Gene-specific Transcriptional Activation Mediated by the p150 Subunit of the Chromatin Assembly Factor 1*§

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Chromatin assembly factor 1 contains three subunits, p150, p60, and p48. It is essential for coupling nucleosome assembly to newly synthesized DNA. Whether chromatin assembly factor 1 subunits have functions beyond escorting histones, which depends on the complex formation of p150 and p60, has been an issue of great interest. This study reveals a novel role of p150, but not p60, in gene-specific transcriptional activation. We found that p150 transcriptionally activated an essential viral promoter, the major immediate early promoter (MIEP) of the human cytomegalovirus, independently of p60. Knocking down p150 decreased the MIEP function in both transfected and virally infected cells. The chromatin immunoprecipitation analysis and the in vitro protein-DNA binding assay demonstrated that p150 used its KER domain to associate with the MIEP from −593 to −574 bp. The N-terminal 244 residues were also found essential for p150-mediated MIEP activation, likely through recruiting the acetyltransferase p300 to acetylate local histones. Domain swapping experiments further showed that the KER and the N terminus of p150 acted as an independent DNA binding and transcriptional activation domain, respectively. Because p60 did not seem involved in the reaction, together these results indicate for the first time that p150 directly activates transcription, independently of its histone deposition function.

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3 The abbreviations used are: CAF1, chromatin assembly factor 1; MIEP, major immediate early promoter; HCMV, human cytomegalovirus; PCNA, proliferating cell nuclear antigen; shRNA, short hairpin RNA; m.o.i., multiplicity of infection; ChIP, chromatin immunoprecipitation; EMSA, electrophoretic mobility shift assay; Ab, antibody; DAPA, DNA affinity protein assay; NLS, nuclear localization sequence; oligo, oligonucleotide; GalDBD, Gal4 DNA binding domain; GalAD, Gal4 activation domain; GFP, green fluorescent protein.

because knocking down either subunit disrupts the activity (2–4). In addition, CAF1 facilitates DNA synthesis depending on the binding of the N-terminal 31 residues of p150 to the proliferating cell nuclear antigen (PCNA), which acts as a sliding clamp to stimulate the processivity of DNA polymerase (5, 6). The internal region of p150 contains two large bunches of charge residues called KER (a region enriched in lysine, glutamic acid, and arginine) and ED (a region enriched in glutamic acid and aspartic acid) domains, which are thought to be the binding sites for acetylated histones H3 and H4 (1, 7). Both p150 and p60 contain a PEST domain, which is believed to mediate the degradation of these two proteins (8, 9). Knocking down p150, in some cases, causes a concomitant loss of p60 protein, presumably due to the rapid turnover of the free subunit (4).

CAF1 is involved in transcriptional regulation. The loss of the p150 homolog CAC1 in yeast impairs the constitutive gene silencing at telomeres and mating-type loci (10–12). p150 regulates the formation of heterochromatin in mammalian cells during replication (Refs. 13, 14 and references therein) and in plants it maintains the transcription of certain subsets of genes (15, 16). Furthermore, p150 exists in a chromatin-remodeling complex WINAC, which coactivates ligand-induced transcription function of the vitamin D receptor (17). Currently, these effects are thought to be controlled by the global chromatin structure that requires CAF1-mediated nucleosome assembly.

Although p150, p60, and p48 co sediment in the fraction that exhibits the nucleosome assembly activity (7), each of the CAF1 subunits also displays its unique distribution pattern, especially during S phase of the cell cycle (18). This implicates that each subunit may have additional roles beyond chromatin assembly, and the additional functions may not require them to complex with one another. In mouse cells, p150, but not p60, regulates progression of the middle-to-late S phase and promotes the replication of pericentric heterochromatin via interacting with the HP1 (heterochromatin protein 1) (19). p150 interacts with HP1 α/γ and the MBD1 (methyl-CpG binding domain protein 1) through its N- and C-terminal regions, respectively, and may guide them to heterochromatin regions (14, 20, 21). p48 associates with the retinoblastoma tumor suppressor protein (22) and serves as an escort of various histone metabolism enzymes (23). Whether CAF1 subunits have other independent functions remains to be explored.

Previously, a yeast two-hybrid assay was used to screen for proteins associating with the immediate early protein 2 (IE2) of
the human cytomegalovirus (HCMV) (24). We identified that the p150 fragment aa 87–938 interacted with the C-terminal half (aa 291–579) of IE2. Because proteins obtained using this strategy, such as Nrfl and Nrf2 (24), antagonized the IE2-mediated transcriptional repression of its own promoter, the major IE promoter (MIEP), we examined if p150 has a similar effect. Unexpectedly, in the absence of IE2, p150, but not p60, increased the MIEP activity in a dose-dependent manner. We demonstrated that p150 associated with the MIEP in transfected cells. Both the potential DNA binding and the transcriptional activation domains on p150 were also characterized. This study reveals a previously unidentified role for p150 as a direct transcriptional activator.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—pcDNA3.1-HA-p150 and pcDNA3.1-HA-p150C, which encode the HA-tagged, full-length p150 or the C-terminal residues 641–938, respectively, were kindly provided by Dr. P. D. Kaufman (25). To construct the plasmid for establishing a stable cell line expressing aa 641–938 of p150, pSEP7-HA-p150C was generated by inserting the corresponding cDNA into pSEP7 between the HindIII and XhoI sites. pcDNA3.1-HA-p60 was constructed by inserting the corresponding p60 cDNA into pcDNA3.1-HA between the XhoI and XbaI sites, pPK38, ΔPEST, ΔKER, and ΔED, which produce the in vitro translated wild-type p150 or p150 mutants with the corresponding internal deletion, were kindly provided by Dr. B. Stillman (1). Plasmids expressing the series of terminally deleted mutants of p150, used for in vitro transcription/translation and for expression in mammalian cells, were generated by inserting the specific PCR-amplified p150 fragment flanked by EcoRI sites into the pcDNA3.1/V5-His-TOPO vector (Invitrogen). To generate the internally deleted mutants of p150, two-step cloning was performed. The cDNAs encoding the p150 fragments N-terminal to the domain to be deleted were first PCR-amplified using primers with 3' EcoRI linker, followed by cloning into pcDNA3.1/V5-His-TOPO (Invitrogen). The resulting plasmids were then digested with EcoRI and ligated with the EcoRI site-flanked cDNAs encoding the p150 fragment C-terminal to the domain to be deleted. The orientation of the inserts was confirmed by DNA sequencing. Plasmids encoding the firefly luciferase gene driven by the HCMV major IE promoter (pMIEP–733/+2) and the MIEP derivatives pMIEP–540/+2 and pMIEP–235/+2 were kindly provided by Dr. Y. H. W. Lee. To generate plasmids encoding the luciferase reporter driven by serially 5’-deleted mutants of MIEP, the specific MIEP fragments were PCR-amplified with primers containing the Nhel or HindIII site and then inserted into pGL2 between the Nhel and HindIII sites. All the serially deleted mutants were checked by DNA sequencing after construction.

To generate recombinant proteins from *Escherichia coli* to perform in vitro binding assays, the PCR-amplified cDNA fragment encoding amino acids 1–296 or 311–445 of p150 was inserted into the pET100D/TOPO vector (Invitrogen), which encodes proteins N-terminally tagged with His6 and Xpress. The plasmids encoding Gal4 activation domain (GalAD)-fused p150 fragments were generated by inserting the PCR-amplified cDNA of GalAD from pACT2 (Clontech) into the HindIII site of pcDNA3.1-p150/311–938-V5/His or pcDNA3.1-p150/505–938-V5/His. pMH100-TK, which contains a luciferase reporter gene driven by the thymidine kinase promoter of herpes simplex virus containing an additional five-Gal4-binding site, was kindly provided by Dr. Ronald Evan. To generate plasmids encoding the Gal4 DNA binding domain (GalDBD) fused to the C-terminal end of p150 fragments, the cDNA encoding GalDBD was PCR-amplified from pCMX-GalDBD and cloned into XbaI site of pcDNA3-p150/1–296-V5/His or pcDNA3-p150/1–641-V5/His. The orientation was checked by DNA sequencing. The plasmid encoding the chimera protein with GalDBD at its N terminus and p150 aa 564–938 at its C terminus was generated by inserting the EcoRI-digested p150 fragment from pcDNA3.1-p150/564–938-V5/His into pCMX-Gal4DBD. The cDNA encoding the GalDBD-p150 fragment was then PCR-amplified and subcloned into pcDNA3.1-V5/His (Invitrogen). The plasmid encoding the KER domain fused to the C terminus of VP16 was generated by inserting the EcoRI-digested p150 fragment from pcDNA3.1-p150 311–445-V5/His into pVP16 (Clontech). The series of plasmid pLKO.1-puro-sh150, which encode several short hairpin RNAs (shRNAs) targeting to different regions of p150, were obtained from the National RNAi Core Facility located at the Institute of Molecular Biology/Genomics Research Center, Academia Sinica, Taiwan. The following sequences are the regions targeted by each shp150: shp150-1 (CHAF1A, TRCN0000074273), 3’-untranslated region, TCTTGTGTAAAA; shp150-2 (CHAF1A, TRCN0000074272), +1523 to +1543, CCACCCGGAATGCAGATATTT; shp150-3 (CHAF1A, TRCN0000074275), +879 to +917, CCTCCGCA-GAATACTAAGAA.

**Cell Culture, Transfection, and Virus Infection**—The human lung adenocarcinoma H1299 and osteosarcoma U2OS cells were obtained from and maintained as instructed by the ATCC. U373MG human glioblastoma astrocytoma cells were obtained from the European Collection of Cell Cultures and cultured in minimal essential medium alpha medium (Invitrogen) containing 10% fetal bovine serum, 100 units/ml penicillin G sodium (Invitrogen), and 100 μg/ml of streptomycin sulfate (Invitrogen). All transfections were carried out using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. 5 × 10⁵ or 3 × 10⁵ cells were seeded, respectively, onto 6-well plates or 10-cm plates 1 day before transfection and harvested 24 h post-transfection. To generate stable lines, 300 μg/ml hygromycin or 25 μg/ml puromycin (both from Sigma) were used to enrich cells containing the plasmid. In transfection/infection experiments, cells were transfected with the MIEP-driven luciferase reporter and infected with HCMV 4 h post-transfection. HCMV strain RC256 (26) was obtained from the ATCC and maintained according to the manufacturer’s instructions. Infections were performed at multiplicity of infection (m.o.i.) of 1 (Fig. 2A) or the indicated amount (Fig. 2B) for 2 h.

**Western and Luciferase Assay**—Luciferase assays and Western analyses were performed as reported (27).

**Chromatin Immunoprecipitation (ChIP)**—ChIP assays were performed as described (27) with some modifications. The precipitated DNAs were analyzed by real time PCR using a LightCycler (Roche Applied Science) and a QuantiTect SYBR Green
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PCR kit (Qiagen) according to the manufacturer’s instructions. The primers and annealing temperatures were as follows: MIE promoter, 5'-CAATATGGCCATTAGCC-3' and 5'-GGGC-TATGACTAATGACC-3' (-733 to -537 bp, annealed at 58 °C); luciferase 1, 5'-TATGGAATTGAACCCCGTG-3' and 5'-CCAACCCAACGGAACATT-3' (+50 to +173 bp, annealed at 60 °C); luciferase 2, 5'-TACCATCATGTCCTGTGA-TCGG-3' and 5'-GTATGGAATTGAACCCCGTG-3' (+545 to +735 bp, annealed at 60 °C); and luciferase 3, 5'-TCTTATCCTAGGATAC-3' and 5'-CATAGTGTCCTGACACATAA-3' (+992 to +1188 bp, annealed at 60 °C).

DNA Affinity Protein Assay (DAPA)—DNAs containing serial deletions of MIEP fragments were PCR-amplified from pMIEP−733/+2 with biotinylated primers and extracted by PCR purification kit (Qiagen). Purified DNAs were incubated with streptavidin beads (Sigma) in the DAPA buffer (20 mM Hepes-KOH, pH 8.0, 10% glycerol, 100 mM KCl, 1 mM MgCl2, 0.2 mM EDTA, 0.1% Nonidet P-40) and extensively washed with DAPA buffer. To prepare lysates for determining the association of the endogenous p150 on MIEP, H1299 cells cultured on 500-cm2 dishes with 80% confluence were trypsinized and lysed in 0.5 ml of binding buffer (20 mM Hepes, pH 8.0, 250 mM NaCl, 0.5% Triton X-100) with mild vortexing at 4 °C for 1 h. After that, the lysates were centrifuged at 16,000 g for 15 min, and the supernatants were then aliquoted at the concentration of 10 mg/ml and stored at -80 °C. 1 mg of lysate was incubated with the DNA-bead complex in 900 μl of DAPA buffer and rotated at 4 °C overnight. The pulled down proteins were washed three times with DAPA buffer containing 200 mM KCl and separated with 10% SDS-PAGE, followed by Western analysis. To map the MIEP binding domain of p150, the indicated p150 mutants were translated in vitro in the presence of [35S]methionine by the TnT® Quick Coupled Transcription/Translation System (Promega). The 35S-labeled proteins were incubated with streptavidin bead-DNA complex overnight and then washed with DAPA buffer three times. Pulled down proteins were analyzed by SDS-PAGE and subjected to autoradiography. 10% of the lysate was loaded as input control.

Electrophoretic Mobility Shift Assay (EMSA)—The recombinant p150 fragments expressed in BL21 Star (DE3) One Shot Chemically Competent E. coli (Invitrogen) were purified using Ni-Sepharose™ 6 Fast Flow (GE Healthcare) according to the procedure described in QIAexpress Detection and Assay Handbook (Qiagen). For probe labeling, the PCR-amplified DNA was radiolabeled using G-25 column (GE Healthcare). DNA binding reactions were set up in 30 μl of binding buffer (20 mM HEPES, pH 7.9, 8% glycerol, 0.5 mM EDTA, 100 mM KCl, 0.5 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride) containing 32P-labeled MIEP DNA fragments and various E. coli-expressing recombinant proteins. After incubation on ice for 20 min, reaction mixtures were separated by 4% or 6% native acrylamide gels in 0.5× TBE containing 5% glycerol and subjected to autoradiography. For antibody supershift assays, the anti-Xpress antibody (Invitrogen), which recognizes the epitope fused to the E. coli-expressing proteins, was added into the reactions before gel electrophoresis.

Antibodies—The primary antibodies used in Western blots are as follows: rabbit anti-p150N antibody (H-300; Figs. 2B and 5F and supplemental Fig. S1), mouse anti-VP16 antibody (1–21; Fig. 5F), mouse anti-GalDBD antibody (RK5C1; Fig. 5F), and mouse anti-PCNA antibody (PC10; Fig. 2B) (all from Santa Cruz Biotechnology); mouse anti-cytomegalovirus antibody against immediate early proteins of cytomegalovirus (MAB810; Fig. 2B), mouse anti-β-tubulin antibody (MAB3408; Figs. 1C, 2A, and 4A and supplemental Fig. S1), mouse anti-actin antibody (MAB1501; Figs. 1B, 2B, 3, 4, and 5E; and 5, D, and E) (all from Millipore); mouse anti-HA antibody (12CA5, Roche Applied Science; Fig. 4A and supplemental Fig. S1; 16B12, Covance; Figs. 1A, 2A, and 4E); mouse anti-V5 antibody (R960-25, Invitrogen; Figs. 1, B and C, 3C, and 5, D and E); mouse anti-p150 antibody (ab7655; Figs. 3A and 4F); and mouse anti-p60 antibody (ab8133; Fig. 1B) (both from Abcam). The primary antibodies used in ChIP are as follows: rabbit anti-RNA polymerase II antibody (sc-585; Fig. 3C); mouse anti-p150N antibody (H-300; Fig. 4C) (both from Santa Cruz Biotechnology); mouse anti-HA antibody (16B12, Covance; Fig. 4B); rabbit anti-acetylated H3 antibody (06599) and rabbit anti-acetylated H4 antibody (06866) (Fig. 6) (both from Upstate).

RESULTS

p150, but Not p60, Activates the HCMV MIEP Promoter in Transfected Cells—p150 was examined for its ability to regulate the HCMV MIEP promoter. H1299 cells were transfected with the construct containing the luciferase reporter gene driven by MIEP in the absence or presence of increasing amounts of p150 plasmid. The luciferase activity was analyzed. Wild-type p150 increased the MIEP activity in a dose-dependent manner (Fig. 1A, lanes 2–4). HA-tagged p150 behaved similarly (Fig. 1A, lane 6). In contrast, the expression of either the HA-tagged C-terminal third of p150 from aa 641 to 938 (p150C) or p60 down-regulated the MIEP activity (Fig. 1A, compare lanes 7 and 8 with lane 5). The Western using the HA Ab shows the successful expression of each effector protein. Overexpression of p150C has been shown to degrade the endogenous p150 and thus exerts a dominant negative effect (25). Consistently, we observed a reduced level of the endogenous p150 in the presence of p150C (supplemental Fig. 1, compare lane 3 with lane 1). The fact that instead of increasing the MIEP activity, HA-p60 down-regulated the MIEP function suggests that p150 mediated the MIEP activation independently of the chromatin assembly function. HA-p60 likely titrated out the endogenous p150 from the MIEP promoter. To further exclude the possibility that p60 was involved in p150-mediated MIEP activation, the MIEP luciferase activity was assayed in the absence or presence of p150 in cells with p60 knocked down by small interfering RNA. As shown in Fig. 1B, the MIEP activity was increased in cells containing reduced levels of p60 (compare lane 3 with lane 1). Importantly, p150 with a V5 tag at its C terminus still activated the MIEP activity in the absence of p60 (Fig. 1B, lanes 3 and 4). These data strongly support the notion that the MIEP-activating function of p150 did not require p60. The p150-mediated MIEP activation was gene-specific because the activity of
human PCNA, p16, or viral SV40 promoter was not affected (Fig. 1C).

**p150 Regulates the MIEP Activity during HCMV Infection**—We next examined if the MIEP activity could be modulated by p150 during HCMV infection. To this end, the MIEP-driven luciferase construct was transfected into U373MG cells stably expressing HA-p150C, followed by HCMV infection. As shown in Fig. 2A, the MIEP activity was impaired in infected cells with a reduced level of p150. To this, the p150 constitutively knocked down U2OS cells were generated using shRNA strategy. Three lines with a differential level of p150 were infected with HCMV. As shown in Fig. 2B, only shp150-1, but not shp150-2 or -3, decreased the expression of the endogenous p150. Consistently, after HCMV infection, the expression of viral IE1 and IE2 proteins, which are driven by the MIEP, was only reduced in cells with shp150-1 (Fig. 2B, compare lanes 2 and 3 with lane 1). The phenomenon was also observed in cells infected with a higher titer of HCMV (Fig. 2B, lanes 4–9). These results indicate that p150 contributed to the activation of the HCMV MIEP promoter both in transfected and infected cells.

**N-terminal 244 Residues Are Important for p150 to Activate MIEP**—The MIEP transcriptional activation domain within p150 was explored. Deletion mutants of p150 were expressed in cells and assayed for their ability to stimulate the MIEP activity. As shown in Fig. 3A, full-length p150 activated the MIEP activity (lane 2). Consistent with what we described in Fig. 1, the p150 mutant defective in p60 binding (aa 1–641) (1) still retained the ability to stimulate the MIEP function (Fig. 3A, lane 3). In contrast, the p150 fragments lacking aa 1–244 or 1–310 lost the ability (Fig. 3A, lanes 4 and 5). One might argue that the two p150 fragments, similar to HA-p150C, must have induced the degradation of the endogenous p150, and thus they failed to increase the MIEP activity. To exclude this possibility, the expression of the cellular p150 in the presence of each exogenously added p150 molecule was compared. As shown by Western blot (Fig. 3A, lower panel, marked with an asterisk), the protein level of the endogenous p150 remained the same in all cases. These results suggest that the N-terminal 244 residues were essential for the MIEP activation function of p150. Consistently, the ChIP assay shows that the recruitment of RNA polymerase II to the transcription start site of the MIEP was increased only in the presence of the full-length p150 (Fig. 3B, I–938–V5), but not the p150 mutant lacking the N-terminal 310 residues (311–938–V5). Next we investigated whether the puta-
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The minimum p150-responsive region on MIEP from −733 to −540 bp was further dissected. To this, a series of terminally deleted MIEP mutants were generated and analyzed by reporter assay in the absence (Fig. 4E, white bar) or presence (black bar) of HA-p150. HA-p150 only stimulated the activity of MIEP containing the region from −593 to −574 bp (Fig. 4E, lanes 1–14), whereas the MIEP mutants without this region failed to be activated by HA-p150 (lanes 15–20). Next we examined whether this 20-bp sequence was required for p150 binding. The biotin-labeled MIEP deletion mutants with or without this sequence were linked to streptavidin beads and incubated with cell lysates followed by precipitation and Western blot using Ab recognizing the endogenous p150. Consistently, p150 did not bind to MIEP fragments lacking the region from −593 to −574 bp (Fig. 4F, compare lanes 5 and 6 with 2, 3, and 4). Thus we conclude that p150 activated the MIEP activity through physical association with the MIEP from −593 to −574 bp.

p150 Binds to MIEP through its KER Domain—To map the MIEP binding domain of p150, a series of terminally or internally deleted p150 mutants were translated in vitro and examined for their ability to bind to the biotin-labeled MIEP fragment from −733 to −537 bp (Fig. 5A). All p150 mutants lacking the KER domain (aa 311–445) failed to be pulled down by the MIEP fragment, whereas all others containing KER were precipitated. We then analyzed if KER domain alone mediates the MIEP binding or without this domain sequence were linked to streptavidin beads and incubated with cell lysates followed by precipitation and Western blot using Ab recognizing the endogenous p150. Consistently, p150 did not bind to MIEP fragments lacking the region from −593 to −574 bp (Fig. 4F, compare lanes 5 and 6 with 2, 3, and 4). Thus we conclude that p150 activated the MIEP activity through physical association with the MIEP from −593 to −574 bp.

p150 Physically Associates with the MIEP from −593 to −573 bp—To understand the mechanism by which p150 activated MIEP, first we examined the promoter region responding to p150-mediated activation. Deletion fragments of MIEP were fused to the luciferase reporter and transfected into cells in the absence or presence of HA-p150, followed by the luciferase assay. HA-p150 only up-regulated the activity of MIEP containing the region between −733 and −541 bp (Fig. 4A), suggesting this region harbors the p150-responsive element. To further study if p150 physically associates with this region, ChIP assays were performed with anti-HA Ab to precipitate DNAs associated with HA-p150. The IgG was used as a control. The ChIPed lysates were harvested 24 h postinfection for analyzing IE protein expression. PCNA and actin were used as loading controls.

FIGURE 2. p150 is required for the MIEP activity during HCMV infection. A, p150C antagonizes the activated MIEP-driven reporter activity during HCMV infection. U373MG cells stably expressing HA-p150C were transfected with pMIEP −733/+2, followed by infection with HCMV at an m.o.i. of 1. Cell lysates were harvested 72 h postinfection for analyzing luciferase activity (upper panel) and protein expression (lower panel). B, knocking down p150 impairs the synthesis of the HCMV IE proteins. Three independent p150 constitutively knocked shp150-1, shp150-2, and shp150-3 were infected with HCMV at the indicated m.o.i. Cell lysates were harvested 72 h postinfection for analyzing luciferase activity (upper panel) and protein expression (lower panel).
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641)) failed to bind to MIEP DNA. Furthermore, p150 without the KER domain was defective in MIEP binding (ΔKER), whereas deletion of other regions had no effect (ΔPEST, Δ445–505, Δ296–311, and Δ505–750). It was concluded that KER was the only domain of p150 to associate with the MIEP.

To exclude the possibility that the KER-mediated MIEP association was through other components in the rabbit reticulocyte lysate, the E. coli-expressed, Xpress-tagged KER (X-KER) was examined for its binding to the 32P-labeled MIEP fragment from −733 to −537 bp by the EMSA. We found that a protein-DNA complex was formed when the MIEP fragment was coincubated with X-KER, but not the Xpress-tagged p150 domain containing aa 1–296 (X-1–296) (Fig. 5B, compare lanes 2 and 3 with 1). The presence of X-KER in the protein-DNA complex was further confirmed by the super-shifted band observed upon addition of the anti-Xpress Ab into the binding reaction (Fig. 5B, lane 4). In contrast, adding anti-Xpress Ab into the mixture containing the DNA probe and X-1–296 did not result in any super-shifted band (Fig. 5B, lane 5). The Coomassie Blue staining shows that the two p150 fragments used here were equally expressed at the right size (supplemental Fig. 2). We further tested if KER binding requires the 20-bp sequence from −593 to −574 bp of MIEP. As shown in Fig. 5C, X-KER caused a band shift of 32P-labeled MIEP oligonucleotides from −593 to −537 bp, leaving very little amount of unbound free probe (lane 2). Furthermore, the protein-DNA complex was super-shifted by the anti-Xpress Ab, confirming the presence of X-KER in the shifted complex (Fig. 5C, lane 5). In contrast, most of the DNA fragments containing −573 to −537 bp, which lost the 20-bp p150-responsive sequence, remained un-shifted in the presence of KER (Fig. 5C, lane 4) or together with anti-Xpress Ab (lane 6), indicating the importance of the 20-bp sequence in KER binding.

We next determined whether the KER domain of p150 indeed modu-
expected, the KER domain (Fig. 5D, lane 2), but not other p150 fragments (lanes 3 and 4), decreased the activity of MIEP, presumably through competing with the endogenous p150 for MIEP binding. To further address whether KER can direct other activation domains to act on MIEP, the N-terminal 310 or 504 residues of p150 were replaced with the GalAD. We then examined the ability of the chimera proteins to activate MIEP. As shown in Fig. 5E, GalAD fused to the p150 fragment containing aa 311–938, but not aa 505–938, up-regulated the MIEP activity. Note that only the former fragment contains the KER domain. This suggests that KER might be able to function as an independent DNA binding domain. The possibility was further tested by fusing KER to the VP16 activation domain (VP16AD) from herpes simplex virus. As shown in Fig. 5F, the VP16AD-KER fusion protein activated the MIEP activity (lane 3), whereas GalAD-VP16AD failed to do so (lane 4). As a control, we tested the ability of these two chimera proteins to activate the promoter containing five Gal4-binding sites and found that VP16AD-KER did not stimulate the Gal4-dependent activation, whereas GalAD-VP16 greatly activated the promoter (supplemental Fig. 3). These results show that p150 directly bound to MIEP via the association of KER domain with the MIEP from −593 to −574 bp, and this was critical for the MIEP activity in cells.

p150 Recruits p300 to MIEP by Its Activation Domain—Next the potential activation mechanism of p150 was examined. By ChIP, we found that acetylation of histones H3 and H4 on MIEP region near the p150-binding site was increased in the presence of full-length p150, but not the p150 mutant lacking the N-terminal activation domain (Fig. 6, top and middle panels). We also found that the coactivator p300, a histone acetyltransferase, was recruited to this region when full-length p150 was overexpressed (Fig. 6, bottom panel). Histone acetylation has been shown to increase the transcriptional activity by either changing the DNA-histone interaction or serving as a signal to recruit other transcriptional regulators (Ref. 28 and references therein). These results suggest that the activation domain of p150 recruited p300 to acetylate histones around MIEP and activated MIEP.

DISCUSSION

Whether CAF1 subunits have functions beyond chromatin assembly remains to be explored. In this study, we show that p150 transcriptionally increased the activity of the HCMV MIEP...
FIGURE 5. Characterization of the DNA binding domain of p150. A, KER domain (residues 311–445) is critical for p150 binding to MIEP. In vitro DAPA experiments similar to Fig. 4 were performed except that the MIEP oligo on beads was incubated with various in vitro translated, 35S-labeled p150 fragments. The GFP sequence and two nuclear localization sequences (NLS) from SV40 large T protein (GFP/2LTNLS) were fused to the KER domain (GFP/KER) or ED domain (GFP/505–641). Main panel, schematic diagram of the p150 deletion mutants. Right panel, autoradiography results. PEST, domain enriched in proline (P), glutamic acid (E), serine (S), and threonine (T); KER, domain enriched in lysine (K), glutamic acid (E), and arginine (R); ED, domain enriched in glutamic acid (E) and aspartic acid (D).

B, E. coli-expressing KER domain binds to MIEP region from 733 to 537 bp. The E. coli-expressing His-Xpress-fused aa 311–445 (X-KER, lanes 3 and 4) or 1–296 (X-(1–296), lanes 2 and 5) of p150 was incubated with 32P-labeled MIEP DNA fragments from 733 to 537 bp, followed by EMSA. The anti-Xpress antibody was used for super-shifting the DNA-protein complex. The reaction mixtures were separated on a 4% native acrylamide gel that was then subjected to autoradiography.

C, MIEP region from 593 to 574 bp is required for KER binding to MIEP in vitro. The EMSA experiment similar to B was performed except that 32P-labeled MIEP DNA fragment from 593 to 574 bp (lanes 1, 2, and 5) or 573 to 537 bp (lanes 3, 4, and 6) was used, and the reaction mixtures were separated on a 6% native acrylamide gel.

D, overexpression of KER suppresses the MIEP activity in cells. H1299 cells were transfected with pMIEP/733/2 together with vector or the plasmid encoding two NLS sequence-fused aa 311–445 (2NLS-KER-V5, lane 2), 445–564 (2NLS-445–564-V5), or 505–641 (2NLS-505–641-V5) of p150. Cell lysates were harvested 24 h post-transfection and analyzed for luciferase activity (upper panel) and protein expression (lower panel).

E, KER domain is required to guide the activation domain of Gal4 protein (GalAD) to act on MIEP. H1299 cells were transfected with pMIEP–733/+2 together with vector or the plasmid encoding the GalAD fused to the p150 fragment from aa 311–938 (lane 2) or 505–938 (lane 3) and analyzed for luciferase activity (upper panel) and protein expression (lower panel). F, KER alone directs the activation domain of VP16 protein (VP16AD) to act on MIEP. H1299 cells were transfected with pMIEP–733/+2 and the Renilla reporter driven by SV40 promoter, together with vector or the plasmid encoding the full-length p150-V5 (lane 2), the VP16AD fused to KER (VP16AD-KER, lane 3), or VP16AD fused to the DNA binding domain of Gal4 protein (GalDBD-VP16AD, lane 4) and analyzed for luciferase activity (upper panel) and protein expression (lower panel).
Transcriptional Activation by p150

p150 activated MIEP. Because distinct functions have been implicated for each subunit of the CAF1 complex based on the earlier fractionation study, which shows the unique distribution pattern of the three subunits during S phase of the cell cycle (18), it was not too surprising to find p150 alone modulated the MIEP transcription independently of p60.

The KER domain was important for p150 to associate with the MIEP. This was supported by the in vitro DAPA (Fig. 5A) and EMSA (Fig. 5, B and C) experiments. The results were consistent no matter whether the KER used was translated in vitro from the rabbit reticular lysate or expressed in E. coli cells. Thus it remains an intriguing question whether other KER-containing proteins show DNA binding activity. Therefore, we searched the data base (nonredundant SwissProt sequences of basic local alignment search tool at the NCBI web site) for proteins containing homologous sequences to KER. Two potential proteins were identified, which are BAZ2B (bromodomain adjacent to zinc finger domain protein 2B) and translation initiation factor IF-2 from Pseudoalteromonas haloplanktis, a marine bacterium. BAZ2B is a member of novel bromodomain-containing protein family (29), whose KER-like domain shares 43% of the identity with the p150 KER region from aa 338 to 396. P. haloplanktis IF-2 also shares 41% of the identity with the p150 KER region from aa 312 to 370. To date, no function has been assigned to the KER-like domain of these two proteins. By computer modeling (ModWeb Modeling Server), we found that KER of both p150 and BAZ2B likely forms a helical coiled-coil structure. However, it is not clear whether the structure could form a leucine zipper-like DNA binding domain.

The MIEP region from −593 to −574 bp, 5′-TTGATTATGACTAGTTATT′-3′, was identified to be the p150-responsive element (Fig. 4). Because of the high AT content (80%) in the sequence, the binding of KER to MIEP in EMSA resulted in the smearing pattern (Fig. 5), similar to the AT binding of other proteins such as the high mobility group protein 1 (30). It is likely that the DNA-binding mechanism mediated by p150 in this case is distinct from that used for p150 when it binds to p60 in the CAF1 complex and nonspecifically associates with the replicating chromatin. The latter case depends largely on the association of p150 and PCNA (5, 6) for DNA replication foci targeting and both KER and ED domains for full chromatin assembly activity, presumably through interacting with histones (1). In our study, the ED domain was not required for p150 to bind MIEP (Fig. 5A).

In summary, we have provided evidence to show that p150 may directly regulate transcription independently of its chromatin assembly activity. Similarly, another histone chaperon SET, a member of the NAP1 family, was also reported to function as a transcription factor to modulate the activity of the p450c17 promoter (31, 32). Thus, chromatin assembly factors may not just simply escort histones. To explore potential p150-regulated genes, sequence alignment was performed with basic local alignment search tool at the NCBI web site. The 20-bp p150-responsive element-like motif was found in 2995 loci in the human genome. Among them, 76 are located within 10 kb of the 5′ upstream regulatory region of functionally known genes. Most proteins encoded by these genes are involved in signaling transduction, gene regulation, metabolism, viability,
and development. Whether p150 exerts its transactivation function on these genes requires further investigation.

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