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Distinct Regions of the Chicken p46 Polypeptide Are Required for Its *in Vitro* Interaction with Histones H2B and H4 and Histone Acetyltransferase-1

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We cloned cDNA encoding the chicken p46 polypeptide, chp46, homologous to the p48 subunit of chicken chromatin assembly factor-1, chCAF-1p48. It comprises 424 amino acids including a putative initiation Met, is a member of the WD protein family, with seven WD repeat motifs, and exhibits 90.3% identity to chCAF-1p48 and 94.3% identity to the human and mouse p46 polypeptides (hup46 and mop46). The *in vitro* immunoprecipitation experiment established that chp46 interacts with histones H2B and H4 and chicken histone acetyltransferase-1, chHAT-1, whereas hup46 interacts with histones H2A and H4 and chHAT-1 and chCAF-1p48 with histone H4 and chHAT-1. The *in vitro* immunoprecipitation experiment, involving truncated mutants of chp46, revealed not only that two regions comprising amino acids 33–179 and 375–404 are necessary for its binding to H2B, but also that two regions comprising amino acids 1–32 and 405–424 are necessary for its binding to H4. Furthermore, the GST pulldown affinity assay, involving truncated mutants of chp46, revealed that a region comprising amino acids 359–404 (in fact, 375–404) binds to chHAT-1 *in vitro*. Taken together, these results indicate not only that chp46 should participate differentially in a number of DNA-utilizing processes through interactions of its distinct regions with chHAT-1 and histones H2B and H4, but also that the proper propeller structure of chp46 is not necessary for its interaction with chHAT-1. © 2000 Academic Press

Chromatin assembly factor-1 (CAF-1) is preferentially involved in chromatin assembly in eukaryotes, comprises three subunits of 48 kDa (p48), 60 kDa (p60), and 150 kDa (p150) (1–3), and assembles new nucleosomes through a two-step reaction during DNA replication (4, 5). As the first step, histones H3 and H4 are deposited through a reaction that is dependent upon CAF-1, and later histones H2A and H2B are added to this immature nucleosome precursor, even in the absence of CAF-1 (3, 5–7). In recent years, knowledge concerning the characteristics of the p48 subunit of CAF-1 (CAF-1p48), which has seven WD repeat motifs and is a member of the WD protein family, in numerous DNA-utilizing processes has been accumulated (8). CAF-1p48 was identified as a polypeptide that is tightly associated with the catalytic subunit of human histone deacetylase-1 and -2 (HDAC-1 and -2) (9). The small subunit of *Drosophila* CAF-1, p55, is a homolog of the mammalian factor, Rbap48, associated with HDACs, and is also an integral subunit of the nucleosome remodeling factor (NURF). In addition, the mammalian homologs are contained in large complexes involved in transcription repression, chromatin disruption, chromatin remodeling, and histone acetylation and deacetylation (10–16).

We have found that chicken CAF-1p48, chCAF-1p48, interacts with chHDAC-1 and 2 *in vivo,* in an immunoprecipitation experiment followed by Western blotting (17). Furthermore, the GST pulldown affinity assay, involving deletion mutants of both chCAF-1p48 and chHDAC-2, revealed not only that chCAF-1p48 binds to two regions of chHDAC-2 comprising amino acids 82-180 and 245-314, respectively, but also 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 *in vitro* interaction. Recently, using the gene targeting technique for the chicken DT40 B cell line (18, 19), we established not only that chCAF-1p48 is essential for cell viability, but also that a deficiency of it causes chromosome aberrations, such as decreased DNA synthesis, chromosome instability surrounding newly replicated DNA, increased UV sensi-

Abbreviations used: CAF-1, chromatin assembly factor-1; chCAF-1p48, p48 subunit of chicken CAF-1; HDAC, histone deacetylase; HAT-1, histone acetyltransferase-1; chHAT-1, chicken HAT-1; p46, p46 polypeptide; chp46, chicken p46; hup46, human p46; mop46, mouse p46; GST, glutathione *S*-transferase; HA, hemagglutinin.

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tivity, and nuclear and microtubule abnormalities including chromosome hypercondensation, prior to cell death (Takami *et al.*, in preparation). These chromosome aberrations accompanied by chCAF-1p48 depletion should not be directly related with each other, indicating that the p48 subunit probably participates differentially in a number of DNA-utilizing processes.

On the other hand, the p46 polypeptide (p46), which is a CAF-1p48 homolog, together with the latter, is contained in repressor complexes with HDAC-1 and 2, and mSin3 (the mammalian homolog of Sin3), Rb (the retinoblastoma protein), or Mi2 plus MeCP2, to repress transcription (12–16). Interestingly, p46 associates with a cytoplasmic histone acetyltransferase-1, HAT-1, and this complex has been found to possess the ability to acetylate particular Lys residues, Lys-5 and Lys-12, of histone H4 (20). In addition, p46, like CAF-1p48, has been reported to bind itself to histone H4 (and H2A) (6, 21). To systematically assess the role of each of p46 and p48, recently, we cloned and sequenced cDNA encoding chicken HAT-1 (chHAT-1). It comprises 408 amino acids including a putative initiation Met and exhibits 80.4% identity to the human homolog. The mechanism of the interaction of chHAT-1 with chCAF-1p48 has also been described in detail (Ahmad *et al.*, submitted).

In this study we cloned cDNA encoding the chicken p46, chp46, comprising 424 amino acids including a putative initiation Met, exhibiting 90.3% homology to chCAF-1p48, and carrying seven WD repeat motifs. The immunoprecipitation experiment showed that chp46 binds to histones H2B and H4, and chHAT-1, although human p46, hup46, binds to histones H2A and H4, and chHAT-1, but not to H2B. Furthermore, two regions of chp46 comprising amino acids 33–179 and 375–404 are necessary for its binding to H2B, and two different regions comprising amino acids 1–32 and 405–424 are necessary for its binding to H4. On the other hand, the GST pulldown affinity assay revealed not only that chp46, as well as chCAF-1p48, associates with chHAT-1, but also that a C-terminal region, comprising amino acids 359–404 (in fact, 375–404), is essential for this interaction. These results suggest not only that the proper propeller structure of chp46, probably due to its WD repeat motifs, should not be necessary for its *in vitro* interaction with chHAT-1, but also that chp46 should be involved in a number of DNA-utilizing processes in distinct manners.

MATERIALS AND METHODS

Materials. pBluescript II SK(−) and pCite4a(−) were purchased from Stratagene and Novagen, respectively. Glutathione–Sepharose beads were a product of Amersham Pharmacia Biotech, and anti-FLAG M2 beads were a product of Eastman Kodak, Co.

Cloning and sequencing of cDNA encoding chp46. A PCR product of 435 bp, corresponding to a part of the coding region of hup46, was first prepared from chicken DT40 cDNA, using sense and antisense oligonucleotide primers containing sequences 5′-CTGATGATCAGAAACTTATGATATGGG-3′ and 5′-AGCAAATGACCCAAGGTTCATTGGG-3′, respectively, which were constructed based on the amino acid sequences (DDQKLMIWD and PNEPWVICS) in hup46 deduced from its cDNA (22). To obtain full-length chp46 cDNA, using the resultant PCR product as a probe, we screened the DT40 lambda ZAP II cDNA library (17, 18) as described (23). The entire nucleotide sequences of both strands of the largest cDNA insert were sequenced by the dye terminator method (Applied Biosystems Division, Perkin–Elmer).

Plasmid construction. We constructed the pCiteFLAGp46 plasmid carrying the gene encoding chp46 as described (17, 24). First a sense primer containing *Nco*I (5′) with the sequence 5′-CCATGGCGAGTAAGGAAGTGCTGGAGG-3′ and an antisense primer containing *Sal*I (3′) with the sequence 5′-CTCGAGTTACTGTCCTTGACCTTCCAG-3′ were constructed. Next we prepared the DNA fragment encoding the full-length coding region of chp46 by PCR using the parental plasmid [pB(II)SKp46] carrying the full-length chp46 cDNA (Takami *et al.*, in preparation) as a template with these primers, followed by digestion with *Nco*I plus *Sal*I. We replaced the resultant *Nco*I/*Sal*I fragment with the *Nco*I/*Sal*I fragment of the pCiteFLAGp48 plasmid carrying the gene encoding chCAF-1p48 (Ahmad *et al.*, submitted).

We generated deletion mutants of chp46 as follows. To generate pCiteFLAGp46-(1–404) and pCiteFLAGp46-(1–374), respectively, we first used or constructed a sense 5′-Cite primer containing the sequence 5′-GGGGACGTGGTTTTCCTTTGA-3′ (Novagen) and an antisense primer containing *Sal*I (3′) with the sequences 5′-GGAAGGACTCGAGCTACATTTGCCATATCTGCAT-3′ and 5′-GAAGAAGCTCGAGCTACTTAGCAGTGTGTCCTCC-3′. Next we prepared two DNA fragments lacking the fragments encoding amino acids 405–424 and 375–424, respectively, by PCR using pCiteFLAGp46 as a template with these primers, followed by digestion with *Nco*I plus *Sal*I. The *Nco*I/*Sal*I fragments obtained were each replaced with the *Nco*I and *Sal*I fragment of the pCiteFLAGp46 plasmid. To generate pCiteFLAGp46-(33–424) and pCiteFLAGp46-(180–424), we first prepared two DNA fragments encoding amino acids 33–424 and 180–424, respectively, by PCR using pCiteFLAGp46 as a template with a sense primer containing *Nco*I (5′) with the sequences 5′-CTTTCCATGGTGATGACACACGCGCTGGAG-3′ and 5′- CCTTCCATGGTTAACCATGAAGGTGAAGTG-3′, and an antisense 3′-Cite primer containing the sequence 5′-CGATCAATAACGGTCGCCTGA-3′ (Novagen), followed by digestion with *Nco*I plus *Xho*I. The *Nco*I/*Xho*I fragments obtained were each replaced with the *Nco*I and *Xho*I fragment of the pCiteFLAGp46 plasmid. To generate pCiteFLAGp46-(359–404), we first used or constructed a sense 5′-Cite primer containing the sequence 5′-GGGGACGTGGTTTTCCTTTGA-3′ (Novagen) and an antisense primer containing *Xho*I (3′) with the sequence 5′-AATGCTCGAGCTATGCCATTTGCCAGACTTG-3′. Next we prepared the DNA fragment lacking the fragments encoding amino acids 1–358 and 405–424, by PCR using pCiteFLAGp46-(1–404) as a template with these primers, followed by digestion with *Nco*I plus *Xho*I. The *Nco*I/*Xho*I fragment was introduced between the *Nco*I and *Xho*I sites of the pCiteFLAGp46 plasmid.

To generate the pCiteFLAGhup46 vector expressing FLAG-tagged hup46, the entire coding sequence of the hup46 gene was obtained by RT-PCR from mRNA derived from the Hep2 cell line, and then cloned into the pCite-4a vector. We constructed the pCiteHAH2A, pCiteHAH2B, pCiteHAH3, and pCiteHAH4 plasmids as follows. pGEX-2TKH2A, pGEX-2TKH2B, pGEX-2TKH3, and pGE-X2TKH4 (Takami *et al.*, in preparation) were digested with *Nde*I plus *Sal*I. The *Nde*I/*Sal*I fragments encoding the full-length chicken H2A, H2B, H3, and H4 cDNAs were each replaced with the *Nde*I and *Sal*I fragment of the pCiteHAHAT-1 plasmid (Ahmad *et al.*, submitted). The previously constructed pGEX-2TKchHAT-1 (Ahmad *et al.*, submitted) was also used.

Immunoprecipitation experiment. To produce [^{35}S]Met-labeled full-length chp46, a set of truncated mutants of it, hup46 and chCAF-

1p48, the Single Tube Protein System 3 (Novagen) was used. The *in vitro* immunoprecipitation experiment was performed, with 5 μl of [^{35}S]Met-labeled full-length FLAG-tagged chp46, its mutants, hup46 or chCAF-1p48, and 5 μl of [^{35}S]Met-labeled HA-tagged histones H2A, H2B, H3, and H4 and chHAT-1 in 200 μl of bead-binding buffer (50 mM potassium phosphate buffer, pH 7.5, 450 mM KCl, 10 mM $MgCl_2$, 10% glycerol, 1% Triton X-100, 1% BSA). After standing for 60 min, 20 μl of the reaction mixture was removed as an input sample, and the remaining mixture was added to 20 μl of anti-FLAG M2 beads (Eastman Kodak Co.), followed by gentle rotation for 60 min at 4°C. The affinity beads were collected by centrifugation at 12,000 rpm for 10 s, and then washed with 1 ml of the bead-binding buffer containing 0.1% 4-(2-aminoethyl)-benzosulfonyl fluoride (AEBSF) three times. The beads were suspended in 30 μl of 2× SDS sample buffer and then boiled for 5 min. Aliquots (15 μl) of the resultant eluates were analyzed by 15% SDS–PAGE, and then the gels were washed with a fluorographic reagent (Amersham Pharmacia Biotech), dried, and subjected to fluorography.

GST pulldown affinity assay. The *in vitro* binding assays were performed, using 5 μl of [^{35}S]Met-labeled full-length FLAG-tagged chp46 or one of its mutants, and 4 μg of the GST–chHAT-1 fusion protein (Ahmad *et al.,* submitted) or 6 μg of GST, prepared as described (17), in 200 μl of the bead-binding buffer. After standing for 60 min, the reaction mixture was added to 20 μl of a 50% slurry of glutathione-Sepharose beads containing 4 μg of EtBr, followed by gentle rotation for 60 min at 4°C. The affinity beads were collected by centrifugation at 3500 rpm for 2 min and then washed with 1 ml of the bead-binding buffer without BSA and EtBr, but containing 0.1% AEBSF five times. The beads were suspended in 30 μl of 2× SDS sample buffer and then boiled for 5 min. Aliquots (15 μl) of the resultant eluates were analyzed by 12 or 15% SDS–PAGE, and then the gels were stained, dried, and subjected to fluorography.

RESULTS AND DISCUSSION

Cloning of cDNA Encoding chp46

To study the characteristics of chp46, we cloned and sequenced cDNA encoding it. Based on conserved amino acid sequences in hup46 and mouse p46 (mop46) (11, 22), we prepared the 435-bp PCR fragment, corresponding to a part of cDNAs encoding hup46 and mop46, by PCR using cDNAs from DT40 cells. Our screening, using the resultant PCR product, of a DT40 lambda ZAP II cDNA library yielded five positive cDNA clones. Sequence analysis of the largest cDNA insert of 1799 bp among them revealed that the clone contained both an initiation codon and a 3′ poly(A) tail, and also appeared to contain the full-length chp46 cDNA sequence. The amino acid sequence deduced from the nucleotide sequence, comprising 424 residues including a putative initiation Met, together with those of chCAF-1p48 (17), and of the human and mouse counterparts of these two polypeptides (6, 9, 22), is shown in Fig. 1. This chicken polypeptide exhibits 94.3% identity in amino acid sequences to hup46 and mop46, and 90.4% identity to chCAF-1p48, which was completely identical with the human and mouse homologs (Takami *et al.,* in preparation). Moreover, it possesses seven copies of the WD motif, a motif of 37–61 amino acids, including a WD, FD, or WN dipeptide. Therefore, this chicken p46 polypeptide (chp46) (Accession No. AF279275) is also a member of the WD protein family and is expected to act as a scaffold upon which multimeric complexes can be built through association with other proteins (25–28).

In Vitro Interaction of chp46 with Histones H2B and H4 and chHAT-1

We have revealed that chCAF-1p48 binds to chHDACs (17) and chHAT-1 (Ahmad *et al.,* submitted) *in vivo* and *in vitro.* On the other hand, it has been reported that a heterodimer of hup46 and HAT-1 is involved in the chemical modification of core histones with acetyl groups, especially in the acetylation of Lys-5 and Lys-12 of histone H4 (20). In addition, the GST pulldown affinity assay revealed that hup46 binds to both histones H4 and H2A (21).

Therefore, to determine whether or not chp46, together with chCAF-1p48 and hup46 as a control, bound to core histones and chHAT-1, we carried out an *in vitro* immunoprecipitation experiment, using [^{35}S]Met-labeled FLAG-tagged chp46, chCAF-1p48, or hup46, and [^{35}S]Met-labeled HA-tagged chicken histone H2A, H2B, H3, or H4 or chHAT-1. Each sample was separated by 15% SDS–PAGE, and then the gels were treated with the fluorographic reagent and subjected to fluorography. As shown in Figs. 2C and 2D, chCAF-1p48 bound to histone H4 but not to other core histones, and this agreed in part with results reported for the human p48, i.e., with the GST pulldown affinity assay, one group has found that the human p48 associates with histones H4 and H2A (21), but another group has reported that it only associates with histone H4 (29). The reason for this discrepancy remains unclear. On the other hand, interestingly, chp46 bound to histones H2B and H4, but not to histones H2A and H3 (Figs. 2B and 2D), although hup46 bound to histones H2A and H4 (Figs. 2A and 2D). This result concerning hup46 agreed with that reported previously for the human one, with the GST pulldown assay (21).

The immunoprecipitation experiment also revealed that chp46, as well as hup46 and chCAF-1p48, could bind to chHAT-1 *in vitro* (Figs. 2A–2C). To confirm these results, we carried out the GST pulldown affinity assay. First, chp46 was translated *in vitro* in the presence of [^{35}S]Met, and then its ability to interact with GST–chHAT-1 was assayed. The sample was separated by 12% SDS–PAGE, and the proteins were stained with Coomassie blue (data not shown), followed by fluorography. As shown in Fig. 3C, the GST–chHAT-1 fusion protein bound to chp46, as well as to chCAF-1p48 (data not shown), whereas under the same conditions β-galactosidase did not bind to it.

Regions of chp46 Required for Its Binding Ability as to Histones H2B and H4 and chHAT-1

Next we determined the region(s) of chp46 necessary for its binding ability as to three proteins, histones

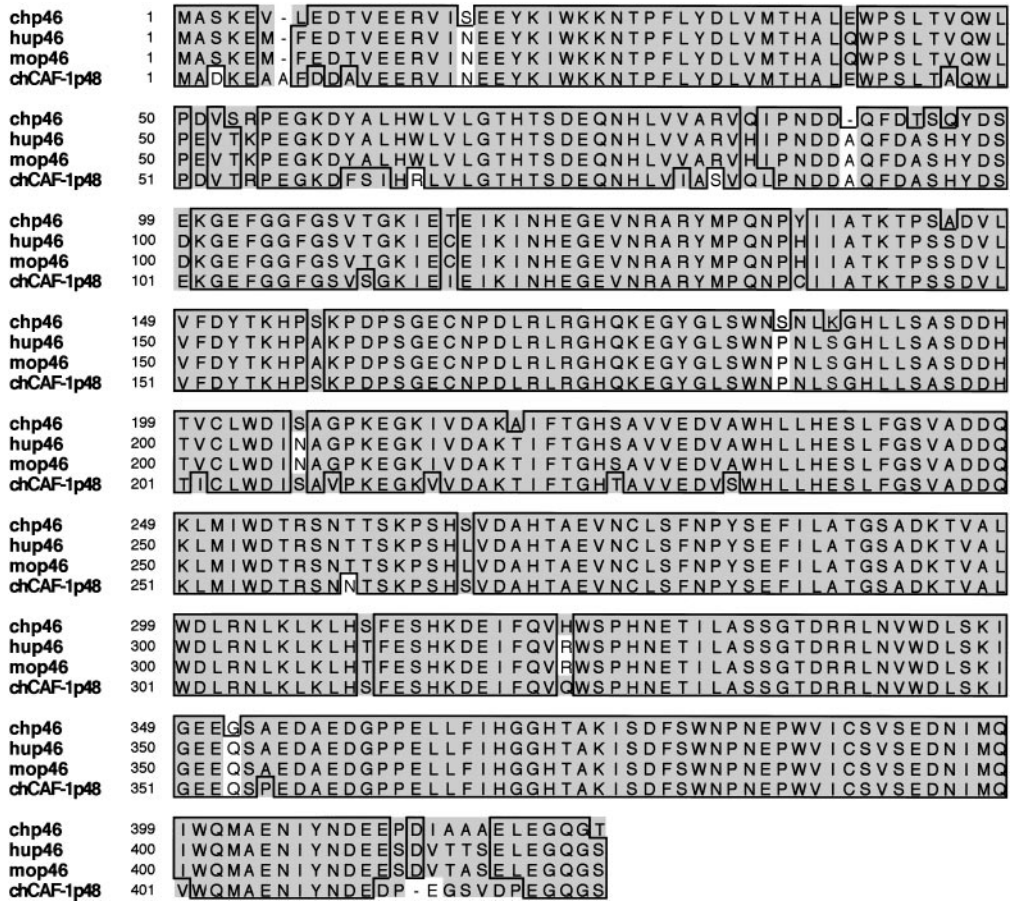


FIG. 1. Comparison of the aligned amino acid sequences of p46 polypeptides and the p48 subunit of chicken CAF-1. The complete amino acid sequences of four proteins, chp46, hup46, and mop46 and chicken CAF-1p48 (chCAF-1p48), are shown. Amino acid residues that are identical in three or four proteins are boxed, and analogous amino acids are indicated by shading. Dashes indicate gaps inserted to maximize the alignment of the sequences.

H2B and H4, and chHAT-1, because the interaction of p46 with one or more of these proteins could be thought to play a key role in the DNA-utilizing processes. We constructed C-terminal and N-terminal truncated mutants of FLAG-tagged chp46, and studied their *in vitro* interaction with HA-tagged histones H2B and H4, and the GST–chHAT-1 fusion protein.

We first performed an *in vitro* immunoprecipitation assay, involving these p46 mutants, to determine the binding region(s) of chp46 as to histones H2B and H4. [^{35}S]Met-labeled HA-tagged histones H2B and H4, respectively, were incubated with the *in vitro* translated [^{35}S]Met-labeled FLAG-tagged chp46 and its derivatives, followed by immunoprecipitation with anti-FLAG antibody-coupled beads. One C-terminal truncated protein, Δp46-(1–404), exhibited similar binding ability toward H2B to that of the parental chp46 protein (Figs. 3A and 3D). However, the other one, Δp46-(1–374), exhibited no binding ability. These findings suggested that the C-terminal region of chp46, comprising amino acids 375–404, is necessary for its binding ability toward histone H2B. On the other hand, one N-terminal truncated protein, Δp46-(180–424), exhibited no binding ability, although the other one, Δp46-(33–424), exhibited similar ability to that of the parental protein, indicating that a region of chp46, comprising amino acids 33–179, is really necessary for its binding ability toward histone H2B. Both chp46 and hup46 exhibit extensive homology (about 98%) in an approximately 250 C-terminal amino acid sequence, but relatively low homology (about 90%) in this region of amino acids 33–179 (Fig. 4A). Therefore, the difference between the interaction of chp46 with histone H2B and that of hup46 with histone H2A should be mainly due to the difference in the amino acid sequence of this particular region of these two p46 polypeptides.

On the other hand, two C-terminal truncated proteins, Δp46-(1–404) and Δp46-(1–374), exhibited no binding ability toward histone H4 (Figs. 3B and 3D). In addition, one N-terminal truncated protein, Δp46-(33–424), together with another N-terminal mutant, Δp46-(180–424), exhibited no binding ability. Taken together, these results indicated that two regions of chp46, the N-terminal 1–32 amino acid sequence and

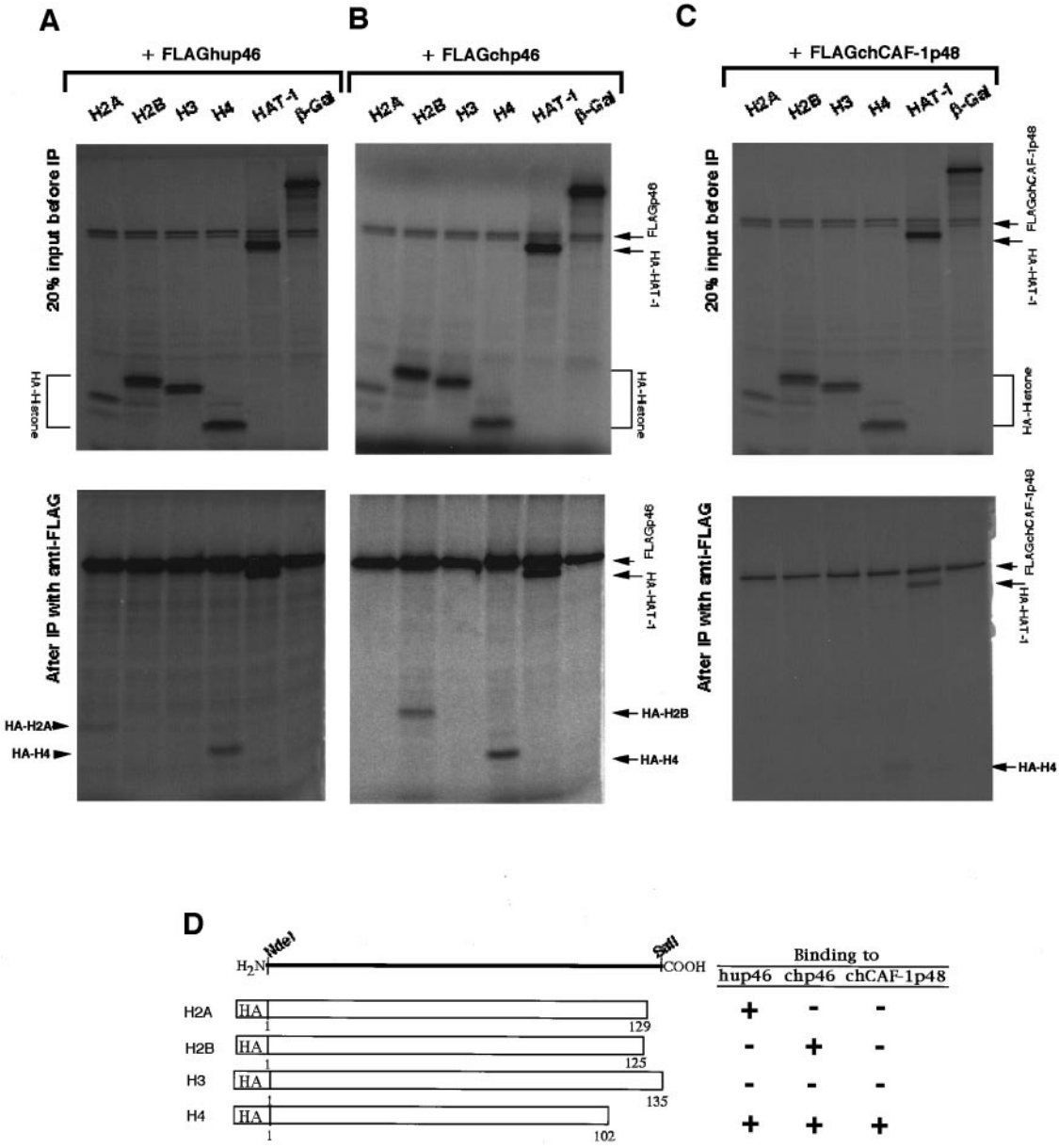
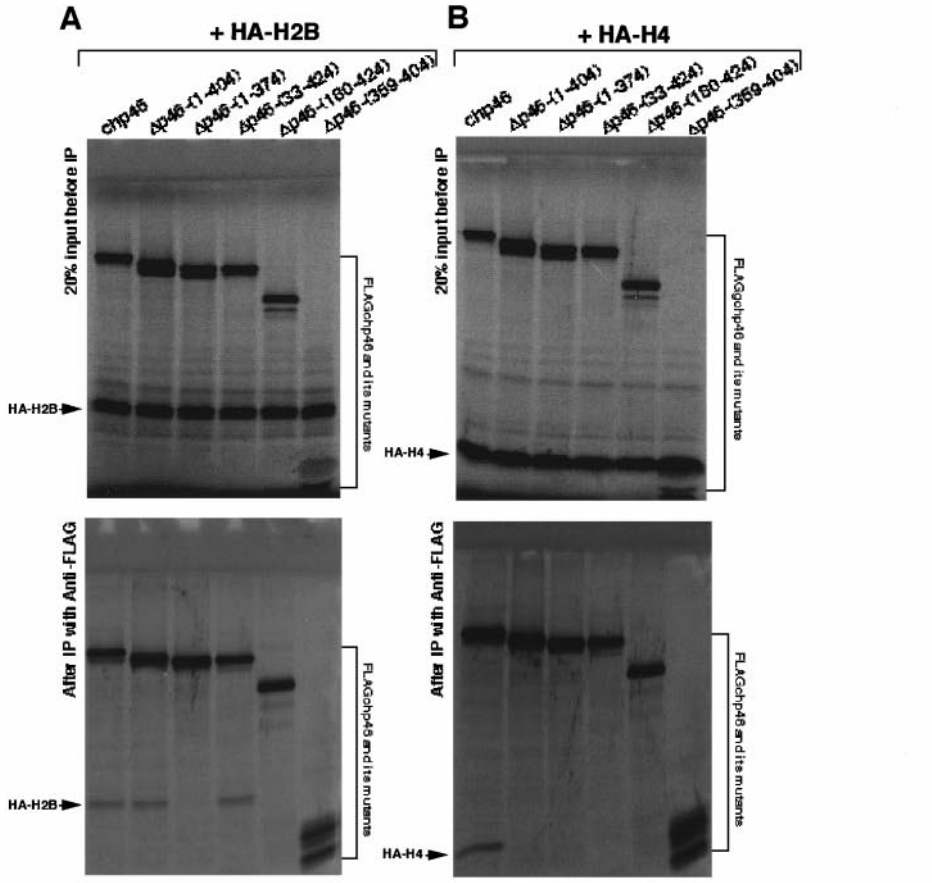
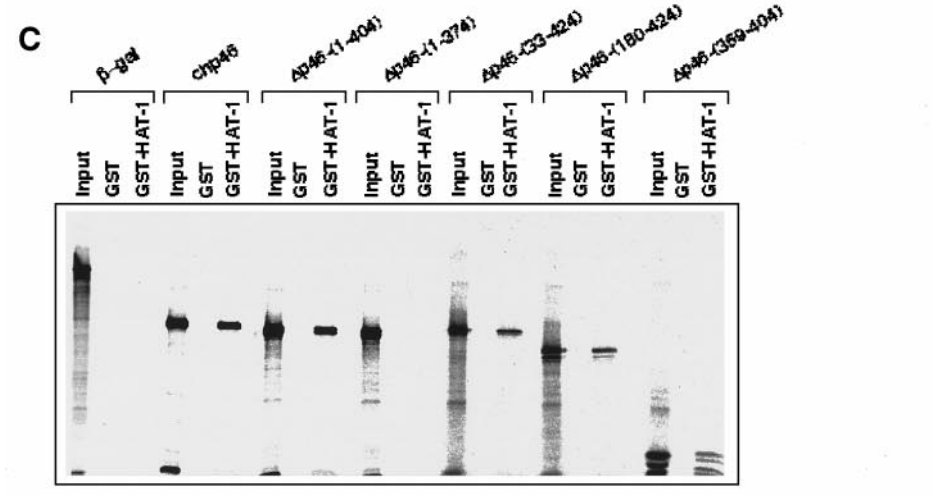


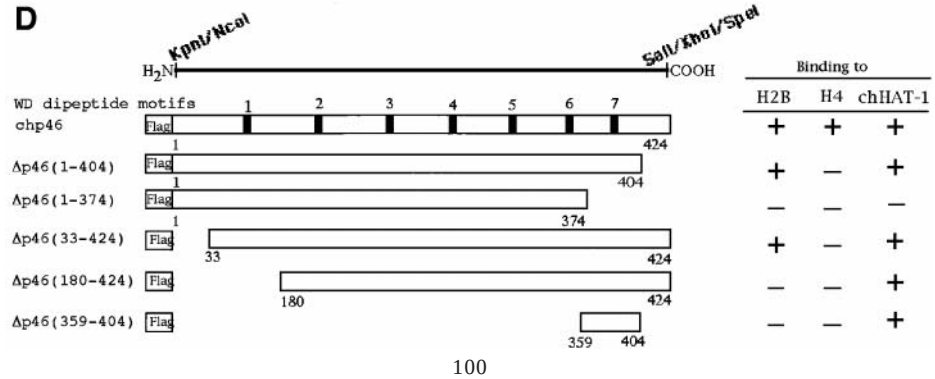
FIG. 2. Immunoprecipitation experiment on the *in vitro* interaction of chp46, hup46, or chCAF-1p48 with HA-tagged core histones H2A, H2B, H3, and H4 and chHAT-1. An *in vitro* immunoprecipitation experiment was carried out, using [^{35}S]Met-labeled FLAG-tagged hup46 (A), chp46 (B), or chCAF-1p48 (C), and [^{35}S]Met-labeled histones H2A, H2B, H3 and H4, and chHAT-1, together with β-galactosidase (β-gal) as a negative control, followed by the addition of anti-FLAG M2 beads. Immunoprecipitated samples (lower panels) and input samples, i.e., portions (20%) of the reaction mixtures before the immunoprecipitation (upper panels), were separated on 15% SDS–PAGE, and then the [^{35}S]-labeled proteins were detected by fluorography. The results concerning core histones in A, B, and C are summarized in D.

the C-terminal 405–424 amino acid sequence, are necessary for its binding ability toward histone H4. These two regions were extremely consistent with those of the human (29) and chicken (Takami *et al.*, in preparation) p48 subunits necessary for the interaction with histone H4.

To determine the binding region(s) of chp46 as to chHAT-1, we examined the *in vitro* interaction of truncated mutants of FLAG-tagged chp46 with the GST–chHAT-1 fusion protein (Figs. 3C and 3D). One C-terminal truncated protein, Δchp46-(1–374), exhibited no binding activity, although the other one, Δchp46-(1–404), exhibited similar binding activity to that of the parental chp46 protein. On the other hand, two N-terminal truncated proteins, Δp46-(33–424) and Δp46-(180–424), exhibited similar binding ability. These findings suggested that the C-terminal region of chp46, comprising amino acids 375–404 and carrying the seventh WD (WN) dipeptide motif, is necessary for its *in vitro* binding activity as to chHAT-1.


A
+ HA-H2B
B
+ HA-H4
chp46
Δp46(1-404)
Δp46(1-374)
Δp46(33-424)
Δp46(180-424)
Δp46(359-404)
20% input before IP
After IP with Anti-FLAG
FLAGchp46 and its mutants
HA-H2B
HA-H4


C
β-gal
chp46
Δp46(1-404)
Δp46(1-374)
Δp46(33-424)
Δp46(180-424)
Δp46(359-404)
Input
GST
GST-HAT-1


D
KpnI/NcoI
SalI/XhoI/SpeI
H2N
COOH
WD dipeptide motifs
chp46
Δp46(1-404)
Δp46(1-374)
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Δp46(180-424)
Δp46(359-404)
Flag
Binding to
H2B
H4
chHAT-1

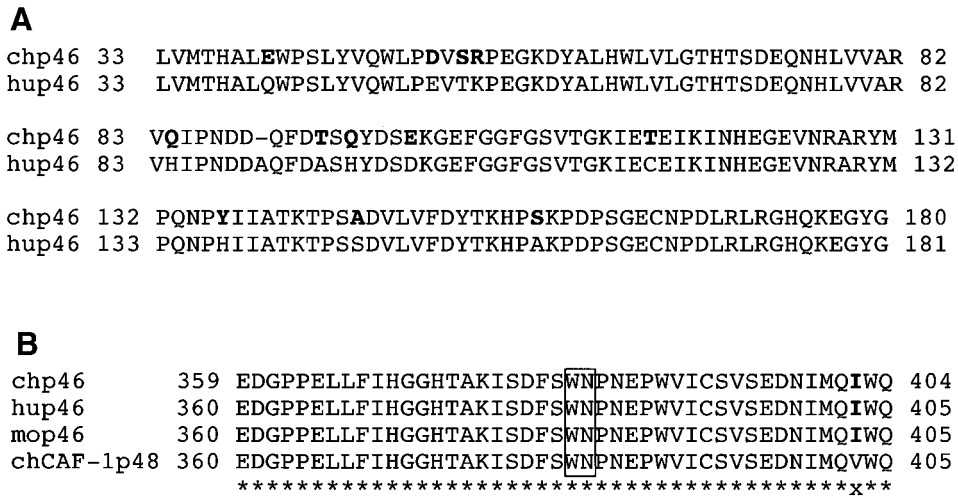

FIG. 4. The amino acid sequences of regions of chp46 essential for its binding activity as to histone H2B and chHAT-1. The amino acid sequences of the essential region for the binding of chp46 to histone H2B and the corresponding one of hup46 are shown (A). Amino acid residues altered in chp46 compared to in hup46 are indicated by bold letters. The amino acid sequence of the essential region for the binding of chp46 to chHAT-1 and the corresponding ones of hup46 and mop46, and chCAF-1p48 are shown (B). The seventh WD (WN) dipeptide motif is boxed. Ile at position 402 of p46s, which is substituted with Val at the corresponding position, 403, of chCAF-1p48, is indicated by a bold letter.

Finally, to confirm these results, we constructed the mutant, Δp46-(359–404), with simultaneous deletion of both the N-terminal 1-358 region and the C-terminal 405–424 region of chp46, and then assayed its binding activity toward chHAT-1. As shown in Figs. 3C and 3D, the region definitely interacted with chHAT-1, indicating that this region is enough for the *in vitro* interaction of chp46 with chHAT-1. However, as expected, the region comprising amino acids 359–404 exhibited no binding activity toward histones H2B and H4 (Figs. 3A, 3B, and 3D).

Very recently, using the *in vitro* GST pulldown affinity assay, involving truncated and missense mutant proteins of chCAF-1p48, we established not only that a region of chCAF-1p48, comprising amino acids 376–405 and carrying the seventh WD (WN) dipeptide motif, tightly bound to chHAT-1 *in vitro,* but also that a double Ala substitution mutation as to the motif (WN7AA) had no influence on this *in vitro* interaction (Ahmad *et al.*, submitted). As shown in Fig. 4B, the binding region of chp46 toward chHAT-1 contained only one amino acid change (Ile to Val) relative to that of chCAF-1p48 (17) toward chHAT-1. Taken together, these results indicate that the proper propeller structure of chp46, like that of chCAF-1p48, expected to be a platform for protein-protein interactions that should participate in DNA-utilizing processes, may not be necessary for its *in vitro* interaction with chHAT-1.

In summary, chp46 should be involved in numerous DNA-utilizing processes in different manners, i.e., the interaction of chp46 with chHAT-1 and histones H2B and H4 probably occurs in distinct regions of it, such as the region of amino acids 359–404 (in fact, 375–404) for chHAT-1, two regions of amino acids 33–179 and 375–404 for histone H2B and two regions of amino acids 1–32 and 405–424 for histone H4, whereas the proper propeller structure of chp46 is not necessary for the interaction with at least chHAT-1.

ACKNOWLEDGMENT

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FIG. 3. *In vitro* interaction of chp46 and truncated mutants of it with histones H2B and H4, and chHAT-1. An *in vitro* immunoprecipitation experiment was carried out, using [^{35}S]Met-labeled FLAG-tagged chp46 or its truncated mutants, and [^{35}S]Met-labeled HA-tagged histones H2B (A) and H4 (B), followed by the addition of anti-FLAG M2 beads. Immunoprecipitated samples (lower panels) and input samples, i.e., portions (20%) of the reaction mixtures before the immunoprecipitation (upper panels), were separated on 15% SDS–PAGE, and then the [^{35}S]-labeled proteins were detected by fluorography. The binding activity of chp46 or one of its truncated mutants labeled *in vitro* with [^{35}S]Met as to the GST–chHAT-1 fusion protein was examined by means of the GST pulldown affinity assay. As a control, β-gal was used. Each sample was resolved by 12% [or 15% SDS–PAGE in the case of Δp46-(359–404)], and then the proteins were stained with Coomassie blue (data not shown), followed by fluorography (C). The results in A, B, and C are summarized in D.

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