WD Repeats of the p48 Subunit of Chicken Chromatin Assembly Factor-1 Required for \textit{in Vitro} Interaction with Chicken Histone Deacetylase-2*

(Received for publication, November 9, 1998, and in revised form, March 1, 1999)

Ahyar Ahmad, Yasunari Takami, and Tatsuo Nakayama‡

From the Department of Biochemistry, Miyazaki Medical College, Kihara, Kiyotake, Miyazaki 889-1692, Japan

Chromatin assembly factor-1 (CAF-1) is essential for chromatin assembly in eukaryotes, and comprises three subunits of 150 kDa (p150), 60 kDa (p60), and 48 kDa (p48). We cloned and sequenced cDNA encoding the small subunit of the chicken CAF-1, chCAF-1p48. It consists of 425 amino acid residues including a putative initiation Met, possesses seven WD repeat motifs, and contains only one amino acid change relative to the human and mouse CAF-1p48s. The immunoprecipitation experiment followed by Western blotting revealed that chCAF-1p48 interacts with chicken histone deacetylases (chHDAC-1 and -2) \textit{in vivo}. The glutathione S-transferase pulldown affinity assay revealed the \textit{in vitro} interaction of chCAF-1p48 with chHDAC-1, -2, and -3. We showed that the p48 subunit tightly binds to two regions of chHDAC-2, located between amino acid residues 82–180 and 245–314, respectively. We also established that two N-terminal, two C-terminal, or one N-terminal and one C-terminal WD repeat motif of chCAF-1p48 are required for this interaction, using deletion mutants of the respective regions. These results suggest that chCAF-1p48 is involved in many aspects of DNA-utilizing processes, through alterations in the chromatin structure based on both the acetylation and deacetylation of core histones.

Understanding the process of chromatin assembly in eukaryotes is a fundamental goal, because alterations in the chromatin structure have been thought to be predominantly involved in DNA-utilizing processes, such as replication, recombination, repair, and gene expression (1–9). Chromatin assembly factor-1 (CAF-1)\(^1\) was originally purified from human cells and promotes chromatin assembly on replicating SV40 DNA in the presence of a cytosol replication system (10, 11). CAF-1 is a complex of three polypeptides of 150 kDa (p150), 60 kDa (p60), and 48 kDa (p48) (12). During DNA replication, CAF-1 assembles new nucleosomes through a two-step reaction (10, 13). Coupled to DNA replication, as the first step, histones H3 and H4 are deposited through a reaction that is preferentially dependent upon CAF-1, but histones H2A and H2B are added later to this immature nucleosome precursor, even in the absence of CAF-1 (12, 14, 15). These results indicate that CAF-1 interacts preferentially with H3 and H4, whereas NAP-1 binds to H2A and H2B (2, 16).

The WD protein family members, which are made up of highly conserved WD repeating units, found in eukaryotes, but not in prokaryotes, are involved in numerous biological processes such as signal transduction, RNA processing, gene expression, vesicular trafficking, and cell division (17–19). Thus, most of them seem to be regulatory, and none is an enzyme. The consensus core of the repeating unit, the WD repeat, usually ends with the characteristic sequence, Trp-Asp (WD), and such a conserved unit occurs four to ten times within each polypeptide (19). Each repeat comprises a region of variable length preceding a conserved core of about 30 amino acids (maximum range, 23–41 amino acids), ending with Gly-His (GH) and WD dipeptide residues. In addition, the number of amino acids from WD to the next downstream GH is very variable (6–94 amino acids), although shorter sequences are more common (112 residues long). All of these WD repeat proteins have been proposed to fold into propellers in which the internal \(\beta\)-strands form a rigid skeleton that is fleshed out on the surface by specialized loops to which other proteins bind (19–22). The amino acid residues in the WD repeats of Tup1, the yeast repressor, that are required for the interaction of Tup1 with homeodomain protein \(\alpha\)2 have been genetically identified (23). Point mutations in the WD40 domains of the Eed (embryonic ectoderm development) protein block its interaction with Ezh2, a mammalian homolog of the \textit{Drosophila enhancer of zeste \(E(z)\)} (24).

CAF-1p48, with seven WD repeat motifs, is a member of this WD repeat protein family. In recent years, knowledge concerning the characteristics of CAF-1s in the DNA-utilizing processes has been rapidly accumulated (9). For instance, CAF-1p48 was identified as a polypeptide that is tightly associated with the catalytic subunit of human histone deacetylase-1 (HDAC-1) (25). In addition, the smallest subunit of \textit{Drosophila} CAF-1, p55, is homologous to a mammalian factor, RbAp48, associated with HDAC-26. Interestingly, \textit{Drosophila} p55 was reported to be an integral subunit of the nucleosome remodeling factor (NURF) (27). However, there was little information concerning the detailed mechanisms for the protein-protein interaction of CAF-1p48 in higher eukaryotes.

In this study we first cloned the cDNA encoding chicken CAF-1p48, chCAF-1p48, and demonstrated that it tightly binds to chHDACs \textit{in vivo} and \textit{in vitro}. We describe the \textit{in vitro} interaction of chCAF-1p48 with two regions of chHDAC-2, comprising amino acid residues 82–180 and 245–314, respectively. We also describe that this interaction requires two N-terminal, two C-terminal, or one N-terminal plus one C-terminal WD repeat of chCAF-1p48, as deletion of the respective regions results in a loss of the binding activity.

\* This work was supported in part by a grant-in-aid from the Ministry of Education, Science, Sports and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

\^1 The abbreviations used are: CAF-1, chromatin assembly factor-1; ch, chicken; GST, glutathione S-transferase; HDAC, histone deacetylase; PCR, polymerase chain reaction; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis; IPTG, isopropyl \(\beta\)-D-thiogalactopyranoside; BSA, bovine serum albumin.
INTERACTION OF CHCAF-1P48 WITH CHHDAC-2

EXPERIMENTAL PROCEDURES

Materials—In this study the XL-1-Blue MRF² Escherichia coli strain, E. coli SORL strain (Stratagene), and E. coli BL-21 strain (Amersham Pharmacia Biotech) were used. pBluescript II SK (−) and pCE4a (−) were purchased from Promega. The pGEX-2TK gene fusion vector and glutathione-Sepharose beads were products of Amersham Pharmacia Biotech.

Cloning and Sequencing of cDNA Encoding chCAF-1p48—Based on conserved amino acid sequences (LVMTHALEWP and PNEPWVICSV) in the mouse and human CAF-1p48s, using the resultant PCR product as a probe, we screened the chicken DT40 cDNAs using the two degenerate primers. To obtain part of the coding regions of mammalian CAF-1p48s, was prepared from the chicken DT40 cDNAs using the two degenerate primers. To obtain full-length chCAF-1p48 cDNAs, using the resultant PCR product as a probe, we screened the DT40 AZAP II cDNA library constructed by us,² using poly(A) mRNA prepared from the chicken DT40 cell line, essentially as described (28). The entire nucleotide sequences of both strands of the largest cDNA insert were sequenced by the dye terminator method (Applied Biosystems Division, Perkin-Elmer).

Immunoprecipitation and Western Blotting—To construct a vector, designated pET-HAchCAF-1p48, expressing chCAF-1p48 under the control of the tetracycline operon (tetO) and cytomegalovirus minimal promoter, cDNA encoding influenza H1A epitope-tagged chCAF-1p48 was amplified by PCR using a full-length chCAF-1p48 cDNA, which was prepared from the chicken DT40 cDNAs using the two degenerate primers. To obtain full-length chCAF-1p48 cDNAs, using the resultant PCR product as a probe, we screened the DT40 AZAP II cDNA library constructed by us,² using poly(A) mRNA prepared from the chicken DT40 cell line, essentially as described (28). The entire nucleotide sequences of both strands of the largest cDNA insert were sequenced by the dye terminator method (Applied Biosystems Division, Perkin-Elmer).

Cloning and Sequencing of cDNA Encoding chCAF-1p48—Based on conserved amino acid sequences (LVMTHALEWP and PNEPWVICSV) in the mouse and human CAF-1p48s deduced from their cDNAs (15, 21), conserved amino acid sequences (LVMTHALEWP and PNEPWVICSV) in the mouse and human CAF-1p48s were determined. The resultant PCR product to yield pCiteHAp48 encoding amino acids 1–19 of chCAF-1p48 and amino acids 12–19 of HA. Next we prepared the plasmid pCiteHAp48-(181–425), using an ECL kit according to the manufacturer’s instructions.

Cells (1.5 × 10⁸) were lysed in 1 ml of RIPA buffer (25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride) with occasional inversion. After standing for 30 min at 4°C, the lysate was centrifuged at 15,000 rpm for 10 min, and the supernatant obtained was divided into two equal portions. To each portion, 5 μg of anti-HA antibody (12CA5, Roche Molecular Biochemicals) or anti-FLAG antibody (Eastman Kodak Co.) were added. After standing for 60 min on ice, protein G-Sepharose beads (40 μl) were added to the mixture. Following gentle rotation, 5 h at 4°C, the protein G-Sepharose beads were pelleted by centrifugation at 3,500 rpm for 2 min and washed with the lysis buffer plus 0.1% Triton X-100 and 0.1% phenylbenzosulfonyl fluoride four times, then with phosphate-buffered saline buffer once. The GST fusion protein-bound beads were collected by gentle rotation. The GST fusion protein-bound beads were collected by gentle rotation. The GST fusion protein-bound beads were collected by gentle rotation. The GST fusion protein-bound beads were collected by gentle rotation.

We generated deletion mutants of chCAF-1p48 as follows. pCiteHAp48-(1–367) and pCiteHAp48-(1–375), respectively, were generated by digestion of the XcmI/SalI fragment encoding amino acids 268–425 and the EcoRV/BamHI fragment encoding amino acids 376–425 from pCiteHAp48 with digestion by XcmI plus SalI and EcoRV plus BamHI, followed by religation after blunt-end trimming with Klenow polymerase. To generate pCiteHAp48-(1–326), the XcmI/SalI fragment of chCAF-1p48 cDNA was excised from pCiteHAp48 and introduced between the XcmI and SalI sites of the same plasmid. pCiteHAp48-(55–425), pCiteHAp48-(139–425), and pCiteHAp48-(268–425), respectively, were generated by digestion of the SalI/NcoI fragment encoding amino acids 1–81, amino acids 376–488, and amino acids 181–488 of chCAF-1p48 and amino acids 1–19 of HA. Next we prepared the plasmid pCiteHAp48-(181–425), using an ECL kit according to the manufacturer’s protocol (Amerham Pharmacia Biotech). Horseradish peroxidase-conjugated anti-mouse IgG for HA antibody (Dako) and horseradish peroxidase-conjugated anti-rabbit IgG for chCAF-1p48 antibody were used as secondary antibodies.

Plasmid Construction—To construct the pGEX-2TKchCAF-1p48 plasmid, the NcoI/SalI fragment containing the full-length chCAF-1p48 cDNA was excised from the parental plasmid (pBluescriptII/IISKp84), blunt-ended with T4 polymerase, and then subcloned into the SalI site of the pGEX-2TK plasmid. We used the pGEX-

2TKchCAF-1p48-2 as follows. The parental chimeric plasmid (pBluescriptII/IISKhHDAC-2) carrying the full-length chCAF-1p48 cDNA was digested with KpnI, followed by treatment with T4 polymerase. From the resultant blunt-ended chCAF-1p48 cDNA, the KpnI blunt-ended XhoI fragment containing the full-length chCAF-1p48 cDNA was excised and subcloned between the BamHI (blunt-ended by T4 polymerase) and XhoI sites of the pGEX-2TK plasmid.

We constructed the pCiteHDAC-1, -2, and -3 plasmids as follows. The three parental plasmids, pBluescriptII/IISKhHDAC-1, -2, and -3,² were digested with ClaI, blunt-ended with T4 polymerase, and then digested with NcoI. The resultant blunt-ended ClaI/NcoI fragments, carrying the full-length chCAF-1p48 cDNA, were subcloned between the NcoI and SalI sites of the pCite4a(−) plasmid.

DELETION MUTANTS OF CHHDAC-2—Deletion mutants of chCAF-1p48 were constructed as follows. The StuIPstI fragment encoding amino acids 82–488, the HindIII/PstI fragment encoding amino acids 115–488, the XcmI/PstI fragment encoding amino acids 181–488, the NcoI/SalI fragment encoding amino acids 315–488, and the FspIPstI fragment encoding amino acids 371–488 of chCAF-1p48, respectively, were digested by deletion of pCiteHAp48 encoding amino acids 1–19 and amino acids 12–19 of HA. Next we prepared the plasmid pCiteHAp48-(1–326), using an ECL kit according to the manufacturer’s instructions.

Expression and Purification ofGST Fusion Proteins—In this study the XL-1-Blue MRF² Escherichia coli BL-21 cells were transformed with pGEX-2TKchCAF-1p48 and pGEX-2TKchHDAC-2, respectively, harboring the full-length chCAF-1p48 and chHDAC-2 cDNAs and grown to an A₅₅₀ ≈ 0.2 in 400 ml of LB medium supplemented with 200 μg/ml ampicillin. Upon induction with 50 μM isopropyl β-D-thiogalactopyranoside (IPTG) overnight at 20°C, the cells were collected by centrifugation and suspended in 10 ml of lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) containing 0.1% (v/v) phenylbenzoquinol fluoride and 1 mg/ml lysozyme, in liquid N₂ for 2 min, followed by ultrasonication for 3 min. Continuously, the cell lysate was added to Sarkosyl to a final concentration of 1%. After standing for 60 min, Triton X-100 was added to a final concentration of 1%, and then the cell lysate was allowed to stand for an additional 30 min. The cell homogenate was subsequently put on a 300,000 g supernatant, mixed with 1 ml of a 50% slurry of glutathione-agarose beads for 4 h with gentle rotation. The GST fusion protein-bound beads were collected by centrifugation at 3,500 rpm for 2 min and washed with the lysis buffer containing 1% Triton X-100 and 0.1% phenylbenzoquinol fluoride four times, then with phosphate-buffered saline buffer once. The GST fusion proteins were eluted with 4 ml of 20 mM glutathione in 50 mM Tris-HCl,
pH 8.5, and the resultant eluate was concentrated with a Millipore membrane, followed by the addition of glycerol to a final concentration of 20%. The samples obtained were resolved by 10% SDS-PAGE, essentially as described (28).

GST Pulldown Affinity Assay—To produce [35S]Met-labeled full-length chCAF-1p48 and chHDAC-1, -2, and -3, a set of truncated mutants of chCAF-1p48, and a set of truncated mutants of chHDAC-2, the TNT-coupled transcription-translation system (Promega) was used. In vitro binding assays were performed using 5 mlof [35S]Met-labeled protein fractions and 4 mg of the GST fusion proteins or 6 mg of GST, prepared as described, in 200 ml of bead-binding buffer (50 mM potassium phosphate buffer, pH 7.5, 450 mM KCl, 10 mM MgCl₂, 10% glycerol, 1% Triton X-100, 1% BSA). After 1 h of standing, the reaction mixture was added to 20 ml of a 50% slurry of glutathione-agarose beads, containing 10 mg/ml BSA and 4 mg of EtBr, followed by gentle rotation for 1 h at 4°C. The affinity beads were collected by centrifugation at 3,500 rpm for 2 min and then washed with 1 ml of GST bead-binding buffer without BSA and EtBr but containing 0.1% phenylbenzosulfonyl fluoride five times. The beads were suspended in 30 ml of 2x SDS sample buffer and then boiled for 5 min. Aliquots (15 ml) of the resultant eluates were analyzed by 12% SDS-PAGE, and then the gels were stained, dried, and subjected to fluorography.

RESULTS

Cloning of cDNA Encoding chCAF-1p48—To determine unequivocally the identification of chCAF-1p48 as a novel subunit of CAF-1, we cloned and sequenced its cDNA. Based on conserved amino acid sequences (the corresponding sequences in the chicken homolog are underlined in Fig. 1A), the seven WD (including PD and WN) dipeptide motifs are boxed. Two regions corresponding to those in the human and mouse CAF-1p48s, were underlined. B, the WD repeat motifs of chCAF-1p48. The characteristic GH and WD dipeptide motifs are boxed. Each WD repeat contains a variable region of 5-29 amino acid residues and a core of 32 amino acid residues.
precipitated with anti-HA or anti-FLAG antiserum. Aliquots of proteins were probed with anti-chHDAC-1 or -2 antiserum (12% SDS-PAGE. After being transferred to a nitrocellulose membrane, noprecipitated with anti-HA or anti-FLAG antiserum were subjected to the lysates of DT40 and tetHAp48 cells and tetHAp48 samples immunoprecipitated with anti-HA or anti-FLAG antiserum to determine whether or not chCAF-1p48 binds to these chHDACs in vivo. Western blotting, using anti-chHDAC-1 and -2 antisera, against, respectively, recombinant chHDAC-1 and -2 C-terminal peptide-GST fusion proteins was carried out. DT40 cells were first co-transfected with p6tet-HAchCAF-1p48 and pTACbleo, and the transfected cells that express the HA-tagged chCAF-1p48 were selected with phleomycin. A cell line (tetHAp48) overexpressing the HA-tagged protein in the absence of tetracycline was established. Proteins in the lysate of tetHAp48 cells were immunoprecipitated with anti-HA or anti-FLAG antiserum. The immunoprecipitated samples, together with the HA-p48, HA-tagged chCAF-1p48, IgG-H, mouse IgG heavy chain.

Therefore, chCAF-1p48 is a member of the WD repeat protein family and possesses seven copies of the WD motif, a motif in 37–61 amino acid residues, including a WD, FD, or WN dipeptide (Fig. 1A). Like most proteins containing WD repeats, chCAF-1p48 is expected not only to physically associate with other proteins but also to act as a scaffold upon which multimolecular complexes can be built.

In Vivo Interaction of chCAF-1p48 with chHDACs—To determine whether or not chCAF-1p48 binds to these chHDACs in vivo, Western blotting, using anti-chHDAC-1 and -2 antisera, against, respectively, recombinant chHDAC-1 and -2 C-terminal peptide-GST fusion proteins was carried out. DT40 cells were first co-transfected with p6tet-HAchCAF-1p48 and pTACbleo, and the transfected cells that express the HA-tagged chCAF-1p48 were selected with phleomycin. A cell line (tetHAp48) overexpressing the HA-tagged protein in the absence of tetracycline was established. Proteins in the lysate of tetHAp48 cells were immunoprecipitated with anti-HA or anti-FLAG antiserum. The immunoprecipitated samples, together with the HA-p48, HA-tagged chCAF-1p48, IgG-H, mouse IgG heavy chain.

In contrast, anti-chHDAC-1 antiserum recognized the protein band corresponding to chHDAC-1 in the cell lysates of both tetHAp48 and DT40 cells (Fig. 2B). Furthermore, as expected, the same (chHDAC-1) band showed up for the sample immunoprecipitated with anti-HA antiserum but not that immunoprecipitated with anti-FLAG antiserum. Similarly, anti-chHDAC-2 antiserum recognized the band corresponding to chHDAC-2 in the lysates of both tetHAp48 and DT40 cells, as well as the sample immunoprecipitated with anti-HA antiserum (Fig. 2C). Reasonably, no band showed up for the sample immunoprecipitated with anti-FLAG antiserum. These results indicate clearly that both chHDAC-1 and -2 were present in the sample immunoprecipitated with anti-HA antiserum, suggesting that the two enzymes bind tightly to chCAF-1p48 in vivo.

Expression and Purification of GST-chCAF-1p48 Fusion Protein in E. coli—To construct a chimeric plasmid, pGEX-2TKchCAF-1p48, expressing the GST-chCAF-1p48 fusion protein, the chCAF-1p48 cDNA was subcloned into the pGEX-2TK plasmid in frame. GST fusion proteins were synthesized in E. coli, extracted, and purified essentially as described above. As shown in Fig. 3, the electrophoretic patterns on SDS-PAGE of whole cell lysates before and after the induction with IPTG revealed that GST-chCAF-1p48 fusion proteins of approximately 74 kDa were dramatically accumulated in E. coli BL-21 cells containing the pGEX-2TKchCAF-1p48 plasmid. In addition, the GST-chCAF-1p48 fusion proteins were purified to more than 95% homogeneity, using glutathione-agarose beads (see lane 3 in Fig. 3).

In Vivo Interaction of chCAF-1p48 with chHDAC-1, -2, and -3—We cloned and sequenced three cDNAs, encoding chH- DAC-1, -2, and -3, respectively, which comprise 480, 488, and 428 amino acid residues, including a putative initiation Met. Compared with chHDAC-1 and -2, the C-terminal region of chHDAC-3 is about 50 amino acids shorter. However, in the corresponding regions all three chHDACs exhibit extensive homology (95%). Furthermore, we recently developed E. coli systems with which all of chHDAC-1, -2, and -3 could be dramatically synthesized. These recombinant chHDAC-1, -2, and -3, like recombinant maize HDAC-2 expressed in E. coli (30), exhibited little enzymatic activity in vitro (data not shown). As
Two Regions of chHDAC-2 Required for in Vitro Interaction with chCAF-1p48—To determine the putative binding region(s) of chCAF-1p48, we constructed a series of C-terminal truncated mutants of HA-tagged chCAF-1p48 and studied the in vitro interaction with the GST-chHDAC-2 fusion protein, essentially as described above. Two truncated proteins, ΔchCAF-1p48-(1–328) and ΔchCAF-1p48-(1–267), exhibited no binding activity, although the other one (ΔchCAF-1p48-(1–375)) exhibited binding activity similar to that of the parental chCAF-1p48 protein (Fig. 6). These findings suggested that the deletion of the region comprising amino acids 329–375, in addition to the C-terminal region comprising amino acids 376–425, resulted in the loss of the binding activity.

Next we constructed a series of N-terminal truncated mutants of HA-tagged chCAF-1p48 and studied their binding abilities as to chHDAC-2, essentially as described above. As shown in Fig. 6, the abilities of two truncated mutant proteins, ΔchCAF-1p48-(55–375) and ΔchCAF-1p48-(139–425), were the same as that of the parental chCAF-1p48 protein. On the other hand, two other truncated proteins, ΔchCAF-1p48-(181–425) and ΔchCAF-1p48-(268–425), did not exhibit the ability. These results indicated that the deletion of the region comprising amino acids 139–180, in addition to the N-terminal region comprising amino acids 1–138, resulted in the loss of the binding ability.

Moreover, we constructed two truncated mutants, respectively, with simultaneous deletion of both the N-terminal and C-terminal regions of chCAF-1p48 and assayed their binding activities essentially as described above. Fig. 5 shows that ΔchHDAC-2-(245–370), like ΔchHDAC-2-(82–370) and ΔchHDAC-2-(162–370), exhibited binding activity, although it even lacks the region comprising amino acids 115–180. In addition, ΔchHDAC-2-(315–488), lacking the N-terminal region comprising amino acids 1–314, exhibited no activity, whereas ΔchHDAC-2-(1–314) bearing the same N-terminal region exhibited the binding ability.

These results suggested that two possible binding regions of chHDAC-2 as to chCAF-1p48 are located between amino acids 115–180 and amino acids 245–314, respectively, and that either is enough as the binding region. Finally, to confirm these results we constructed two mutant proteins, ΔchHDAC-2-(82–180) and ΔchHDAC-2-(245–314), and studied their binding activities. As expected, the two regions definitely interacted with chCAF-1p48, indicating that either is enough for the in vitro interaction with chCAF-1p48. Moreover, the findings that both regions were extensively conserved within chHDAC-1 and -3, as in homologs from other organisms, and that chCAF-1p48 bound to the two chHDACs (see Fig. 4) suggest the possible involvement of the two corresponding domains of all HDACs in their in vitro interactions with CAF-1p48.
425) and ΔchCAF-1p48-(55–375), exhibited the binding activity but ΔchCAF-1p48-(139–375) did not revealed that the simultaneous lack of an N-terminal WD repeat and a C-terminal one also causes loss of the binding ability. Taken together our results indicate that among the seven WD repeats of chCAF-1p48, two N-terminal, two C-terminal, or one N-terminal and one C-terminal, one is necessary for the in vitro interaction of the p48 subunit with chHDAC-2.

**DISCUSSION**

Eukaryotic HDACs, like acetylpolysine amidohydrolases, are members of a deacetylase superfamily of proteins that not only recognize an acetylaminopropyl group and catalyze the removal of an acetyl group by cleaving a non-peptide amide bond but also that share nine blocks, 1 to 9, exhibiting considerable sequence homology (31). All of them are located in the approximately two-thirds N-terminal region of each of three
Two binding domains, designated as BD1 and BD2, comprising amino acids 82–180 (in fact, probably 115–180) and amino acids 245–314 of chHDAC-2 (Fig. 5), respectively, contain blocks 3, 4, and 5 plus approximately half of the N-terminal region of block 6 and approximately half of the C-terminal region of block 8 plus block 9. BD1 and BD2 are also both located in the corresponding regions of chHDAC-1 and -3 as in those of other mammalian HDAC homologs (25, 32, 33). To determine how this interaction of BD1 and/or BD2 with chCAF-1p48 is involved in DNA-utilizing processes, such as replication, recombination, repair, and gene expression, further studies must be performed.

The amino acid sequence of chCAF-1p48 shows that it is a member of the superfamily of WD repeat proteins (Fig. 1). All of the seven WD repeats found in it well match those in the small subunits of CAF-1s in most eukaryotes (15, 25, 27). chCAF-1p48 exhibits binding ability as to chHDAC-2 (and chHDAC-1 and 3) when the second WD dipeptide motif and the shorter region of 13 amino acids preceding it remain, but the p48 subunit loses the ability when both the first and second WD repeats with the variable region of 27 amino acids just behind the second WD dipeptide motif are lacking (Fig. 6). On the other hand, the binding ability of chCAF-1p48 disappears when the region of 16 amino acids preceding the sixth WD dipeptide motif, together with this motif and the seventh one, is lacking. Conversely, the ability does not change even when the shorter region of 6 amino acids preceding the seventh WD dipeptide motif, together with both this motif and the C-terminal region of the protein, is lacking. Interestingly, even when the first WD repeat plus the region of 44 amino acids just behind the first WD dipeptide motif, or the shorter region of 6 amino acids preceding the seventh WN dipeptide motif, plus the
Acknowledgment—We are grateful to Dr. C. M. Tiree for the pAS1 plasmid.

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