Inositol 1,3,4-Trisphosphate 5/6-Kinase Associates with the COP9 Signalosome by Binding to CSN1*

Received for publication, August 26, 2002, and in revised form, September 23, 2002
Published, JBC Papers in Press, September 24, 2002, DOI 10.1074/jbc.M208709200

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The COP9 signalosome (CSN) is a complex of eight proteins first identified as a repressor of plant photomorphogenesis. A protein kinase activity associated with the COP9 signalosome has been reported but not identified; we present evidence for inositol 1,3,4-trisphosphate 5/6-kinase (5/6-kinase) as a protein kinase associated with the COP9 signalosome. We have shown that 5/6-kinase exists in a complex with the eight-component COP9 signalosome both when purified from bovine brain and when transfected into HEK 293 cells. 5/6-kinase phosphorylates the same substrates as those of the COP9 signalosome, including IxBo, p53, and c-Jun but fails to phosphorylate several other substrates, including c-Jun 1–79, which are not substrates for the COP9-kinase-associated kinase. Both the COP9 signalosome-associated kinase and 5/6-kinase are inhibited by curcumin. The association of 5/6-kinase with the COP9 signalosome is through an interaction with CSN1, which immunoprecipitates with 5/6-kinase. In addition, the inositol kinase activity of 5/6-kinase is inhibited when in a complex with CSN1. We propose that 5/6-kinase is the previously described COP9 signalosome-associated kinase.

The most abundant soluble inositol phosphates found in eukaryotic cells are inositol 1,3,4,5,6-pentakisphosphate (InsP5) and inositol hexakisphosphate (InsP6) (1–3). In the InsP6 biosynthetic pathway, inositol 1,3,4-trisphosphate (InsP3) is a key regulatory enzyme at the branch point for the synthesis of InsP4 isomers, InsP5, and InsP6 (4, 5). 5/6-kinase utilizes inositol 1,3,4-trisphosphate as a substrate and generates two distinct products, inositol 1,3,4,5,6-pentakisphosphate and inositol 1,3,4,5,6-tetrakisphosphate and inositol 1,3,4,5-tetrakisphosphate, in the ratio of 5:1 (5). In addition, inositol 3,4,5,6-tetrakisphosphate can be phosphorylated by 5/6-kinase to produce InsP7 (7). 5/6-kinase, purified from calf brain or expressed recombinantly from Escherichia coli, preferentially generates InsP5 (8). The enzyme is conserved from plants to humans and is found even in Entamoeba histolytica (8, 9).

During the purification of 5/6-kinase from calf brain, a large complex of proteins was noted to co-purify with 5/6-kinase (5); this complex was identified as the COP9 signalosome (CSN) by amino-terminal sequence analysis of the largest subunit (CSN1) and Western blot analysis of the fifth subunit (CSN5) (10). The COP9 signalosome is composed of eight distinct proteins (CSN1 to CSN8) and was first identified in plants defective for photomorphogenesis (11). The COP9 mutants develop a light-induced phenotype when grown in the dark; the molecular defect appears to result from decreased degradation of HY5, a positive regulator of light signaling (12, 13). The largest subunit of the COP9 signalosome (CSN1), also known as G-protein suppressor 1 (GPS1), was first identified in an extragenic suppressor screen in Saccharomyces cerevisiae for its ability to rescue mutants defective for the Ga subunit (20). Overexpression of full-length CSN1 inhibits c-Jun N-terminal kinase (JNK1) activity as well as Jun-dependent promoter activation (21). The carboxyl-terminal region of CSN1 has been shown to be responsible for incorporating CSN1 into the CSN complex, whereas the amino-terminal domain has been shown to suppress Fos transcription activation (22).

Two known functions associated with the COP9 signalosome are de neddylation and phosphorylation (14). Nedd8 is a member of the ubiquitin family that may modulate the ubiquitination machinery (15, 16); de neddylation has been postulated to control ubiquitin E3 ligase functions. The COP9 signalosome also exhibits a serine/threonine protein kinase activity toward c-Jun, IxBo, p105, and p53 (17–19); however, when expressed alone, none of the eight CSN subunits exhibits protein kinase activity, and the enzyme responsible for this kinase activity has not been identified. Because no intrinsic kinase activity was found with the complex, the unknown enzyme was referred to as a COP9 signalosome-associated kinase. We have previously shown that 5/6-kinase purified from bovine brain and expressed recombinantly in SF21 cells exhibit in vitro protein kinase activity toward c-Jun and ATF-2 (10). Because the COP9 signalosome was found to co-purify with 5/6-kinase, we postulated that 5/6-kinase may be the enzyme responsible for the protein kinase activity associated with the COP9 signalosome (10).

We now report that 5/6-kinase associates with the COP9 signalosome. A purified fraction of bovine COP9 signalosome and 5/6-kinase co-migrate upon gel filtration. Of the eight subunits of the complex, only CSN1 co-immunoprecipitates with Myc-tagged 5/6-kinase. Consistent with the reported activity of the protein kinase associated with the COP9 signalosome, 5/6-kinase phosphorylates IxBo, c-Jun, and p53 but not c-Jun 1–79 or NF-xB p52 subunit. Curcumin, a potent anti-tumor agent, was shown to inhibit the COP9-associated kinase activity in vitro and in vivo (19). We show that curcumin...
inhibits both 5/6-kinase protein and inositol kinase activities. In HEK 293 cells overexpressing 5/6-kinase, the level of CSN5/Jab1 increases, suggesting that 5/6-kinase can modulate the levels of at least one component of the COP9 signalosome. In addition, overexpression of CSN1 inhibited 5/6-kinase activity, suggesting that interaction with the COP9 signalosome can alter this activity.

MATERIALS AND METHODS

Reagents—All chemicals and reagents were obtained from Sigma unless otherwise specified. Goat polyclonal antibody against human CSN5, mouse monoclonal antibody against human α-tubulin, rabbit polyclonal antibody against human CSN1, and 1xSDS, purified GST-p53, GST-C-Jun 1–79, JNK1, and NF-E B p52 were obtained from Santa Cruz Biotechnology. Rabbit polyclonal antibodies against CSN1 to CSN8 were obtained from Affinity Research Products (Devon, United Kingdom). Recombinant His-tagged, full-length c-Jun was obtained from Promega. Curcumin was obtained from Sigma and dissolved at 0.2% in Me2SO. Restriction enzymes were purchased from New England Biolabs.

DNA Constructs—Myc-tagged human 5/6-kinase containing six repeats of the Myc epitope at the amino terminus was generated as follows. Full-length 5/6-kinase was excised from pBARK46–5/6-kinase (10) with EcoRI and EcoRV and inserted into pCS-MT2 vector (kindly provided by Dr. Yunfeng Feng) to contain the Myc epitope with EcoRI and Smal. A carboxyl-terminally tagged His6-5/6-kinase was constructed in pTrcHis2B (Stratagene) and inoculated in 2 l of BL21 (Stratagene). After isopropyl-1-thio-D-galactopyranoside (IPTG) and induced at 30°C for 4 h, cells were harvested, and recombinant protein was purified using glutathione-agarose (Amersham Biosciences). Amino-terminus-tagged human 5/6-kinase with FLAG and His6 epitopes was expressed in SF21 cells as reported previously (10). The recombinant protein was purified on a TALON resin column (Clontech) followed by an M2 anti-FLAG monoclonal antibody column (Sigma), according to the manufacturer’s instructions. A His-tagged 5/6-kinase construct was transformed into BL21 (Stratagene) and inoculated in 2 x YT medium (170 mM NaCl, 10 g of yeast extract, and 16 g/liter tryptone) overnight. Cells were harvested and incubated with 25 mM Tris, pH 7.4, 150 mM NaCl, 1 mM β-mercaptoethanol, 1 mg lysozyme/ml, and 1 mM phenylmethylsulfonyl fluoride for 30 min at 4°C. The lysate was sonicated, and the supernatant was purified on a 1-mL TALON resin column.

Kinase Assays—In vitro protein kinase assays using recombinant 5/6-kinase purified from SF21 cells were as described (10). Briefly, 5 μg of enzyme was added to 1 μg of substrate in the presence of 10 μM [γ-32P]ATP (ICN Pharmaceuticals) in kinase buffer (20 mM Tris, pH 7.6, 1 mM β-mercaptoethanol, 1 mM MgCl2, and 10% glycerol). Ten-microliter reactions were incubated at 37°C for 20 min and terminated by the addition of 4x SDS sample buffer. The protein samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride (Millipore) membranes for autoradiography. Curcumin inhibition of 5/6-kinase activity was performed by preincubation of 5/6-kinase purified with curcumin (50 μM) for 10 min at 37°C before the addition of substrates. Inositol 1,3,4-triphosphate 5/6-kinase assays were performed as described (5). Curcumin was preincubated with purified 5/6-kinase for 10 min at 37°C before assaying for inositol kinase activity.

Gel Filtration Chromatography—A fraction of the preparation of bovine brain 5/6-kinase (5) containing both the signalosome and 5/6-kinase was used for gel filtration. A 600 × 7.5 mm Bio-Sil SEC-250 high performance liquid chromatography gel filtration column (Bio-Rad) equilibrated with Buffer A (20 mM HEPES, pH 7.6, 50 mM NaCl, 10% (v/v) glycerol, 1 mM dithiothreitol, and 1 mM ATP) was run at 0.5 ml/min, and 2-ml fractions were collected. The molecular markers were separated on 10–40% glycerol gradients, and 2-ml fractions were collected. Eluted proteins were precipitated with trichloroacetic acid, resolved by SDS-PAGE, and subjected to Western blot analysis using antibodies to CSN1 and CSN8.

Transfection and Immunoprecipitation—HeLa or HEK 293 cells (2 × 10⁶ cells) were plated on 60-mm dishes 24 h prior to transfection. Lipofectamine 2000 (Invitrogen) was used for each transfection according to the manufacturer’s instructions. Cells were harvested 48 h after transfection, washed with phosphate-buffered saline, pH 7.4, followed by lysis in Buffer B and brief sonication. For immunoprecipitation, total lysate was first precleared using protein A-agarose, and 300 μg of lysate was incubated for 4 h at 4°C with 10 μl of anti-Myc antibody (9E10) or anti-CSN1 antibody-protein A-agarose (Sigma). Agarose beads were washed four times with Buffer C (10 mM sodium phosphate, pH 7.4, 0.1% Nonidet P-40, and 250 mM NaCl), boiled in SDS sample buffer, and the proteins were separated by SDS-PAGE for Western blot analysis.

Phospho-amino Acid Analysis—HEK 293 cells stably expressing 5/6-kinase were grown to confluence in a 60-mm plate and washed twice with phosphate-free media. Orthophosphate 32P (1 mCi) in phosphate-free and serum-free media was added to cells for 4 h before harvesting in Buffer B. Anti-5/6-kinase antibody (2 μg) was added to cell lysates and incubated overnight at 4°C followed by 40 μl of a 50% suspension of Protein A-agarose for 1 h. The immunoprecipitate was washed with 25 mM Tris–HCl containing 500 mM NaCl and separated by SDS-PAGE, and the labeled protein was detected by autoradiography. The 5/6-kinase was excised from the gel, electroeluted, and prepared for phospho-amino acid analysis as described previously (10).

RESULTS

Bovine 5/6-Kinase Co-migrates with COP9 Signalosome upon Gel Filtration—We previously demonstrated that two subunits of the COP9 signalosome, CSN1 and CSN5, co-purify with calf-brain 5/6-kinase (10). We now show that all eight subunits of the COP9 signalosome and 5/6-kinase were readily detected in the complex (Fig. 1A). A Coomassie Blue stain of this fraction demonstrated that the COP9 signalosome subunits are the major proteins present (10).

We sought to determine whether the COP9 signalosome subunits existed as an intact complex by using gel filtration to separate any dissociated subunits from the complex. Free His-5/6-kinase eluted in fraction 35 with an apparent molecular mass of 50 kDa. All eight subunits of the COP9 signalosome were observed to elute together in fractions 17–23 when stained with Coomassie Blue (data not shown). Western blot analysis using antibodies against CSN1 and CSN8 showed that the elution position of the COP9 signalosome corresponds to an apparent molecular mass of 600 kDa (Fig. 1B), similar to the plant COP9 signalosome (18). Free subunits of the COP9 signalosome were not detected in the gel filtration experiment. 5/6-kinase was also detected in fraction 19 (Fig. 1B). Therefore, purified bovine COP9 signalosome and 5/6-kinase co-migrated on a gel filtration.

5/6-Kinase Interacts with Endogenous CSN1—We sought to determine which subunit(s) of the COP9 signalosome interacts with 5/6-kinase by using HEK 293 cells transiently transfected with Myc-tagged human 5/6-kinase. Endogenous CSN1 was detected bound to the myc-5/6-kinase agarose as shown (Fig. 2A), whereas protein A-agarose alone did not bind CSN1. Furthermore, neither CSN8 nor CSN5 was detected bound to the myc-5/6-kinase agarose as shown (Fig. 2A).
5/6-Kinase Associates with the COP9 Signalosome Subunit CSN1

A. WB: CSN1, CSN2, CSN3, CSN4, CSN5, CSN7, 5/6-kinase

B. 700kDa Thyroglobulin 160kDa G-globulin 44kDa Myoglobin

Fractions: 17 19 21 23 25 27 29 31 33 35 37 39 41

Antibodies: CSN1, 5/6-kinase, His-5/6-kinase

Fig. 1. 5/6-Kinase associates with the COP9 signalosome. A, purified COP9 signalosome and 5/6-kinase were separately analyzed by Western blot analysis (WB) using antibodies to each subunit of the signalosome and 5/6-kinase (shown above each panel). B, bovine brain fraction containing purified 5/6-kinase and COP9 signalosome were applied to a Bio-Sil column. Each fraction was analyzed by Western blotting using antibodies against CSN1, CSN8, or 5/6-kinase. His-tagged 5/6-kinase was applied to the column to indicate the position of 5/6-kinase alone.

The interaction between 5/6-kinase and CSN1 was confirmed using an anti-CSN1 antibody for the immunoprecipitation in HeLa cells. Anti-CSN1 antibody was added to the lysates of HeLa cells, transiently transfected containing 5/6-kinase, and followed by the addition of protein A-agarose beads. Myc-tagged-5/6-kinase was detected bound to CSN1 as shown in Fig. 2B; 10% of the Myc-immunoprecipitate and 50% of the CSN1-immunoprecipitate were loaded on the gel. All eight subunits of the COP9 signalosome were bound to anti-CSN1 antibody-agarose as determined by SDS-PAGE followed by Coomassie Blue staining (data not shown). Protein A agarose alone did not bind any Myc-tagged 5/6-kinase (Fig. 2B). In addition, anti-Myc-agarose also failed to bind any 5/6-kinase in untransfected HeLa cells (data not shown). These results show that, in HeLa cells, Myc-tagged human 5/6-kinase can interact with endogenous CSN1 within the COP9 signalosome.

Because endogenous CSN1 can co-immunoprecipitate 5/6-kinase, we attempted to confirm the interaction between CSN1 and 5/6-kinase by co-transfection of HeLa cells with plasmids containing HA-tagged CSN1 and Myc-tagged 5/6-kinase. Immunoprecipitation using HeLa cell lysates indicates that anti-HA monoclonal antibody-agarose pulled down CSN1 (Fig. 2C). Western blot analysis using anti-CSN8 antibody demonstrated that another COP9 signalosome subunit was also isolated using the anti-HA antibody-agarose. Because CSN8 is found predominantly in the complex, although in plants CSN8 has been reported to exist only in the complex, CSN1 expressed in NIH 3T3 has been shown to exist in the complex as well as free subunits (22). Therefore, it is possible for 5/6-kinase to interact with free CSN1 as well as with that in the complex.

The 5/6-Kinase Protein Kinase Profile Is Similar to That of the COP9 Complex-associated Kinase—IxBa, c-Jun, and p53 have been identified as substrates for the COP9 signalosome-associated kinase (18, 19). FLAG-His-5/6-kinase (FH-5/6-kinase) expressed in Sf21 cells was purified to homogeneity as described previously (10). In an in vitro protein kinase assay, purified FH-5/6-kinase was autophosphorylated in the presence of γ-32P-labeled ATP. In addition, FH-5/6-kinase also phosphorylated full-length His-tagged c-Jun, GST-tagged IxBa, and GST-tagged p53 (Fig. 3A), which is consistent with the substrate profile reported for the COP9 signalosome-asso-
COP9 signalosome protein kinase activity (19). To test whether thophorylated (Fig. 3 cells. 5/6-kinase purified from Sf21 cells was prominently au-

kinase expressed in HEK 293 cells shows similar substrate

/H18528 c-Jun. Thus, Myc-tagged 5/6-

protein kinase assays with I

/H9260 c-Jun. 79, a c-Jun amino-termi-

GST B a panel of substrates (Fig. 3

c-Jun, p53, and JNK1 in

in vitro protein kinase activity of 5/6-kinase. A, in vitro protein kinase assays with IxBo, c-Jun, c-Jun 1–79, p53, and JNK1 in the absence or presence of purified 5/6-kinase. B, in vitro protein kinase assays with IxBo, c-Jun, c-Jun 1–79, and p53 in the presence of Myc-5/6-kinase immunoprecipitates from HEK 293 cells expressing Myc-5/6-kinase. Samples were resolved by SDS-PAGE and exposed for autoradiography. Phosphorylated proteins are indicated in the left of the panel. Representative of four independent experiments. Nonspecific bands are denoted by an asterisk (*).

Curcumin Can Inhibit Both 5/6-Kinase Inositol and Protein Kinase Activity—Curcumin has been reported to inhibit the COP9 signalosome protein kinase activity (19). To test whether curcumin inhibits 5/6-kinase, the protein kinase assays were repeated on c-Jun, p53, and IxBo in the presence of 50 μM curcumin. 5/6-kinase treated with curcumin exhibited 75% inhibition of activity toward all substrates and also showed a reduction in autophosphorylation (Fig. 5A compared with Fig. 3A). Nonspecific bands on the autoradiograph such as the ones present in the lane containing c-Jun 1–79 were not affected by the presence of curcumin. As shown in Fig. 5B, curcumin inhibited 5/6-kinase protein kinase activity in a dose-dependent manner (Fig. 5C is a graph of the relative intensity of IxBo in Fig. 5B). The addition of 50 μM curcumin to 5/6-kinase inhibited inositol kinase activity by 25% (not shown).

Overexpression of 5/6-Kinase Increases CSN5 Expression—Overexpression of CSN2 increases de novo COP9 signalosome complex formation (17); therefore, we tested whether 5/6-kinase overexpression would affect the levels of any of the proteins in the complex. Stable HEK 293 cell lines expressing 5/6-kinase or vector were generated (10), and the levels of endogenous COP9 signalosome subunits were measured by Western blotting. Of the eight subunits tested, only the level of CSN5 was shown to have increased 1.48 ± 0.1-fold (average of seven experiments) in cells that overexpress 5/6-kinase (Fig. 6). An increase in the levels of CSN5 was observed in stable cell lines overexpressing 5/6-kinase in the absence of induction with tetracycline. In these cells there is a leak of 5/6-kinase expression corresponding to levels 3-fold over that of endogenous 5/6-kinase. Overexpression of CSN1 Inhibits 5/6-Kinase Activity—We sought to determine the effect of CSN1 binding to 5/6-kinase by co-expression of HA-CSN1 and Myc-5/6-kinase in HeLa cells. 5/6-kinase activity was measured from cells transfected with plasmids containing either vector Myc-5/6-kinase, HA-CSN1, or both. As shown in Fig. 7A, the first-order rate constant k for 5/6-kinase activity (k−1) is -1200 min⁻¹ mg⁻¹. In the presence of CSN1, the rate constant drops to -300 min⁻¹ mg⁻¹. The transfection of plasmids containing vector (k−1 = 8 min⁻¹ mg⁻¹) or HA-CSN1 (k−1 = 9 min⁻¹ mg⁻¹) alone had negligible effect on 5/6-kinase. The level of expression of Myc-tagged

\[ S/S_0 = e^{-kt}, \text{ where } k^{-1} \text{ is the first order rate constant.} \]
5/6-kinase and HA-tagged CSN1 in co-transfected cells is equivalent to the cells expressing only 5/6-kinase or CSN1 as shown in Fig. 7B.

**DISCUSSION**

The COP9 signalosome complex consists of eight proteins that are highly conserved from plants to mammals (11). We previously demonstrated that this signalosome co-purifies with 5/6-kinase, a key enzyme in the synthesis of the higher phosphorylated forms of inositol (2). In this report, we confirm that 5/6-kinase associates with the COP9 signalosome using several criteria. 5/6-kinase co-elutes with the COP9 signalosome upon gel filtration, whereas in the absence of the complex it behaves as a monomer. Of the eight subunits present in the complex, only CSN1 coimmunoprecipitates with 5/6-kinase.

The COP9 signalosome has been shown to associate with the 26 S ubiquitin proteasome and may regulate protein stability (25). The structural organization of the COP9 signalosome, as revealed by electron microscopy, resembles the lid of the 19 S regulatory particle of the 26 S proteasome (26, 27). The COP9 signalosome has been shown to interact with ubiquitin E3-ligase and exhibit deneddylase activity (28, 30).

A reported function of the COP9 signalosome is an associated protein kinase activity, which has not been identified. Purified COP9 signalosome was reported to exhibit protein kinase activity toward c-Jun, IκBα, p105 (NF-κB precursor), and p53 (17–19). However, none of the eight subunits exhibited protein kinase activity when expressed alone; therefore, the kinase activity has been attributed to a COP9-associated protein kinase (18).

We reported previously that 5/6-kinase can phosphorylate c-Jun and ATF-2 (10). We show here that 5/6-kinase can also phosphorylate IκBα and p53, both known substrates for the COP9 signalosome-associated kinase. In addition, 5/6-kinase did not phosphorylate the p52 subunit of NF-κB or an amino-terminal peptide of c-Jun, proteins that are not substrates for the COP9 associated kinase (17, 18). c-Jun phosphorylation by the COP9 signalosome appeared to be independent of the JNK pathway, and the phosphorylation sites on c-Jun were mapped.
to the amino-terminal residues serine 63 and serine 73 (17, 18). Because c-Jun lacking its carboxyl-terminal (c-Jun 1–79) was not phosphorylated by the purified COP9 signalsome, Seeger et al. (18) proposed that the putative kinase only recognizes c-Jun dimers, which require the carboxyl-terminal region for dimerization. 5/6-kinase also exhibited the same specificity, being active only toward full-length c-Jun but not toward c-Jun 1–79. These results implicate 5/6-kinase as a candidate for the protein kinase activity reported to be associated with the COP9 signalsome.

The protein kinase activity of the COP9 signalsome has been reported to be inhibited by curcumin (19). Studies using curcumin to inhibit the kinase activity reveal differential regulation of substrate stability. In the presence of curcumin, p53 levels were enhanced, whereas those of c-Jun were suppressed (17, 31), suggesting that phosphorylation by the COP9-associated kinase may affect the stability of these transcription factors. These studies, using curcumin as an inhibitor of the COP9 signalsome-associated kinase, led to the hypothesis that the phosphorylation activity of the associated kinase may also modulate the association between the COP9 complex and the ubiquitin machinery (25). We show here that both the insoluble and protein kinase activities of 5/6-kinase are inhibited by curcumin, consistent with the reported inhibition of the COP9 signalsome-associated kinase.

Overexpression of the subunits of the COP9 signalsome has been reported to alter the composition of the signalsome. Transient transfection of CSN2 increases the level of the complex, whereas CSN5 overexpression does not change the level of signalsome subunits (17). We show that 5/6-kinase expression in HEK 293 cells increases the levels of CSN5, which has been reported to enhance AP-1 activation and mediate the nuclear export and degradation of the cyclin-dependent kinase inhibitor p27 (p27Kip1) (6). Because CSN5 is known to exist in a complex-bound and a complex-free form (23), the increase in CSN5 is likely restricted to the complex-free CSN5. 5/6-kinase induction of CSN5 offers a possible regulatory mechanism for CSN5 activity.

In addition to the protein kinase activity of 5/6-kinase, we have shown that 5/6-kinase can interact with the COP9 signalsome. Bovine brain 5/6-kinase was found to be present in the purified COP9 signalsome, and immunoprecipitation studies showed that CSN1 can interact with 5/6-kinase (Figs. 1 and 2). Because the carboxyl-terminal domain of CSN1 has been shown to be sufficient for incorporating CSN1 into the COP9 signalsome (32), it is likely that 5/6-kinase interacts with the amino-terminal domain of CSN1, which has been shown to inhibit c-Fos expression and suppress activation of an AP-1 promoter (22). The association of 5/6-kinase with CSN1 inhibits 5/6-kinase inositol kinase activity. Co-expression of 5/6-kinase and CSN1 resulted in a significant inhibition of 5/6-kinase activity, suggesting that a large portion of 5/6-kinase is associated with CSN1. These results also provide evidence for a link between inositol phosphate metabolism and the COP9 signalsome.

The COP9 signalsome has been proposed to act as a scaffold complex for bringing different molecules together (25, 29). Using yeast two-hybrid screens and electron microscopy, CSN1 is thought to position next to CSN5 (24). Because 5/6-kinase associates with CSN1, and CSN5 recruits p53, it is conceivable that one subunit of the complex brings different substrates to 5/6-kinase for phosphorylation. In addition, CSN1 can inhibit 5/6-kinase activity, suggesting that the interaction may alter 5/6-kinase enzyme activity under some conditions.

Acknowledgments—We thank Dr. Shao-chun Chang, Dr. Marina Kisseleva, John Verbsky, and Heidi Rayala for helpful and critical reading of the manuscript.

REFERENCES

1. York, J. D., Guo, S., Odom, A. R., Spiegelberg, B. D., and Stolz, L. E. (2001) Adv. Enzyme Regul. 41, 57–71
2. Shears, S. B. (2001) Cell. Signal. 13, 151–158
3. Irvine, R. F., and Schell, M. J. (2001) Nat. Rev. Mol. Cell Biol. 2, 327–338
4. Verbsky, J. W., Wilson, M. P., Kisseleva, M. V., Majerus, P. W., and Wente, S. R. (2002) J. Biol. Chem. 277, 31857–31862
5. Wilson, M. P., and Majerus, P. W. (1996) J. Biol. Chem. 271, 11904–11910
6. Tomoda, K., Kubota, Y., Arata, Y., Mori, S., Maeda, M., Tanaka, T., Yoshida, M., Yoneda-Kato, N., and Kato, J. Y. (1998) J. Biol. Chem. 273, 2392–2398
7. Yang, X., and Shears, S. B. (2000) Biochem. J. 351, 551–555
8. Field, J., Wilson, M. P., Mai, Z., Majerus, P. W., and Samuelson, J. (2000) Mol. Biochem. Parasitol. 108, 119–123
9. Wilson, M. P., and Majerus, P. W. (1997) Biochem. Biophys. Res. Commun. 232, 678–818
10. Wilson, M. P., Sun, Y., Can, L., and Majerus, P. W. (2001) J. Biol. Chem. 276, 40988–41004
11. Wei, N., and Deng, X. W. (1998) Photochem. Photobiol. 68, 237–241
12. Wei, N., and Deng, X. W. (1992) Plant Cell 4, 1507–1518
13. Wei, N., Chamovitz, D. A., and Deng, X. W. (1999) Cell 97, 117–124
14. Lyapina, S., Cope, G., Sherechenko, A., Serino, G., Teugue, T., Zhou, C., Wolf, D. A., Wei, N., Sherechenko, A., and Deshaies, R. J. (2001) Science 292, 1382–1385
15. Yeh, E. T., Gong, L., and Kamitani, T. (2000) Gene 248, 1–14
16. Ohb, M., Kim, W. Y., Mosleh, J. J., Chen, Y., Chau, V., Read, M. A., and Kaelin, W. G. Jr. (2002) EMBO Rep. 3, 177–182
17. Naumann, M., Bech-Otschir, D., Huang, X., Ferrell, K., and Dubiel, W. (1999) J. Biol. Chem. 274, 35297–35300
18. Seeger, M., Kraft, R., Ferrell, K., Bech-Otschir, D., Dundaye, R., Schade, R., Gordon, C., Naumann, M., and Dubiel, W. (1998) FASEB J. 12, 469–478
19. Bech-Otschir, D., Kraft, R., Huang, X., Henklein, P., Kapelari, B., Pollmann, C., and Dubiel, W. (2001) EMBO J. 20, 1630–1639
20. Spiro, E. H., Boldtsh, K. S., Paral, A. R., Staub, S. F., Koo, D., Chang, C. Y., Xie, W., and Colicelli, J. (1996) Mol. Cell. Biol. 16, 6698–6706
21. Schwechheimer, C., and Deng, X. W. (2001) Trends Cell Biol. 11, 420–426
22. Tege, T., Matsui, M., and Wei, N. (2001) J. Mol. Biol. 305, 1–9
23. Chamovitz, D. A., and Segal, D. (2001) EMBO Rep. 2, 96–101
24. Fu, H., Reis, N., Lee, Y., Glickman, M. H., and Vierstra, R. D. (2001) EMBO J. 20, 7096–7107
25. Bech-Otschir, D., Seeger, M., and Dubiel, W. (2002) J. Cell Sci. 115, 467–473
26. Kapelari, B., Bech-Otschir, D., Hegerl, R., Schade, R., Dundaye, R., and Dubiel, W. (2000) J. Mol. Biol. 300, 1169–1178
27. Henke, W., Ferrell, K., Bech-Otschir, D., Seeger, M., Schade, R., Jungblut, P., Naumann, M., and Dubiel, W. (1999) Mol. Biol. Rep. 26, 29–34
28. Schwechheimer, C., Serino, G., Callis, J., Crosby, W. L., Lyapina, S., Deshaies, R. J., Gray, W. M., Estelle, M., and Deng, X. W. (2001) Science 292, 1379–1382
29. Chamovitz, D. A., and Glickman, M. (2002) Curr. Biol. 12, R232
30. Kubo, H., Kubo, H., Suzuki, T., Iwai, K., Yamanaka, K., Minato, N., Suzuki, H., Shimbara, N., Hidaka, Y., Osaka, F., Omata, M., and Tanaka, K. (2001) EMBO J. 20, 4003–4012
31. Polleman, C., Huang, X., Mall, J., Bech-Otschir, D., Naumann, M., and Dubiel, W. (2001) Cancer Res. 61, 8416–8421
32. Wang, X., Kang, D., Feng, S., Serino, G., Schwechheimer, C., and Wei, N. (2002) Mol. Biol. Cell 13, 646–655
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J. Biol. Chem. 2002, 277:45759-45764.
doi: 10.1074/jbc.M208709200 originally published online September 24, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M208709200

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