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Constitutive phosphorylation of IκBα by casein kinase II

(NF-κB/Rel/transcription/PEST/protein purification)

CHARLENE F. BARROGA, JENNIFER K. STEVENSON, EDWARD M. SCHWARZ, AND INDER M. VERMA

Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800

Communicated by Renato Dulbecco, The Salk Institute for Biological Studies, La Jolla, CA, April 28, 1995 (received for review April 11, 1995)

ABSTRACT **The NF-κB/Rel proteins are sequestered in the cytoplasm in association with the phosphorylated form of IκBα. Upon induction with a wide variety of agents, the activity of NF-κB/Rel proteins is preceded by the rapid degradation of IκBα protein. We report the identification and partial purification of a cellular kinase from unstimulated or stimulated murine cells, which specifically phosphorylates the C terminus of IκBα. There are several consensus sites for casein kinase II (CKII) in the C-terminal region of IκBα. Additionally, the activity of the cellular kinase is blocked by antibodies against the α subunit of CKII. No phosphorylation of the C-terminal region of IκBα can be detected if the five possible serine and threonine residues that can be phosphorylated by CKII are mutated to alanine. A two-dimensional tryptic phosphopeptide map of IκBα from unstimulated cells was identical to that obtained by *in vitro* phosphorylation of IκBα with the partially purified cellular kinase. We propose that constitutive phosphorylation of IκBα is carried out by CKII.**

The NF-κB/Rel family of transcription factors are key regulators of a variety of genes involved in the immune and inflammatory responses, growth, differentiation, and development (reviewed in refs. 1–3). NF-κB/Rel proteins are sequestered in an inactive form (NF-κB complex) in the cytoplasm through their association with an inhibitory protein called IκB (4–7). Stimulation of the cells with a variety of agents results in a rapid degradation of IκB, allowing the translocation of NF-κB/Rel proteins to the nucleus where they can activate the transcription of target genes containing the decameric κB DNA-binding site (8–14). This rapid mechanism of activation makes members of the NF-κB/Rel family of transcription factors potent and pleiotropic gene activators.

The diversity of agents that can activate NF-κB complex ranges from cytokines, such as tumor necrosis factor α, interleukin 1, phorbol esters, bacterial lipopolysaccharide (LPS), DNA-damaging agents, double-stranded RNA, hydrogen peroxide, and chemicals that generate reactive oxygen intermediates (reviewed in refs. 1 and 15). Although many of these activators may use different signal-transduction pathways, they all converge on one common target, the disruption of NF-κB/IκB interactions, resulting in the loss of IκBα proteins. Partially purified NF-κB complexes can be activated *in vitro* by phosphorylation with protein kinase C, cAMP-dependent protein kinase, heme-regulated eukaryotic initiation factor 1 (eIF-1) kinase, raf-1 kinase, etc. (16–19). The assumption was that the IκBα protein present in the complex becomes phosphorylated, resulting in its release from NF-κB/Rel proteins. With the molecular cloning and subsequent availability of antibodies to IκBα (a prototypic member of the IκB family), it came as a surprise that the IκBα protein was completely degraded before the DNA-binding activity of NF-κB could be detected (8–12, 14). Furthermore, prior to degradation, a slow-migrating form of the IκBα protein was detected and shown to be due to phosphorylation (8–10, 12). Since mammalian IκBα and its homologue in chicken, pp40, are found in the cell as phosphoproteins (18, 20), agents that activate NF-κB could also induce a kinase that hyperphosphorylates IκBα, leading to its degradation. Consequently, there may be two specific kinase. One of them constitutively phosphorylates IκBα and this phosphorylated form associates with NF-κB proteins sequestering them in the cytoplasm. The second kinase further phosphorylates the phosphorylated form of IκBα in response to external signals, leading to its eventual degradation and the release of NF-κB.

MATERIALS AND METHODS

IκBα Deletion Mutants and Glutathione *S*-Transferase (GST)-Fusion Proteins. Mutants were generated by PCR and confirmed by sequencing. The pT7GT fusion-protein expression plasmids were transformed into the *Escherichia coli* strain BL21 (DE3) (Novagen). Bacterial cultures (1 liter) were grown to an OD_{600} of 1.0 and then induced for 3 h with 0.5 mM isopropyl β-D-thiogalactoside (IPTG). Cells were resuspended in 30 ml of phosphate-buffered saline (PBS; 10 mM Na_2HPO_4/150 mM NaCl/2.7 mM KCl) containing 0.1% Triton X-100, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg of aprotinin and 20 μg of leupeptin (Sigma) per ml, and sonicated on ice. The soluble fractions were incubated with 1.0 ml of glutathione (GSH)-Sepharose beads (1:1) (Pharmacia) for 1 h at 4°C, after which they were washed four times with 50 ml of the above lysis buffer.

Purification Protocol. The mouse pre-B cell line 70Z/3 stably harboring CD14 (LPS receptor), 70Z/3-hCD14 (21), kindly provided by R. J. Ulevitch of Scripps Reserach Institute, was grown in RPMI 1640 medium containing 10% (vol/vol) fetal calf serum, 100 μg of penicillin, 100 μg of streptomycin, and 800 μg of G418 per ml, and 50 μM 2-mercaptoethanol. The cells were grown to confluency, harvested, and resuspended in PBS containing 0.1% Triton X-100, 1 mM DTT, 10 μg of leupeptin and 10 μg of aprotinin per ml, 0.1 mM PMSF, and 0.2 mM Na_3VO_4. Whole-cell extracts (WCE) were cleared by centrifugation at 10,000 × *g* for 10 min and precipitated with 50% ammonium sulfate. The pellet was resuspended in Tris buffer [20 mM Tris·HCl, pH 7.5/100 mM NaCl/10% (vol/vol) glycerol/10 mM $MgCl_2$/1 mM DTT/0.1 mM EDTA/0.1 mM PMSF] and was passed through a Blue Sepharose column equilibrated in Tris buffer. The flow through and the first two washes were loaded onto a Q Sepharose column equilibrated in Tris buffer and developed with 200 ml of a 0–1.0 M NaCl gradient in Tris buffer. Fractions having IκBα kinase activity were pooled, brought up to 0.2 M NaCl, loaded onto a phosphocellulose column equilibrated with Tris buffer containing 0.2 M NaCl (22, 23), and eluted with a 200-ml gradient of 0.2–1.0 M NaCl in Tris buffer.

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Abbreviations: CKII, casein kinase II; LPS, lipopolysaccharide; WCE, whole-cell extracts; GSH, glutathione; GST, glutathione *S*-transferase; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol.

Kinase Assays. *In vitro kinase binding assay.* Cell extracts (100 μg) were diluted to 1.0 ml in 1× PBS/0.1% Triton X-100/0.1% Tween 20/1 mM DTT. To this was added 10 μg of GST or GST-fusion proteins bound to GSH-Sepharose beads. The mixture was rotated at 4°C for 1 h, after which the beads were pelleted and washed two times with 1 ml of the PBS binding buffer, and then three times with 1 ml of the Hepes kinase buffer without ATP (20 mM Hepes·KOH, pH 7.4/10 mM $MgCl_2$/10 mM $MnCl_2$/1 mM DTT). After being washed, the beads were resuspended in 20 μl of the kinase buffer containing 20 μM ATP and 2 μCi of [γ-^{32}P]ATP (1 Ci = 37 GBq). After incubation for 15 min at 37°C, the reaction was terminated by washing three times with 1 ml of the kinase buffer without ATP. The phosphorylated proteins were eluted from the beads with 20 μl of 2× Laemmli sample buffer, resolved by SDS/10% PAGE, and visualized by autoradiography.

In vitro kinase solution assay. GST or GST-fusion proteins were eluted from the GSH-Sepharose beads with 20 mM GSH/20 mM Tris·HCl, pH 8.0/100 mM NaCl. Five micrograms of the soluble GST or GST-fusion proteins was mixed with 10 μg of WCE or 5 μl of the biochemically purified fractions. To this was added 20 μl of the Hepes kinase buffer containing 20 μM ATP (or GTP) and 2 μCi of [γ-^{32}P]ATP (or [γ-^{32}P]GTP) and incubated for 15 min at 37°C.

Antibody competition. One microliter of the Q Sepharose fraction 31 (Q31) was preincubated with varying amounts of the casein kinase II (CKII) antibody for 15 min at room temperature prior to the addition of the substrate GST–C-Term and the Hepes kinase buffer containing 20 μM ATP and 2 μCi of [γ-^{32}P]ATP. The reaction was allowed to proceed as above, and the phosphorylated proteins resolved SDS/PAGE. Other antibodies used (5 μl) in these studies were against the following kinases: Pelle (kindly provided by S. Wasserman, Texas Southwestern Medical Center, Dallas), Raf (Oncogene Science), and CDC2 (Upstate Biotechnology, Lake Placid, NY). GST–ELK and p44^{MAPK} were kindly provided by R. Fukunaga and T. Hunter (The Salk Institute).

Immunokinase assays. One hundred microliters of the Q Sepharose fraction was incubated with 10 μl of the CKII antibodies and 40 μl of Protein A-Sepharose in 350 μl of RIPA buffer (20 mM Tris·HCl, pH 8.0/100 mM NaCl/0.2% Triton X-100/0.2% deoxycholate/0.2% Nonidet P-40/1 mM PMSF). The mixture was rotated at 4°C overnight and immune complexes were washed two times with 1 ml of RIPA buffer and three times with 1 ml of the Hepes kinase buffer without ATP. GST–C-Term fusion protein and Hepes kinase buffer containing 20 μM ATP and 2 μCi [γ-^{32}P]ATP were added for the kinase assay.

^{32}P-*in Vivo* Labeling and Immunoprecipitation. 70Z/3-hCD14 cells (2.8×10^7) were incubated for 4 h at 37°C in 3 ml of phosphate-free Dulbecco's modified Eagle's medium (DMEM) containing 10% (vol/vol) dialyzed fetal calf serum (GIBCO/BRL), 1 mM sodium pyruvate, 100 μM nonessential amino acids, and 0.29 mg of L-glutamine, 800 μg of G418, and 0.8 mCi ^{32}P orthophosphate per ml. Cytoplasmic extracts were prepared, and the IκBα protein was immunoprecipitated by using a 1:50 dilution of anti-mIκBα serum raised against the N-terminal 60 amino acids of IκB. Immunoprecipitated proteins were fractionated by electrophoresis through an SDS/15% polyacrylamide gel, and the labeled IκBα protein was eluted from the gel and subjected to tryptic peptide mapping according to Boyle *et al.* (24).

GST–IκBα protein was labeled with [γ-^{32}P]ATP by using the purified cellular kinase from the phosphocellulose column (P18). The *in vitro*-labeled GST–IκBα was gel purified on an SDS/15% polyacrylamide gel, eluted, and subjected to tryptic peptide mapping as above.

RESULTS

Constitutive IκBα Kinase in Both Unstimulated and Stimulated Cells. WCE from 70Z/3 cells transfected with the gene for CD14 (70Z/3-hCD14) which were either unstimulated or stimulated with LPS were prepared and mixed with GSH-Sepharose beads containing GST or GST-fused IκBα (GST–IκBα) or various portions of the IκBα protein (Fig. 1*A*). The bound proteins were pelleted, washed extensively, incubated in the kinase buffer containing [γ-^{32}P]ATP, and analyzed by SDS/PAGE. The data in Fig. 1*B* show the phosphorylation of GST–IκBα (lanes 2 and 8) but not of GST alone (lanes 1 and 7), indicating that there is a cellular kinase that associates specifically with GST–IκBα, resulting in its phosphorylation. This cellular kinase is present in both the unstimulated and LPS-stimulated WCE (compare Fig. 1*B*, lanes 2 and 8), suggesting a constitutive IκBα kinase. In the experiments shown here, we have used CD14 (LPS) receptor gene-transfected 70Z/3 cells to increase the efficiency of stimulation

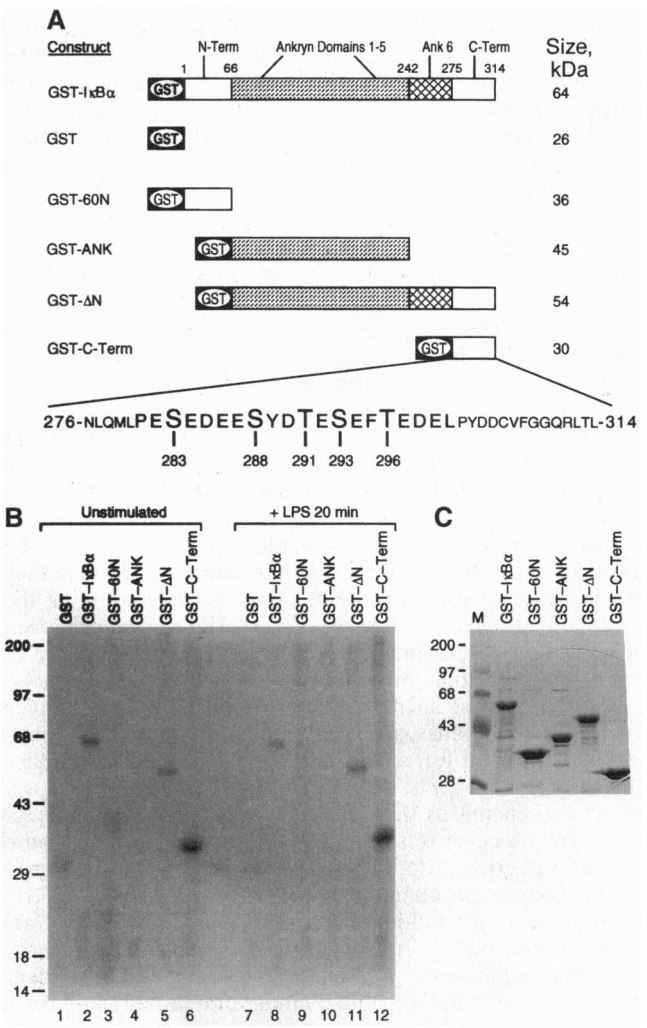

FIG. 1. Identification of a constitutive IκBα kinase. (*A*) Schematic drawing of IκBα constructs fused to GST. The IκBα C-terminal 39 amino acid sequence containing CKII consensus sites is shown. The serine and threonine residues are in bold letters. (*B*) *In vitro* kinase-binding assays. Ten micrograms of bacterially produced GST or GST-fusion proteins bound to GSH-Sepharose beads was incubated with WCE from unstimulated or LPS-stimulated 70Z/3-hCD14 cells. Phosphorylated proteins were resolved by SDS/10% PAGE and visualized by autoradiography. An identical pattern of phosphorylation is detected using WCE from either unstimulated cells (lanes 1–6) or LPS-stimulated cells (lanes 7–12). (*C*) Coomassie blue-stained gels of the GST–IκBα and GST–IκBα deletion mutant proteins.

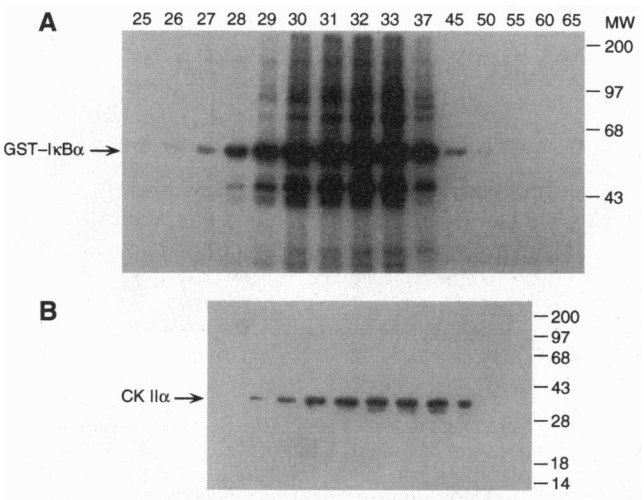

FIG. 2. Partially purified kinase from the Q Sepharose column contains CKII activity. (*A*) WCE from unstimulated 70Z/3-hCD14 cells were fractionated as described in *Materials and Methods*, and fractions from the Q Sepharose column were assayed by *in vitro* kinase solution assay by using soluble GST–IκBα protein as the substrate. (*B*) Q Sepharose fractions exhibiting IκBα kinase activity were immunoblotted with CKII antibodies recognizing 44-kDa α (catalytic) subunit of CKII (Upstate Biotechnology). The peak of IκBα kinase activity correlates with the peak of the CKII immunoreactive bands. MW, molecular weight ($\times 10^{-3}$).

with LPS, but similar results were obtained from nontransfected 70Z/3 and Jurkat cells (data not shown).

To determine which region of the IκBα protein is phosphorylated, we generated fusion proteins containing GST–60N (N-terminal region), GST-ANK (containing the first five ankyrin repeats), GST–ΔN (N-terminal 60 aa are deleted), and GST–C-Term (aa 276–314) and used them in the *in vitro* kinase binding assays with WCE. Fig. 1*B* shows that only those chimeric proteins which contain the C-terminal region are phosphorylated (lanes 2, 5, 6, 8, 11, and 12) by cellular kinases present in both the uninduced and induced WCE. The same pattern of phosphorylation can be observed when fusion proteins were eluted from the GSH-Sepharose beads and used in an *in vitro* solution kinase assay as described in *Materials and Methods* (data not shown). Fig. 1*C* shows the Coomassie blue-stained gel of the GST-fusion proteins.

Partially Purified Constitutive IκB Kinase Has CKII Properties. Fig. 2*A* shows the profile of the kinase activity from the Q Sepharose column when using soluble GST–IκBα protein as a substrate. A number of other phosphorylated proteins copurify with the kinase activity. Examination of the C terminal amino acid sequence of IκBα (Fig. 1*A*) reveals that it contains serine and threonine residues flanked by many acidic amino acids, reminiscent of the CKII consensus site. We therefore reasoned that the fractionated kinase activity from the Q Sepharose column may be related to CKII. Immunoblotting active fractions with CKII antibodies shows the presence of the 44-kDa α subunit of CKII (22) (Fig. 2*B*). The peak of IκBα kinase activity corresponds to the peak of the CKII subunit. We conclude that the cellular kinase fraction capable of phosphorylating IκBα contains CKII.

Purified CKII and Q Sepharose-Purified Kinase Phosphorylation of IκB C Terminus. Fig. 3*A* shows that the pattern of phosphorylation of the various IκBα proteins is identical between commercially available CKII (Upstate Biotechnology) and Q31 (fraction 31 from Q Sepharose in Fig. 2*A*). There are fewer nonspecific phosphorylated proteins observed when purified CKII was used in the assays (Fig. 3*A*, lanes 1–6). To further confirm that the kinase activity in the Q31 fraction is

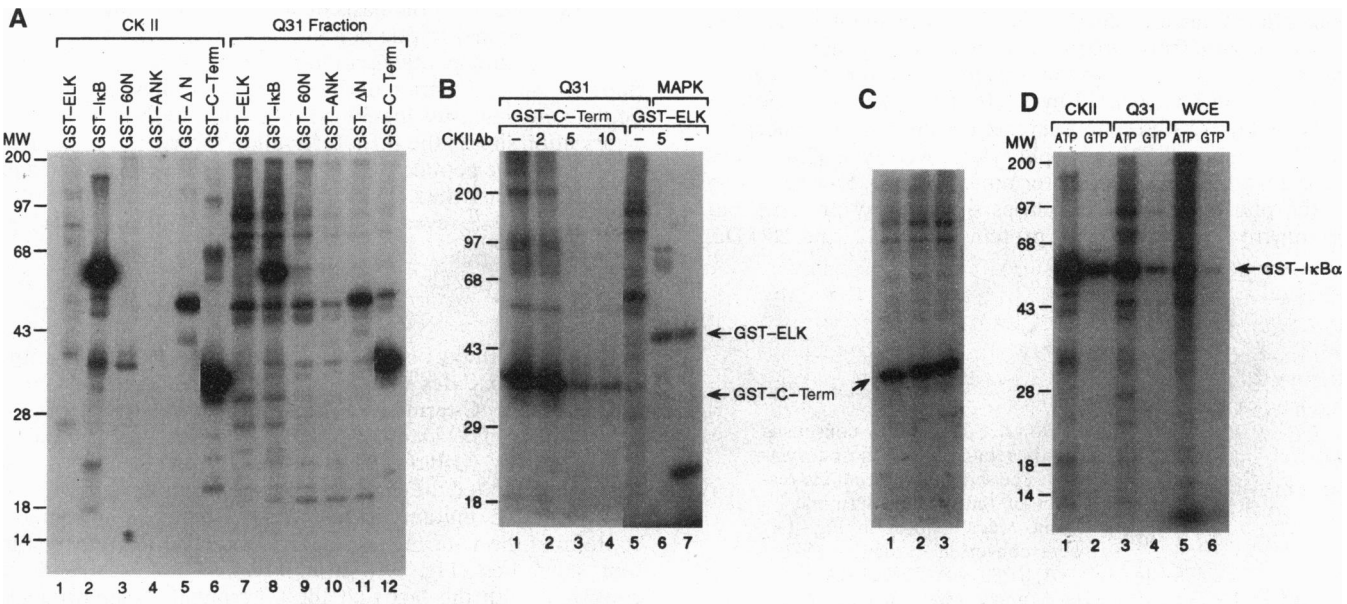

FIG. 3. Characterization of the partially purified kinase from Q Sepharose as CKII. (*A*) Purified CKII and Q Sepharose-purified kinase exhibit identical phosphorylation of the various IκBα constructs. Ten nanograms of purified CKII (Upstate Biotechnology) (lanes 1–6) or 5 μl of fraction Q31 (lanes 7–12) was incubated with 5 μg of soluble GST–IκBα protein or various deletion constructs in a solution kinase assay. Phosphorylated proteins were resolved on an SDS/10% polyacrylamide gel and visualized by autoradiography. (*B*) CKII antibodies can specifically block the IκBα C-terminal kinase activity from the Q Sepharose fractions. The Q31 fraction was incubated with different amounts of CKII antibodies (CKIIAb) prior to the addition of the substrate, GST–C-Term, and the subsequent kinase assay. One microliter of the Q31 fraction was incubated without CKII antibody (lane 1), or with 2 μl (lane 2), 5 μl (lane 3), or 10 μl (lane 4) of CKII antibody. $p44^{MAPK}$ was also incubated with 5 μl of CKII antibodies (lane 6) or without CKII antibody (lane 7), prior to the addition of its substrate, GST–ELK. The Q31 fraction was also incubated with GST–ELK (lane 5). (*C*) Immunoprecipitation of a C-terminal IκBα kinase activity from fraction Q31 with CKII antibodies. CKII antibodies were incubated with fraction Q31, and the immunoprecipitate was used to phosphorylate GST–C-Term. (*D*) The constitutive kinase from the unstimulated 70Z/3-hCD14 WCE and the Q Sepharose fractions can utilize GTP, similar to the purified CKII. Purified CKII, the Q31 fractions, and WCE were incubated with soluble GST–IκBα protein and Hepes kinase buffer containing either [γ-^{32}P]ATP (lanes 1, 3, and 5) or [γ-^{32}P]GTP (lanes 2, 4, and 6). Phosphorylated proteins were resolved on an SDS/10% polyacrylamide gel and visualized by autoradiography. MW, molecular weight ($\times 10^{-3}$).

due to CKII, we incubated fraction Q31 with CKII antibodies prior to the addition of the substrate and the kinase buffer. The CKII antibodies were raised against residues 70–91 of the catalytic domain (α subunit) of CKII and should inhibit CKII activity (25). Fig. 3*B* shows that with increasing concentrations of the CKII antibodies, phosphorylation of the C terminus of IκBα decreased (compare lanes 1–4). This competition is specific for CKII, since the CKII antibodies had no effect on the ability of the $p44^{MAPK}$ to phosphorylate its substrate GST–ELK (Fig. 3*B*, lanes 6 and 7). Moreover, there was no effect on the Q31 kinase activity when antibodies against the Pelle, Raf, and CDC2 kinases were used (data not shown). To further demonstrate that CKII is the IκB kinase present in the Q31 fraction, the Q31 fraction was subjected to immunoprecipitation using CKII antibodies and protein A-Sepharose. The immunoprecipitates were washed extensively prior to the addition of the substrate GST–C-Term and the kinase buffer containing [γ-^{32}P]ATP. Fig. 3*C* shows that a C-terminal IκBα kinase activity can be immunoprecipitated using CKII antibodies. Lanes 1–3 show three successive rounds of immunoprecipitation of the same fraction.

One of the hallmarks of CKII activity is that, unlike other kinases, it can also utilize GTP to transfer phosphate to the substrate. The data in Fig. 3*D* show that like CKII, both the Q31 fraction and the WCE can phosphorylate GST–IκBα protein when using either [γ-^{32}P]ATP (lanes 1, 3, and 5) or [γ-^{32}P]GTP (lanes 2, 4, and 6).

CKII Consensus Site Is Required for Phosphorylation. CKII recognizes the phosphorylation site motif (Ser/Thr)-XaaXaaZaa where Xaa is often an acidic residue and Zaa must be either an acidic residue or a phosphorylated serine or threonine residue (25, 26). The C-terminus of IκBα has five possible serine and threonine residues that could be phosphorylated by CKII. These residues, Ser-283, Ser-288, Thr-291, Ser-293, and Thr-296, are highly conserved between IκBα from human, rat, and chicken. We therefore mutated the three serines and the two threonines to alanine to generate IκBα-Ala-C-Term. Fig. 4 shows that this mutant C terminus can no longer be phosphorylated by CKII (lane 3). Additionally, a more highly purified cellular kinase obtained from a phosphocellulose column, P18, phosphorylates wild-type C terminus (lane 1), but not mutated C terminus (lane 2). Silver staining of the phosphocellulose fractions shows many proteins, but prominent among them are proteins of 44, 42, and 28 kDa,

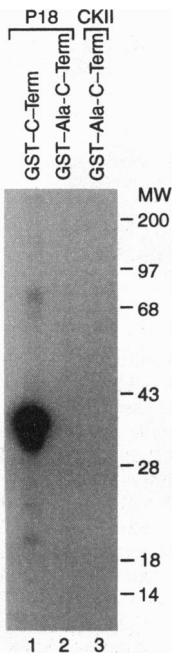

FIG. 4. The CKII consensus site is required for phosphorylation. The serine and threonine residues of the IκBα C-terminal 39 amino acids (shown in Fig. 1*A*) were converted to alanine (S-283 → A, S-288 → A, T-291 → A, S-293 → A, and T-296 → A) to generate GST–Ala-C-Term. Five microliters of the P18 fraction of the cellular kinase purified from the phosphocellulose column was incubated with GST–C-Term (lane 1) or GST–Ala-C-Term (lane 2), and 10 ng of purified CKII was incubated with GST–Ala-C-Term (lane 3) and soluble kinase assays were performed. Neither CKII nor the kinase from the P18 fraction utilizes GST–Ala-C-Term as a substrate. MW, molecular weight ($\times 10^{-3}$).

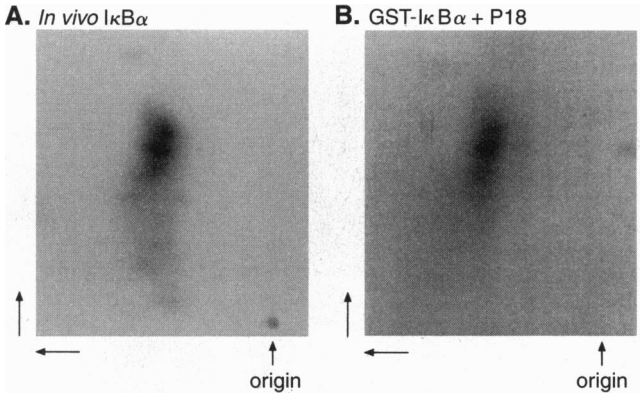

FIG. 5. Tryptic peptide mapping of *in vivo* and *in vitro* labeled IκBα. Tryptic fragments were run with buffer, pH 8.9, for the electrophoresis dimension and isobutyric acid for the chromatography dimension (24). The position of the origin is indicated by a short arrow. (*A*) Endogenous IκBα tryptic fragments labeled *in vivo* with ^{32}P. (*B*) Tryptic fragments from GST–IκBα labeled *in vitro* using [γ-^{32}P]ATP and the purified kinase from the phosphocellulose column, P18.

corresponding to the α, α', and β subunits of CKII (refs. 22 and 23; data not shown).

Consensus CKII Site in the C Terminus of IκBα Is Phosphorylated *in Vivo*. To determine whether the IκBα C-terminal sites that were phosphorylated by CKII and the purified cellular kinase from unstimulated 70Z/3-hCD14 cells are the *in vivo* constitutive phosphorylation sites, we generated a phosphotryptic peptide map of IκBα from unstimulated 70Z/3-hCD14 cells. The cells were labeled with orthophosphate for 4 h, and the endogenous IκBα protein was immunoprecipitated by using mouse IκBα antibodies. The phosphotryptic peptide map of IκBα reveals two closely migrating peptides (Fig. 5*A*), which, on the basis of size and charge, are likely to be the C-terminal tryptic peptide of 49 amino acids. The two closely migrating peptides could represent different combinations of phosphorylation of the five possible sites. This tryptic peptide encompasses the 39 amino acids and the CKII phosphorylation sites in the IκBα C terminus shown in Fig. 1*A*. The phosphotryptic peptide map of GST–IκBα that was labeled *in vitro* with the purified cellular kinase from the phosphocellulose column, P18, reveals a similar tryptic peptide pattern (Fig. 5*B*).

DISCUSSION

We have identified CKII as the cellular kinase that constitutively phosphorylates some or all of the serine and threonine residues in the C-terminal 39 amino acids of IκBα in 70Z/3-hCD14 cells. The identity of this kinase was confirmed by several means: (*i*) Pure CKII and the biochemically purified fractions exhibited the same pattern of phosphorylation on the IκBα deletion mutant proteins (Fig. 3*A*). Fusion proteins containing the C-terminal 39 amino acids of IκBα were the best substrates (Fig. 3*A*, lanes 6 and 12). This result is consistent with the fact that the C-terminal 39 amino acids contain multiple consensus sites for CKII phosphorylation (Fig. 1*A*). (*ii*) Fractions from the Q Sepharose and phosphocellulose columns that exhibited the C-terminal IκBα kinase activity contained a polypeptide that specifically immunoreacted with the antibodies directed against the conserved lysines of the catalytic domain of CKII (Fig. 2). (*iii*) These antibodies specifically inhibited the CKII kinase activity that was present in the Q Sepharose fractions, while it had no effect on the activity of MAP kinase to phosphorylate its substrate GST–ELK (Fig. 3*B*). (*iv*) CKII has the unique ability to utilize GTP in addition to ATP as a phosphate donor, although with a lower efficiency (K_m for GTP ≈30–40 μM, while K_m for

ATP ≈ 10 μM) (reviewed in ref. 27). This distinct specificity distinguishes CKII from a host of other Ser/Thr kinases. Since pure CKII and the biochemically purified fractions were both efficient in utilizing GTP (Fig. 3*D*), this confirmed that the kinase in the fractions is CKII. (*v*) When all the serine and threonine residues in the consensus CKII sites present in the C-terminal domain were changed to alanine, the resulting mutant, GST–IκB-Ala-C-Term was no longer phosphorylated by either CKII or the purified fraction (Fig. 4). (*vi*) The two-dimensional mapping experiments demonstrate that the major phosphopeptides from the *in vivo* phosphorylated endogenous IκBα correspond to the major phosphopeptides produced from GST–IκBα phosphorylated *in vitro* by the purified kinase (Fig. 5 *A* and *B*). No phosphopeptides were identified when the mutant IκB-Ala-C-Term was used as the substrate (data not shown). We conclude that the C-terminal 39 amino acids is the *in vivo* constitutive phosphorylation site and that CKII is the *in vivo* constitutive kinase of IκBα.

CKII is a ubiquitous, highly conserved kinase, located in both the nucleus and the cytoplasm (25). It is thought to be involved in mitogenesis because its activity is modulated by treatment of different cell types with growth-stimulatory factors (28, 29). However, the precise regulation of CKII activity has not been deciphered. In LPS-stimulated 70Z/3-hCD14 cells, neither the amount nor the activity of CKII was stimulated compared with unstimulated cells (Fig. 1*B*), and it can be detected in almost equal amounts in the cytoplasm and the nucleus (data not shown). CKII has been shown to phosphorylate a variety of nuclear oncoproteins implicated in cell proliferation, such as Fos, Jun, Myc, Myb, p53, adenovirus E1A protein, human papilloma virus E7 protein, and the simian virus 40 large T antigen, in addition to a variety of other proteins (25, 29). The effect of CKII phosphorylation varies depending on the protein. Phosphorylation by CKII at the N-terminal site of Myb, near its DNA-binding domain, reversibly inhibits the sequence specific binding of Myb to DNA (30). In E1A, T antigen, and Myc proteins, the regions that are required for transforming function are targets for CKII phosphorylation (31). More often, CKII phosphorylation has no discernible effect on the function of the protein but rather makes accessible the subsequent phosphorylation by another kinase (25, 32–34).

All of the proteins mentioned above share the characteristic of being highly regulated because of their potent transforming potential. They are also extremely short-lived, and contain PEST sequences that have been implicated in the degradation of unstable proteins (35). The CKII phosphorylation sites in IκBα comprise part of the PEST sequence, as noted by Ernst *et al.* (36). It is possible that phosphorylation of the PEST sequences has a function in the rapid degradation of IκBα. However, phosphorylation by CKII in itself is not the signal for IκBα degradation. The role of this constitutive phosphorylation could be to facilitate inducible phosphorylation or degradation of IκBα. Consequently, without the basal phosphorylation, further phosphorylation or degradation may be inefficient. Identification of the inducible kinase will help in the elucidation of the precise role of CKII phosphorylation in the regulation of IκBα activity.

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