The Crystal Structure of CCG1/TAF\textsubscript{II}250-interacting Factor B (CIB)*

Received for publication, November 6, 2003, and in revised form, December 4, 2003
Published, JBC Papers in Press, December 11, 2003, DOI 10.1074/jbc.M312165200

Balasundaram Padmanabhan‡‡, Takashi Kuzuhara‡, Naruhiko Adachi‡¶, and Masami Horikoshi‡¶

From the ‡Horikoshi Gene Selector Project, Exploratory Research for Advanced Technology, Japan Science and Technology Corporation, 5-9-6 Tokodai, Tsukuba, Ibaraki 300-2635, and the ¶Laboratory of Developmental Biology, Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

The general transcription initiation factor TFIID and its interactors play critical roles in regulating the transcription from both naked and chromatin DNA. We have isolated a novel TFIID interactor that we denoted as CCG1/TAF\textsubscript{II}250-interacting factor B (CIB). We show here that CIB activates transcription. To further understand the function of this protein, we determined its crystal structure at 2.2-Å resolution. The tertiary structure of CIB reveals an α/β-hydrolase fold that resembles structures in the prokaryotic α/β-hydrolase family proteins. It is not similar in structure or primary sequence to any eukaryotic transcription or chromatin factors that have been reported to date. CIB possesses a conserved catalytic triad that is found in other α/β-hydrolases, and our in vitro studies confirmed that it bears hydrolase activity. However, CIB differs from other α/β-hydrolases in that it lacks a binding site excursion, which facilitates the substrate selectivity of the other α/β-hydrolases. Further functional characterization of CIB based on its tertiary structure and through biochemical studies may provide novel insights into the mechanisms that regulate eukaryotic transcription.

Eukaryotic gene expression is mainly regulated at the level of transcription initiation from chromatin templates (1–3). This regulation involves the orchestrated interplay of chromatin factors, gene-specific DNA-binding factors, general transcription factors, and transcription machinery elements and their interactors. Eukaryotic transcription is essentially regulated in three distinct steps. The first step is the alteration of chromatin structure through the modification and remodeling of chromatin components. A major target of the structural changes in chromatin is the nucleosome, which is the fundamental repeating unit of chromatin that consists of histones and DNA in a precise stoichiometric balance. Histone modification enzymes, ATP-dependent remodeling factors, and histone chaperones are involved in regulating the histone-DNA and histone-histone interactions that lead to particular chromatin structures. Of these chromatin regulators, the tertiary structures of histone acetyltransferase (HAT), 1 histone deacetylase homologues, histone methyltransferases, and histone chaperones have been solved (4–7). The second step of eukaryotic transcription regulation is the binding of regulatory transcription factors to promoter/enhancer elements. The tertiary structures of numerous types of DNA-binding domains that are present in these factors have been determined, and their DNA binding interactions have also been well characterized and classified. However, structures of most transcriptional activation/regulatory domains are not yet available. This may be because such unstable activation/regulatory domains are induced to fit to the basal transcription machinery (8). The third step is the regulation of RNA polymerization by RNA polymerase II and several general transcription initiation and elongation factors (9, 10). One of these general transcription initiation factors is TFIID, which is known to interact with various kinds of transcription/chromatin factors and to activate transcription initiation. The tertiary structures of RNA polymerase II and general transcription initiation and elongation factors, including domains of TATA box-binding protein (TBP), TBP-associated factor (TAF), TFIIA, TFIIB, TFIIE, TFIIF, and S-II (TFIIS), have recently been determined (11). Nevertheless, the mechanisms by which the activated general transcription initiation and elongation factors regulate RNA polymerization are not yet fully understood.

TFIID is a multiprotein complex that is composed of TBP (12) and TAFs (13, 14), and it plays a central role in both basal and regulatory transcription from the naked DNA template. TFIID binds to the TATA box of the core promoter and initiates transcription by forming the preinitiation complex with other general transcription initiation factors and RNA polymerase II (9, 10). It also interacts with various kinds of regulatory transcription factors as well as cofactors and activates transcription in cooperation with these elements (15–17). Some studies suggest that TFIID also plays a key role in regulating transcription from a chromatin DNA template. In one of these studies, it was found that the binding of TFIID to the TATA box of the core promoter during nucleosome assembly potentiates the subsequent initiation by RNA polymerase II (18). Moreover,

* This work was supported by a grant-in-aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan, by the New Energy and Industrial Technology Development Organization, and by Exploratory Research for Advanced Technology of the Japan Science and Technology Corporation, 5-9-6 Tokodai, Tsukuba, Ibaraki 300-2635, and the Laboratory of Developmental Biology, Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan.

‡ Present address: Protein Structure Team, RIKEN Genomics Sciences Center, 1-7-22 Suehiro, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan.

¶ To whom correspondence should be addressed. Tel.: 81-3-5841-8469; Fax: 81-3-5841-8468; E-mail: horikosh@iam.u-tokyo.ac.jp.

1 The abbreviations used are: HAT, histone acetyltransferase; TBP, TATA box-binding protein; CCG1, cell cycle arrest in G1; TAF, TBP-associated factor; TAF\textsubscript{II}, TAF with a molecular weight of 250 kDa; MOZ, monocytic leukemia zinc finger protein; Tip60, Tat-interactive protein 60; MYST, MOZ-Ybf2/Sas3-Sas2-Tip60; CIA, CCG1-interacting factor A; CIB, CCG1-interacting factor B; aa, amino acid(s); HCR, highly conserved region; EGFP, enhanced green fluorescent protein.
Crystallographic analyses have revealed that there are multiple histone-fold pairs in TFIID (19, 20). In addition, a biochemical study has suggested that a subset of four yeast histone-fold TAFs forms a histone-fold octamer (21).

The largest subunit of TFIID (22, 23) is CCG1/TAFII250. CCG1 and TAFII250 are acronyms. CCG1 stands for cell cycle arrest in G1, and TAFII250 stands for TATA box-binding protein-associated factor with a molecular weight of 250 kDa. It has been reported that CCG1 has three enzymatic activities, namely, HAT activity (24), histone H1-specific ubiquitin-activating/conjugating activity (25), and protein kinase activity (26). CCG1 has two bromodomains that are also found in various chromatin factors and that bind to the acetylated amino-terminal tails of histones (27). These structural and functional observations indicate that the function of CCG1 is closely related to the alteration of chromatin structure. In addition, cell lines that express CCG1 bearing an amino acid substitution suffer cell cycle arrest that leads to apoptosis, which suggests that CCG1 plays a role in growth and apoptosis (28).

Considering the characteristics of TFIID described above, we speculated that the molecules that interact with TFIID are likely to be chromatin factors and/or transcriptional regulators/cofactors. Indeed, when we isolated and characterized various TFIID interactors, we found that one of the TAF interactors is a MOZ-Ybf2/Sas3-Sas2-Tip60 (MYST)-type HAT denoted as Tip60 (Tat-interactive protein 60) (29–32) and that one of the CCG1 interactors is a histone chaperone denoted as CIA (CCG1-interacting factor A) (33–37). These proteins are highly conserved among various species, and they regulate several nuclear events, including DNA replication, DNA repair, transcription, silencing, and apoptosis, which indicates that they play important roles in regulating chromatin.

When we screened for proteins that interact with the conserved HAT domain of CCG1 by using the yeast two-hybrid system, we found that one of the TAF interactors is a MOZ-Ybf2/Sas3-Sas2-Tip60 (MYST)-type HAT denoted as Tip60 (Tat-interactive protein 60) (29–32) and that one of the CCG1 interactors is a histone chaperone denoted as CIA (CCG1-interacting factor A) (33–37). These proteins are highly conserved among various species, and they regulate several nuclear events, including DNA replication, DNA repair, transcription, silencing, and apoptosis, which indicates that they play important roles in regulating chromatin.

When we screened for proteins that interact with the conserved HAT domain of CCG1 by using the yeast two-hybrid system, we found that one of the TAF interactors is a MOZ-Ybf2/Sas3-Sas2-Tip60 (MYST)-type HAT denoted as Tip60 (Tat-interactive protein 60) (29–32) and that one of the CCG1 interactors is a histone chaperone denoted as CIA (CCG1-interacting factor A) (33–37). These proteins are highly conserved among various species, and they regulate several nuclear events, including DNA replication, DNA repair, transcription, silencing, and apoptosis, which indicates that they play important roles in regulating chromatin.

When we screened for proteins that interact with the conserved HAT domain of CCG1 by using the yeast two-hybrid system, we found that one of the TAF interactors is a MOZ-Ybf2/Sas3-Sas2-Tip60 (MYST)-type HAT denoted as Tip60 (Tat-interactive protein 60) (29–32) and that one of the CCG1 interactors is a histone chaperone denoted as CIA (CCG1-interacting factor A) (33–37). These proteins are highly conserved among various species, and they regulate several nuclear events, including DNA replication, DNA repair, transcription, silencing, and apoptosis, which indicates that they play important roles in regulating chromatin.

When we screened for proteins that interact with the conserved HAT domain of CCG1 by using the yeast two-hybrid system, we found that one of the TAF interactors is a MOZ-Ybf2/Sas3-Sas2-Tip60 (MYST)-type HAT denoted as Tip60 (Tat-interactive protein 60) (29–32) and that one of the CCG1 interactors is a histone chaperone denoted as CIA (CCG1-interacting factor A) (33–37). These proteins are highly conserved among various species, and they regulate several nuclear events, including DNA replication, DNA repair, transcription, silencing, and apoptosis, which indicates that they play important roles in regulating chromatin.
EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screen—We isolated the cDNA of CIB (70–210 aa) during a yeast two-hybrid screen (38) that aimed to search for factors that interact with CCG1 (GenBankTM/EBI accession number Q96IU4). A full-length clone (1–210 aa) of CIB was isolated by plaque hybridization using a partial CIB clone as a probe. The coding sequence of its cDNA was also identified in the mouse expressed sequence tag data bases (accession number Q8VCR7). DNA encoding a highly conserved region (HCR) of the human CCG1 protein (447–1112 aa) was cloned in-frame with the Gal4 DNA-binding domain in pAS1-CYH2 using NdeI and BamHI sites to make the pAS1-CYH2-CCG1-HCR construct. A human peripheral lymphocyte cDNA library fused to the Gal4 activation domain in pACT at the XhoI site was co-transformed with the pAS1-CYH2-CCG1-HCR plasmid into Y190 yeast cells in which the His3 and /H9252-galactosidase genes are integrated under the GAL4 element as the reporter. The yeast transformants were selected on minimal medium lacking tryptophan, leucine, and histidine and containing 25 mM 3-aminotriazole. The resultant colonies were tested for /H9252-galactosidase activity to detect the specific interactions. The /H9252-galactosidase assay was performed as described previously (39).

Direct Interaction between CIB and CCG1 Proteins—The CIB and CCG1 (447–788 or 777–1111 aa) proteins were expressed in Escherichia coli and purified to near homogeneity. The full-length CIB protein or buffer was immobilized with CNBr-activated Sepharose and used in the pull-down assay. Thus, 0.2 µg of CCG1 (447–788 or 777–1111 aa) protein was mixed with either 1 µg of CIB protein that had been immobilized with Sepharose or with Sepharose alone in the binding buffer (25 mM Hepes-KOH, pH 7.5, 2 mM MgCl2, 2 mM CaCl2, 10% glycerol, 150 mM KCl, 20 mM ZnCl2) and incubated for 1 h at 4 °C. This was followed by three washes with the binding buffer. The pellet was then subjected to SDS-PAGE and Western blotting using an anti-His tag antibody.

Genomic Southern Blot Hybridization—Hybridization was performed for 18 h at 65 °C in 5× saline/sodium phosphate/EDTA, 2% SDS, 0.1× SSC, 0.1% SDS at room temperature and two washes for 20 min in 0.1× SSC, 0.1% SDS at 60 °C. Autoradiography was performed for 48 h at −80 °C. Human genomic DNA (Clontech, Inc.) was digested with EcoRI, BamHI, HindIII, or PstI. The digested DNA was then subjected to 0.7% agarose electrophoresis, transferred to a nylon membrane, and fixed with ultraviolet light. The DNA probe used was the same as that used in the Northern blot hybridization assay.

Detection of CIB mRNA Transcripts in Human Tissues—A multiple human tissue mRNA blot (Clontech, Inc.) was hybridized with DNA probes that had been radiolabeled by [32P]dCTP using a random priming kit (Amersham Biosciences). The DNA probe was generated by XhoI digestion of CIB cDNA. Hybridization was performed for 18 h at 42 °C in 50% formamide, 5× saline/sodium phosphate/EDTA, 2% SDS, 10× Denhardt’s solution, followed by three washes for 10 min in 2× SSC, 0.05% SDS at room temperature and two washes for 20 min in 0.1× SSC, 0.1% SDS at 60 °C. Autoradiography was performed for 48 h at −80 °C. Human genomic DNA (Clontech, Inc.) was digested with EcoRI, BamHI, HindIII, or PstI. The digested DNA was then subjected to 0.7% agarose electrophoresis, transferred to a nylon membrane, and fixed with ultraviolet light. The DNA probe used was the same as that used in the Northern blot hybridization assay.

Detection of CIB mRNA Transcripts in Human Tissues—A multiple human tissue mRNA blot (Clontech, Inc.) was hybridized with DNA probes that had been radiolabeled by [32P]dCTP using a random priming kit (Amersham Biosciences). The DNA probe was generated by XhoI digestion of CIB cDNA. Hybridization was performed for 18 h at 42 °C in 50% formamide, 5× saline/sodium phosphate/EDTA, 2% SDS, 10× Denhardt’s solution, followed by three washes for 10 min in 2× SSC, 0.05% SDS at room temperature and two washes for 20 min in 0.1× SSC.
SSC, 0.1% SDS at 50 °C. Autoradiography was performed for 48 h at 80 °C.

Cellular Localization of CIB—CIB was cloned in-frame with green fluorescent protein in pEGFP. pEGFP-CIB was transfected into COS cells by Lipofectin (Invitrogen) according to the manufacturer’s instructions. The COS cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. Thirty-six h after transfection, the cells were examined under a fluorescent microscope (Zeiss) equipped with fluorescein isothiocyanate and ultraviolet filters.

Transcriptional Activation Assay—CIB (70–210 aa) was cloned in-frame with the Gal4 DNA-binding domain in pAS1-CYH2 using NdeI and XhoI sites to make the pAS1-CYH2-CIB construct. pAS1-CYH2-CIB was transformed in Y190 yeast cells in which the -galactosidase gene is integrated under the GAL4 element as the reporter. The transcriptional activation assay was performed by the -galactosidase assay to detect transcriptional activation activity.

Expression, Purification, and Crystallization of Human CIB—The expression, purification, and crystallization of human CIB have been described elsewhere (40, 41).

Structure Determination—All diffraction data were collected at the beamline BL18B of the Photon Factory, Tsukuba, Japan. The structure was solved by the multiple isomorphous replacement method at 2.8-Å resolution and refined to 2.2 Å with data from a native crystal (Table I).

The Crystal Structure of CIB

**Table 1**

| Summary of data collection and refinement statistics | Native | Uranyl acetate | KAu (CN)₄ |
|-----------------------------------------------------|--------|--------------|-----------|
| Data collection                                     | PF BL18B | PF BL18B | PF BL18B |
| Source                                              |         |             |           |
| Heavy atom concentration                            | 2.2     | 2.8         | 3.5       |
| Soaking time                                        | 94      | 88          | 65        |
| Resolution (Å)                                      | 9.8 (9.6) | 8.2 (7.1) | 12.7 (9.3) |
| Unique reflections                                  | 10,809  | 5048        | 1969      |
| Completeness (%)                                     | 3.9 (10.5) | 6.0 (12.4) | 8.3 (13.4) |
| Rmerge (%)                                          | 4.5     | 24.5        | 19.4      |
| Rfree (%)                                           | 0.62    | 0.61        | 0.61      |
| Refinement statistics                                | 30.0-2.2 |             |           |
| Resolution (Å)                                      | 18.9 (19.3) |             |           |
| σ cutoff                                            | 0       |             |           |
| Reflections                                         | 10,801  |             |           |
| No. of protein residues                              | 208     |             |           |
| No. of water molecules                               | 191     |             |           |
| No. of sulfate ions                                  | 1       |             |           |
| Rcryst (%)                                          | 23.5 (26.4) |             |           |
| Rfree (%)                                           | 18.9 (19.3) |             |           |
| Average B factors (Å²)                              | 20.90   |             |           |
| Protein                                             | 36.86   |             |           |
| solvent                                             | 0.097   |             |           |
| Root mean square deviations                         | 1.39    |             |           |

* PF, Photon Factory.
* Numbers in parentheses are values in the highest resolution shell.
* Rmerge = Σ||Fobs|−|<I>/Σ|Fobs| summed over all observations and reflections.
* Rfree = Σ||Fobs|−|<I>/Σ|Fobs|, where Fobs and F are the derivative and native structure factors, respectively.
* Rcryst = Σ|<Fobs|−|<I>/Σ|Fobs|, for centric reflections, and F is the calculated heavy atom structure factor.
* Rfree calculated with 5% of data omitted from refinement.

Fig. 5. **Stereo view of the Cβ trace of the CIB structure.** The tertiary structure of CIB shows that CIB possesses an α/β-hydrolase fold. The numbers of residues are indicated. The catalytic triad (Ser-111, His-188, and Asp-162) is shown by ball-and-stick representation.

**Table I**

| Summary of data collection and refinement statistics | Native | Uranyl acetate | KAu (CN)₄ |
|-----------------------------------------------------|--------|--------------|-----------|
| Data collection                                     | PF BL18B | PF BL18B | PF BL18B |
| Source                                              |         |             |           |
| Heavy atom concentration                            | 2.2     | 2.8         | 3.5       |
| Soaking time                                        | 94      | 88          | 65        |
| Resolution (Å)                                      | 9.8 (9.6) | 8.2 (7.1) | 12.7 (9.3) |
| Unique reflections                                  | 10,809  | 5048        | 1969      |
| Completeness (%)                                     | 3.9 (10.5) | 6.0 (12.4) | 8.3 (13.4) |
| Rmerge (%)                                          | 4.5     | 24.5        | 19.4      |
| Rfree (%)                                           | 0.62    | 0.61        | 0.61      |
| Refinement statistics                                | 30.0-2.2 |             |           |
| Resolution (Å)                                      | 18.9 (19.3) |             |           |
| σ cutoff                                            | 0       |             |           |
| Reflections                                         | 10,801  |             |           |
| No. of protein residues                              | 208     |             |           |
| No. of water molecules                               | 191     |             |           |
| No. of sulfate ions                                  | 1       |             |           |
| Rcryst (%)                                          | 23.5 (26.4) |             |           |
| Rfree (%)                                           | 18.9 (19.3) |             |           |
| Average B factors (Å²)                              | 20.90   |             |           |
| Protein                                             | 36.86   |             |           |
| solvent                                             | 0.097   |             |           |
| Root mean square deviations                         | 1.39    |             |           |
RESULTS

Interaction of CIB with the CCG1-HAT Domain and Transcriptional Activation Activity of CIB—We used a yeast two-hybrid system employing the HAT domain of CCG1 as bait to screen for proteins that interact with human CCG1 and identified two novel proteins named CIA (33–37) and CIB (40, 41). To confirm that CIB interacts specifically and directly with CCG1-HAT, we performed two-hybrid and pull-down assays. The two-hybrid assay shows that CIB does interact specifically with CCG1-HAT (Fig. 1A), whereas the pull-down assay (Fig. 1B) indicates that CIB protein binds directly and specifically to the 777–1111-aa domain of CCG1 but not to the 447–788-aa domain. To confirm that the CIB protein is encoded in the human genome, we performed genomic Southern blot hybridization using CIB cDNA as a probe. This indicated that CIB is indeed a human factor (Fig. 2A). Northern blot hybridization analysis also shows that CIB mRNA is expressed in almost all human tissues (Fig. 2B), which indicates that CIB is ubiquitously expressed. The CIB protein is found both in the nucleus and the cytosol (Fig. 3A), which suggests that it is involved in both nuclear and cytosolic reactions. As CIB interacts with the CCG1-HAT domain of the TFIID subunit, it is likely that CIB is involved in transcriptional regulation. Moreover, Fig. 3B indicates that CIB has transcriptional activation activity. All these results indicate that the CIB protein is indeed a general factor in humans that interacts with CCG1-HAT.

The Overall Structure of CIB—Because the primary structure of CIB differs from that of all other known eukaryotic transcription factors, we chose to resolve its tertiary structure in the hope that this might reveal its function. The structure of CIB protein was solved by multiple isomorphous replacement and refined to a crystallographic R-factor of 18.9% at 2.2-Å resolution (Figs. 4 and 5 and Table I). The tertiary structure has an α/β-fold (Fig. 6). The β-sheet consists of seven parallel β-strands (β1, β3, β4, β5, β6, β7, and β8) and one antiparallel amino-terminal β-strand (β2), and it is flanked by helices α1 and α6 on one side and by helices α2, α3, α4, and α5 on the other side. The β-strands form a twisted β-sheet. A long loop comprising 69–90 aa connects β-strand β4 and helix α2. The outer two strands, β1 and β8, are oriented nearly perpendicular to each other, which results in a left-handed superhelical twist in the β-sheet. The first two strands, β1 and β2, run antiparallel to each other and are connected by a hairpin turn. The remaining β-strands in the β-sheet run parallel to strand β1.

Structure-based Functional Analyses of CIB—To elucidate the function of CIB, its structure was compared with the protein structures in the Protein Data Bank using the program DALI (49). The search revealed good structural similarity with prokaryotic bromoperoxidase (Fig. 7A) (50, 51), 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (52), soluble epoxide hydrolase (53), and carboxylesterase (54) with z-scores of 18.3, 18.5, 16.5, and 15.3, respectively. Superposition of the Ca-
The Active Site of CIB-A

The active site is located in a deep cleft running across the carboxyl ends of the central β-sheet (Fig. 9A). A long flexible loop comprising 69–90 aa that runs across one side of the cleft contains mainly hydrophobic residues. This inverted “V” shape-like loop may move away to make room for a substrate that is being bound. At one side of the cleft, the hydrophobic residues Ile-41, Leu-112, Ile-84, Gly-85, and Leu-87 line up, probably so that they can form hydrophobic interactions with a substrate moiety. Arg-42 and Lys-141 lie at the edge of the cleft region and may potentially interact electrostatically with the substrate moiety. Because electrostatic surface potential analysis shows that the active site region is predominantly composed of a hydrophobic pocket (Fig. 10), the candidate substrate is likely to be hydrophobic.
The general protein surface of CIB is also mainly hydrophobic (Fig. 10). The total number of negatively charged aa (Asp and Glu) and positively charged aa (Arg and Lys) is 17 and 13, respectively. Such low numbers of charged aa in CIB indicate that the surface is rather hydrophobic. The hydrophobicity of CIB is high when compared with bromoperoxidase (51), 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (52), soluble epoxide hydrolase (53), and carboxylesterase (54) (Fig. 10). This appreciable difference of CIB relative to the other hydrolases may indicate a unique feature of CIB and suggests that this hydrophobic region might be responsible for protein-protein interactions in transcriptional regulation.

**Biochemical Activities of CIB**

The comparative studies of the primary and tertiary structures of CIB suggest it may have hydrolase activity. To test this, we used p-nitrophenyl butyrate, a typical substrate for hydrolases, and purified the recombinant CIB protein to near 95% homogeneity (Fig. 11 A). CIB induced the hydrolysis of p-nitrophenyl butyrate into p-nitrophenol in a dose-dependent manner (Fig. 11 B). Thus, as predicted, CIB possesses hydrolase activity. The specific activity of

---

**Fig. 8. Structure-based amino acid sequence alignment of CIB with other α/β-hydrolases.** The red characters indicate homologous aa. The white characters on the red background indicate aa that show complete homology between four sequences. The alignment was produced by ClustalW (73) and was manually modified. The sequences used in this figure (produced by ESPript (74)) were obtained from the Entrez data base: Protein Data Bank code 1BRT, Swiss-Prot accession number P29715 (bromoperoxidase: 1–128, 214–277 aa); Protein Data Bank code 1C4X, Swiss-Prot accession number D78322 (2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase: 1–139, 222–283 aa); and Protein Data Bank code 1A0U, Swiss-Prot accession number S79600 (carboxylesterase: 1–55, 81–216 aa). The secondary structures of CIB are shown by arrows and helices. Catalytic triads are indicated with triangles.
CIB was about 50 units/mg, which is comparable with \textit{E. coli} esterase (58). To our knowledge, CIB is the first eukaryotic transcriptional factor/cofactor that has been found to be a hydrolase that bears a bromoperoxidase-related \(\alpha/\beta\)-hydrolase fold.

\section*{DISCUSSION}

\textbf{Significant Features of the Structure of CIB}—The structure of CIB differs from the structures of all other eukaryotic transcription factors. Instead, it bears a structure that resembles the \(\alpha/\beta\)-hydrolase fold found in several prokaryotic enzymes. Interestingly, unlike most other hydrolases, CIB lacks a binding site excursion in its \(\alpha/\beta\)-hydrolase fold structure. The binding site excursion, which acts as a lid, is believed to support the substrate binding activity of a hydrolase as it is positioned above the substrate-binding site (54). How CIB recognizes its substrate is thus unclear. One possibility is that a binding partner complements the lack of a binding site excursion in CIB. This notion is supported by the presence of a long flexible loop near the active site region of CIB (Fig. 5). When the binding partner of CIB binds, it may induce the loop to undergo a conformational change that then facilitates substrate binding. We speculate that CCG1, more specifically, its HAT domain, could be a candidate binding partner. Supporting this notion is that CIB and CCG1-HAT may share the same substrate because they interact with each other. That substrate could be acetyl-CoA because when we incubated CIB with acetyl-CoA and subjected the mix to time-of-flight mass spectrometry analysis, we detected a peak that corresponds to an acetyl-CoA-CIB complex (data not shown). Thus, CCG1-HAT may play an important role in the substrate binding of CIB (discussed further in the next section).

\textbf{Prediction of the CIB Substrate That Is Involved in Transcriptional Regulation}—The identification of the native substrate of CIB and analysis of complex crystal structures of CIB

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig9.png}
\caption{\textbf{Putative active site of CIB and its electrostatic surface.} \textbf{A}, region near the active site triad that is composed of Ser-111, Asp-162, and His-188. This was produced by Molscript (72). Secondary structure elements are labeled as shown in Fig. 8. \textbf{B}, superposition of CIB structure (green) with \(\alpha/\beta\)-domain of prolyl aminopeptidase (Protein Data Bank code 1QTR) (blue); the catalytic triads are Ser-111/Ser-113, Asp-162/Asp-268, and His-188/His-296. The figures are generated by Molscript (72).}
\end{figure}
with its substrate and/or with CCG1 will most likely reveal a novel transcription-regulatory mechanism, as CIB is a nuclear hydrolase enzyme and is the first such enzyme shown to be involved in transcription. As suggested above, acetyl-CoA may be the substrate that is bound by CIB. This is because the likely binding partner of CIB is CCG1, the largest subunit of TFIID, which consists of many functional domains including a HAT domain (517–976 aa) (24, 62). In addition, the HAT domain of CCG1 maps to the central and most conserved portion of CCG1. We showed in this report that CIB interacts with CCG1-HAT directly (Fig. 1B). That CIB binds to this conserved domain suggests that it is involved in regulating histone acetylation and chromatin organization along with CCG1. Supporting this notion is that a single amino acid mutation of CCG1 in hamster cell lines results in their arrest in G1 phase of the cell cycle followed by apoptosis. As CIB is the binding partner of CCG1, this observation suggests that CCG1 and CIB both play a role in cell proliferation and apoptosis (28).

It is likely that further investigation of the relationship between TFIID and CIB will help to reveal the full extent of the biological activities of CIB. That these two molecules interact at a physiological level is suggested by the fact that TFIID and CIB both exist in the nucleus and that they interact directly with each other in vitro (Figs. 1B and 3A). This is supported by the fact that CIB is expressed in almost all tissues as shown by our Northern blot hybridization analysis (Fig. 2). That CIB binds to this conserved domain suggests that it is involved in regulating histone acetylation and chromatin organization along with CCG1. Supporting this notion is that a single amino acid mutation of CCG1 in hamster cell lines results in their arrest in G1 phase of the cell cycle followed by apoptosis. As CIB is the binding partner of CCG1, this observation suggests that CCG1 and CIB both play a role in cell proliferation and apoptosis (28).
histone H3 and probably contributes to transcription through its histone chaperone activity (33). In addition to transcription, CIA functions in DNA replication, DNA repair, cell cycle, and apoptosis (34, 66). CIA has a genetic link with and interacts physically with MYST-HAT and blocks the acetylation activity of its complex (67). CIA also interacts with the SWI/SNF chromatin-remodeling complex (68). Acetylation involves the hydrolysis of an acetyl-CoA, whereas chromatin remodeling requires the hydrolysis of ATP. Thus, through its putative acetyl-CoA- and ATP-hydrolyzing activity, CIA may cooperate with or inhibit CIA and CIA interactors, thus contributing to the regulation of chromatin organization.

Another example of a CCG1 interactor is the retinoblastoma tumor suppressor protein Rb, which regulates the cell cycle, tumor formation, cell differentiation, and senescence (69). Rb interacts directly with CCG1 through multiple domains and inhibits the intrinsic kinase activity of CCG1 (70). Rb is regulated by its phosphorylation and dephosphorylation, which involves the hydrolysis of a phosphoryl bond. It would be of interest to investigate whether CIA could regulate the phosphorylation state of Rb through its hydrolase activity.

Finally, as CIA directly interacts with CCG1, which is involved in a wide range of biological phenomena, including gene expression, chromatin organization, and cell cycle, through its biochemical activities, which include HAT, kinase, and ubiquitin activities, further analyses of the function of CIA in relation to the activities of CCG1 and CCG1 interactors are likely to yield novel insights into the physiological roles that are played by each of these elements.

Acknowledgments—We thank Drs. A. Paeche, S. Sugio, and T. Senda for providing time and expertise at various stages of the project. We are also grateful to Drs. T. Umehara, A. Kimura, T. Chimura, and T. Chimura and to all our lab members for helpful discussions. In addition, we thank Drs. N. Sakabe, M. Suzuki, and N. Inagaki for help with data collection at the Photon Factory (Proposal No. 00G119).

REFERENCES

1. Workman, J. L., and Kingston, R. E. (1998) Annu. Rev. Biochem. 67, 545–579
2. Korshunov, R. D., and Lorch, Y. (1999) Cell 98, 285–294
3. Roth, S. Y., Denu, J. M., and Allis, C. D. (2001) Curr. Opin. Struct. Biol. 11, 549–561
4. Workman, J. L., and Roeder, R. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9336–9338
5. Muller, P., and Sauer, F. (2000) Structure 8, 595–605
6. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
7. Workman, J. L., and Roeder, R. G. (1987) Cell 49, 318–330
8. Pham, A. D., and Sauer, F. (2000) Science 289, 2357–2360

The Crystal Structure of CIA