Major Vault Protein, in Concert with Constitutively Photomorphogenic 1, Negatively Regulates c-Jun–Mediated Activator Protein 1 Transcription in Mammalian Cells

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Abstract

Constitutively photomorphogenic 1 (COP1), a RING finger ubiquitin ligase with substrates including c-Jun and p53, was recently found to be overexpressed in a number of breast and ovarian tumor samples. In addition to its E3 activity, COP1 was also shown to be able to inhibit activator protein 1 (AP-1) transcription. Through an affinity purification method, we have identified major vault protein (MVP) as a novel interacting partner for COP1 in mammalian cells. MVP, also known as lung resistance protein, is the main component of a ribonucleoprotein organelle called vault, and has been implicated in multiple drug resistance in many cancer cell lines and primary tumor samples. The interaction between COP1 and MVP is detectable at the endogenous level and occurs mostly in the cytoplasm. Similar to COP1, MVP inhibits c-Jun accumulation and AP-1 transcription activity. MVP knockout or knockdown cells contain elevated amount of c-Jun and increased AP-1 transcription activity. UV irradiation enhances MVP tyrosine phosphorylation, causes dissociation of COP1 from MVP, and alleviates the inhibitory activity of MVP on AP-1 transcription. Taken together, we propose that MVP, most likely through its interaction with COP1, suppresses c-Jun–mediated AP-1 transcription under unstressed conditions, thereby preventing cells from undergoing stress response. (Cancer Res 2005; 65(13): 5835-40)

Introduction

Constitutively photomorphogenic 1 (COP1), an evolutionarily conserved protein, contains an NH_2-terminal RING finger motif, followed by a coiled-coil domain and seven WD40 repeats. Initial genetic studies in *Arabidopsis* revealed that COP1 is essential for plant development and is required to suppress the light-signaling cascade in the dark (1, 2). *Arabidopsis cop1* mutants display light-grown phenotypes even under continuous darkness and exhibit gross misregulation of light-controlled genome expression (1, 3). In recent years, COP1 has been shown to contain E3 ubiquitin ligase activity, and among its substrates are light inducible transcription factors LAF1 and HY5, and the major photoreceptor under far-red light, phytochrome A (4–6). Therefore, it has been widely accepted that plant COP1 likely exerts its repression effect on photomorphogenesis by targeting critical positive regulators and/or the photoreceptors for ubiquitination and degradation.

COP1 homologues have been identified in animals (7–9), but their physiologic function remains to be elucidated, considering that animals do not undergo photomorphogenesis. Initial characterizations suggested that mammalian COP1, like its plant counterpart, is involved in ubiquitination and is itself a substrate (9, 10). Later studies showed that mammalian COP1 interacts directly with c-Jun and p53, targeting them for ubiquitination and degradation (10–12).

The p53 tumor suppressor facilitates the repair or elimination of severely damaged cells through cell cycle arrest, DNA repair, cellular senescence, differentiation, or apoptosis in response to cellular stresses (13). On the other hand, the c-Jun proto-oncogene has been implicated in cell proliferation, survival, differentiation, transformation, and apoptosis (14). UV irradiation results in rapid and dramatic accumulation of both p53 and c-Jun (15, 16). In response to UV-induced DNA damage, p53 causes cell cycle arrest through the transcriptional activation of p21, whereas c-Jun attenuates p53-dependent cell cycle arrest by negatively regulating p53 transcription activity (17). It was suggested that mammalian COP1 recruits c-Jun to an SCF-like complex composed of DET1, DDB1, Cul4A, and Rbx1, which polyubiquitinates c-Jun and targets it for proteasome-mediated degradation (11). Mammalian COP1 can also function as a monomeric E3 ubiquitin ligase to directly ubiquitinate p53 (12). The dual roles of mammalian COP1 in the degradation of c-Jun and p53 by different mechanisms highlight the versatility of COP1 in modulating the balance of p53 and c-Jun in response to specific physiologic cues. In addition, COP1 was reported to repress the transcription activity of c-Jun independent of proteolysis (10). More recently, COP1 was found overexpressed in breast and ovarian adenocarcinomas (18), suggesting COP1 may be involved in tumorigenesis, possibly through its regulation of p53, c-Jun, and likely other unidentified factors.

To further understand the role of mammalian COP1, we set out to identify additional *in vivo* COP1-interacting partners through affinity purification. Here we report that major vault protein (MVP) specifically associates with COP1 and negatively regulates c-Jun level and activator protein 1 (AP-1) transcription activity under unstressed conditions.

Materials and Methods

DNA constructs and antibodies. pFlag-hCOP1 was generated by inserting the full-length *HsCOP1* open reading frame from a single EST

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clone (GenBank accession no. BI461685) into the pFLAG-CMV II vector. MVP-Flag was a generous gift from Dr. Bennett (Department of Pharmacology, Yale University School of Medicine, New Haven, CT) (19). pAP-1-luc and pRL-TK are as described by the manufacturer (Clontech, Palo Alto, CA).

Purified COP1 and MVP polyclonal antibodies were previously described (9, 20). Anti-Flag (Sigma, St. Louis, MO), anti-MVP (LRP, BD Transduction Laboratories, San Diego, CA), anti-MVP (LRP56, Kamiya, Seattle, WA), anti-MVP (LRP56, Calbiochem, La Jolla, CA), anti-P-Tyr (Sigma), anti–c-Jun (SC-45, Santa Cruz, Biotech, Inc., Santa Cruz, CA), anti–P-c-Jun (S63, Cell Signaling, Beverly, MA), anti–tubulin (Sigma), and anti-Hsp70 (BD Transduction Laboratories) antibodies were used according to the recommendations of the manufacturers.

Cell culture, transfection, and generation of stable cell lines. HEK293 and HeLa cells were cultured in high-glucose DMEM with 10% fetal bovine serum. $MVP^{+/+}$ and $MVP^{-/-}$ mouse embryonic fibroblasts (MEFs) were maintained as previously described (20). Transfection was done using Lipofectamine 2000 (Invitrogen, San Diego, CA) according to the instructions of the manufacturer. To generate stable lines, HEK293 cells were transfected with Flag-hCOP1 or Flag-CMV-2 vector together with pBABE. Puromycin (3 mg/L) was used for selection.

Affinity purification of Flag-hCOP1–associated proteins. HEK293 cells stably expressing Flag-hCOP1 or empty vector were harvested and washed with PBS. The cell pellets were lysed in lysis buffer [20 mmol/L Tris-Cl (pH 7.4), 250 mmol/L NaCl, 0.4% NP40, 10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1× Protease Inhibitor Cocktail], incubated on ice for 30 minutes, and vortexed regularly. The lysates were then cleared by centrifugation twice at 15,000 × *g* for 30 minutes. The supernatants were collected and precipitated with anti-Flag M2 affinity resin (Sigma) overnight at 4°C. The resin was first washed with high-salt wash buffer [20 mmol/L Tris-Cl (pH 7.4), 550 mmol/L NaCl, 0.4% NP40, 10% glycerol, 1 mmol/L PMSF, 1× Protease Inhibitor Cocktail], then washed thrice with lysis buffer, and finally washed twice with elution buffer [25 mmol/L Tris-Cl (pH 7.4), 150 mmol/L NaCl, 0.01% NP40, 10% glycerol, 1 mmol/L EDTA]. Proteins were eluted with 1 mg/mL Flag peptide in elution buffer. Eluted proteins were precipitated with trichloroacetic acid, resuspended in 2× SDS sample buffer, and subjected to SDS-PAGE. Coomassie or silver stain was used to visualize the protein bands. The protein bands of interest were excised, digested by trypsin, and analyzed by matrix-assisted laser desorption/ionization-mass spectrometry and liquid chromatography-mass spectrometry.

Cell fractionation and immunoprecipitation analysis. HEK293 cells stably expressing Flag-hCOP1 were harvested, washed with PBS, and dounce homogenized in a hypotonic buffer [10 mmol/L HEPES (pH 7.4), 10 mmol/L NaCl, 1.5 mmol/L $MgCl_2$, 2 mmol/L $CaCl_2$, 0.5 mmol/L DTT, 0.5 mmol/L PMSF, 1× Protease Inhibitor Cocktail]. After centrifugation at 600 × *g* for 5 minutes, the supernatant was adjusted to 250 mmol/L NaCl, centrifuged again at 15,000 × *g* for 30 minutes, and was designated as the cytoplasmic fraction. The pellet was washed twice with the hypotonic buffer, resuspended in lysis buffer, incubated on ice for 30 minutes with regular vortexing, and then centrifuged at 15,000 × *g* for 30 minutes. The resulting supernatant was designated as the nuclear fraction.

Whole cell lysates and cytoplasmic and nuclear fractions were incubated with the indicated antibody at 4°C for 4 hours to overnight. Immunocomplexes are precipitated with Protein A or Protein G resins by rotating at 4°C for 2 hours. Immunoprecipitates are washed five times with lysis buffer, resuspended in SDS sample buffer, and subjected to Western blot analysis.

RNA interference and luciferase reporter assay. A mixture of two RNA oligos with 3′ dTdT overhangs were used for MVP: AAG AGU AUG UGC CAU CUG CCA and AAG UGC UGC AGG CCA AGC UAA (Dharmacon, Lafayette, CO). Cells were transfected thrice with Lipofectamine 2000 (Invitrogen) at 24-hour intervals according to the instructions of the manufacturer. Cells used in AP-1 reporter assays were transfected for the third time with the appropriate oligos along with pAP-1-luc and pRL-TK at a 10:1 ratio. Thirty hours following the third transfection, the transcription activities were assessed by Promega dual luciferase assay kit and measured by Wallac Victor 2 plate reader (Perkin-Elmer). UV treatment when required was carried out 6 hours before the cells were harvested.

Results

Affinity purification of constitutively photomorphogenic 1–associated proteins from mammalian cells. COP1 exists as large protein complexes in mammalian cells (6). To isolate COP1-associated proteins from mammalian cells, we generated HEK293 cell lines stably expressing Flag-tagged hCOP1. Flag-hCOP1 and its associated proteins were affinity purified from the lysates of these cells by using anti-Flag antibody (M2) coupled beads. After extensive washing, the beads were eluted with Flag peptide and the eluted proteins were separated by SDS-PAGE. Compared with controls, two unique protein bands of molecular weights 110 and 90 kDa were consistently obtained from Flag-hCOP1–expressing cells (Fig. 1*A*). The 90-kDa band was confirmed to be Flag-hCOP1 by both Western blot and mass spectrometry analysis (data not shown), and the 110-kDa protein band was identified by mass spectrometry analysis as MVP (Fig. 1*B*).

MVP is the core component of "vault," a 13-MDa ribonucleoprotein organelle of unknown function (21). MVP, also known as lung resistance protein (LRP), is expressed at elevated levels in many cancer cell lines and various primary tumor samples,

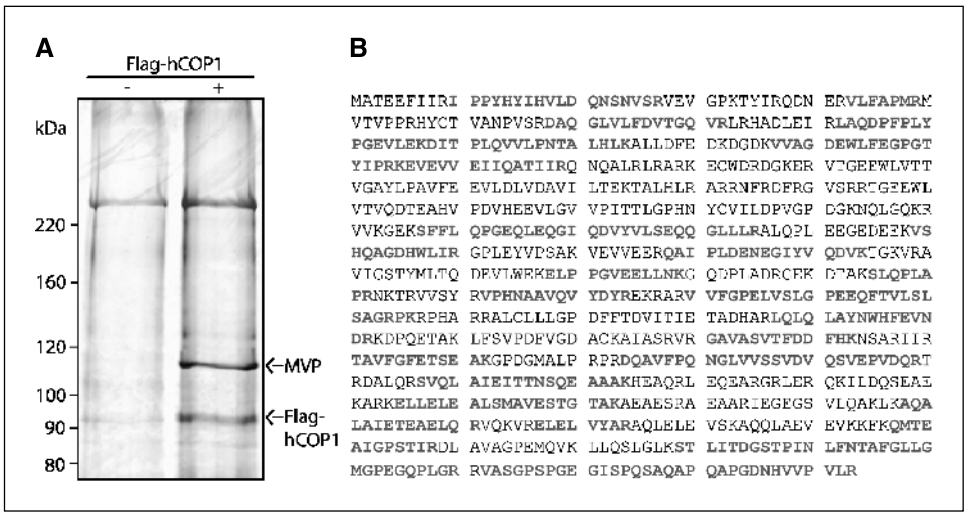

Figure 1. Copurification of MVP with Flag-hCOP1 from mammalian cells. *A,* silver-stained SDS-PAGE gel from Flag-peptide elution after affinity purification using Flag-antibody coupled beads. Flag purification was done using HEK293 cells or HEK293 cells stably expressing Flag-hCOP1 as indicated above each lane. Unique protein bands were marked by arrows and their identities revealed by mass spectrometry analysis. *B,* amino acid sequence of the MVP protein with matching peptides identified by mass spectrometry highlighted in bold.

especially those resistant to chemotherapy, and was suggested to play a role in multiple drug resistance (22–24).

Specific interaction of major vault protein and constitutively photomorphogenic 1 in the cytosol of mammalian cells. To ensure the specificity of the MVP/Flag-hCOP1 interaction, Flag-hCOP1–expressing HEK293 cell lysates were subjected to immunoprecipitation with preimmune, anti-COP1 or anti-MVP antibodies. Anti-COP1 and anti-MVP antibodies pulled down both Flag-hCOP1 and MVP, whereas neither protein was precipitated by the preimmune serum (Fig. 2*A*). More importantly, we were able to detect the endogenous COP1-MVP interaction in HeLa cells without any transgene (Fig. 2*B*).

To further dissect the subcellular location of this interaction, we first fractionated the HEK293 cells expressing Flag-hCOP1 into cytoplasmic and nuclear fractions, and then incubated each fraction with anti-Flag affinity resin. Western blot analysis showed that in contrast to c-Jun, which binds to Flag-hCOP1 primarily in the nucleus (Fig. 2*C*), MVP interacts with Flag-hCOP1 predominantly in the cytoplasm (Fig. 2*C*), consistent with the previous findings of MVP as a mostly cytoplasmic protein (25). Interestingly, overexpression of COP1 causes a dramatic increase of MVP protein level in HEK293 cells (Fig. 2*D*), indicating COP1 may regulate the expression of MVP.

The interaction between constitutively photomorphogenic 1 and major vault protein is reduced after UV exposure. In mammalian cells, the two known COP1 substrates, c-Jun and p53, are both involved in the UV stress response (17), strongly hinting at the relevance of COP1 in this pathway. Therefore, we investigated the effect of UV irradiation on the interaction between COP1 and MVP. Following exposure to 40 J/m^2 of UV, HEK293 cells stably expressing Flag-hCOP1 were incubated with fresh medium for additional periods of time before the cells were harvested and subjected to immunoprecipitation with anti-MVP antibodies. As shown in Fig. 3 (*top*), within 4 hours following UV exposure, the amount of Flag-hCOP1 pulled down by anti-MVP antibody gradually decreases as the incubation period increases. Interestingly, this coincides with elevated tyrosine phosphorylation of MVP, which peaks at 6 hours after UV irradiation (Fig. 3, *top*). In a reciprocal immunoprecipitation experiment, the amount of MVP associated with anti-Flag immunocomplexes also decreases post UV treatment (Fig. 3, *middle*). No apparent changes of the total protein level of either MVP or Flag-hCOP1 in the cells were observed after UV irradiation, indicating no significant proteolysis of either protein occurred (Fig. 3, *bottom*).

Major vault protein, in conjunction with constitutively photomorphogenic 1, represses activator protein 1 transcription activity under unstressed conditions. It has been reported that COP1 negatively regulates AP-1 transcriptional activity (10, 11). We examined whether MVP is involved in the regulation of this pathway by utilizing wild-type ($MVP^{+/+}$) and *mvp*-deficient ($MVP^{-/-}$) MEFs (20). Western blot analysis of whole cell lysates showed readily detectable amounts of MVP protein in $MVP^{+/+}$ MEFs, but none in $MVP^{-/-}$ MEFs (Fig. 4*A*). To measure the AP-1 transcriptional activity, we transfected $MVP^{+/+}$ and $MVP^{-/-}$ MEFs with pAP-1-luc (Clontech), a reporter construct that contains multiple copies of the AP-1 enhancer fused to a TATA-like promoter, followed by the coding sequence of firefly luciferase reporter gene. Because the binding of transcription factors to AP-1 initiates the transcription of luciferase reporter gene, the luciferase intensity thus reflects the level of AP-1 transactivation. By using this reporter system, we discovered that the basal AP-1 transcription activity in *mvp*-deficient cells was significantly enhanced compared with wild-type cells (Fig. 4*A*). This corresponded with an elevated amount of c-Jun in *mvp* knockout cells (Fig. 4*A*). Introduction of a Flag-tagged MVP construct into $MVP^{-/-}$ MEFs restored the AP-1 transcription activity to near wild-type level, whereas exogenous expression of MVP-Flag in wild-type cells further reduced the AP-1 transcription activity (Fig. 4*A*). These results indicate that MVP, like COP1, is a strong inhibitor of AP-1 transactivation in unstimulated cells.

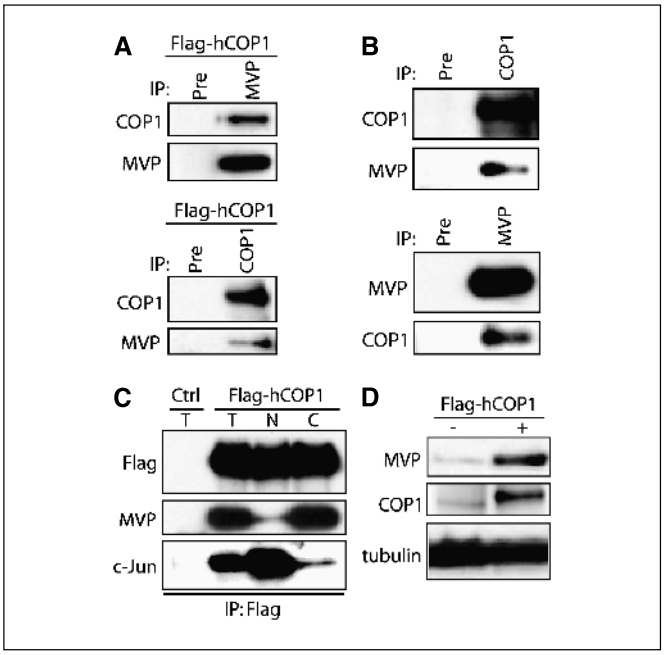

Figure 2. Specific interaction of MVP with exogenous and endogenous COP1, and cellular location of this interaction. *A,* coimmunoprecipitation of Flag-hCOP1 and endogenous MVP. HEK293 cells stably expressing Flag-hCOP1 were immunoprecipitated with preimmune, anti-MVP and anti-COP1 antibodies, and subjected to Western blot with anti-COP1 and anti-MVP antibodies. *B,* endogenous COP1 interacts with endogenous MVP. HeLa cells were immunoprecipitated with preimmune, anti-MVP and anti-COP1 antibodies, and subjected to Western blot with anti-COP1 and anti-MVP antibodies. *C,* COP1 interacts with MVP predominantly in the cytoplasm. HEK293 cells stably expressing Flag-hCOP1 were first separated into nuclear and cytoplasmic fractions and then subjected to immunoprecipitation with Flag-antibody coupled beads. Flag immunoprecipitation from whole cell lysate of untransfected HEK293 cells was used as negative control. Equal amounts of Flag precipitates from control (*Ctrl*), whole cell lysate (*T*), nuclear fraction (*N*), and cytoplasmic fraction (*C*) were loaded to the SDS-PAGE and immunoblotted with anti-COP1, anti-MVP, and anti–c-Jun antibodies. *D,* overexpression of COP1 causes increased MVP level. Western blot analysis was done using anti-MVP, anti-COP1, and anti–tubulin antibodies against equal amount of HEK293 cell lysates stably expressing empty Flag vector or Flag-hCOP1 as indicated.

Because COP1 and MVP dissociate from each other in response to UV (Fig. 3), we asked whether UV influences the effect of MVP on AP-1 transcription activity. Cells were treated with 40 J/m^2 of UV and incubated for additional 6 hours before the AP-1 transcription activity was measured. In concert with previous findings, UV irradiation stimulated AP-1 transcription activity, increased the amount of c-Jun, and induced c-Jun phosphorylation in wild-type cells (ref. 16; Fig. 4*B*). However, this response was much less dramatic in $MVP^{-/-}$ cells (Fig. 4*B*). The c-Jun quantity and the AP-1 transcription activity in unstressed $MVP^{-/-}$ cells were similar to those in UV-irradiated wild-type cells, and UV treatment did not significantly further increase the c-Jun amount and the AP-1 transcription activity in *mvp* null

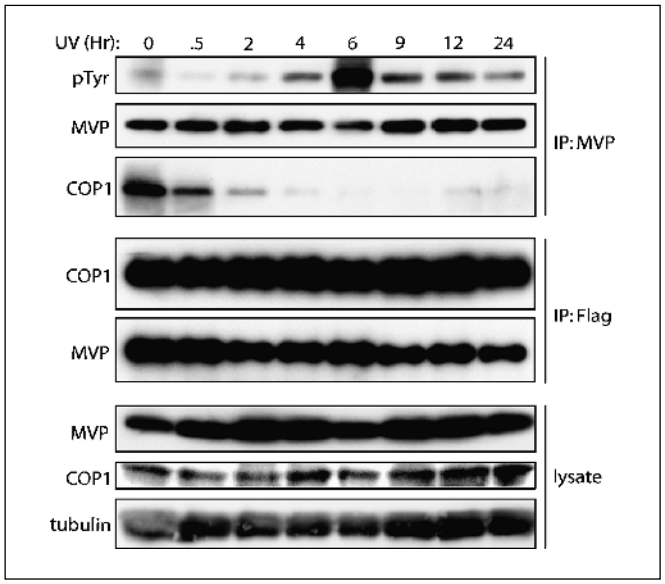

Figure 3. UV irradiation causes dissociation of COP1 from MVP and increased tyrosine phosphorylation of MVP. HEK293 cells stably expressing Flag-hCOP1 were irradiated with UV (40 J/m^2) and harvested at different time points as indicated following UV treatment. Cell lysates were first immunoprecipitated with anti-MVP (*top*) or anti-Flag (*middle*) antibodies, or directly blotted (*bottom*) with indicated antibodies.

cells (Fig. 4*B*). These data indicate that the AP-1 pathway is constitutively active in *mvp* knockout cells. Similar results were obtained in small interfering RNA (siRNA)–mediated MVP knockdown cells. Reduced amounts of MVP resulted in increased c-Jun level and elevated AP-1 transcription activity (~5-fold) under normal conditions (Fig. 4*C*). However, in MVP knockdown cells, UV irradiation did not significantly alter c-Jun levels and caused less than 2-fold increase in AP-1 transcription activity (Fig. 4*C*). Further MVP siRNA experiment showed that the amount of MVP protein in the cells inversely correlates with both c-Jun levels and AP-1 transcription activity in a dose-dependent manner (Fig. 4*D*).

Major vault protein represses activator protein 1 transactivation under unstressed conditions probably by enhancing the interaction between constitutively photomorphogenic 1 and c-Jun. Collectively, our data suggest that MVP represses the AP-1 pathway under unstressed conditions, most likely through its interaction with COP1. After UV irradiation, MVP becomes phosphorylated, releases COP1, and ceases to inhibit AP-1 transcription. Because COP1 had been previously shown to down-regulate c-Jun–mediated AP-1 transactivation through direct physical interaction with c-Jun (10), we decided to test if there is any correlation between the inhibitory effect of MVP on AP-1 transcription and the interaction between COP1 and c-Jun, and how UV exposure affects this interaction. For this purpose, we did Flag immunoprecipitation using cell lysates from either untreated or UV-irradiated HEK293 cells stably expressing Flag-hCOP1 as described above. Subsequent immunoblotting showed that in equal amounts of Flag-hCOP1 precipitates, reduced amount of MVP was pulled down from UV-exposed cell extracts compared with wild-type cells (Fig. 5), consistent with a reduction of MVP-COP1 interaction after UV exposure (Fig. 3). Furthermore, we detected significantly lesser c-Jun in Flag-hCOP1 immunoprecipitates from UV-treated cells, although the total c-Jun level was in fact higher in these cells than that of untreated cells (Fig. 5). Therefore, our data indicate that a strong COP1-MVP interaction in the cytoplasm directly correlates to a tight COP-c-Jun interaction in the nucleus (Figs. 2*C* and 5). This enhanced COP1-c-Jun interaction results in less c-Jun available to activate target gene transcription (Figs. 4 and 5).

Discussion

Our study has shown that in mammalian cells, MVP associates with COP1, and similar to COP1, it also suppresses

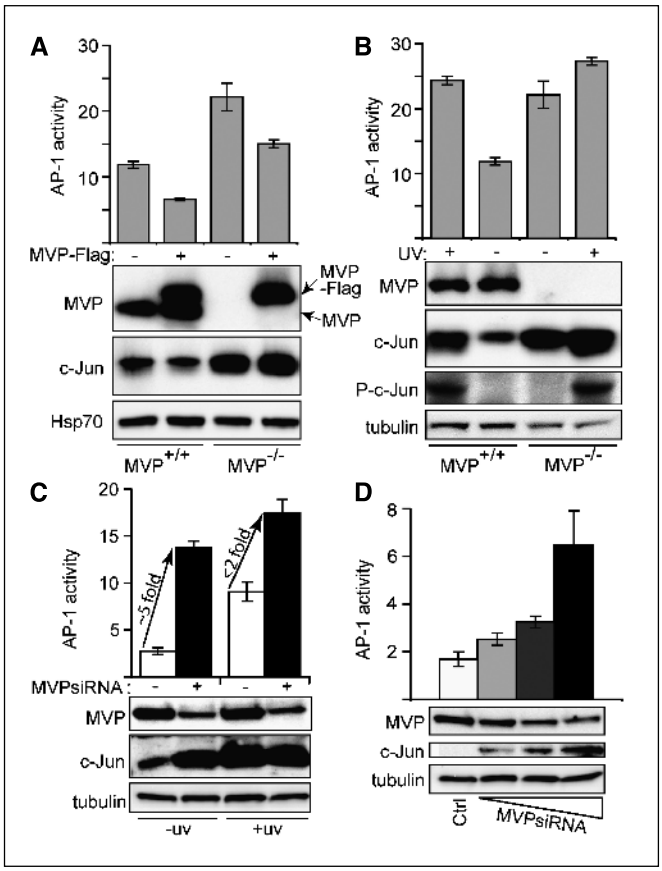

Figure 4. MVP negatively regulates c-Jun level and AP-1 transcription activity under unstressed conditions. *A,* MVP-deficient cells contain elevated c-Jun level and increased basal AP-1 transcription activity. Wild-type ($MVP^{+/+}$) or MVP-deficient ($MVP^{-/-}$) MEFs were transfected with empty Flag vector or MVP-Flag, together with reporter pAP-1-luc and an internal control construct, pRL-TK. Thirty-six hours after transfection, cells were lysed and analyzed for luciferase activity (*top*) or subjected to Western blot analysis with indicated antibodies (*bottom*). *Columns,* average values (n = 4); *bars,* SD. *B,* $MVP^{-/-}$ cells have diminished UV response in c-Jun induction and AP-1 activation. Wild-type ($MVP^{+/+}$) or *mvp*-deficient ($MVP^{-/-}$) MEFs were transfected with reporter pAP-1-luc and internal control pRL-TK. Thirty hours following transfection, cells were either left untreated (−) or exposed to 40 J/m^2 of UV (+). After 6 hours, cells were harvested and analyzed for luciferase activity (*top*) or subjected to Western blot analysis with indicated antibodies (*bottom*). *Columns,* average values (n = 4); *bars,* SD. *C,* siRNA-mediated MVP reduction causes c-Jun accumulation and enhances AP-1 transcription activity under unstressed conditions. $MVP^{+/+}$ cells transfected with control siRNA (−) or MVP-specific siRNA (+) together with reporter pAP-1-luc and internal control pRL-TK. Cells were either left untreated (−UV) or exposed to 40 J/m^2 of UV (+UV). Six hours later, cells were harvested, analyzed for luciferase activity (*top*), or subjected to Western blot analysis with indicated antibodies (*bottom*). *Arrows,* fold differences between the samples and treatments. *Columns,* average values (n = 4); *bars,* SD. *D,* siRNA-mediated MVP reduction causes c-Jun accumulation and enhances AP-1 transcription activity in a dose-dependent manner. $MVP^{+/+}$ cells were transfected with control siRNA (*Ctrl*) or increased amount of MVP-specific siRNA, together with equal amount of reporter pAP-1-luc and internal control pRL-TK. Cell lysates were analyzed for luciferase activity (*top*) or subjected to Western blot analysis with indicated antibodies (*bottom*). *Columns,* average values (n = 4); *bars,* SD.

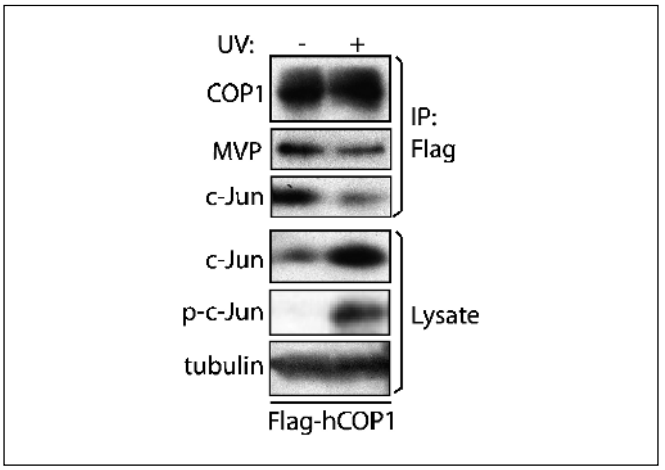

Figure 5. UV exposure reduces the COP1-MVP and COP1-c-Jun interactions. HEK293 cells stably expressing Flag-hCOP1 were either left untreated or irradiated with UV (40 J/m^2) and harvested 9 hours following UV treatment. Cell lysates were first immunoprecipitated with anti-Flag affinity resin (*top*) or directly blotted (*bottom*) with indicated antibodies.

c-Jun–mediated AP-1 transactivation. Because no direct interaction between MVP and c-Jun was detected (data not shown) and MVP localizes primarily to the cytoplasm (21), MVP probably inhibits c-Jun–mediated AP-1 transactivation through COP1. One plausible mechanism is that MVP might function by promoting the nuclear import of COP1, given that COP1 most likely represses AP-1 transcription by binding to transcription factors such as c-Jun in the nucleus. However, compared with wild-type cells, no significant change in COP1 subcellular distribution was observed in *mvp*-deficient or UV-treated cells (data not shown), thus arguing against this possibility.

Another likelihood is that MVP may recruit COP1 to vault, which in addition to its major constituent, MVP, also contains at least three other minor components, vault poly(ADP-ribose) polymerase (VPARP), telomerase-associated protein 1, and one or more small vault RNAs (24). Once recruited onto vault, COP1 may be modified by a certain component of the vault complex (Fig. 6). This yet unidentified modification may enhance the binding affinity of COP1 to c-Jun, preventing c-Jun from binding to AP-1 promoter and thereby repressing AP-1 transcription (Fig. 6). Upon UV exposure, however, MVP becomes phosphorylated, and COP1 is no longer able to associate with vault to be modified, thus failing to interact with c-Jun (Fig. 6). "Freed" c-Jun in turn binds to AP-1 promoter and activates AP-1 transcription (Fig. 6). In agreement with this model, we found that a reduced amount of c-Jun could be pulled down with Flag-hCOP1 from UV-treated cell extracts, despite that a higher level of c-Jun was actually present in these cells compared with untreated cells (Fig. 5).

The most likely component of the vault complex that could potentially modify COP1 is VPARP, a member of a protein family

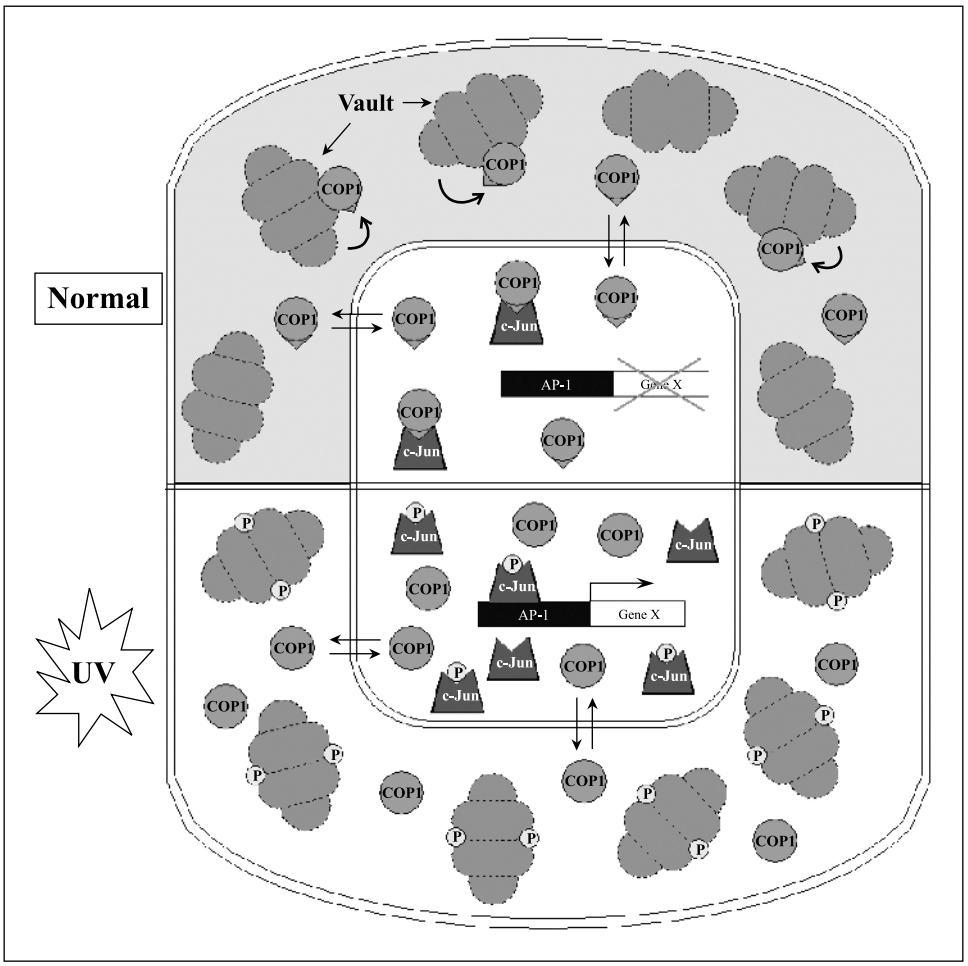

Figure 6. A working model of how MVP (vault), in concert with COP1, represses c-Jun–mediated AP-1 transcription. The upper half of the outer rounded double rectangles represents a cell under normal conditions (*gray*), whereas the lower half represents a cell exposed to UV (*white*). The inner rounded double rectangles represent the nucleus. In both type of cells, COP1 (*circles*) shuttles between the nucleus and the cytoplasm (*arrows*). In the normal cell, the vault complexes interact with COP1 and add yet unknown modification (*triangles*) to COP1. Modified COP1 binds to c-Jun (*trapezoids*) with high affinity in the nucleus, prevents it from binding to the AP-1 promoter (*black*), and thus inhibits AP-1 transactivation. In the UV-treated cell, MVP becomes phosphorylated and no longer interacts with and modifies COP1. Unmodified COP1 is less capable of interacting with c-Jun and targeting it for degradation. The free phosphorylated c-Jun binds to the AP-1 promoter and activates the transcription of downstream genes.

characterized for their ability to catalyze poly(ADP-ribosyl)ation, a covalent protein modification involving a series of transfer of ADP-ribose moieties from coenzyme NAD+ to an aspartate, glutamate, or lysine residue on the surface of the acceptor protein (26). The two most well-studied poly(ADP-ribose) polymerases, PARP-1 and PARP-2, are strongly activated by single-strand DNA breaks (26, 27). The chromatin proteins around the site of breakage are rapidly modified and dissociate from the DNA, making it accessible to repair enzymes (26, 27). In addition to their roles in DNA repair and chromatin remodeling, PARP-1 and PARP-2 also regulate gene expression through physical and functional interactions with specific transcription factors (28). The function of VPARP remains elusive (29). However, it was found to be able to ADP-ribosylate itself and MVP (30). Because a small amount of VPARP seems to copurify with Flag-COP1 (data not shown), it would be interesting to test if COP1 is also a substrate of VPARP, and how such modification, if any, affects COP1 activity.

Our data and previous reports have shown that both MVP and COP1 are required to repress the AP-1 pathway under unstressed (unstimulated) conditions in mammalian cells (Fig. 4; refs. 10, 11). The constitutively active AP-1 pathway in *mvp*-deficient cells is reminiscent of the constitutive photomorphogenic phenotypes displayed by *Arabidopsis cop1* mutants in which light-induced genes are expressed even in the dark (Fig. 4*B*; ref. 3). Therefore, it is tempting to speculate that under normal conditions, MVP, via interaction with COP1, prevents mammalian cells from undergoing stress response (by inhibiting the AP-1 pathway and possibly other unidentified pathways), analogous to the role of plant COP1 in repressing photomorphogenesis in the dark.

A recent study of COP1 expression using ovarian and breast cancer tissue microarrays has found that COP1 is significantly overexpressed in 81% (25 of 32) of breast and 44% (76 of 171) of ovarian adenocarcinoma, suggesting that COP1 may play an important role in oncogenesis (18). On the other hand, MVP has long been known to be overexpressed in ovarian, breast, as well as many other types of tumors, particularly among those that are resistant to anticancer drugs (24). The physical and functional interactions between COP1 and MVP shown in this study raise the possibility that COP1 and MVP may function collaboratively in cancer progression and drug resistance.

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