Centrosomal P4.1-associated Protein Is a New Member of Transcriptional Coactivators for Nuclear Factor-κB*

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Nuclear factor-κB (NF-κB) is a transcription factor important for various cellular events such as inflammation, immune response, proliferation, and apoptosis. In this study, we performed a yeast two-hybrid screening using the N-terminal domain of the p65 subunit (RelA) of NF-κB as bait and isolated centrosomal P4.1-associated protein (CPAP) as a candidate for a RelA-associating partner. Glutathione S-transferase pull-down assays and co-immunoprecipitation experiments followed by Western blotting also showed association of CPAP with RelA. When overexpressed, CPAP enhanced NF-κB-dependent transcription induced by tumor necrosis factor-α (TNFα). Reduction of the protein level of endogenous CPAP by RNA interference resulted in decreased activation of NF-κB by TNFα. After treatment with TNFα, a portion of CPAP was observed to accumulate in the nucleus, although CPAP was found primarily in the cytoplasm without any stimulation. Moreover, CPAP was observed in a complex recruited to the transcriptional promoter region containing the NF-κB-binding motif. One hybrid assay showed that CPAP has the potential to activate gene expression when tethered to the transcriptional promoter. These data suggest that CPAP functions as a coactivator of NF-κB-mediated transcription. Since a physiological interaction between CPAP and the coactivator p300/CBP-mediated transcription was observed and synergistic activation of NF-κB-mediated transcription was achieved by these proteins, CPAP-dependent transcriptional activation is likely to include p300/CBP-binding protein.

Nuclear factor-κB (NF-κB)† is a Rel transcription factor that regulates the expression of a wide variety of genes involved in cellular events such as inflammation, immune response, proliferation, and apoptosis (1–3). Rel family members form hetero- and homodimers that possess distinct specificities and functions. In mammals, five Rel family members have been identified: c-Rel, RelA/p65, RelB, NF-κB1 (p50/p105), and NF-κB2 (p52/p100). In the canonical NF-κB signaling pathway, the prototypical NF-κB complex composed of p50 and RelA subunits is sequestered in the cytoplasm through its assembly with a family of NF-κB inhibitors (IκB) at steady state. When cells are stimulated by signals such as tumor necrosis factor-α (TNFα) and interleukin-1, IκB is phosphorylated by the IκB kinase complex, marking it for ubiquitination and subsequent degradation. The liberated NF-κB heterodimer rapidly translocates into the nucleus and activates target genes by binding directly to κB regulatory elements present in the target loci.

Although these cytoplasmic signaling events are understood in detail, the subsequent nuclear events that regulate the strength and duration of NF-κB-mediated transcriptional activation remain poorly defined (4). RelA contains a transactivation domain (TAD) in its C-terminal region that is known to be responsible for transcriptional activation. TAD has so far been reported to interact with various transcriptional and basal transcription factors that recruit RNA polymerase II, including TATA-binding protein, transcription factor IIB, TAFII105 (TATA-binding protein-associated factor II105), and TLS (translocated in liposarcoma) (5–8). In addition, general coactivators such as cAMP response element-binding protein (CREB)-binding protein (p300/CBP) (9, 10), p300/ CBP-associated factor, and ACTR (coactivator for nuclear hormone receptors) are recruited to the NF-κB transcriptional complex and enhance NF-κB-mediated transcriptional activation (11, 12).

The N-terminal domain of RelA is also known to play important roles in the regulation of NF-κB-mediated transcriptional activation. For example, stimulus-coupled phosphorylation of RelA is known to change its transcriptional activity (4, 10, 13–16), and two of the four serine phosphoacceptor sites present in RelA are in the N-terminal domain. In addition, association with p300/CREB has been reported to occur not only via TAD, but also through the N-terminal domain of RelA. RelA phosphorylation at Ser276 by the catalytic subunit of cAMP-dependent protein kinase (14) or mitogen- and stress-activated protein kinase-1 (15) or at Ser311 by protein kinase Cζ (16) was shown to enhance the binding of p300/CREB to RelA. Moreover, p300/CREB has also been reported to acetylate RelA at three sites in the N-terminal domain: Lys318, Lys321, and Lys316. Acetylation is thought to regulate the transcriptional activity of RelA by increasing its DNA-binding affinity for the κB enhancer or by preventing its association with IκBα (4, 17–20). Finally, BRCA1 also associates with the N-terminal domain of NF-κB via an interaction with its DBD, and BRCA1 null cells exhibit increased NF-κB DNA-binding activity. Although the mechanism of NF-κB activation remains poorly understood, it is now clear that the interaction of NF-κB with these coactivators is critical for the transcriptional activity of NF-κB.
Plasmid Construction—pEFr-FLAG-CPAP was a kind gift from Dr. J. E. Visvader (Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) (23). The cDNA fragment consisting of the entire open reading frame of CPAP was generated by PCR using a human spleen cDNA library (Clontech, Palo Alto, CA) as the template and primers 5'-CGCCGATCCATGTTCCTGAGCCACACTC-3' and 5'-TTTCTTTTCTGGCC ACATGTCAGCCCTGCTGTC-3'. The fragment was inserted into the BamHI-NoI sites of the pDNA3-Myc vector to generate pDNA3-Myc-CPAP and blunt-end cloned into the pCMV-FLAG vector (24) to generate pCMV-CPAP. The series of plasmids encoding deletion mutants of CPAP, pcDNA3-Myc-CPAP-(1–1149), pcDNA3-Myc-CPAP-(1150–1338), and pcDNA3-Myc-CPAP-(967–1338), was constructed by inserting fragments generated by PCR using appropriate synthetic oligonucleotides as primers and pcDNA3-Myc-CPAP as the template. pcDNA3-HA-RelA (where HA is hemagglutinin), pcDNA3-Myc-CPAP, and pcDNA3-Myc-CPAP-(967–1338) were generated in a similar manner using appropriate synthetic oligonucleotides as primers and pcDNA3-Myc-CPAP as the template. PCAP is an NF-kB coactivator of NF-κB that binds to the N-terminal region of RelA, possibly activating transcription through CBP.

EXPERIMENTAL PROCEDURES

RNA Interference Technique—A 21-nucleotide small interfering RNA (siRNA) duplex (5'-AAUGGAAUGCAAGGACAGAUG-3') containing 3'-dTdT overhanging sequences was synthesized (Qiagen Inc.). siRNA transfection was performed using Oligofectamine reagent (Invitrogen) according to the manufacturer's instructions.

RNA Isolation and Reverse Transcription-PCR—Total RNA was isolated from cultured cells using Sepasol RNA I Super (Nacalai Tesque, Kyoto, Japan) according to the manufacturer's instructions. The relative expression of each mRNA was evaluated by semiquantitative reverse transcription-PCR using a One-Step RNA PCR kit (Takara). Glyceraldehyde-3-phosphate dehydrogenase mRNA was used as an internal control. The primers used were as follows: CPAP, 5'-AAAGG-GACACAGATGACG-3' and 5'-TGAATCTACTGCAAGCTGGAG-3'; interferon-β, 5'-CAAGAGCTCTTCCATGAG-3'; 5'-AGGCCCTGTCGATACG-3'; and 5'-CTCTACTTCCATTGCAAG-3'.

Indirect Immunofluorescence Analysis—Indirect immunofluorescence analysis was performed as described previously (29). Cells were permeabilized with 0.1% Triton X-100 after fixation and treated with anti-RelA primary antibody F-6 (and rabbit antisera against CPAP (22)). Rabbit antibodies conjugated to Alexa 488 and Alexa 568 (Molecular Probes, Inc.) were used to visualize primary antibody distribution. Nuclei were stained with 4',6-diamidino-2-phenylindole (Sigma).

DNA-Protein Complex Immunoprecipitation Assay—293T cells treated with 10 nm TNFα were transfected with plasmids. After crosslinking with 1% formaldehyde for 15 min, cells were lysed; sonicated; and subjected to immunoprecipitation using anti-FLAG or anti-RelA antibody. DNA-protein complexes were isolated with proteinase K digestion. Total RNA was extracted from the immunocomplexes with phenol and precipitated with ethanol. The primers used for detection of recovered DNA were 5'-ACGCGAACGCGGCGAGATCCGCACTC-3' and 5'-GCC-TCCTCCAGGGTGTCAC-3' for pNF-κB-luc and 5'-CTACGAAAATAGGCAGCTCC-3' and 5'-CTTATGTTTGGCGTATTCC-3' for pNF-kB-luc.

Cell Culture and Transfection—293T and MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (Nissui) supplemented with 10% fetal bovine serum and L-glutamine. Transfection of plasmids into cells was performed with FuGene 6 transfection reagent (Roche) according to the manufacturer's recommendations.

Immunoprecipitation—Cells were lysed in immunoprecipitation buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Nonidet P-40). After centrifugation, the supernatant was incubated with anti-FLAG antibody M2 (Sigma), anti-RelA antibody F-6 (Santa Cruz Biotechnology, Inc.), anti-c-Myc antibody 9E10 (Santa Cruz Biotechnology, Inc.), or anti-4.1R antibody-1, Calbiochem). The rabbit antisem against CPAP was kindly provided from Dr. T. K. Tang (Institute of Biomedical Sciences, Taipei, Taiwan, Republic of China) (22).

Reportor Assay—Cell extracts were prepared in reporter lysis buffer (Promega) 48 h after transfection. After removal of cell debris, the luciferase activity in the extracts was measured with a luciferase assay kit (Promega) and a Berthold Lumat LB 9507 luminometer according to the manufacturer's instructions.
RESULTS

Identification of CPAP as a Factor That Interacts with RelA—To identify cellular factors that interact with the N-terminal region of RelA, a yeast two-hybrid screen was performed using a human leukemia cDNA library as bait and the N-terminal 427-amino acid region of RelA as prey. From 1.6 × 10^6 L40 yeast transformants, 64 clones were obtained that appeared to interact with RelA. Among them, three independent clones were revealed to encode portions of CPAP.

To confirm the interaction of CPAP with RelA, we performed an immunoprecipitation assay using 293T cells co-transfected with RelA and CPAP. Flag-tagged CPAP was detected in cell lysates in the immunocomplex formed with anti-RelA antibody (Fig. 1A, left upper panel, lane 2), but not with normal mouse IgG (lane 3). The interaction between Flag-tagged CPAP and endogenous RelA was seen without considerable alteration both before and after treatment with TNFα (Fig. 1A, upper and lower left panels, respectively). This seemed to imply that TNFα-induced phosphorylation of RelA is not essential for the interaction with CPAP. Actually, we found that Flag-tagged CPAP was co-immunoprecipitated with a RelA mutant in which one of the TNFα-induced phosphorylation target sites (Ser276) was replaced with alanine (data not shown). This may support the above idea. This interaction was also seen in a GST pull-down assay. Under conditions in which in vitro translated CPAP was not pulled down with GST-bound Sepharose beads (Fig. 1B, lane 2), we found it in a pellet with recombiant GST-RelA-bound Sepharose beads (lane 3). These results suggest that CPAP interacts specifically with RelA. In addition, to examine the region of CPAP responsible for interaction with RelA, we performed a GST pull-down assay as described above using several deletion mutants of CPAP. The in vitro synthesized fragments of CPAP spanning amino acids 1150–1338 and 967–1338, but not amino acids 1–1149, were co-purified with GST-RelA (Fig. 1B). This indicates that the region of CPAP responsible for interaction with RelA resides within amino acids 1150–1338, including a series of 21 nonamer repeats (G-box region). This result was also obtained with the immunoprecipitation assay. In the lysates of 293T cells producing RelA and Myc-tagged CPAP-C (C-terminal amino acids 967–1338 of CPAP), exogenous RelA was efficiently detected in immunocomplexes formed with anti-Myc antibody (Fig. 1A, right panel, lane 6), but not with normal mouse IgG (lane 5). The region of RelA that interacts with CPAP was similarly assessed. The in vitro synthesized fragments of RelA spanning amino acids 1–427 and 201–427, but not amino acids 428–551, 1–312, or 313–427, were co-purified with GST-CPAP-C (Fig. 1C). These results indicate that the central region of RelA is necessary and sufficient for interaction with CPAP.

CPAP Augments NF-κB-dependent Gene Expression—Because CPAP has been reported to activate STAT5-mediated transcription (23), we examined the effect of this protein on RelA-mediated transcription using a reporter assay. When CPAP was ectopically expressed, NF-κB-responsive reporter gene expres-
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Fig. 2. CPAP increases NF-κB-induced gene expression. A, enhancement of NF-κB-dependent reporter gene expression by ectopic expression of CPAP. 293T cells were transfected with 25 ng of pNF-κB-luc (containing wild-type NF-κB-binding sites upstream of the luciferase gene; upper panel) or pNF-κB-mt-luc (containing mutated NF-κB-binding sites; lower panel) together with the indicated amounts of pCMV-FLAG-CPAP. After 42 h of transfection, cells were mock-treated (bars 1, 3, and 5) or treated with 10 ng/ml TNFα (bars 2, 4, and 6) and harvested after an additional incubation for 6 h. Values represent the relative luciferase activity expressed as the mean ± S.E. of three independent transfections. B, suppression of endogenous CPAP production by siRNA. The 21-nucleotide RNA duplex (siRNA) directed against the CPAP sequence was transfected into MCF-7 cells (lane 1). The levels of CPAP protein were evaluated by immunoblotting 48 h post-transfection. As a negative control, total cell extracts from MCF-7 cells with no siRNA treatment (lane 1) and with treatment with control siRNA (lane 2) were used. The relative protein levels of CPAP (upper panel) and α-tubulin as a positive control (lower panel) are shown. Molecular mass markers are shown on the left. The arrow indicates intact CPAP protein. The arrowhead shows the putative degraded form of CPAP. C, effects of CPAP siRNA on NF-κB-dependent reporter gene expression. MCF-7 cells were transfected with control siRNA (gray bars) or CPAP siRNA (black bars). At 24 h post-transfection, 25 ng of pNF-κB-luc, 25 ng of pHK-LTR-luc, and 200 ng of either pKS'-CMV (bars 1–4) or pcDNA3-RelA (bars 5 and 6) were transfected into cells (upper panel). The same experiment using pNF-κB-mt-luc instead of pNF-κB-luc was also carried out (lower panel). An additional 18 h later, cells were treated with (bars 3 and 4) or without (bars 1, 2, 5, and 6) 10 ng/ml TNFα for 6 h. Values represent the luciferase activity expressed as the mean ± S.E. of three independent transfections. D, effects of CPAP siRNA on endogenous gene expression induced by TNFα. MCF-7 cells were transfected with control (lanes 1 and 3) or CPAP siRNA (lane 2 and 4). The cells were treated for 6 h with 10 ng/ml TNFα (lanes 3 and 4). Semiquantitative reverse transcription-PCR was performed to estimate the amounts of interferon-β (IFNβ), TNF receptor-associated factor-1 (TRAF1), CPAP, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs.

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sion was enhanced by up to 2–3-fold in a dose-dependent manner (Fig. 2A, upper panel). In contrast, reporter activity from the plasmid containing mutated NF-κB-binding sites in the promoter region was not affected by ectopically expressed CPAP (Fig. 2A, lower panel). These data suggest that CPAP can specifically up-regulate NF-κB-mediated transcription.

To investigate the contribution of endogenous CPAP to transcriptional activation, we examined the effect of CPAP siRNA, which was designed to specifically knock down the expression of CPAP, on NF-κB-dependent transcriptional activation in MCF-7 breast cancer-derived cells. We confirmed that endogenous CPAP protein levels were significantly reduced by transfection with CPAP siRNA, whereas the levels of other cellular proteins such as α-tubulin were not changed (Fig. 2B). The level of NF-κB-mediated transcription induced by either TNFα or RelA in CPAP siRNA-treated cells was decreased to <50% of that in cells transfected with control siRNA (Fig. 2C, upper panel). In contrast, reporter activity from the plasmid containing mutated NF-κB-binding sites was not affected by knocking down CPAP (Fig. 2C, lower panel). These findings indicate that endogenous CPAP is required for full activation of NF-κB-dependent reporter gene expression.

Next, we examined whether CPAP affects expression of endogenous target genes. After treatment with TNFα, total RNA was isolated from MCF-7 cells transfected with either control or CPAP siRNA and analyzed by reverse transcription-PCR to detect the mRNA levels of interferon-β and TNF receptor-associated factor-1, which are known to be induced by NF-κB. As shown in Fig. 2D, transfection with CPAP siRNA, but not control siRNA, down-regulated TNFα-induced expression of interferon-β and TNF receptor-associated factor-1 mRNAs. These results indicate that CPAP plays an important role in NF-κB-mediated transcriptional activation in cells.

Translocation of CPAP into the Nucleus upon TNFα Treatment—RelA is translocated from the cytoplasm into the nucleus upon stimulation by specific cytokines. To determine
whether the localization of CPAP is similarly altered by activation of the NF-κB pathway, we examined the subcellular localization of CPAP in MCF-7 cells by indirect immunofluorescence analysis with or without TNFα treatment. As reported previously (23), CPAP was found to localize primarily in the cytoplasm, although some protein was also detectable in the nucleus without stimulation (Fig. 3a). As CPAP was immunoprecipitated with RelA from the cytoplasmic fraction of such cells (data not shown), it seemed likely that a cytoplasmic complex is present before TNFα stimulation. However, following TNFα treatment for 20 min, a portion of CPAP was observed to accumulate in the nucleus (Fig. 3e), similar to RelA (b and f). These results suggest that at least a portion of cytoplasmic CPAP enters the nucleus in a TNFα-dependent manner.

**Recruitment of CPAP to the NF-κB-binding Motif**—The increase in NF-κB-dependent transcriptional activation by CPAP, the nuclear accumulation of CPAP in response to TNFα stimulation, and the physical interaction of CPAP with RelA all suggested the possibility that CPAP, together with RelA, is recruited to the transcriptional promoters of NF-κB target genes. To examine this possibility, we performed a DNA-protein complex immunoprecipitation assay. As shown in Fig. 4 (upper panel, lanes 1–3), a DNA fragment containing an NF-κB-binding motif was detected by PCR in complexes specifically immunoprecipitated by either anti-FLAG or RelA antibodies from lysates of 293T cells transfected with pNF-κB-luc, FLAG-tagged CPAP, and RelA expression plasmids. In contrast, no DNA fragment was amplified from cells transfected with pNF-κB-mt-luc instead of pNF-κB-luc (Fig. 4, lower panel, lanes 2 and 3). These data suggest that CPAP is recruited to the transcriptional promoter region containing an NF-κB-binding motif via association with RelA.

**CPAP Can Activate Gene Expression When Tethered to a Transcriptional Promoter**—We showed above that CPAP interacted with RelA, up-regulated NF-κB-mediated transcription, and formed part of the complex binding to a transcriptional promoter containing NF-κB-binding motifs. These data suggest that CPAP acts as a transcriptional coactivator of NF-κB. We examined this possibility using a one-hybrid assay system with fusion proteins consisting of the Gal4 DNA-binding domain (DBD) and full-length CPAP or its C-terminal region in mammalian cells. As demonstrated in Fig. 5 (second bar), Gal4 DBD-fused CPAP up-regulated luciferase expression from pFR-luc, a reporter plasmid containing a Gal4-responsive transcriptional promoter. In contrast, CPAP by itself had no effect on the same promoter (Fig. 5, third bar). No difference in luciferase levels was observed between the exogenous Gal4 DBD-containing constructs (data not shown). This suggests that CPAP has a transactivation capacity when tethered to the transcriptional promoter. This activity is likely to be located in the C-terminal part of CPAP because this region showed higher transcriptional promoter activity compared with full-length CPAP (Fig. 5, fourth and second bars, respectively). Together with our results above, these data suggest that CPAP acts as a transcriptional coactivator in the NF-κB transcriptional complex.

**Interaction between CPAP and CBP**—To obtain insights into the mechanism of CPAP-dependent transcriptional activation, we assessed whether CPAP can recruit known coactivators to the transcriptional promoter. First, we examined the physical association of CPAP with CBP, p300, steroid receptor coactivator-1 (SRC-1), and transcription intermediary factor-2 using a GST pull-down assay. In vitro synthesized CBP or p300, but not SRC-1 or transcription intermediary factor-2, was co-purified with GST-CBP-C, whereas none could be pulled down by normal mouse IgG (Fig. 6a) (data not shown), suggesting that CPAP interacts with CBP and p300. To narrow down the region of CBP required for interaction with CPAP, deletion analysis was carried out using five CBP fragments, CBP1–CBP5 (27), which were produced and metabolically labeled in cells. Only CBP4, which contains the C/H5 domain, could be co-purified with GST-CBP-C (Fig. 6b), suggesting that CPAP associates with CBP through this region of CBP.

Next, we performed a reporter assay to examine the effect of CPAP on NF-κB-mediated transcription. As shown in Fig. 6c, overexpression of either CBP or CPAP in cells enhanced TNFα-induced NF-κB-dependent transcription by −2–3-fold.
partly by binding CBP and recruiting it to the cellular transcriptional machinery. After 48 h of transfection, cells were harvested and subjected to DNA-protein complex including RelA and an NF-κB transcriptional machinery. We also observed that CPAP exists in a likely to activate transcription when presented to the promoter. Moreover, we found that the transcriptional coactivator CBP and can activate transcription when tethered to a promoter.

In this study, we have presented data showing that CPAP is a transcriptional coactivator.

**DISCUSSION**

We identified CPAP as a molecule that associates with RelA and contributes to RelA-mediated transcriptional activation. Although CPAP was previously identified as a centrosomal protein (22), its function was not clear. Recently, however, CPAP was reported to interact with STAT5 to enhance STAT5-mediated transcription (23). However, the mechanism of CPAP-mediated transcriptional activation remained unclear.

In this study, we have presented data showing that CPAP is a component of the NF-κB transcriptional activation complex and can activate transcription when tethered to a promoter. Moreover, we found that the transcriptional coactivator CBP contributes at least in part to transcriptional activation by association with CPAP.

Because CPAP fused with Gal4 DBD, but not CPAP itself, could induce reporter gene expression under the control of a promoter with Gal4-responsive elements (Fig. 5), CPAP is likely to activate transcription when presented to the transcriptional machinery. We also observed that CPAP exists in a DNA-protein complex including RelA and an NF-κB-responsive element, suggesting that CPAP binds to the promoter of NF-κB target genes in association with RelA to activate transcription. Enhancement of STAT5-mediated transcription by CPAP could be explained by a similar mechanism because CPAP was shown to interact with STAT5a/b as well as RelA. These data suggest that CPAP is a transcriptional coactivator.

The C-terminal 372 amino acids of CPAP exhibited transcriptional activation capability when fused with Gal4 DBD, suggesting that this region is responsible for transcriptional activation. This region associates with CBP, indicating that CBP may be involved in the transcriptional activation potential of CPAP. CBP is known to activate transcription by two mechanisms. CBP functions as a molecular bridge between the basal transcriptional machinery and transcription factors recruited to specific enhancer elements. In addition, the histone acetyltransferase activity of CBP plays an essential role in opening up chromatin structure to allow for efficient transcriptional activity (30, 31). Previous work also showed that p300/CBP binds to RelA and supports NF-κB-mediated transcriptional activation (9, 10). Here, we have shown that CPAP can associate with p300/CBP as well as RelA, indicating that these three proteins may form a complex. The breast cancer-related BRCA1 has been proposed to function as a scaffolding protein that tethers several factors, including RelA, CBP, and RNA polymerase II, to transcriptional promoter elements (21). By analogy with BRCA1, it seems likely that CPAP supports the transactivating effects of CBP by acting as a scaffolding protein that stabilizes CBP within the NF-κB transcriptional complex. We have shown that the C-terminal domain of CPAP interacts with the C-terminal region of p300/CBP containing the C/H3 and glutamine-rich domains. It was previously reported that the C-terminal TAD of RelA interacts mainly with the N-terminal region of p300/CBP containing the C/H1 and KIX domains (9, 10), which is distinct from the region that interacts with CPAP. These results may provide a structural framework for the formation of a complex including these three factors. However, the interactions are likely rather more complex because the C-terminal region of CPAP has also been identified as aRelA-interacting region. Furthermore, we already found that CPAP forms multimer (data not shown). Therefore, stoichiometric analysis will be required to unveil the functional structure of this mysterious complex as well as to better understand the molecular mechanism of CPAP-dependent transcriptional activation.

It is well known that some CBP-associated transcriptional coactivators such as p300/CBP-associated factor, SRC-1, and ACTR have histone acetyltransferase activity (32–34). Some members of the p300/CBP-associated factor-related family with strong histone acetyltransferase activity, such as GCN5, have a conserved amino acid sequence called motif A (35), which is considered to be a characteristic structural feature of histone acetyltransferase. However, we have not found any amino acid sequences similar to motif A in CPAP. On the other hand, it has been reported that the histone acetyltransferase domains of SRC-1 and ACTR members of the SRC family with relatively low histone acetyltransferase activity share regions of high glutamine content (33, 34). Because CPAP has multiple glycine or glutamine repeats in the C-terminal region shown to have transcriptional activation capacity, it is possible that CPAP possesses histone acetyltransferase activity in the C-terminal region. To determine whether this is in fact the case, biochemical analysis using purified CPAP is required in the future.

Besides functioning as a transcriptional coactivator, CPAP might also affect interactions between RelA and molecules that inhibit NF-κB-mediated transcription, such as IκB, histone deacetylase-1 (13), and RelA-associated inhibitor (36). Histone deacetylase-1 has been reported to interact directly with the N-terminal region of RelA to exert its corepressor function (13). RelA-associated inhibitor, which binds to the central region of RelA, has also been reported to inhibit RelA-mediated transcriptional activation via an unknown mechanism (36). As we have already detected that IκBα was co precipitated with a CPAP-RelA complex from the cell lysate (data not shown), it may be unlikely that the presence of IκBα precludes the association of RelA and CPAP. Further analysis of the complex including RelA and CPAP under physiological conditions should provide valuable insight into the regulatory mechanism of NF-κB-dependent transcriptional activation.

In addition, we found that the subcellular localization of CPAP was partially altered from the cytoplasm to the nucleus upon TNFα treatment. It was also previously reported that CPAP, which binds to STAT5, translocates from the cytoplasm.
to the nucleus in response to prolactin-mediated activation of the JAK-STAT pathway and enhances STAT5-dependent transcription (23). As it has been reported that CPAP has three putative nuclear localization signals in its C-terminal region (23), it seems possible that CPAP is retained in the cytoplasm somehow in the steady state of cells, but released by particular stimuli. It has been revealed that ACTR, which is located mainly in the cytoplasm with a small portion in the nucleus in most cells, translocates from the cytoplasm to the nucleus upon TNFα/H9251 activation and subsequent phosphorylation by IKB kinase-β (37). Further study on the molecular mechanism of stimulation-dependent nuclear translocation of CPAP may provide new knowledge regarding the fine regulation of gene expression by extracellular stimuli.

In this study, we have shown that CPAP can modulate RelA function in the nucleus. However, CPAP was first identified as a component of the centrosomal complex. The molecular interaction between CPAP and RelA evokes the possibility that RelA exists in centrosomes. Centrosomal location of factors related to transcription, such as p53 (38, 39) and BRCA1 (40, 41), has been reported and may be involved in centrosomal replication in a transcriptional activity-dependent or -independent manner. Further analysis of whether the interaction between CPAP and RelA affects centrosomal function may reveal new biological roles for RelA as well as CPAP.

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