Characterization of Human Constitutive Photomorphogenesis Protein 1, a RING Finger Ubiquitin Ligase That Interacts with Jun Transcription Factors and Modulates Their Transcriptional Activity*

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RING finger proteins have been implicated in many fundamental cellular processes, including the control of gene expression. A key regulator of light-dependent development in Arabidopsis thaliana is the constitutive photomorphogenesis protein 1 (atCOP1), a RING finger protein that plays an essential role in translating light/dark signals into specific changes in gene transcription. atCOP1 binds the basic leucine zipper factor HY5 and suppresses its transcriptional activity through a yet undefined mechanism that results in HY5 degradation in response to darkness. Furthermore, the pleiotropic phenotype of atCOP1 mutants indicates that atCOP1 may be a master regulator of several transcriptional pathways. Here we report the cloning and characterization of the human orthologue of atCOP1. Human COP1 (huCOP1) distributes both to the cytoplasm and the nucleus of cells and shows a striking degree of sequence conservation with atCOP1, suggesting the possibility of a functional conservation as well. In co-immunoprecipitation assays huCOP1 specifically binds basic leucine zipper factors of the Jun family. As a functional consequence of this interaction, expression of huCOP1 in mammalian cells down-regulates c-Jun-dependent transcription and the expression of the AP-1 target genes, urokinase and matrix metalloproteinase 1. The RING domain of huCOP1 displays ubiquitin ligase activity in an autoubiquitination assay in vitro; however, suppression of AP-1-dependent transcription by huCOP1 occurs in the absence of changes in c-Jun protein levels, suggesting that this inhibitory effect is independent of c-Jun degradation. Our findings indicate that huCOP1 is a novel regulator of AP-1-dependent transcription sharing the important properties of Arabidopsis COP1 in the control of gene expression.

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§ The abbreviations used are: atCOP1, Arabidopsis thaliana constitutive photomorphogenesis protein 1; bZIP, basic leucine zipper; CSN, COP9 signalosome; huCOP1, human constitutive photomorphogenesis protein 1; NLS, nuclear localization sequence(s); HA, hemagglutinin; MMP1, matrix metalloproteinase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAb, monoclonal antibody; E1, ubiquitin-activating enzyme; E2, ubiquitin carrier protein; E3, ubiquitin-protein isopeptide ligase; GST, glutathione S-transferase.

RING finger domains are found in a large number of proteins involved in many fundamental cellular processes, such as proliferation, apoptosis, development, oncogenesis, and viral pathogenicity (1–3). The RING domain is characterized by a conserved pattern of cysteine and histidine residues that bind two zinc atoms in a particular cross-brace arrangement. A unifying function for the RING has been difficult to define, given the large number of molecular pathways that are affected by RING proteins. It has been proposed that a general feature of RING domains is the ability to mediate interactions with other RING or non-RING proteins and to contribute to the assembly of macromolecular complexes (1). More recently, a role for RING domains in ubiquitination reactions has been identified, and several RING finger proteins have demonstrated ubiquitin ligase activity, with the ability to transfer ubiquitin to a substrate as well as to themselves (4–6).

Many RING finger proteins have been implicated in regulating transcription through a variety of mechanisms (7), including ubiquitination and degradation of positive (8–12) or negative regulators of transcription (13–15). Pathways independent of protein degradation have also been described and may involve protein sumoylation (16), changes in promotor recruitment (17), interference with components of the transcriptional machinery, such as general transcription factors (18, 19) or RNA polymerase II (20, 21), changes in histone acetylation (22, 23), or chromatin rearrangement (24, 25).

The Arabidopsis thaliana RING finger constitutive photomorphogenesis protein 1 (atCOP1), has been characterized for the profound effect that loss of function mutations have on Arabidopsis photomorphogenic development (26). atCOP1 influences Arabidopsis growth by regulating the pattern of gene expression in seedlings. Work from X. W. Deng’s laboratory (27) demonstrated that under defined extracellular conditions (darkness) atCOP1 accumulates in the nucleus, where it binds one or more transcriptional regulators, such as the basic leucine zipper (bZIP) factor HY5 (28). Degradation of HY5 in the dark is dependent on the presence of wild type atCOP1 in the nucleus (29). In addition, COP1 seems to interact functionally with other transcription factors, such as the basic leucine zipper factor HY5 (28). Degradation of HY5 in the dark is dependent on the presence of wild type atCOP1 in the nucleus (29). In addition, COP1 seems to interact functionally with other transcription factors, such as the basic leucine zipper factor HY5 (28). Degradation of HY5 in the dark is dependent on the presence of wild type atCOP1 in the nucleus (29).
ally with other proteins, because nuclear accumulation of at-COP1 requires the activity of several genes (26), many of which encode subunits of a single multimeric complex, the COP9 signalosome (CSN) (30).

Our laboratory has been investigating the role of the mammalian CSN and its functional regulation in response to extracellular signals, such as intercellular adhesion (31). Because atCOP1 is integral to the function of the plant CSN, we sought to obtain further insight into the function of the mammalian CSN by studying the human COP1 orthologue. In this paper we report the cloning and characterization of human COP1 (huCOP1). Similar to its plant counterpart, huCOP1 contains seven WD40 repeats in the carboxyl terminus and an amino-terminal RING domain, which displays ubiquitin ligase activity in an autoubiquitination assay in vitro. We show that huCOP1 possesses a nuclear localization sequence (NLS) that directs distribution of the protein to the nucleus. In immunoprecipitation assays huCOP1 specifically binds transcription factors of the Jun family through a highly conserved amino acid motif. The functional consequence of this interaction is the down-regulation of c-Jun transcriptional activity by ectopically expressed huCOP1 protein. Our findings indicate that also in human cells COP1 may function as a regulator of gene expression, affecting the AP-1-dependent pathway, which plays a central role in mammalian cell proliferation and differentiation.

EXPERIMENTAL PROCEDURES

Cloning of huCOP1—Translated BLAST search of a human expressed sequence tag data bank revealed several expressed sequence tags with a high degree of identity with A. thaliana COP1. IMAGE clone 1707833 was obtained from Human Genome Mapping Project Resource Center (Cambridge, UK) and used to screen a phage library from NT2/D1 cells (Stratagene). A positive clone was amplified, and phage DNA was extracted. The cDNA insert (2.6 kb) was excised and sequenced. Primer extension experiments and 5′ rapid amplification of cDNA ends-PCR revealed that although huCOP1 cDNA extended further 5′ for 500 nucleotides, an in-frame stop codon was present 150 nucleotides upstream of the identified ATG (GenBankTM accession number: NP_071902). HeLa cell RNA was extracted with the RNAeasy reagent (200 μg of total RNA were precipitated overnight with 40 μg of each of the following 32P-labeled primers: COPI, 16, 5′-GGCT-ACACAGATCGTGGACTCC-3′; and COPI, 17, 5′-GGATAAGAAGCAG- GAGGGCGGAATGTCACCGA-3′. Annealing occurred in hybridization buffer (150 mM KCl, 10 mM Tris, pH 7.5, 1 mM EDTA) at progressively decreasing temperatures (from 80°C to 42°C). Autoradiograms were carried out with the Superscript II enzyme (Invitrogen) at 42°C. After RNAse treatment and phenol/chloroform extraction, the samples were precipitated and resolved on a 10% polyacrylamide sequencing gel. Amplification of 5′ cDNA ends was performed with the above primers and the GeneRacer kit (Invitrogen), following the manufacturer’s protocol. Sequence homologies were determined with the ClustalW multiple alignment tool.

Northern Blot Analysis—huCOP1 cDNA was digested with EcoRI to yield a 900-nucleotide fragment. After gel purification, the probe was labeled with 32P[γ-32P]ATP using a Rediprime II kit (Amersham Biosciences) and hybridized to commercial Northern blot membranes (FirstChoice Northern Human Blot 1 and FirstChoice Northern Human Blot 2; Ambion) containing 2 μg of poly(A) RNA from different human tissues. The membranes were stripped and subsequently probed with a β-actin cDNA probe.

Production of Monoclonal Antibodies and Western Blotting—The amino-terminal fragment of COP1 (amino acids 1–452) was cloned into pGEX4T1 (Pharmacia Corp.). Recombinant GST-COP1 was purified from Escherichia coli by binding to glutathione-Sepharose (Pharmacia Corp.) and used to raise monoclonal antibodies against COP1 (SP212). SP212 recognized overexpressed FLAG-COP1 by Western blotting and by indirect immunofluorescence. Lyssates from transfected COS-7 cells and human cell lines were obtained by boiling samples for 5 min in 2% SDS, followed by the addition of RIPA correction buffer (32). Tissue homogenates from rat embryo (embryonic day 18.5) brains were ob-

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RESULTS

Cloning of Human COP1—To identify human homologues of the atCOP1 protein, we performed a similarity search of a human expressed sequence tag database using the atCOP1 open reading frame, encoding a protein of 731 amino acids with 34% identity, 64% similarity to the atCOP1 protein (35).

Expression of COP1 in Mammalian Tissues—Northern blot analysis of COP1 transcripts showed widespread expression in normal human tissues. Highest levels were detected in testes, placenta, skeletal muscle, and heart (Fig. 1B).

A monoclonal antibody (MoAb) raised against recombinant huCOP1 recognized the FLAG-tagged version of the transfected protein in Western blot analysis (Fig. 2A, left lane), as well as an additional band with a slightly lower molecular weight, compatible with the untagged endogenous protein, present in lysates both from transfected COS7 and untransfected HeLa cells (Fig. 2A). Extensive Western blot analysis of COP1 expression in a panel of cell lines showed that endogenous levels of COP1 are in general low (or undetectable) in lysates derived from human cell lines and cultures (examples are shown in Fig. 2, A and B, right panel). The anti-COP1 MoAb also recognized the murine form of the protein (GenBank access number NP036061; 97% identity with huCOP1) in lysates from embryonic rat brain and in the murine embryonic cell lines analyzed (Fig. 2B, left panel).

Structural Determinants of the Subcellular Localization of huCOP1—To study the role of the different protein domains in determining huCOP1 subcellular localization, we transfected FLAG-tagged versions of full-length huCOP1 and of various huCOP1 deletion constructs in COS7 cells and analyzed the distribution of the proteins by indirect immunofluorescence. Extensive expression studies demonstrated that huCOP1 is present both in the cytoplasm and the nucleus (Fig. 2C), where it has a speckled distribution, with preferential enrichment at the nuclear periphery. Constructs lacking the amino-terminal domain (FLAG-cWD40 and FLAG-WD40; Fig. 2C, bottom row) showed cytoplasmic localization, in contrast to mutants carrying carboxyl-terminal deletions (FLAG-RINGcc and FLAG-RING; Fig. 2C, middle rows), which are preferentially concentrated in the nucleus. This finding implied the existence of a signal for nuclear localization in the amino-terminal portion of the protein. Consistently, point mutations of Arg91 and Arg93 in the putative monopartite NLS are sufficient to prevent nuclear entry not only of the full-length protein but also of the FLAG-RINGcc construct, resulting in a complete relocalization of the protein (FLAG-COP1mNLS and FLAG-RINGccMNLs; Fig. 2C, right panels).

In the role of WD40 repeats in establishing protein-protein interactions (37), it is not surprising that huCOP1 mutants that lack these motifs showed a less localized pattern of nuclear distribution (Fig. 2C, row 2, central panel), indicating that the WD40 repeats may be necessary to retain huCOP1 in the correct subnuclear location. Further deletion of the coiled-coil region resulted in a complete diffused nuclear pattern (FLAG-RING; Fig. 2C, row 3, central panel), suggesting that this region may also be involved in protein-protein interactions.

In Arabidopsis, the coiled-coil domain of atCOP1 has been implicated in the homodimerization of the protein. We tested whether huCOP1 can also dimerize by transfecting HA-tagged full-length huCOP1 together with either FLAG-tagged full-length huCOP1 protein (FLAG-COP1) or one of several COP1 deletion constructs, as indicated (Fig. 3). Only constructs containing the coiled-coil region of the protein (FLAG-COP1, FLAG-RINGcc, and FLAG-cWD40) were co-precipitated with HA-full-length huCOP1 by anti-HA antibodies, confirming the importance of this domain for the self-dimerization of the human protein as well.

HuCOP1 Has Ubiquitin Ligase Activity in Vitro—In Arabidopsis, the coiled-coil domain of atCOP1 has been implicated in the homodimerization of the protein. We tested whether huCOP1 can also dimerize by transfecting HA-tagged full-length huCOP1 together with either FLAG-tagged full-length huCOP1 protein (FLAG-COP1) or one of several COP1 deletion constructs, as indicated (Fig. 3). Only constructs containing the coiled-coil region of the protein (FLAG-COP1, FLAG-RINGcc, and FLAG-cWD40) were co-precipitated with HA-full-length huCOP1 by anti-HA antibodies, confirming the importance of this domain for the self-dimerization of the human protein as well.
In Arabidopsis cells the presence of an intact COP1 protein is necessary to allow proteasome-dependent degradation of the HY5 transcript factor. This interaction is critical for atCOP1-mediated regulation of HY5 transcriptional activity.

HuCOP1 Forms Complexes with Selected bZIP Transcription Factors

FIG. 2. Mammalian COP1 is detectable in human and mammalian cells and localizes to both the nucleus and the cytoplasm. A, anti-COP1 monoclonal antibody recognizes transfected and endogenous huCOP1. COS7 cells were transfected with 2 μg of FLAG-tagged full-length COP1 (left lane) or pCMVFLAG vector (middle lane). Total HeLa cell lysates (right lane) were extracted with 2% boiling SDS buffer. Total cell lysates were blotted with anti-COP1 antibodies raised against recombinant COP1. B, endogenous COP1 expression was analyzed by Western blotting with anti-COP1 antibodies of lysates from human (right panels) or murine (left panel) cell lines and tissues. Cellular content in lysates was estimated by blots raised against recombinant COP1. C, epitope-tagged full-length COP1 (FLAG-COP1) or the indicated deletion constructs were transfected in COS7 cells. Distribution of the transfected proteins was analyzed by indirect immunofluorescence and confocal microscopy. The middle panels in rows 1–3 show enlarged details of the nuclear distribution of the different constructs. Right panels, rows 1 and 2 show expression patterns of full-length COP1 and of the RINGcc truncated construct carrying a mutation (asterisk) in the first predicted NLS. NLS mutants are exclusively localized to the cytoplasm.

FIG. 3. huCOP1 homodimerizes through its coiled-coil region. COS7 cells were transfected with HA-tagged full-length huCOP1 and the indicated FLAG-tagged constructs. The cell lysates were immunoprecipitated (IP) with anti-HA MoAb (12CA5), resolved by denaturing SDS-PAGE, and transferred to membranes. The blots were hybridized with anti-FLAG MoAb to detect co-precipitated huCOP1 proteins (top panel). Expression of HA-COP1 and of the deletion constructs in the different samples was determined by blotting of whole cell lysates with either anti-HA MoAb (middle panel) or with anti-FLAG MoAb (bottom panel). The asterisk indicates immunoglobulin heavy chains.

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to interact with human bZIP transcription factors. We transfected FLAG-COP1 together with a panel of bZIP transcription factors and immunoprecipitated huCOP1 with anti-FLAG antibodies. huCOP1 co-precipitated selectively with Jun family members (c-Jun in Fig. 5A; JunD in Fig. 5B) but not with the other bZIP factors tested (c-Fos, CREB1, and CREM; Fig. 5C). JunD was also independently isolated as an interactor of huCOP1 (fused to the GAL4 DNA-binding domain) in a yeast two-hybrid screen of a human B cell library (not shown).

Co-immunoprecipitation experiments of c-Jun with the different deletion constructs of COP1 showed that the main site of interaction between COP1 and c-Jun lies in the WD40 domain, as reported for the binding of HY5 to atCOP1 (39), although an additional binding site is present in the coiled-coil region of the protein (Fig. 5D).

Although in Arabidopsis dimerization of atCOP1 has been reported to be necessary for the interaction with the HY5 transcription factor, in mammalian cells COP1 seems not to require dimerization to interact with c-Jun, as shown by the ability of the isolated WD40 domain to co-precipitate c-Jun (Fig. 4D) in the absence of homodimerization (Fig. 3).

A consensus motif whose integrity is essential for the interaction with atCOP1 has been identified in Arabidopsis proteins that bind to atCOP1, such as HY5 or STH (40). We found that
this motif is also highly conserved in Jun family proteins (Fig. 6A). To test the relevance of this motif for the interaction between c-Jun and huCOP1, we mutated to Ala two residues central to the motif (Val232-Pro233), resulting in the c-JunVPAA construct (Fig. 6A). This substitution was sufficient to completely abolish binding of c-Jun to COP1 (Fig. 6B), although it did not alter the subcellular distribution of the protein (Fig. 6B). In addition, c-JunVPAA remained transcriptionally active (Fig. 7A).

**HuCOP1 Modulates AP-1-driven Transcription** — To test the functional consequences of the observed interaction between c-Jun and huCOP1, we transfected 293T cells with a luciferase reporter gene under the control of an AP-1 promoter, together with c-Jun and increasing quantities of huCOP1. c-Jun-mediated transactivation was suppressed in a dose-dependent manner by the presence of wild type huCOP1 (Fig. 7A, *hatched bars versus white bar*). The inhibitory effect of huCOP1 depended on the interaction with c-Jun, as demonstrated by the inability of huCOP1 to interfere with the transcriptional activity of c-JunVPAA (Fig. 7A, *bars on the right*) and by the absence of a suppressive effect when the RINGC deletion mutant of COP1, lacking the main c-Jun binding site, was transfected (Fig. 7A). Interestingly, the inhibitory effect required the presence of an intact COP1 RING domain, although the RING domain is not involved in binding of COP1 to c-Jun. The RING mutant of COP1, which carries two Cys to Ala substitutions in the RING domain, did not affect c-Jun transactivation activity (Fig. 7A, *black bars*), although it still bound c-Jun in a co-immunoprecipitation assay (Fig. 7B), indicating that the inhibitory action of huCOP1 on c-Jun transcriptional activity is not due to a simple steric hindrance effect. To test whether huCOP1 could also affect c-Jun-dependent transactivation of a natural AP-1 promoter, we transfected 293T cells with a luciferase reporter gene comprising the basal proximal promoter and the AP-1 enhancer region of the urokinase (u-PA) gene (34) (Fig. 8A, *right bars*). Transfection of huCOP1 (*hatched bars*) reduced c-Jun-dependent transactivation of the AP-1 enhancer but had no effect on the activity of the basal proximal u-PA promoter, which is regulated by Sp family proteins (Fig. 8A, *left bars*) (34). The ability of huCOP1 to modulate c-Jun activity in a physiological promoter context was further tested by the effect of huCOP1 expression on the mRNA levels of known AP-1 target genes, such as u-PA itself and MMP1 (41–43). These
genes were chosen among the characterized c-Jun targets for their c-Jun-dependent inducibility in the cellular systems used in this study (293T or HeLa cells). The cells were transfected in triplicate with c-Jun or c-Jun plus c-Fos to induce AP-1-dependent expression of u-PA or MMP1, respectively. The samples were also transfected with either 0.2 μg of huCOP1, 0.2 μg of the RING mutant COP1, or empty vector. After 48 h, total RNA was extracted from each sample, and the relative mRNA levels for u-PA and MMP1 were estimated by quantitative real time reverse transcriptase-PCR. Overexpression of huCOP1 resulted in decreased inducibility of the analyzed AP-1 target genes (Fig. 8B, hatched bars) but did not alter the levels of the control gene (Fig. 8B, GAPDH). Conversely, expression of mutant COP1 was ineffective in regulating AP-1-dependent expression of the target genes (Fig. 8B, black bars).

**HuCOP1 Does Not Directly Affect c-Jun Ubiquitination—**
Because the integrity of the RING domain is necessary not only for huCOP1 inhibitory activity on AP-1-dependent transcription but also for huCOP1 ubiquitin ligase activity, we tested the hypothesis that c-Jun function may be down-regulated following ubiquitination of c-Jun by huCOP1. In an in vitro assay, the addition of purified E1, UbcH5B, and huCOP1 alone was not sufficient to induce ubiquitination of recombinant, bacterially expressed c-Jun (not shown). In addition, overexpression of huCOP1 in 293T cells did not consistently affect c-Jun steady state levels in cell lysates, nor its half-life, as measured by pulse-chase experiments (not shown), indicating that the transcriptional inhibition activity of huCOP1 may be independent of its ubiquitin ligase properties.

**DISCUSSION**

In this paper we report the cloning and characterization of the human orthologue of the Arabidopsis RING protein at-COP1, which plays a fundamental role in the control of gene expression during plant development (26) by relaying light/dark-derived signals to the transcriptional machinery. Human COP1 shows a high degree of sequence conservation and domain organization with its plant counterpart. We found that transfected huCOP1 distributed to the cytoplasm and the nucleus of cells and that nuclear localization was directed by a NLS located in the amino-terminal region of the protein. This dual distribution may be important for huCOP1 function, because it has been demonstrated to be essential, in Arabidopsis, for regulating COP1 activity in response to extracellular stimuli. Consistently, a partial, chimeric mouse/human COP1 construct responded to light/dark stimuli by changing its subcellular localization pattern when introduced in Arabidopsis cells, pointing to a close functional conservation between the mammalian and the plant protein (44).

The putative coiled-coil region (amino acids 203–305) and the seven WD40 motifs (amino acids 405–731) of huCOP1 represent potential interaction domains for protein partners. The discrete nuclear pattern of huCOP1 depended on the presence of these domains and is compatible with distribution to specific subnuclear compartments, as has been described for other RING proteins, such as PML (45). The coiled-coil region was also involved in huCOP1 homodimerization, a property shared by atCOP1 (59). The ability of huCOP1 to establish several protein-protein interactions is further suggested by our preliminary gel filtration studies (not shown) indicating that COP1 may be present in vivo in a high molecular weight complex. These data are consistent with the notion that the assembly in large macromolecular structures is a frequent characteristic of proteins containing a RING domain (1).

The striking degree of structural conservation of huCOP1 with the Arabidopsis counterpart suggested the interesting possibility of a functional conservation as well. Central to Arabidopsis COP1 biological function in seedling development is the regulation of a bZIP protein, the photomorphogenic transcription factor HY5. The inhibitory activity of atCOP1 on HY5-dependent transcription requires the direct interaction of the two proteins through a specific binding motif (acidic residues followed by VPD/E and a hydrophobic amino acid), present in several COP1-binding proteins (40). We identified a similar amino acid motif in the mammalian bZIP proteins c-Jun and JunD, members of the AP-1 family of transcription factors. AP-1 factors convert multiple extracellular stimuli, such as growth factors, cytokines, and cellular stress, into a variety of transcriptional responses. AP-1 target genes have been implicated in many diverse biological processes, including oncogenesis, cell survival, proliferation, and differentiation (46, 47), making the AP-1 family one of the most complex systems of transcriptional control. Protein-protein interactions play an important role in regulating AP-1-dependent transcription and in controlling the specificity of AP-1 transcriptional responses (41, 48).
The COP1-binding motif we identified in c-Jun and JunD mediated the interaction between these factors and huCOP1 and suggested that this highly conserved pathway is involved in the regulation of Jun-dependent transcription. Consistently, expression of huCOP1 inhibited in a dose-dependent fashion c-Jun-dependent transcription both of synthetic promoter-reporter constructs and of known AP-1 target genes. Further, this effect required the integrity of the COP1-binding consensus.

The molecular basis of Arabidopsis COP1 suppressive activity on transcription are unknown; however, it has been hypothesized that COP1 may function as a ubiquitin ligase that directly targets transcription factors for degradation, based on the presence of the RING finger domain and on the ability of aCOP1 to permit degradation of HY5 in response to the adequate extracellular stimulus (29). We found that the integrity of the RING domain is important for huCOP1 inhibitory activity on AP-1-dependent transcription, and this prompted us to test a possible role of huCOP1 in inducing the degradation of c-Jun.

Overexpression of huCOP1 in two different cellular systems (COS7 and 293T cells) did not result in a decrease of c-Jun steady state levels in total cell lysates, nor in a reduction of c-Jun half-life, as analyzed by pulse-chase experiments (not shown). Consistently, in our in vitro ubiquitination assay we could demonstrate that the RING of huCOP1 has indeed ubiquitinating activity toward COP1 itself, but it is not capable of inducing ubiquitination of a recombinant c-Jun protein, in the presence of purified E1 and UbcH5B (not shown). Our findings raise the possibility that ubiquitination of c-Jun by huCOP1 may occur only in the presence of another yet to be identified component of the ubiquitin ligase complex requiring the integrity of huCOP1 RING finger domain for its function. This hypothesis is supported by the recent findings by Hardtke et al. (49) that the Arabidopsis COP1-interacting protein CIP8 promotes ubiquitination of HY5 in vitro, suggesting that ubiquitination of HY5 in vivo could require the formation of a multi-molecular complex that includes both COP1 and CIP8.

Alternatively, we cannot rule out the possibility that signal-dependent post-translational modifications of c-Jun may be necessary to render the molecule susceptible to ubiquitination or to proteasome-dependent proteolysis. Furthermore, a specific (and still unknown) extracellular cue could be required to trigger COP1-dependent degradation of c-Jun in mammalian cells, in analogy to the dark/light signals that regulate aCOP1 function in A. thaliana.

On the other hand, our observation that huCOP1 interferes with c-Jun transcriptional activity in the presence of constant levels of total c-Jun protein indicates that inhibition of AP-1-dependent transcription occurs independently of protein degradation, as reported for other RING finger proteins implicated in transcriptional regulation (see Refs. 1 and 7 for reviews). The requirement for an intact RING domain, in this case, may reflect the importance of protein-protein interactions established by the RING in mediating transcriptional repression rather than its role in protein ubiquitination.

The identification of huCOP1-interacting proteins will be of great importance to fully understand the function of huCOP1 in regulating c-Jun transcriptional activity. A genetic and functional interaction between COP1 and the CSN has been described in Arabidopsis, where loss of function mutants of the CSN show morphogenic alterations virtually indistinguishable from those of COP1 loss of function mutants (50). The CSN has been implicated in the correct subcellular localization of COP1, because aCOP1 is excluded from the nucleus in CSN mutants (51). It will be of interest to investigate the relationship between huCOP1 and the CSN. In mammalian cells, the CSN has been implicated by different laboratories (52–54) as well as by ours (31) in the modulation of AP-1-dependent transcription. The molecular mechanisms through which the CSN affects c-Jun function are still debated and may include post-translational modifications of c-Jun (55). Whether a functional interaction with huCOP1 plays a role in this aspect of CSN activity is an interesting possibility that still remains to be tested.

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