Identification and Characterization of CKIP-1, a Novel Pleckstrin Homology Domain-containing Protein That Interacts with Protein Kinase CK2

Denis G. Bosc, Kevin C. Graham, Ronald B. Saulnier, Cunjie Zhang, David Prober, R. Daniel Gietz, and David W. Litchfield

The catalytic subunits of protein kinase CK2, CK2α and CK2α', are closely related to each other but exhibit functional specialization. To test the hypothesis that specific functions of CK2α and CK2α' are mediated by specific interaction partners, we used the yeast two-hybrid system to identify CK2α- or CK2α'-binding proteins. We report the identification and characterization of a novel CK2-interacting protein, designated CKIP-1, that interacts with CK2α, but not CK2α', in the yeast two-hybrid system. CKIP-1 also interacts with CK2α in vitro and is co-immunoprecipitated from cell extracts with epitope-tagged CK2α and an enhanced green fluorescent protein fusion protein encoding CKIP-1. EGFP-CKIP-1 when they are co-expressed, CK2 activity is detected in anti-CK2α immunoprecipitates performed with extracts from non-transfected cells indicating that CKIP-1 and CK2 interact under physiological conditions. The CKIP-1 cDNA is broadly expressed and encodes a protein with a predicted molecular weight of 46,000. EGFP-CKIP-1 is localized within the nucleus and at the plasma membrane. The plasma membrane localization is dependent on the presence of an amino-terminal pleckstrin homology domain. We postulate that CKIP-1 is a non-enzymatic regulator of one isoform of CK2 (i.e. CK2α) with a potential role in targeting CK2α to a particular cellular location.

Protein kinase CK2 (CK2) is an essential, highly conserved, protein serine/threonine kinase present in all eukaryotic cells (reviewed in Refs. 1–6). CK2 has been reported to phosphorylate a broad range of cellular proteins located in a variety of cellular compartments (mainly the nucleus and cytoplasm) and is involved in important cellular processes such as transcription, translation, morphogenesis, and cell cycle progression (reviewed in Refs. 1–7). These observations support an important role for CK2 in a variety of cellular functions; however, its specific roles and mode of regulation in cells remain poorly understood. Moreover, these results suggest that CK2 is involved in a complex array of interactions with a wide selection of cellular proteins that are present in a broad variety of cellular locations.

CK2 is a tetrameric protein comprised of two regulatory subunits (CK2β) and two catalytic subunits (CK2α and/or CK2α'). CK2α and CK2α' are the products of separate genes, and their amino acid sequences are highly conserved between higher eukaryotes (reviewed in Ref. 7). In fact, in mammals and birds, CK2α and CK2α' exhibit greater than 90% identity over their 330 amino-terminal amino acids (7). This amino-terminal sequence identity is in stark contrast to the unrelated carboxy-terminal sequences of CK2α and CK2α' that exhibit no obvious similarity (8–10). This sequence divergence between the carboxy-terminal domains of CK2α and CK2α' suggests that important functional differences that exist between the two different catalytic isozymes result from these unique sequences (11).

Previous studies have failed to demonstrate significant catalytic differences between CK2α and CK2α' in vitro (12), a result that likely reflects the high similarity exhibited by the catalytic domains of CK2α and CK2α' (12). By comparison, there is mounting evidence in support of functional specialization of both CK2α and CK2α' in cells (11, 13). For example, in HeLa cells, differences in the localization of CK2α and CK2α' have been observed, with further indications that the localization of both CK2α and CK2α' may be altered in a cell cycle-dependent manner (13). However, these differences in subcellular localization may be cell type-specific, since major differences in the localization of CK2α and CK2α' have not been observed in all cells where they have been examined (14, 15). It has also been demonstrated that CK2α, but not CK2α', is phosphorylated in mitotic cells of mammalian and avian origin (16), suggesting that the functions of the two isoforms are independently regulated during cell division. The mitotic sites of phosphorylation of CK2α were identified as Thr344, Thr360, Ser362, and Ser370, all of which are located within the carboxyl-terminal domain of CK2α (17). There is also a “PXXP” motif adjacent to two of the phosphorylation sites. Interestingly, “PXXP” motifs have been implicated in protein-protein interactions, most notably with SH3 domains of a variety of regulatory proteins (18, 19).

Biochemical studies have localized CK2 activity within the...
cytoplasm, the nucleus, and with other cellular structures including the plasma membrane (1, 2, 20, 21). Immunofluorescence studies have confirmed that CK2 is localized in the nucleus and in the cytoplasm (13–15, 22, 23). The factors or mechanisms that regulate the subcellular distribution of CK2 remain poorly understood. In this regard, it may be notable that there is mounting evidence demonstrating that CK2 interacts with a variety of cellular proteins. For example, CK2β has been reported to interact with FGF-2 (24), A-Raf (25, 26), Nopp140 (27), p53 (28–30), p21WAF1/CIP1 (31), c-MOS (32), and CD5 (33). CK2α and CK2α’ have also been observed to interact with specific protein partners. For instance, both CK2α and CK2α’ have been shown to interact with nucleolin (34), ATF1 (35, 36), whereas only CK2α has been shown to interact with HSP90 (37, 38) and with PP2A (39). Interestingly, the domain of CK2α implicated in the interaction with PP2A was localized to the sequence 169HEHRKL (human and chicken CK2α), which is one of the few regions of non-identity between CK2α and CK2α’ (HQQKKL). Collectively, these observations suggest that the subcellular localization of CK2, and presumably its ability to phosphorylate a number of its target proteins, may be regulated by interactions with specific protein partners. The resulting compartmentalization of CK2 could provide a mechanism for regulating CK2 activity in cells. In fact, this mode of regulation could reconcile the observations that although CK2 is a predominantly nuclear enzyme, it can also phosphorylate cytoplasmic proteins, as well as nuclear proteins.

The possible regulation of CK2 through its interactions with specific protein partners in cells together with the observed functional differences between CK2α and CK2α’ led us to hypothesize that the catalytic subunits of CK2 are involved in isozyme-specific protein-protein interactions and that these interactions may be mediated through the unique carboxyl-terminal domains of CK2α or CK2α’. To examine this hypothesis, we used a yeast two-hybrid system to screen an Epstein-Barr virus-transformed human B-cell library using GAL4 DNA-binding domain fusions encoding full-length CK2α or CK2α’, as well as their respective carboxyl-terminal domains, as bait. In this study, we report the isolation and identification of a novel protein, designated casein kinase interacting protein 1 (i.e. CKIP-1), that binds CK2α but not CK2α’. In addition to providing further indications of functional specialization for CK2α and CK2α’, the identification of this novel protein may provide new insights for understanding how the access of CK2 to some of its cellular targets is regulated. Examination of the predicted amino acid sequence of CKIP-1 reveals the presence of a pleckstrin homology domain that could mediate interactions between CKIP-1 and cellular membranes or proteins as well as motifs (including a leucine zipper and PXXP) that could be involved in protein-protein interactions. Overall, our results raise the possibility that CKIP-1 is a non-enzymatic regulator of CK2 that could play a role in controlling the access of CK2α to specific cellular targets through its ability to sequester CK2 or to recruit CK2 to specific cellular locations.

**EXPERIMENTAL PROCEDURES**

**Materials**

Human osteosarcoma Saos-2 cells and SV40 large T-transformed green monkey kidney COS-7 cells were obtained from ATCC (Manassas, VA) and were maintained in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Life Technologies, Inc.) supplemented with 10% fetal calf serum (Life Technologies, Inc.) and penicillin/streptomycin. The two deletion constructs of CK2 (i.e. CK2a-(332–391) and CK2a-(332–391)(4D)) were amplified from an sk+ CK2α template using the following primers: 5′-CGG GAA TTC GCT CGA ATG GGT TCA TCT 3′ (sense primer with EcoRI site) and 5′- CGG ATC CAC CTC TGC TCA 3′ (antisense primer with BamHI site). Similarly, a 190-base pair fragment representing the carboxyl-terminal domain of CK2α (i.e. CK2α’-(333–350)) was amplified from a sk+ CK2α template using the following primers: 5′- TCC GCC GGT GAC ACT ATA G 3′ (antisense primer with EcoRI site) and 5′- CGG ATC CAC CTC TGC TCA 3′ (antisense primer with BamHI site). The two deletion constructs of CK2 (i.e. CK2α-(332–391)(4D)) were amplified from an sk+ CK2α template using the following primers: 5′- CCG GAA TTC GCT CGA ATG GGT TCA TCT 3′ (antisense primer with EcoRI site) and 5′- CGG ATC CAC CTC TGC TCA 3′ (antisense primer with BamHI site). The respective EcoRI/BamHI fragments were then subcloned into EcoRI- and BamHI-digested pGEX vector.

To generate CK2α-(332–391)(4D), the nucleotides coding for the amino acid residues representing the four mitotic sites of phosphorylation at amino acid residues (Thr344, Thr360, Ser362, and Ser370) within CK2α were replaced with alanines using a double-stranded primer set of the following primers: 5′- TGG CAT AGG TCT CTT GAA ATG CCC TCT GCT CAG 3′ (sense primer with EcoRI site) and 5′- GGA CCT GCA GGC GAT CCA GTG 3′ (antisense primer with BamHI site).

**Plasmid Constructs**

pGBT9 Constructs—Full-length cDNAs encoding CK2α or CK2α’ were subcloned into the BamHI site of the pGEX vector. Appropriate orientation was verified by DNA sequencing. To prepare constructs encoding the carboxyl-terminal domains of CK2α or CK2α’ (i.e. CK2a-(332–391) and CK2α’-(333–350)), the cDNAs encoding the respective carboxyl-terminal domains were amplified by PCR with restriction sites (EcoRI and BamHI) at either end to facilitate subcloning. A 217-base pair fragment representing the carboxyl-terminal domain of CK2α (i.e. CK2α-(332–391)) was amplified from an sk+ CK2α template using the following primers: 5′- CCG GAA TTC GCT CGA ATG GGT TCA TCT 3′ (antisense primer with EcoRI site) and 5′- CGG ATC CAC CTC TGC TCA 3′ (antisense primer with BamHI site). The amplified fragments were then subcloned into EcoRI- and BamHI-digested pGEX vector.
respective NcoI/SalI fragments were subcloned into SalI/NcoI-digested pGBT9-C2α to replace the 3′-coding region of C2α. These constructs were transformed into yeast as described below to ensure that they did not autonomously activate transcription and to verify that the truncated CK2α hybrid proteins still exhibited interactions with pACTII-C2β. The latter test ensures that the deleted proteins maintain the overall integrity of C2α that is required to interact with C2β.

**pEGFP Constructs—**To generate constructs for the expression of enhanced green fluorescent protein fusions of CKIP-1, a 1.5-kb BglII fragment encoding the entire open reading frame encoded by the two-hybrid positive was subcloned into the BglII site of the pEGFP-C2 vector (CLONTECH) to yield the pEGFP-C2-CKIP-1. Alternatively, a cloned GST fusion protein of CK2α with the carboxyl-terminal domain of CK2α was generated as follows. A fragment encoding residues 133–409 (sense primer with 5′-GTA TCA GAA TTC CCC GAG CCA AGA ACC GTA TCT TGG 3′) was generated using the following primers: 5′-GTA TCA GAA TTC CCC GAG CCA AGA ACC GTA TCT TGG 3′ (sense primer with BclI and EcoRI sites) and 5′-GAA TTC CCC GAG CCA AGA ACC GTA TCT TGG 3′ (antisense primer with EcoRI and SalI sites). This fragment was subcloned into pCR-Blunt (Invitrogen) and sequenced. A BclI/BamHI fragment of the insert was then isolated and subcloned into pEGFP-C2-C2KI-1 (308–409) that had been digested with BglII and BamHI to generate pEGF-C2-CKIP-1 (133–409).

**Other Constructs—**A strategy similar to that described in the preceding section was used to subclone the 1.5-kb BglII fragment and the 300-bp BamHI/BglII fragment into pDest-SX to generate glutathione S-transferase (GST) fusion proteins encoding the entire open reading frame (designated GST-C2KI-1) and a GST fusion protein encoding the carboxy-terminal 102 residues of C2KI-1 (designated GST-C2KI-1 (308–409)). A plasmid encoding a GST fusion protein of CK2α was constructed by subcloning the BamHI fragment encoding full-length CK2α into the BglII site of pDest-SX. Constructs encoding C2KI subsunits with HA epitope tags in pBc/CMV (Invitrogen) were previously described (14). Briefly, HA-CK2α and HA-CK2α′ encode amino-termi
ally tagged CK2α and CK2α′, respectively, whereas CK2α-HA encodes CK2α with a carboxy-terminal HA tag. The construct designated HA-CK2α′/n encodes an amino-terminally tagged chimera where the carboxy-terminal domain of CK2α has been attached to the amino-termi
nal domain of CK2α′ as described (14).

**Anti-CKIP-1 Antibodies**
Anti-CKIP-1 antibodies were raised in rabbits by Babco (Richmond, CA) using a GST fusion protein encoding the carboxy-terminal 102 residues of CKIP-1 (i.e. GST-C2KI-1 (308–409)) as the antigen. To characterize these antibodies, lysates were prepared from human osteosarcoma Saos-2 cells using Laemmli sample buffer and were analyzed on Western blots that were developed with pre- or post-immune serum from either of the two immunized rabbits in the presence or absence of GST-C2KI-1 (308–409) or GST. Antiserum from either of the two immunized rabbits, but not the pre-immune serum, recognized a band of approximately 50 kDa in extracts of Saos-2 cells that was blocked by the inclusion of GST-C2KI-1 (308–409), but not by GST, during the incubation with antibody. Affinity-purified anti-GST antibodies were obtained from this rabbit serum by passing the serum through a GST-affinity column that was made by coupling purified GST to Affi-Gel 10 (Bio-Rad) according to the manufacturer’s recommendations. Once this serum had been depleted of anti-GST antibodies, it was passed through a GST-C2KI-1 (308–409) affinity column that was similarly prepared to isolate affinity-purified anti-CKIP-1 antibodies. Antibodies were recovered from affinity columns as described (45).

**Transformation and Maintenance of Yeast**
The yeast strain PJ69-4a (MATα, ade2, trp1-901, leu 2-3, 112 his3–200, 1-133–409) was transformed with the various pGBT9-C2KI constructs using the method of Schiestl and Gietz (41). The transformants were selected on plates of synthetic complete medium minus Trp and minus Leu (Trp-Leu−), as well as on complete medium minus Trp, minus Leu, minus His, and minus Ade (Trp-Leu− His− Ade−) and grown for 3–5 days at 30 °C. Colonies that grew under the latter conditions were selected for further characterization.

**Screening of Yeast Two-hybrid Positives for CK2β**
Since we had previously demonstrated that interactions between CK2α (or CK2α′) and CK2β can be detected using the two-hybrid system (42), we utilized a PCR strategy to examine positive colonies from the screens performed using CK2α or CK2α′ as the bait for the presence of the cDNA encoding CK2β. For this analysis, yeast were grown overnight at 30 °C in liquid culture. Yeast were disrupted by vortexing with glass beads, and plasmid DNA was isolated as described previously (43). The primers used for PCR were 5′-GCC GGG ATC CTG AGC AGC TCA GAG GAG 3′ (sense primer located within the domain of CK2β) that interacts with CK2α and 5′-CCA CCA GAA TTC GCC ATG CCG GTA GAG GTG TGA TCA 3′ (antisense primer located within the ADH-terminator of pACT) (46). Colonies that were positive for CK2β were not further analyzed.

**Isolation of the CKIP-1 cDNA**
After excluding all of the positives that encoded CK2β, one positive from the screen with CK2α was further characterized. Plasmid DNA was obtained from yeast by glass bead preparation and electroporated into the yeast strain of Escherichia coli. To select for those bacteria that harbored the pACT plasmid, bacteria were selected on −Leu plates. Plasmid DNA was then isolated and used to re-test for interactions with Gal4 DNA binding domain fusions of CK2α or CK2α′ in yeast, as described above. Transformations were also performed with a number of control constructs (as indicated). Plasmid DNA was also used as a template for sequencing manually using T7 polymerase or by auto-
mated sequencer (Perkin-Elmer).

**Northern Blot Analysis**
A Human Multiple Tissue Northern blot (CLONTECH) was probed as recommended in the accompanying manual. Probes that were labeled with 32P were as follows: an XhoI fragment of the CKIP-1 cDNA, or the full-length cDNAs encoding CK2α, CK2α′ or CK2β.

**In Vitro Binding Assay**
A GST fusion protein encoding full-length CK2α or GST itself were expressed in bacteria and purified using glutathione-agarose as described previously (17). Purified GST-C2KI and GST were then coupled to Affi-Gel 10 (Bio-Rad) at a concentration of 4 mg/ml according to the manufacturer’s recommendations. Radiolabeled (35S-labeled) CKIP-1 was produced by in vitro transcription and translation using a T-N Kit (Promega) with T7 polymerase according to the manufacturer’s recommendations. For this procedure, a PCR product encoding CKIP-1 was generated using a heat-stable DNA polymerase (Pfu) obtained from Stratagene) from pACT-38 using the following primers: 5′-TAA TAC GAC TCA CTA TAG GGA GAC CATG ATG GAT GAT GTA TAT AAC TAT 3′ (sense primer) and 5′-CCA CCA GAA TTC GCC ATG CCG GTA GAG GTG TGA TCA 3′ (antisense primer). The former primer anneals within the activation domain of Gal4 that is encoded by pACT and introduces a T7 promoter to be used for in vitro transcription, whereas the latter primer anneals to a sequence within the ADH terminator of pACT. Radiolabeled CKIP-1 (5 μl of the reticulocyte lysate reaction) was incubated for 1 h at 4 °C with 5 μl of Affi-Gel 10 to obtain a total volume of 50 μl in interaction buffer (20 mM Hepes, pH 7.4, 150 mM NaCl, 10% glycerol, 0.05% Nonidet P-40) supplemented with protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 1% apropin). After the 1-h incubation, the Affi-Gel beads were collected by centrifugation, and the supernatant was removed, and the beads were washed three times with the interaction buffer. Proteins retained on the Affi-Gel beads were eluted by the addition of Laemmli sample buffer and were subjected to SDS-polyacrylamide gel electrophoresis. Radiolabeled proteins were visualized using a PhosphoImager (Molecular Dynamics).

**Transfection of COS-7 and Saos-2 Cells**
Cells were grown to 80% confluence and then split 1:8 into 100 × 20-mm culture plates or 35 × 20-mm wells containing coverslips. The following day, the media were changed 2–5 h prior to transfection. Transfections were carried using calcium phosphate as described (44) or
Novel PH Domain-containing CK2-interacting Protein

Using Eugene 6 (Roche Molecular Biologicals) as described by the manufacturer. Plasmids encoding various pEGFP constructs were added at concentrations of 5 μg per 100 × 20-mm culture plates or 1–2 μg per 35 × 20-mm wells of a 6-well flat bottom tissue culture plate. The various pBluescript vectors encoding CK2 constructs were added at concentrations of 15–25 μg per 100 × 20-mm culture plate or 5–10 μg per 35 × 20-mm well of a 6-well flat bottom tissue culture plate. For calcium phosphate precipitations, precipitates of DNA were left on cells for 16–18 h after which time the cells were washed thoroughly with PBS, pH 7.4–7.5, to remove the precipitate. Media was added to cells which were incubated a further 24 h. Cells were then washed thoroughly with PBS, the liquid was removed, and cells grown on this occasion were mounted onto glass slides for fluorescence microscopy, whereas cells on the plates were frozen at −80 °C until needed.

Cell extracts were initially prepared by scraping cells from plates in lysis buffer (100 mM Tris-Cl, pH 7.5, 2 mM EDTA, 100 mM NaCl, 1% Triton X-100, 10 μM 4-(2-aminoethyl)benzenesulfonyl fluoride, 1% aprotinin, 2 μg/ml leupeptin, 2 μg/ml pepstatin A), followed by sonication (3 × 10 s bursts) on ice. The resulting protein extracts of approx. 16,000– x g for 10 min. The supernatants were collected and used immediately for immunoprecipitations or alternatively stored at −80 °C. Expression of relevant proteins was confirmed using Western blot analysis.

Immunoprecipitation

Immunoprecipitations were carried out in a 1:1 mixture comprised of transfected cell extract (described above) and binding buffer (200 mM NaCl, 1% Triton X-100, 0.1% SDS) supplemented with protease inhibitors (10 μg/ml leupeptin, 0.1% phenylmethylsulfonyl fluoride, 1% aprotinin) (45). These extracts were clarified by centrifugation as described above and incubated with anti-CKIP-1 antibodies (antiserum at 1:250 or 5 μg of affinity purified antibody) for 2 h at 4 °C. Since it was apparent that the anti-CKIP-1 antibodies recognized a protein in cell extract of approximately 50 kDa, antibodies were initially cross-linked to protein A-Sepharose using dimethylpimelimidate as described (45). Samples were rocked on ice for 1 h. Sepharose beads were then washed with 3 × 500 μl of a 1:1 mixture of lysis buffer/binding buffer and then resuspended in Laemmli sample buffer. Samples were analyzed by SDS-polyacrylamide gel electrophoresis followed by Western blot analysis. Relevant proteins were detected using either anti-GFP antibody (1/2000), anti-CKIP-1 antibody (1:1000), or biotinylated anti-CKIP-1 clones (data not shown). However, using the full-length CK2α and CK2α′, we obtained 243 and 196 positives, respectively. We expected that a number of the positives could be CK2β, the regulatory subunit of CK2 that is known to interact strongly with both CK2α and CK2α′ in yeast two-hybrid assays (42, 46).

RESULTS

Yeast Two-hybrid Screen—In order to identify protein-binding partners of CK2α or CK2α′, we performed independent yeast two-hybrid screens of a human B-cell cDNA library using constructs encoding full-length CK2α and CK2α′ as well as the carboxyl-terminal domains of the respective proteins as bait (Fig. 1A). The carboxyl-terminal domains of CK2α or CK2α′ were each utilized in screens in order to examine the possibility that these unique carboxyl-terminal domains were sufficient for interactions with binding partners. Screens with each of these constructs were performed simultaneously. Neither of the carboxyl-terminal domain constructs yielded any positive clones (data not shown). However, using the full-length CK2α and CK2α′, we obtained 243 and 196 positives, respectively.

Specificity of Interaction of pACT-38 with CK2α—In order to determine whether the protein encoded by the pACT plasmid in colony 38 (designated pACT-38) encoded a protein able to interact specifically with CK2α, we re-examined its ability to interact with CK2α as well as a variety of other proteins in a yeast two-hybrid assay (Fig. 1B). Positive interactions involving pACT-38 were only detected when it was co-expressed with CK2α. Despite the high degree of similarity between CK2α and CK2α′, we did not observe any interaction with CK2α′. This latter result is consistent with the absence of this cDNA arising from the green tissue was performed with CK2α′. In these reconstruction assays, the integrity of the CK2α and CK2α′ constructs was confirmed by testing them successfully for interactions with pACT-CK2β (data not shown). The interaction of the pACT-38-encoded protein with CK2α, but not with CK2α′, suggests that differences existing between CK2α and CK2α′ are sufficient to confer interaction specificity. Moreover,
the observation that the pACT-38-encoded protein was not able to interact with either the CK2α-(332–391) or CK2α’-(333–350) in the yeast two-hybrid system indicates that the carboxy-terminal domains by themselves are not sufficient to mediate
the interaction between CK2α and the protein encoded by pACT-38, which we subsequently designated CKIP-1 (i.e. CK2
interacting protein-1).

To extend further the analysis of interactions between CK2α and the protein encoded by pACT-38, we were interested in determining whether the unique carboxy-terminal domain of CK2α is required for interactions. Consequently, we con-
stucted two deletions of CK2α, CK2α-(2–330) and CK2α’-(2–350). As illustrated in Fig. 1A, both of these deletion constructs contain all of the subdomains that are found in all protein kinase family members. Moreover, to residue 330 of CK2α, the two isoforms of CK2 exhibit approximately 90% identity, and there is no similarity between CK2α and CK2α’ beyond that point. The former construct (i.e. CK2α-(2–330)) completely removes all of the unrelated residues, whereas the latter construct (i.e. CK2α’-(2–350)) generates a form of CK2α that is the same length as CK2α’. In preliminary tests, these deletion constructs failed to activate transcription autonomously indicating that they are suitable for use in two-hybrid assays (data not shown). Furthermore, both constructs exhibited interactions with CK2β in two-hybrid assays suggesting that the overall structural integrity of CK2α had been maintained (data not shown). When these constructs were tested for interactions with pACT-38, their capacity for interactions is similar to that exhibited by CK2α (Fig. 1C). This result suggests that although the major difference between CK2α and CK2α’ lies within their carboxy-terminal domain, the carboxy-terminal domain of CK2α is not required for interactions with CKIP-1. It is noteworthy that protein phosphatase 2A also exhibits interactions with CK2α and not with CK2α’ and that the residues important for this interaction are within the amino-terminal 330 residues of CK2 and not within the carboxy-terminal domain (39). More detailed future analysis of CK2α will be required to identify the specific residues of CK2α that are important for the selective interactions with CKIP-1.

**Interactions between CKIP-1 and CK2α in Vitro**—The ability of CKIP-1 to interact directly with CK2α was tested in vitro using purified GST fusion proteins and the products of *in vitro* transcription and translation. In Fig. 2A, we detected a major radiolabeled translation product of approximately 50 kDa in the lysate containing the pACT-38 DNA (lane designated as 38). This band is not present in the control lysate which did not contain any pACT-38 DNA (lane C). By using GST-CK2α and GST proteins as affinity matrices in pull-down assays, it was evident that the CKIP-1 product interacts with GST-CK2α but...
not with GST (Fig. 2B). Quantitation of the amount of radiactivity that was recovered on Affi-Gel beads (Fig. 2B) or remained in the supernatant (Fig. 2C) indicated that approximately 50% of the CKIP-1 was retained on the GST−CK2a, whereas the amount of CKIP-1 that was retained on GST was negligible. A lower band (i.e. below the 47.5K marker) that is most evident in the lysate (Fig. 2A) and in the GST−CK2 pull down (Fig. 2B) could either be an alternative translation product of CKIP-1 resulting perhaps from a different start codon or the band could be a proteolytic degradation product of CKIP-1. However, the precise identity of this band is not known. As was the case with GST, in vitro translated CKIP-1 exhibited negligible interactions with GST−CK2β (data not shown). Taken together, the results from the yeast two-hybrid assays and the in vitro interaction studies suggest that the CKIP-1 protein is capable of interacting specifically and directly with CK2a. We also demonstrated that in vitro translated CK2a could interact with GST−CKIP-1 (data not shown), further confirming the in vitro interactions between the two proteins.

Nucleotide and Deduced Amino Acid Sequences of CKIP-1—

The nucleotide sequence of CKIP-1 was determined by sequencing the 1469-bp CKIP-1 insert located within the BglII site of pACT. We discovered a large open reading frame (ORF), encompassing nucleotides 1–1391, which was in frame with the GAL−4 activation domain of pACT. The ORF was followed by a TGA stop codon at nucleotides 1392–1394. Near the 5′ end, we also observed two successive putative ATG start codons starting at nucleotide 165, in which the second ATG more closely resembles a Kozak consensus sequence (48). The deduced amino acid sequence of the ORF (bp 165–1391) (Fig. 3) encodes a protein of 409 amino acids with a theoretical molecular mass of 46,336.93 Da. Notably, this predicted size is consistent with that observed for the in vitro translation product previously (Fig. 2).

Screening of the nucleotide sequence of CKIP-1 with the “BLAST” programs identified one significant, partial match. The carboxyl-terminal domain of CKIP-1 (encompassing amino acids 338–409 of the predicted amino acid sequence) displayed 95% identity with the predicted amino acid sequence from a partial cDNA encoding an ORF of 72 amino acids that was identified as a putative c-Jun leucine zipper interactive mouse protein (cDNA JZA-20/pir B46132) (50). This ORF does contain a sequence that resembles a leucine zipper (shown in Fig. 3) that may explain why it exhibited the capacity to interact with the leucine zipper of c-Jun. Isolation of a full-length cDNA and further characterization of the protein encoded by JZA-20 was not performed (50). Consequently, the significance of interactions between JZA-20 and c-Jun remains undefined. Analysis of the deduced amino acid sequence of CKIP-1 by “ProfileScan” and “Pfram” demonstrated the presence of a pleckstrin homology (PH) domain between residues 21 and 132 of the deduced protein sequence (Fig. 3).

Northern Blot Analysis—To determine whether we had isolated a cDNA encoding the full-length CKIP-1 protein and to examine its expression patterns, we probed a human multiple tissue northern blot (CLONTECH) using a fragment of the CKIP-1 cDNA. This probe detected a major band of approximately 1.5 kb as well as an additional band of significantly lower intensity at 4.4 kb. The overall intensity signal of the 1.5-kb band of CKIP-1 was highest in the skeletal muscle and heart lanes. Intermediate signals were observed in brain, placenta, and lung lanes, whereas weaker signals were seen in the liver, kidney, and pancreas lanes. We also probed the same Northern with CK2α, CK2α′, and then CK2β (Fig. 4, B–D, respectively), in order to compare expression levels of the different mRNAs throughout the various human tissues. Al-
The tentative start codon(s) of the ORF that we had identified in CKIP-1. At the 3’ end of EST H14297, we identified an AT-rich region that was not observed in the CKIP-1 nucleotide sequence. Taken together, these results support the conclusion that a cDNA that encoded the full-length CKIP-1 protein had been isolated.

Detection of a 50-kDa Protein with Anti-CKIP-1 Antiserum—In order to study the CKIP-1 protein, polyclonal rabbit antibodies directed against a GST fusion protein encoding the carboxyl-terminal 102 amino acids of CKIP-1 (i.e. GST-CKIP-1(308–409)) were raised as described under “Experimental Procedures.” By immunoblot analysis, anti-CKIP-1 antibodies detect a protein of approximately 50 kDa in lysates and in anti-CKIP-1 immunoprecipitates (Fig. 5A) that is not detected in anti-GST immunoprecipitates. Importantly, the 50-kDa band was not evident when immunoprecipitations were performed with pre-immune sera from either of the rabbits (data not shown). Moreover, the 50-kDa band is effectively competed away when Western blots with anti-CKIP-1 sera are performed in the presence of GST-CKIP-1(308–409) (Fig. 5B) but not when these blots are performed in the presence of GST (not shown). Overall, these results support the conclusion that the anti-CKIP-1 antibodies specifically recognize a protein of 50 kDa. Since the apparent molecular weight of this band is very similar to that predicted for the 409-amino acid ORF of CKIP-1, we believe that the 50-kDa protein is endogenous CKIP-1.

Measurement of CK2 Activity in Anti-CKIP-1 Immunoprecipitates—Immunoprecipitates of CKIP-1 were subsequently analyzed for kinase activity toward the specific CK2 substrate peptide RRRDDDSDDD (64) to determine whether there are interactions between CK2 and CKIP-1 in mammalian cells. As illustrated in Fig. 6, immunoprecipitates performed with anti-CKIP-1 antibodies derived from either of two immunized rabbits displayed CK2 activity that was approximately 10-fold above the background activity that was observed in immunoprecipitates performed with pre-immune serum derived from the same rabbits. Kinase activity toward the CK2 substrate peptide was nearly completely abolished by heparin, a known inhibitor of CK2 (7). Furthermore, inclusion of GTP in the kinase activity markedly diminished 32P incorporation into the substrate peptide. Since CK2 is one of the few protein kinases capable of utilizing GTP as substrate, the latter observation is consistent with the conclusion that the kinase being measured in anti-CKIP-1 immunoprecipitates is CK2. Collectively, these results provide strong evidence for interactions between CK2 and CKIP-1 in mammalian cells. However, we cannot rigorously exclude the possibility that kinases distinct from CK2, but with similar properties to CK2, may have contributed to the activity measured in anti-CKIP-1 immunoprecipitates. The CK2 activity that was measured in anti-CKIP-1 immunoprecipitates represents a relatively low percentage (less than 4%) of the activity that was measured in anti-CK2β immunoprecipitates. It is important to note that this estimate is based on the kinase activities that were measured in immune complex kinase assays performed using antibodies (anti-CKIP-1 or anti-CK2β) that may exhibit differences in immunoprecipitation efficiency. Nevertheless, these results suggest that CKIP-1–CK2 complexes represent a rather small fraction of total cellular CK2.

Expression of EGFP Fusion Proteins Encoding CKIP-1—Based on the evidence from co-immunoprecipitation assays that CKIP-1 and CK2 do indeed interact in mammalian cells, we were interested in examining the subcellular distribution of
Novel PH Domain-containing CK2-interacting Protein

Fig. 6. Measurement of CK2 activity in anti-CKIP-1 immunoprecipitates. A, extracts were prepared from human osteosarcoma Saos-2 cells using Nonidet P-40 lysis buffer and were subjected to immunoprecipitation with antiserum from either of 2 rabbits that were immunized with GST-CKIP-1-(308–409) (designated 1 and 2) or with pre-immune serum obtained from either of the same rabbits (designated as 1(pre) and 2(pre)). Immunoprecipitates were utilized in kinase assays using the specific CK2 substrate peptide RRDRDDDDDDD to measure CK2 activity. Incorporation of [32P]ATP into synthetic peptide from [32P]ATP was determined by P81 filter paper assay as described under “Experimental Procedures.” Results represent the average (± range) of duplicate determinations and are representative of three independent experiments. B, kinase activity in anti-CKIP-1 immunoprecipitates toward the CK2 substrate peptide RRDRDDDDDDD was performed in the presence of heparin or GTP as indicated. Kinase activities are normalized to the activities that were measured in the absence of heparin or GTP and represent the average of triplicate determinations (± S.D.) and are representative of two independent experiments.

CKIP-1 since we speculated that CKIP-1 could function as a protein that recruits CK2 to a particular cellular location. To achieve this objective, we prepared an expression construct encoding CKIP-1 as an EGFP fusion protein using pEGFP-C2, a plasmid that allows for the expression of fusion proteins at the carboxyl terminus of EGFP. An EGFP fusion protein encoding the carboxyl-terminal 102 amino acids of CKIP-1 was also constructed using an internal BamHI site within the CKIP-1 cDNA. The latter fusion protein lacks the PH domain that is located near the amino terminus of CKIP-1 but retains the putative leucine zipper. To effect a more precise deletion of the PH domain of CKIP-1, we also generated a construct encoding EGFP-CKIP-1-(133–409) as described under “Experimental Procedures.” As a prelude to examining the localization of these protein within cells, we examined their expression by Western blot analysis of transfected cell lysates using anti-CKIP-1 antisera or anti-GFP antiserum (Fig. 7). As expected, anti-GFP antibodies (Fig. 7) detected EGFP and each of the fusion proteins encoding CKIP-1 or deletions of CKIP-1 (lanes 2–5). The anti-CKIP-1 antibodies detect bands in lanes 2–4 that appear to be identical to the bands that are detectable with anti-GFP but do not detect GFP (lane 5). The low levels of endogenous CKIP-1 that are detected in COS-7 cells are not visible at the levels of detection that are represented in this figure.

Co-immunoprecipitation of EGFP-CKIP-1 and CK2α-HA—To characterize further the EGFP-CKIP-1 fusion protein, we examined its ability to interact with CK2 in mammalian cells using co-immunoprecipitation assays. To achieve this objective, we co-transfected cells with a construct encoding EGFP-CKIP-1 and a construct encoding epitope-tagged CK2α (i.e. CK2α-HA with a carboxyl-terminal HA tag) or with an empty vector (i.e. pRe/CMV). Anti-HA immunoprecipitates were performed with lysates obtained from these cells and examined on Western blots with anti-CKIP-1 antisera (Fig. 8A). Alternatively, immunoprecipitates performed with anti-CKIP-1 serum were analyzed with biotinylated anti-HA antibodies on Western blots (Fig. 8B). In Fig. 8A, the EGFP-CKIP-1 fusion protein can be visualized in a transfected cell lysates (marked lysate). As in Fig. 7, this band was not observed in a lysate from non-transfected cells (not shown). The EGFP-CKIP-1 protein is also present in anti-HA immunoprecipitates (Fig. 8A) derived from cells co-transfected with EGFP-CKIP-1 and CK2α-HA but not in immunoprecipitates from cells transfected with EGFP-CKIP-1 and the empty vector (marked pRe/CMV). In Fig. 8B, a band corresponding to the CK2α-HA is detected exclusively in lysates of cells transfected with CK2α-HA and in anti-CKIP-1 immunoprecipitates. Biotinylated anti-HA antibodies were utilized to eliminate the appearance of the immunoglobulin heavy chain that confounded the detection of CK2α-HA on immunoblots in a number of preliminary experiments. With these biotinylated antibodies, nonspecific bands are observed in all lysates, but since their migration on polyacrylamide gels is significantly different than that of CK2α-HA, they do not interfere with its detection. Moreover, these nonspecific bands are not observed in immunoprecipitates. Overall, the results of the co-immunoprecipitation assays suggest that the EGFP-CKIP-1 protein retains the capacity to interact with CK2α in mammalian cells.

Subcellular Localization of EGFP-CKIP-1 Constructs by Fluorescence Microscopy—To examine the subcellular localization of CKIP-1 in mammalian cells, we examined COS-7 cells that had been transfected with constructs encoding EGFP, EGFP-CKIP-1, EGFP-CKIP-1-(133–409), or EGFP-CKIP-1-(308–409) by fluorescence microscopy. With EGFP, we observed general cellular fluorescence that included prominent nuclear fluorescence (Fig. 9D). By comparison, EGFP-CKIP-1 does not exhibit the general cellular fluorescence and appears to localize chiefly to discrete areas of the cell consistent with plasma membrane and/or membrane ruffles (Fig. 9A). The deletion mutant, EGFP-CKIP-1-(308–409), displays a general cytosolic distribution similar to that observed with EGFP (Fig. 9G) but
Novel PH Domain-containing CK2-interacting Protein

After transfection of EGFP-CKIP-1 and CK2 into human osteosarcoma Saos-2 cells, EGFP-CKIP-1 together with either a pRc/CMV construct encoding CK2 or a carboxyl-terminal HA tag (designated CK2α-HA) or with an empty vector (pRc/CMV) as indicated. A, cell extracts were prepared and immunoprecipitations performed with anti-HA (i.e., 12CA5) antibodies. Cell lysates (as indicated) and immunoprecipitates (lanes marked IP) were analyzed on immunoblots with biotinylated anti-HA antibodies as described under “Experimental Procedures.” The position of CK2 was also marked (designated arrow). B, cells were co-transfected with pEGFP-C2-CKIP-1 and either a pRc/CMV construct encoding CK2α-HA or with an empty vector (pRc/CMV) as in A. Cell extracts were prepared, and immunoprecipitations were performed with anti-CKIP-1 antibodies. Lysates and immunoprecipitates (lanes marked IP) were analyzed on immunoblots with biotinylated anti-HA antibodies as described under “Experimental Procedures.” The position of CK2α-HA is indicated by an arrow. Non-specific bands detected in all lysates with biotinylated anti-HA antibodies are also marked (designated NS).

We also examined the localization of EGFP-CKIP-1 fusion proteins in human osteosarcoma Saos-2 cells because these cells exhibit higher level of endogenous CK1 than do COS-7 cells. In Saos-2 cells, EGFP-CKIP-1 exhibits prominent staining of the cell periphery as is the case in COS-7 cells. Moreover, a proportion, but not all, of the transfected Saos-2 cells also exhibited prominent nuclear localization of CK1 (Fig. 10B). Based on the prediction that CK1-IP-1 could be a non-enzymatic regulator of CK2 that controls the cellular activity of CK2 through its ability to sequester CK2 or to target CK2 to specific cellular locations, we were also interested in determining where CK2 and CKIP-1 exhibit co-distribution within cells. For these experiments, endogenous CK2 was examined in these cells by indirect immunofluorescence. Fluorescent proteins were detected by confocal microscopy. As expected, in Saos-2 cells (Fig. 10A) and also in COS-7 cells (not shown), CK2 exhibits a predominantly nuclear localization with some non-nuclear staining. As noted above, in Saos-2 cells, EGFP-CKIP-1 was detected at the cell periphery and in the nucleus (Fig. 10B). Co-distribution between EGFP-CKIP-1 and CK2 in the nucleus is clearly illustrated by the presence of the yellow color that is seen in the overlay (Fig. 10C). However, with the resolution of these techniques, we could not conclusively determine whether or not a small proportion of the CK2 exhibited co-distribution with the EGFP-CKIP-1 that is at the cell periphery. Similarly, in COS-7 cells where EGFP-CKIP-1 is predominantly localized to the cell periphery, we could not conclusively determine whether EGFP-CKIP-1 and CK2 are co-distributed. In light of the predominant nuclear localization of CK2 and the undoubtedly complexity of interactions between CK2 and its many cellular targets (including substrates), these latter observations are perhaps not surprising.

**DISCUSSION**

In this study, we report the identification of a novel protein of unknown function that we have designated CKIP-1 on the basis that we initially identified it as a CK2-interacting protein. This protein was originally isolated as a CK2-interacting protein using the yeast two-hybrid system, but interactions between CK2 and CKIP-1 were also observed in *vitro* using GST pull-down assays and using co-immunoprecipitation assays from both transfected and non-transfected cells. Furthermore, using the yeast two-hybrid system we have observed that CKIP-1 is able to interact with the CK2α, but not the CK2α’, isoform of protein kinase CK2 suggesting that CKIP-1 exhibits isoform specificity. Based on this observation, it is tantalizing to speculate that the isoform specificity of CKIP-1 may in part contribute to the functional specialization that has been observed for CK2α and CK2α’ (11, 13, 51).

Our results from yeast two-hybrid assays also suggest that, although the major difference between CK2α and CK2α’ lies within their unrelated carboxyl-terminal domains, this domain does not appear to be required for interactions between CK2α and CKIP-1. It is noteworthy that protein phosphatase 2A also exhibits isoform specificity and interacts with CK2α but not CK2α’ (39). Interestingly, the residues that are critical for interactions between protein phosphatase 2A and CK2α reside within the amino-terminal region of CK2α and do not involve its unique carboxyl-terminal domain. Future investigation will be required to identify the precise residues within the amino-terminal 330 amino acids of CK2α that are involved in interactions with CKIP-1.

On the basis that our two-hybrid screens to identify proteins that interact with the catalytic subunits of CK2 yielded predominantly the regulatory β subunit of CK2, our screens must be classified as successful. Furthermore, since the CKIP-1 cDNA was isolated from the screen using the same criteria that yielded CK2β, it is not surprising that CKIP-1 appears to be a physiologically relevant CK2-interacting protein. In utilizing the yeast two-hybrid system to identify proteins that interact with CK2, we anticipated that we could isolate novel substrates or regulators of CK2. Regulators of CK2 could be classified as activators or inhibitors of CK2 (or indirect regulators that interact with the catalytic subunits of CK2) or direct regulators (i.e., activators or inhibitors of CK2) or indirect regulators that could have the potential to control the cellular activity of CK2 by controlling its localization in cells or recruiting the enzyme to its substrates. In this regard, we have examined the possibility that CKIP-1 is a substrate for CK2, but we have been unable to observe any significant phosphorylation of either GST-CKIP-1 or GST-CKIP-1-(308–409) by purified CK2 in *vitro* under conditions that yield extensive phosphorylation of casein (data not shown). Similarly, neither GST-CKIP-1 nor GST-CKIP-1-(308–409) alters the activity of purified CK2 in *vitro* (data not shown). These results suggest that CKIP-1 is neither a substrate nor direct regulator of CK2.
activity. Consequently, the notion that CKIP-1 may be an indirect regulator of CK2 may be reasonable.

The presence of a putative PH domain suggested that CKIP-1 may interact with the plasma membrane since the PH domains of other proteins have been implicated in localizing these proteins to the plasma membrane (52–55). This prediction is supported by the observations made with EGFP-CKIP-1 in COS-7 cells (Fig. 9) and in Saos-2 cells (Fig. 10). In fact, the location of EGFP-CKIP-1 in COS-7 cells appears to be remarkably similar to that reported for the amino-terminal PH domain of pleckstrin (53). In that study, Ma et al. (53), demonstrated that the amino-terminal PH domain expressed in COS-1 cells associated with peripheral membrane ruffles and dorsal membrane projections, and that the presence of the amino-terminal PH domain of pleckstrin is critical for targeting pleckstrin to the plasma membrane.

The prominent nuclear localization of EGFP-CKIP-1 that is observed in a significant proportion of the Saos-2 cells suggests that the regulation and/or functions of CKIP-1 may be rather complex. In particular, since not all of the Saos-2 cells exhibited the nuclear localization of EGFP-CKIP-1, we speculate that its nuclear distribution is perhaps regulated in a cell cycle-dependent manner or in response to particular stimuli. We have also observed nuclear localization of EGFP-CKIP-1 in MC-3T3 cells and in Rat-1 fibroblasts (data not shown) but not in the nucleus of COS-7 cells (Fig. 9). As yet, we do not have any precise insights into why CKIP-1 is observed in the nucleus of some cells but not in others. Based on the observation that we observe EGFP-CKIP-1 in the nucleus of Saos-2 cells, we cannot exclude the possibility that CKIP-1 has a role in facilitating interactions between CK2 and its many nuclear targets. Moreover, the observation that EGFP-CKIP-1 resides in the nucleus of some, but not all, Saos-2 cells raises the intriguing possibility that CKIP-1 has a role in regulating the import or perhaps the export of CK2 from the nucleus. Clearly, a thorough understanding of CKIP-1 and its possible role(s) in regulating the cellular functions of CK2 will require a better understanding of how CKIP-1 and its localization or interactions with CK2 are regulated.

It is intriguing to speculate that CKIP-1 may act to target or sequester CK2 to specific cellular locations and that CKIP-1 indirectly regulates the cellular functions of CK2 by controlling access to its cellular targets. This speculation is supported by our evidence that CK2 and CKIP-1 exhibit interactions in a number of assays including the yeast two-hybrid system, in

**FIG. 9.** EGFP-CKIP-1 co-distributes with the plasma membrane in COS-7 cells. COS-7 cells were transfected with pEGFP-C2-CKIP-1 (A–C), with pEGF-C2 (D–F), or with pEGFP-C2-CKIP-1(308–409) (G–I) and stained with rhodamine-labeled concanavalin A. EGFP fluorescence (A, D, and G) and rhodamine fluorescence (B, E, and H) were visualized by confocal microscopy as described under “Experimental Procedures.” Optical slices of the double labeled cells were overlaid (C, F, and I). Regions of overlap between the EGFP and rhodamine fluorescence are displayed in yellow. Examples of codistribution observed between EGFP-CKIP-1 and rhodamine concanavalin A are marked with arrows (A–C).

**FIG. 10.** Co-distribution of EGFP-CKIP-1 and endogenous nuclear CK2 in Saos-2 cells. Human osteosarcoma Saos-2 were transfected with pEGFP-CKIP-1, and then indirect immunofluorescence was carried out to visualize endogenous CK2 using rhodamine-conjugated secondary antibodies as described under “Experimental Procedures.” Rhodamine fluorescence (A) and EGFP fluorescence (B) were detected by confocal microscopy, and optical slices of these cells were overlaid (C). The nuclei of two transfected cells that were observed in the field are marked with arrows.
co-immunoprecipitations, and that they exhibit co-distribution in the nucleus of Saos-2 cells. Although a precise understanding of how CKIP-1 could be involved in the regulation of CK2 must await a more thorough understanding of how the subcellular distribution of CKIP-1 is regulated, it is not inconceivable that CKIP-1 could recruit CK2 to specific cellular locations. For example, this suggestion would be consistent with a recent report demonstrating that CK2 is associated with the plasma membrane (21, 33), along with reports of plasma membrane-localized CK2 substrates (including spectrin (56), insulin receptor (57), caveolin (58), IGF-II (59), dynamin (60), IRS-1 (61), and most recently CD5 (33)). These findings could implicate both CKIP-1 and CK2a in membrane-associated cellular processes such as receptor-mediated signaling, vesicle transport, and/or cytoskeleton organization. In this regard, CK2 has been previously implicated in insulin signaling (62–64), although the nature of its involvement is not clear and somewhat controversial (7, 65, 66). IRS-1 is a key intracellular regulatory protein that transduces the insulin receptor signal to a variety of proteins (67). It is interesting to note that CK2, the insulin receptor, IRS-1, and dynamin have all been co-localized to caveolae (58, 68–71) and that, like CKIP-1, IRS-1 and dynamin have all been co-localized to the nature of its involvement is not clear and somewhat controversial (7, 65, 66). IRS-1 is a key intracellular regulatory protein that transduces the insulin receptor signal to a variety of proteins (67). It is interesting to note that CK2, the insulin receptor, IRS-1, and dynamin have all been co-localized to caveolae (58, 68–71) and that, like CKIP-1, IRS-1 and dynamin both possess PH domains. In addition to the possibility that CK1 targets CK2 to the plasma membrane, it is also possible that, through its PH domain, CK1 mediates interactions between CK2 and proteins involved in vesicle transport or cytoskeletal organization. Interestingly, likely physiological targets for CK2 include a number of proteins that regulate intracellular trafficking such as p65 (synaptotagmin) (72), furin (73, 74), and dynein (75), and most recently CD5 (33)). These findings could implicate both CKIP-1 and CK2a in membrane-associated cellular processes such as receptor-mediated signaling, vesicle transport, and/or cytoskeleton organization. In this regard, CK2 has been previously implicated in insulin signaling (62–64), although the nature of its involvement is not clear and somewhat controversial (7, 65, 66). IRS-1 is a key intracellular regulatory protein that transduces the insulin receptor signal to a variety of proteins (67). It is interesting to note that CK2, the insulin receptor, IRS-1, and dynamin have all been co-localized to caveolae (58, 68–71) and that, like CKIP-1, IRS-1 and dynamin both possess PH domains. In addition to the possibility that CK1 targets CK2 to the plasma membrane, it is also possible that, through its PH domain, CK1 mediates interactions between CK2 and proteins involved in vesicle transport or cytoskeletal organization. Interestingly, likely physiological targets for CK2 include a number of proteins that regulate intracellular trafficking such as p65 (synaptotagmin) (72), furin (73, 74), and dynein (75). The observations that CK2a is implicated in the polarized growth of both fibroblast and budding yeast cells (76, 77), as well as neutriogenesis in mouse neuroblastoma (N2A) cells (78), also suggests a role for CK2 in the organization of the actin cytoskeleton. In fact, many proteins that interact with the cytoskeletal regulat...
54. Falasca, M., Logan, S. K., Lehto, V. P., Baccante, G., Lemmon, M. A., and Schlessinger, J. (1998) *EMBO J.* 17, 414–422
55. Lemmon, M. A., Falasca, M., Ferguson, K. M., and Schlessinger, J. (1997) *Trends Cell Biol.* 7, 237–242
56. Wei, T., and Tao, M. (1993) *Arch. Biochem. Biophys.* 307, 206–216
57. Grande, J., Perez, M., and Barte, E. (1988) *FEBS Lett.* 232, 130–134
58. Sargiacomo, M., Scherer, P. E., Tang, Z., Casanova, J. E., and Lisanti, M. P. (1994) *Oncogene* 9, 2589–2595
59. Corvera, S., Roach, P. J., DePaoli-Roach, A. A., and Czech, M. P. (1988) *J. Biol. Chem.* 263, 3116–3122
60. Robinson, P. J., Sontag, J. M., Liu, J. P., Fyske, E. M., Slaughter, C., McMahon, Sudhof, T. C. (1993) *Nature* 365, 107–108, 163–166
61. Litchfield, D. W., Dobrowolska, G., and Krebs, E. G. (1994) *Cell. & Mol. Biol. Res.* 40, 373–381
62. Sun, X. J., Rothenberg, P., Kahn, C. R., Backer, J. M., Araki, E., Wilden, P. A., Cahill, D. A., Goldstein, R. J., and White, M. F. (1991) *Nature* 352, 73–77
63. Goldberg, R. I., Smith, R. M., and Jarett, L. (1987) *J. Cell Physiol.* 133, 203–212
64. Smith, R. M., Harada, S., Smith, J. A., Zhang, S., and Jarett, L. (1998) *Cell. Signal.* 10, 355–362
65. Henley, J. R., Krueger, E. W., Oswald, B. J., and McNiven, M. A. (1998) *J. Cell Biol.* 141, 85–99
66. Oh, P., McIntosh, D. P., and Schnitzer, J. E. (1998) *J. Cell Biol.* 141, 101–114
67. Davletov, B., Sontag, J. M., Hata, Y., Petrenko, A. G., Fyske, E. M., Jahn, R., and Sudhof, T. C. (1995) *J. Biol. Chem.* 268, 6816–6822
68. Jones, B. G., Thomas, L., Molloy, S. S., Thulin, C. D., Fry, M. D., Walsh, K. A., and Thomas, G. (1995) *EMBO J.* 14, 5869–5883
69. Dittie, A. S., Thomas, L., Thomas, G., and Tooze, S. A. (1997) *EMBO J.* 16, 4859–4870
70. Karki, S., Tokito, M. T., and Holzbayr, E. L. F. (1997) *J. Biol. Chem.* 272, 5887–5891
71. Rethinaswamy, A., Birnbaum, M. J., and Glover, C. V. C. (1998) *J. Biol. Chem.* 273, 5869–5877
72. Snell, V., and Nurse, P. (1994) *EMBO J.* 13, 2066–2074
73. Ulloa, L., Diaz-Nido, J., and Avila, J. (1993) *EMBO J.* 12, 1633–1640
74. Tapon, N., and Hall, A. (1997) *Curr. Opin. Cell Biol.* 9, 86–92
75. Hubbard, M. J., and Cohen, P. (1993) *Trends Biochem. Sci.* 18, 172–177
76. Faux, M. C., and Scott J. D. (1996) *Cell 85*, 9–12
77. Kalchman, M. A., Graham, R. K., Xia, G., Koide, H. B., Hodgson, J. G., Graham, K. C., Goldberg, Y. P., Gietz, R. D., Pickart, C. M., and Hayden, M. R. (1996) *J. Biol. Chem.* 271, 19385–19394
78. Chen, G., Ray, R., Dubik, D., Shi, L., Cizeau, J., Bleackley, R. C., Saxena, S., Gietz, R. D., and Greenberg, A. H. (1997) *J. Exp. Med.* 186, 1975–1983
79. Paetkau, D. W., Riese, J. A., MacMorran, W. S., Woods, R. A., and Gietz, R. D. (1994) *Genes Dev.* 8, 2035–45
