Sp1 interaction, the mouse thymidine kinase promoter carrying the HLTF and E2F interaction. In these examples for E2F/Sp1 interaction, the mouse thymidine kinase promoter carrying the HLTF and E2F interaction.
Functional Interactions between Sp1 or Sp3 and the Helicase-like Transcription Factor Mediate Basal Expression from the Human Plasminogen Activator Inhibitor-1 Gene

Hao Ding, Abderrafi M. Benotmane, Guntram Suske, Désiré Collen and Alexandra Belayew

J. Biol. Chem. 1999, 274:19573-19580.
doi: 10.1074/jbc.274.28.19573

Access the most updated version of this article at http://www.jbc.org/content/274/28/19573

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 61 references, 27 of which can be accessed free at http://www.jbc.org/content/274/28/19573.full.html#ref-list-1