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Characterization of the tumor suppressor protein p53 as a protein kinase C substrate and a S100b-binding protein

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ABSTRACT **We report here that the negative cell cycle regulator protein p53 is an *in vivo* and *in vitro* substrate for protein kinase C, a cellular receptor for the tumor-promoter phorbol esters. We also demonstrate that p53 interacts in a calcium-dependent manner with S100b, a member of the S100 protein family involved in cell cycle progression and cell differentiation, and that such an interaction inhibits *in vitro* p53 phosphorylation by protein kinase C. The interaction between p53 and S100b was utilized for the purification of cellular and recombinant murine p53 by affinity chromatography with S100b-Sepharose. Furthermore, and of particular interest, we have shown that purified p53 undergoes temperature-dependent oligomerization and that the interaction between S100b and p53 not only induces total inhibition of p53 oligomerization but also promotes disassembly of the p53 oligomers. We suggest that these effects result from the binding of S100b to the multifunctional basic C-terminal domain of p53 and propose that p53 may be a cellular target for the S100 protein family members involved in the control of the cell cycle at the G_0–G_1/S boundary.**

The cell cycle is composed of a series of steps that can be negatively or positively regulated by various factors. Chief among the negative regulators is the p53 protein (1). The p53 gene was initially proposed to be an oncogene, but more recent work has shown that only mutant forms of p53 elicit neoplastic transformation *in vitro* and that wild-type p53, on the contrary, could inhibit oncogene-mediated focus formation, thus acting as a tumor suppressor gene (2). Several properties of p53 have been described (for review, see ref. 3). p53 is a cytosolic phosphoprotein that has to translocate to the nucleus for cellular activity (4). p53 can be phosphorylated by cdc2 kinase (5) and casein kinase II (6). p53 can also form large oligomers and p53 oligomerization has been directly linked to the cellular function of the protein (7). Analysis of the p53 primary structure reveals that the protein has an extended basic C-terminal region that is predicted to form an amphipathic helix-like structure and is probably responsible for its binding to DNA and the formation of stable p53 oligomers (8, 9). This putative DNA-binding and oligomerization-promoting domain on p53 shows a striking similarity with predicted protein kinase C (PKC) phosphorylation sites (10). In addition, this domain resembles in some respects the PKC phosphorylation and calmodulin (CaM)-binding domain on the myristoylated alanine-rich C kinase substrate (MARCKS) protein (11) (see also Fig. 7). MARCKS is a major PKC substrate rapidly phosphorylated in a wide variety of cell types in response to growth factor stimulation (11). *In vitro* MARCKS phosphorylation is strongly inhibited by calcium binding proteins of the "EF-hand" type, S100b, and to a lesser extent by CaM (12). This inhibition has been explained through the binding of the calcium binding proteins to the PKC phosphorylation site domain (13). Because of the similarities between the basic C-terminal domain of p53 and the PKC phosphorylation site domain on MARCKS protein, we investigated the possibility that p53 might also be a PKC substrate that interacts with S100b and CaM.

EXPERIMENTAL PROCEDURES

Cell Lines and Culture. Murine erythroleukemia cells (MELCs), clone F4NWO, were maintained in culture in minimum essential medium (MEM) supplemented with 10% (vol/vol) fetal calf serum and 1 mM 5% (wt/vol) glutamine. cDNA cloning of the p53 mRNA in our MELC strain has revealed no mutation in the five conserved domains of the protein where the transforming mutations generally occur (1), but a 9-amino acid deletion (residues 255–262) was found when compared to the wild-type protein (unpublished data).

Protein Purification and Antibodies. Rat-brain PKC isoforms were purified as described (14). Bovine brain S100b protein was purified as described (15). S100b and CaM-Sepharose columns were prepared as in ref. 16. Monoclonal antibodies PAB-240, PAB-248, PAB-242, and PAB-421 are from hybridoma cell supernatants (17, 18).

Metabolic Labeling, Immunoprecipitation, and Autoradiography. Approximately 3×10^7 exponentially growing MELCs were preincubated in 50 ml of phosphate-free MEM with 5% glutamine for 90 min. The cells were pelleted by centrifugation and resuspended in 5 ml of phosphate-free MEM plus [^{32}P]orthophosphate (1 mCi/ml; 1 Ci = 37 GBq). Cells were labeled for 90 min, split 1:2, and treated with 2 μl of dimethyl sulfoxide or with 2 μl of a phorbol 12-myristate 13-acetate (PMA) solution (3 mM) in dimethyl sulfoxide. After 10 min, cells were washed twice with phosphate-buffered saline (PBS) and lysed in 1 ml of lysis buffer [40 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM NaF/aprotinin (10 μg/ml)/0.5% Triton X-100]. After sonication and centrifugation to remove insoluble material, p53 was immunoprecipitated with a mixture of monoclonal antibodies PAB-242, PAB-240, and PAB-248. The immunoprecipitate was analyzed by SDS/PAGE on an 8% gel and autoradiography.

***In Vitro* PKC Phosphorylation Assays.** Standard phosphorylation assays were performed in 40 mM Tris·HCl (pH 7.5) at 35°C. The reaction mixture (100 μl) contained 1 μM p53, 80 μM [^{32}P]ATP, and 3 mM $MgCl_2$. $CaCl_2$ (0.2 mM), phosphatidylserine (2 μg), and diolein (0.25 μg) were included in the assay or omitted as indicated. The reaction was stopped by adding SDS stop solution [5% (wt/vol) SDS/25% (vol/vol) glycerol/500 mM Tris·HCl, pH 6.8]. The proteins were separated by SDS/PAGE on an 8% gel and the gels were

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Abbreviations: MELC, murine erythroleukemia cell; PKC, protein kinase C; MARCKS, myristoylated alanine-rich C kinase substrate; CaM, calmodulin; PMA, phorbol 12-myristate 13-acetate.

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fixed and processed for autoradiography. ^{32}P incorporation into p53 was quantified by liquid scintillation counting of the protein excised from the gel.

The Binding of Cellular p53 to S100b-Sepharose. Approximately 2.7×10^8 MELCs were lysed at 4°C in 8 ml of 50 mM Tris·HCl, pH 7.5/2 mM EGTA/200 mM NaCl/50 mM NaF/1% Triton X-100/aprotinin (60 μg/ml)/1 mM phenylmethyl sulfonyl fluoride and sonicated. After centrifugation at 15,000 × *g* for 15 min, the supernatant was collected, diluted 1:10 with buffer A (50 mM Tris·HCl, pH 7.5/4 mM dithiothreitol), and loaded onto a DE52 column (2 × 10 cm) equilibrated with buffer A. After washing the column with 100 ml of buffer A, the bound proteins were eluted with buffer A plus 0.4 M NaCl. The eluted protein fractions were pooled (50 ml), diluted with an equal volume of buffer A, adjusted to 1 mM $CaCl_2$, and loaded onto an S100b-Sepharose column (1 × 8 cm) equilibrated with buffer A containing 200 mM NaCl and 1 mM $CaCl_2$. The flow-through fractions were recycled once on the column and the column was washed with 10 column volumes of equilibration buffer. The bound proteins were subsequently eluted with buffer A containing 200 mM NaCl and 2 mM EGTA; 1-ml fractions were collected.

Purification of Recombinant Murine p53. Approximately 3.8×10^8 Sf9 cells were infected with recombinant baculovirus vEV55p53 (19). Forty-eight hours after infection, the cells were harvested, washed once with PBS, lysed in 100 ml of deionized purified water containing 2 mM EGTA (pH 7.6) and 4 mM dithiothreitol, sonicated, and immediately frozen at −20°C. After thawing, the cell extract was maintained at 4°C, 1 M Tris·HCl (pH 7.6) was added to 40 mM (final concentration), and the extract was briefly sonicated and centrifuged at 15,000 × *g* for 15 min. The supernatant fraction was loaded on a Q-Sepharose fast flow column (2 × 10 cm) equilibrated with buffer B (40 mM Tris·HCl, pH 7.6/5 mM dithiothreitol). The column was serially washed with buffer B plus 0.1 M NaCl, 0.2 M NaCl, and 0.4 M NaCl. Immunoblot analysis revealed that p53 was eluted with buffer B containing 0.4 M NaCl. The p53-containing fractions were pooled (40 ml) and diluted 1:2 with buffer B. Half of the protein solution (40 ml) was adjusted to 1 mM $CaCl_2$ and loaded on a S100b-Sepharose column (1.5 × 10 cm) previously equilibrated with buffer B containing 0.15 M NaCl and 1 mM $CaCl_2$. The column was washed with equilibrating buffer, and p53 protein was eluted with buffer B containing 2 mM EGTA and 0.15 M NaCl. The final purification step consisted of FPLC chromatography on a Mono Q column using a linear gradient from 0 to 0.7 M NaCl. p53 was eluted from the Mono Q column as a symmetrical protein peak at 0.3 M NaCl. The protein was dialyzed against 40 mM Tris·HCl (pH 7.5) and stored at −20°C until used. p53 protein concentrations were determined using the Bio-Rad protein assay system and bovine serum albumin as standard.

Overlay Procedures and Immunodetection of Covalently Cross-Linked p53-S100b Complexes. Triplicate samples of purified human p53 (0.5 μg) were electrophoresed on 8% SDS/PAGE gel and electrotransferred to an Immobilon membrane. After blocking the membrane with 3% (wt/vol) bovine serum albumin in buffer C (20 mM Tris·HCl, pH 7.5/150 mM NaCl), the membrane was cut in three pieces and incubated 15 min with either buffer C or S100b (2 μM) in buffer C in the presence of 1 mM Ca^{2+} or in the presence of 1 mM EGTA. After washing the membranes with corresponding buffers to remove unbound S100b, the p53-S100b complexes were then cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Pierce; 2 mM in buffer C). The p53-S100b complexes were then visualized by immunodetection using a rabbit anti-S100b serum produced in our laboratory (at a 1:50 dilution) and phosphatase-conjugated goat anti-rabbit IgG.

Temperature-Dependent Oligomerization of p53. p53 (0.7 mg/ml) in 40 mM Tris·HCl, pH 7.6/0.16 mM $CaCl_2$ was maintained in an ice-water bath and then transferred to a thermostated cell at 30°C that contained an equal volume of buffer or different concentrations of S100b dialyzed against 40 mM Tris·HCl (pH 7.6). The increase of A_{350} was recorded in a trace mode as a function of time.

RESULTS

Characterization of p53 as a PKC Substrate. The tumor promoter phorbol ester PMA is an *in situ* activator of intracellular PKC. To assess the role of PKC in phosphorylating p53, we compared the effect of PMA (0.1 μM) stimulation of Friend erythroleukemia cells on ^{32}P labeling of p53 with that of control cultures. A constant increase in ^{32}P incorporation could be observed in immunoprecipitated p53 from PMA-treated cells compared to control (Fig. 1*A*). The increase in ^{32}P incorporation varied between 1.5- and 2-fold, by densitometry scanning of the autoradiograms.

In vitro phosphorylation studies of p53 using purified rat brain PKC confirmed p53 as PKC substrate (Fig. 1*B*). Among the different rat brain PKC isoforms (types I–III), the type II and type III phosphorylated p53 significantly, whereas type I PKC was much less efficient (Fig. 1*B*). Although the amounts of PKC isoforms used in the experiments presented in Fig. 1*B* were not equivalent, as revealed by differences in their autophosphorylation signal (Fig. 1*B*), a 1:2 to 1:5 dilution of the type II PKC preparation had no significant effect on the extent of p53 phosphorylation, suggesting that differences in p53 phosphorylation by the various PKC isoforms might reflect differences in specificity for p53.

In the absence of added kinase, we observed that the purified recombinant p53 incorporated ^{32}P when incubated with [^{32}P]ATP to a maximum stoichiometry of 0.05 mol of phosphate per mol of protein (Figs. 1*B* and 2*A*). This p53 phosphorylation could not be stimulated by Ca^{2+} and phospholipids and, therefore, did not result from contamination of

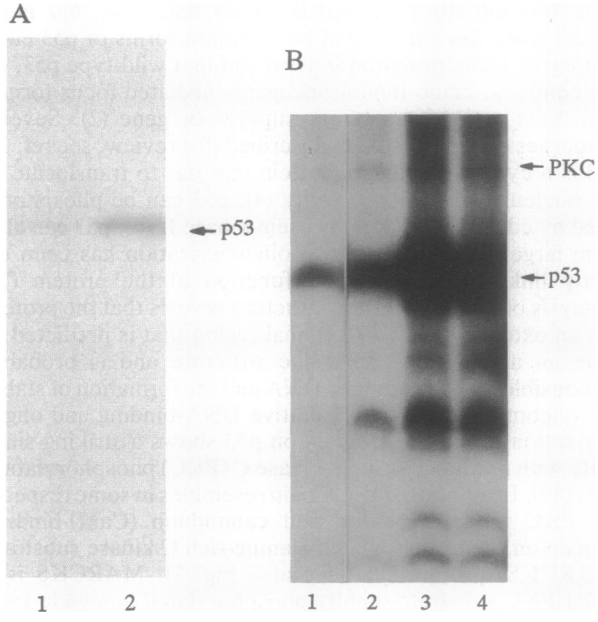

FIG. 1. Phosphorylation of p53 by PKC. (*A*) Immunoprecipitation of phosphorylated p53 from ^{32}P-labeled MELCs. Lanes: 1, control cells; 2, cells treated with PMA. (*B*) *In vitro* phosphorylation of purified p53 by the rat-brain PKC isoforms. Purified p53 was incubated for 30 min in standard PKC assay conditions in the absence (lane 1) or in the presence of type I, type II, or type III PKC isoforms (lanes 2–4, respectively). Positions of the phosphorylated p53 and of the autophosphorylated PKC are indicated.

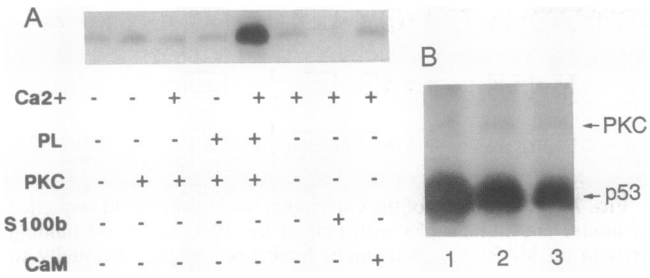

FIG. 2. *In vitro* phosphorylation of p53 by type II PKC: The effects of S100b. (*A*) Recombinant p53 was incubated 5 min in standard phosphorylation assay conditions in the absence (−) or presence (+) of type II PKC and PKC cofactors, Ca^{2+} and phospholipid (PL), as indicated. Comparison of the effect of S100b and CaM on p53 phosphorylation in the absence of added kinase is also shown. (*B*) Recombinant p53 (1 μM) was incubated 5 min in standard PKC phosphorylation assay conditions (plus Ca^{2+} and phospholipase) with type II PKC in the absence (lane 1) and in the presence of S100b at 0.9 μM (lane 2) or 1.8 μM (lane 3).

the p53 with PKC. Whether this phosphorylation of p53 results from an intrinsic autophosphorylation process or is due to a contaminating kinase that copurifies with p53 remains to be determined. When an equimolar concentration of S100b or CaM was added to p53 plus 0.1 mM Ca^{2+} prior to incubation with [^{32}P]ATP, a total inhibition of the p53 phosphorylation occurred only in the presence of S100b; CaM had no effect (Fig. 2*A*).

As expected phosphorylation of p53 by type II PKC was exclusively Ca^{2+}- and phospholipid-dependent (Fig. 2*A*). In our assay conditions, the cofactor-dependent phosphorylation of p53 was rapid at 35°C and was maximal by 10 min with an apparent stoichiometry of 0.65 mol of phosphate incorporated per mol of protein, suggesting a single phosphorylation site on the protein.

When S100b was present in the phosphorylation assay, a 70% inhibition of the Ca^{2+}/phospholipid-dependent PKC phosphorylation of p53 was observed for an S100b/p53 molar ratio of 1.8 (Fig. 2*B*). Because S100b has no effect on PKC autophosphorylation (Fig. 2*B*) and does not inhibit the phosphorylation of other substrates [e.g., histone (16)], it is unlikely that this inhibition is due to an effect of S100b on the kinase itself but rather is a direct consequence of the interaction of S100b with p53. Unlike S100b, CaM had no significant effect on p53 phosphorylation by PKC (data not shown).

The Binding of p53 to S100b. To confirm the binding of p53 to S100b protein, an affinity chromatography procedure was developed using MELC extract and S100b Sepharose (Fig. 3). All the p53 in the MELC extract was retained on the S100b column in the presence of Ca^{2+} and subsequently eluted when EGTA replaced Ca^{2+} (Fig. 3*B*). Silver staining of the p53-containing fractions did not detect p53 due to its low abundance and revealed that several other proteins also bound to the S100b column (Fig. 3*A*); most of these proteins have been identified as PKC substrates (data not shown).

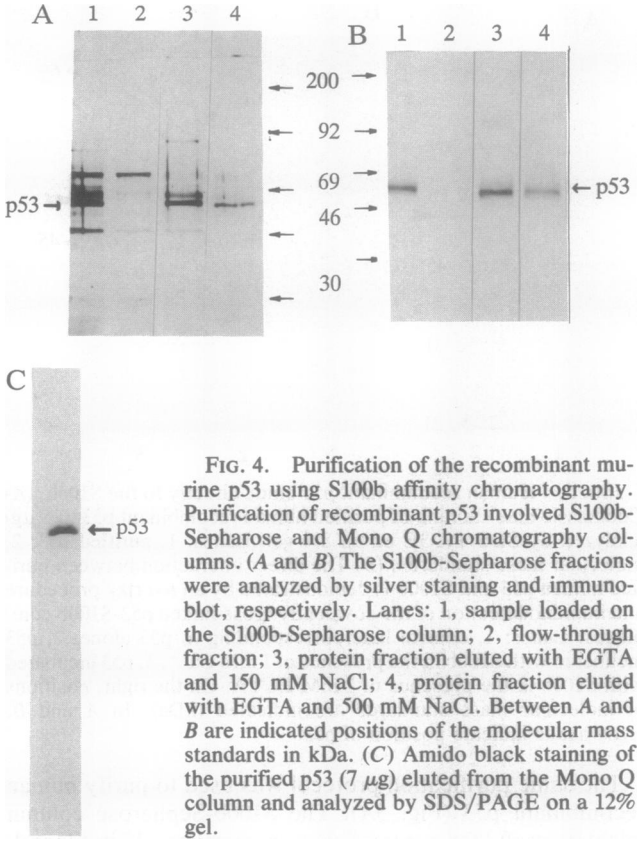

FIG. 4. Purification of the recombinant murine p53 using S100b affinity chromatography. Purification of recombinant p53 involved S100b-Sepharose and Mono Q chromatography columns. (*A* and *B*) The S100b-Sepharose fractions were analyzed by silver staining and immunoblot, respectively. Lanes: 1, sample loaded on the S100b-Sepharose column; 2, flow-through fraction; 3, protein fraction eluted with EGTA and 150 mM NaCl; 4, protein fraction eluted with EGTA and 500 mM NaCl. Between *A* and *B* are indicated positions of the molecular mass standards in kDa. (*C*) Amido black staining of the purified p53 (7 μg) eluted from the Mono Q column and analyzed by SDS/PAGE on a 12% gel.

For comparison, we also tested the binding of cellular p53 to CaM by affinity chromatography. Although significant calcium-dependent binding of p53 on the CaM-Sepharose column was observed, most of the p53 was recovered in the flow-through fractions, suggesting higher affinity of p53 for Ca^{2+}–S100b than for Ca^{2+}–CaM (data not shown).

The S100b affinity chromatography method was then used to purify murine p53 expressed in SF9 insect cells infected with recombinant baculovirus vEV55p53 (19). Recombinant murine p53 bound to the S100b column in the presence of Ca^{2+} and could be eluted with EGTA-containing buffer (Fig. 4). A significant amount of p53 (≈5%) remained bound to the S100b-Sepharose in the presence of EGTA and 150 mM NaCl but was eluted with a higher NaCl concentration (500 mM), suggesting that it can interact with S100b in the absence of Ca^{2+}. Subsequent purification by anion-exchange chromatography on a Mono Q column resulted in purified p53 protein judged to be >95% pure when analyzed on an SDS/polyacrylamide gel and amido black staining (Fig. 4*C*).

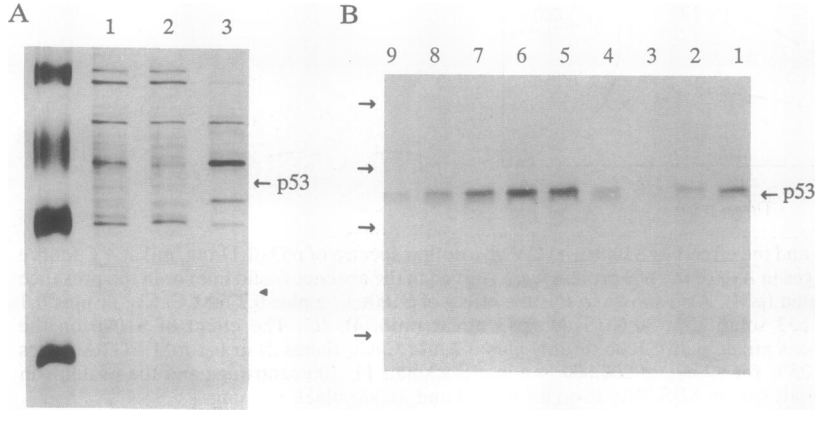

FIG. 3. Cellular p53 from MELCs bind to an S100b-Sepharose column. Analysis of fractions from the S100b-Sepharose column chromatography by SDS/PAGE on an 8% gel (5 μg of protein per lane) followed by either silver staining (*A*) or immunoblot analysis with PAB-421 monoclonal antibody (1:200 dilution) (*B*). (*A*) Lanes: 1, sample loaded on the S100b-Sepharose column; 2, flow-through fraction; 3, fraction eluted with EGTA. (*B*) Lanes: 1, sample loaded on the S100b-Sepharose column; 2, first flow-through fraction; 3, second flow-through fraction; 4–9, fractions eluted with EGTA. The silver-stained band on the left side of *A* and the arrows in *B* correspond to the molecular mass standards 30, 46, 69, and 92 kDa, respectively.

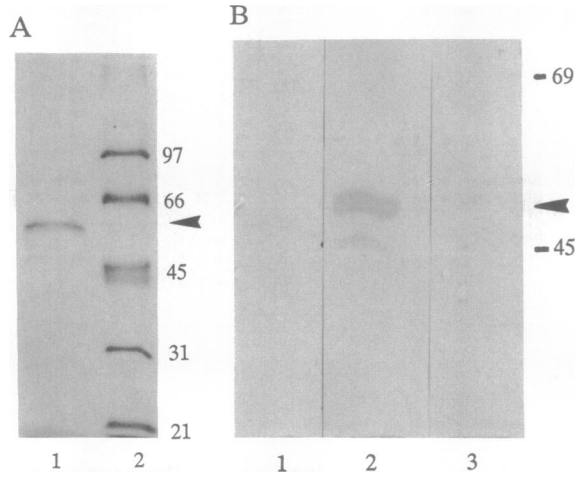

FIG. 5. Human recombinant p53 binds directly to the S100b. (*A*) Coomassie blue staining of purified human recombinant p53 (0.5 μg) analyzed by SDS/PAGE on an 8% gel. Lanes: 1, purified p53; 2, molecular mass standards. (*B*) The direct interaction between purified human p53 and S100b is demonstrated by an overlay procedure and immunodetection of the covalently cross-linked p53-S100b complexes. Lanes: 1, control lane corresponding to p53 alone; 2, p53 incubated with S100b in the presence of 1 mM Ca^{2+}; 3, p53 incubated with S100b in the presence of 1 mM EGTA. On the right, positions of molecular mass standards are indicated (kDa). In *A* and *B*, arrowheads indicate position of p53.

The same purification protocol was used to purify human recombinant p53 (Fig. 5*A*). The S100b-Sepharose column might be useful for purification of mammalian p53 in general.

The binding of p53 to the S100b-Sepharose column through a direct interaction with S100b was confirmed by an overlay procedure and covalent crosslinking of the p53-S100b complexes (Fig. 5). As expected the p53-S100b complexes formed in the presence of Ca^{2+} but not in the presence of EGTA.

The Interaction Between p53 and S100b Regulates p53 Oligomerization *in Vitro*. At 30°C the UV absorption spectrum

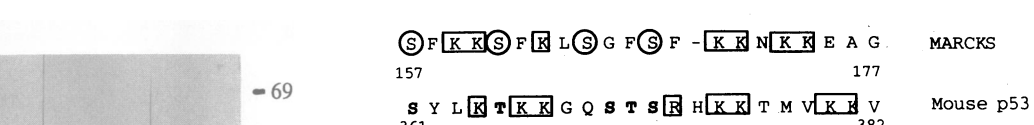

FIG. 7. Comparison of the C-terminal basic amino acid sequence of mouse and human p53 with that of the PKC and CaM-binding domain on MARCKS. Sequences have been aligned manually to compare positions of the putative serine/threonine PKC phosphorylation sites on p53 (boldface type) with that of the phosphorylated serine residues (circled) on MARCKS. Note the striking similarity in position of the basic amino acids that are essential for PKC phosphorylation but also presumably promote association with the acidic CaM or S100b molecules as demonstrated for other proteins (20, 21).

of purified p53 in 40 mM Tris·HCl (pH 7.6) showed abnormal light scattering relative to that of a solution at 4°C, indicating temperature-dependent aggregation (Fig. 6*A*). In Fig. 6*B* the time-dependent changes in light scattering at 350 nm are presented for a p53 solution when the temperature was shifted from 4°C to 30°C. In parallel experiments, we found that the p53 aggregation was independent of the ionic strength, although larger p53 oligomers were formed in the presence of 0.3 M NaCl than in its absence. ATP or GTP has no effect on the oligomerization of p53.

The p53 oligomers formed at 30°C were sufficiently large to be sedimented at 200,000 × *g* (Fig. 6*C*).

When increasing amounts of S100b were added to p53, in the presence of 0.1 mM Ca^{2+}, prior to shifting the temperature from 4°C to 30°C, the oligomerization of p53 was inhibited in a concentration-dependent manner (Fig. 6*B*) and p53 could not be sedimented by centrifugation (Fig. 6*C*). Furthermore, when S100b plus Ca^{2+} was added to oligomerized p53 at 30°C, light scattering of the p53 protein solution at 350 nm decreased within 2 min to reach the level recorded at 4°C (Fig. 6*B*). These results indicate that S100b can interact with p53 to prevent its oligomerization and that it can also induce disassembly of p53 oligomers, perhaps by sequester-

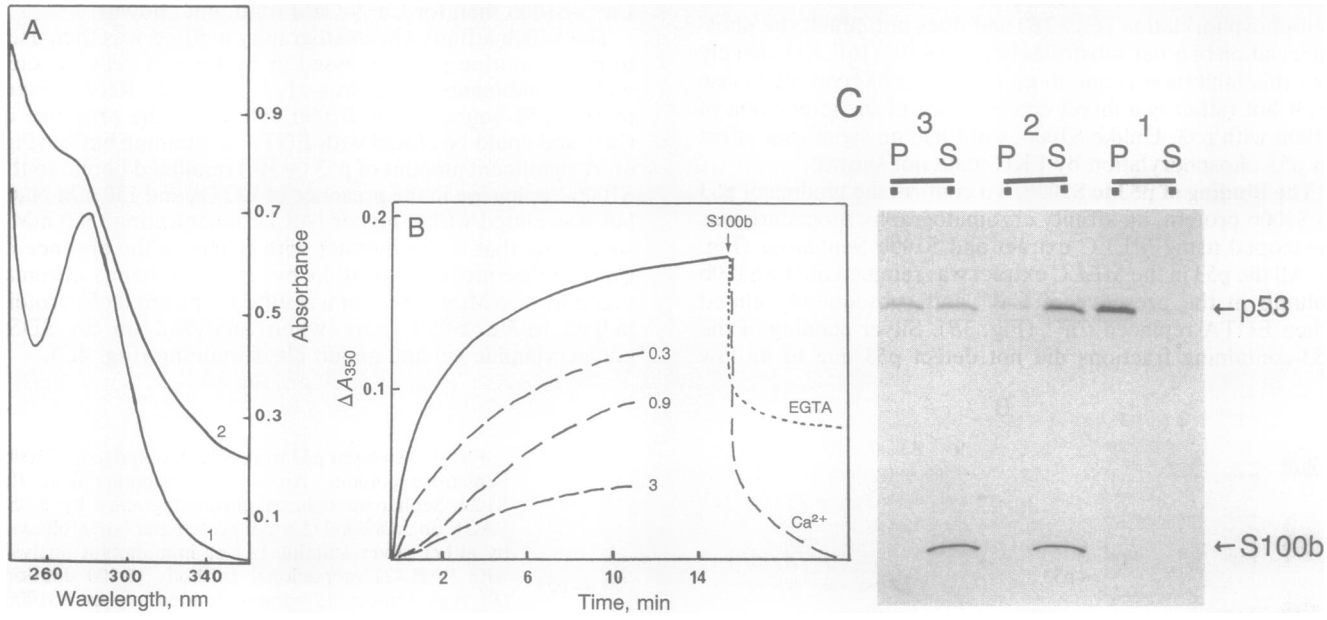

FIG. 6. Temperature-dependent oligomerization of p53 and the effects of S100b. (*A*) UV absorption spectra of p53 (0.11 mg/ml) at 4°C (curve 1) and 30°C (curve 2). (*B*) The temperature-dependent changes in A_{350} of the p53 protein were studied in the absence (solid line) or in the presence of various concentrations of S100b (dashed lines) as indicated (μM). Also shown in *B* is the effect of S100b (↓) plus 0.2 mM $CaCl_2$, or plus 0.1 mM EGTA on the changes in A_{350} of the oligomerized p53 solution at 30°C (S100b/p53 molar ratio, 4). (*C*) The effect of S100b on the sedimentation of the p53 oligomers. p53 (2 μM) (lanes 1) was mixed with S100b (8 μM) plus 0.2 mM $CaCl_2$ (lanes 2) or 0.1 mM EGTA (lanes 3). After 11 min at 25°C, the proteins were centrifuged at 25°C for 30 min at 200,000 × *g* in a Beckman TL 100 centrifuge and the proteins in the supernatant fractions (S) and in the pellets (P) were analyzed by SDS/PAGE on an 8% gel and amido black staining.

ing p53 monomers. Using this approach we also confirmed that, in the absence of NaCl, the interactions between S100b and p53 can occur in the absence of Ca^{2+} but are strengthened when Ca^{2+} is present. S100b induced partial disassembly of p53 oligomers in the presence of 0.1 mM EGTA, as indicated by a decrease in light scattering of the protein solution (Fig. 6*B*) and a reduced sedimentation of p53 oligomers (Fig. 6*C*).

DISCUSSION

The characterization of p53 as a PKC substrate that also binds to S100b lends support to our observation that the C-terminal basic domain of p53 is similar in certain respects to the PKC- and CaM/S100b-binding domain on the MARCK protein (Fig. 7).

Phosphorylation of p53 by PKC has been demonstrated *in situ* and *in vitro*. The limited stimulation of p53 phosphorylation in MELCs by PMA suggests that *in situ* phosphorylation of p53 will not only depend on the activation of the enzyme but also on the accessibility of p53 to the enzyme that could be regulated by intracellular factors and/or by its appropriate cellular localization as observed for other PKC substrates (22, 23). Although we have not yet been able to determine the PKC phosphorylation site on p53, preliminary studies have shown that V8 proteolysis of the phosphorylated p53 generated a single phosphopeptide whose sequence began at residue 350, thus including the predicted PKC phosphorylation site. Further work should be done on the biological meaning of the PKC phosphorylation of p53 in a region of the protein that is suspected to sustain several functional properties such as oligomerization (9), nuclear transport (24), and DNA binding (8).

The observed binding of cellular and recombinant p53 to S100b-Sepharose in the presence of calcium, and to a lesser extent to CaM-Sepharose, further supports the idea that the basic C-terminal domain on p53 and the PKC domain on MARCKS are functionally related. Indeed, as was shown for MARCKS (13), it is probably this domain on p53 that also serves in binding S100b. The S100b-mediated inhibition of both p53 phosphorylation and oligomerization can, therefore, be explained through the same basic process—i.e., the binding of S100b to the C-terminal basic amphiphilic amino acid sequence on p53, blocking the accessibility of the phosphorylation site for the activated PKC, and preventing interactions between p53 monomer (9).

The observed interaction between p53 and S100b might also provide a clue for understanding, at the molecular level, the relationship of Ca^{2+}, cell-cycle-associated calcium-binding proteins of the S100 family, and the G_0–G_1/S transition (for reviews, see refs. 25 and 26).

S100b synthesis has been correlated with the arrest of cell proliferation and cell differentiation (27) and the protein has been found maximally expressed in the G_1 phase of the cell cycle (28), suggesting that it might contribute to the Ca^{2+} dependency of the G_1 phase. S100b has been shown to interact with most of the PKC substrates so far identified in brain tissues (12, 16, 29) and with p36 (calpactin I) (30), a major substrate for the oncogene tyrosine kinase $pp60^{src}$ that is part of the protein complex associated with DNA polymerase α (31). It has therefore been suggested that the various demonstrated effects of the S100b proteins in cell cycle progression and cell-type differentiation may, in fact, be mediated by the same basic processes—i.e., interaction with protein kinase substrates and modulation of target protein kinase substrate phosphorylation (26, 29). The observation that S100b interacts with p53 in a calcium-dependent manner and that such an interaction inhibits p53 phosphorylation by PKC further supports this hypothesis.

It would be worthwhile to study the possible association of S100b or of the other members of the S100 protein family with p53 in living cells and to investigate whether these interactions function in regulating p53 oligomerization in the cytoplasm and/or in controlling its nuclear transport (4). In this respect it is significant to note that the putative S100-binding domain on p53 overlaps with two nuclear localization signals that mediate the migration of p53 to the cell nucleus (24).

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