Protein phosphatase 1 interacts with p53BP2, a protein which binds to the tumour suppressor p53

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Abstract The p53 binding protein, termed p53BP2, was identified as a protein interacting with protein phosphatase 1 (PP1) in the yeast two hybrid system. The interaction was confirmed by co-immunoprecipitation of p53BP2 with epitope-tagged PP1 in vitro. The p53BP2-PP1 complex was stable to NaCl at concentrations which dissociate the p53-p53BP2 complex, and the binding of PP1 and p53 to p53BP2 was mutually exclusive. The region required for interaction with PP1 was shown to be contained within amino acids 297–431 of p53BP2 , which includes two ankyrin repeats. The phosphorylase phosphatase activity of PP1 was inhibited by p53BP2 at nanomolar concentrations. These results suggest that PP1 may be involved in dephosphorylation and regulation of p53 through interaction with p53BP2.

Key words: p53; Protein phosphatase; Two-hybrid system; Human

1. Introduction

The p53 protein acts as a tumour suppressor, mutations in the gene encoding it being the most common genetic alteration in human cancer [1,2]. At the molecular level, p53 functions as a transcription factor which binds to DNA in a sequence-specific manner and can be activated by DNA damaging agents and oxidative stress. A number of genes have binding sites for p53 and the mRNA levels of certain genes, such as $p21^{WAF1}$ [3,4] have been shown to increase in a p53 dependent manner, while transcription of other genes is inhibited. Increases in the level of p53 lead to growth arrest, suggesting that the protein acts as a checkpoint control in the cell cycle regulating entry from G1 phase into S phase. $p21^{WAF1}$, which has been implicated in the inhibition of chromosomal DNA replication, cdk2 cyclin-dependent kinases and cyclin D1 are likely to be involved in this process [5,6]. There is evidence that p53 is regulated by phosphorylation. Mutation of a phosphorylation site in the amino-terminal region reduces the ability of p53 to inhibit cell cycle progression [7], while mutation of the penultimate residue to the carboxy-terminus, which is phosphorylated by casein kinase II, inactivates the growth suppressor activity of p53 in rodent cell lines [8]. Protein kinase C and casein kinase II can activate the latent sequence-specific DNA binding function of p53 in vitro [9].

Protein phosphatase 1 (PP1) interacts with a variety of regulatory subunits which may target it to specific locations in the cell, modulate its substrate specificity and allow its activity to be regulated by extracellular signals. These include the glycogen binding subunits in skeletal muscle [10] and liver [11] which mediate the regulation of PP1 by the hormones insulin and adrenalin, the myosin binding subunits of skeletal [12] and smooth muscle [13], the cytosolic inhibitor proteins inhibitor-1, inhibitor-2 and DARPP-32 [14], and the nuclear proteins sds22 [15] and NIPP-1 [16]. Ribosomal protein L5 has also recently been identified as a molecule that interacts with PP1 [17]. In an attempt to identify important novel regulatory subunits of PP1, we carried out a yeast two hybrid screen using the catalytic subunit of $PP1\gamma_1$. This identified several proteins, including human inhibitor-2 reported recently [18] and p53BP2 which we describe here. p53BP2 is one of two p53 binding proteins (designated p53BP1 and p53BP2), which were identified using expressed p53 in the yeast two hybrid system and shown to bind to wild type but not mutant p53 in vitro [19]. In this communication we show that p53BP2 forms a near stoichiometric complex with PP1 in vitro and modulates its activity towards certain substrates.

2. Materials and methods

2.1. General methods and the yeast two hybrid analysis

Microbial strains, growth conditions and methods for the yeast two hybrid screen were as previously described [18]. Oligonucleotides were synthesized by Miss Audrey Gough (University of Dundee) on an Applied Biosystems model 394 DNA synthesizer. DNA sequencing of expression constructs was performed on an Applied Biosystems 373A automated DNA sequencer using Taq dye terminator cycle sequencing.

2.2. Expression of GST-p53BP2 fusion proteins

*Eco*RI fragments from plasmids pACT-H1 and pACT-H8 containing p53BP2 coding sequence were ligated into the *Eco*RI site of plasmid pGEX-3X [20] to generate plasmids pGEX-H1 and pGEX-H8 containing all the p53BP2 coding sequence present in the pACT plasmids. After sequencing the constructs, they were transformed into *E. coli* BL21 (DE3) pLysS cells. Single colonies were grown to an A_{595} of 0.5 in LB medium (500 ml) containing ampicillin (100 μg/ml) and chloramphenicol (25 μg/ml) at 37°C. IPTG was added to a final concentration of 30 μM and growth continued overnight at 28°C. Cells were harvested by centrifugation at 5000 × *g* for 10 min at 4°C, then resuspended in 10 ml of buffer A (50 mM Tris-HCl pH 7.5, 1 mM DTT, 1 mM EDTA, 0.1 mM EGTA, 100 mM NaCl, 1 mM benzamidine, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 0.1 mM tosylphenylalanylchloromethylketone, 1% (v/v) Triton X-100) and sonicated for 4 × 10 s on ice using a Jencons probe sonicator (model GE 50). The lysate was centrifuged at 48,000 × *g* for 20 min at 4°C, then the supernatant decanted and added to 1 ml of GSH-agarose (Sigma) pre-equilibrated in the same buffer. After mixing for 1 h at 4°C, the suspension was added to a Poly-Prep column (Bio-Rad) and washed with the above buffer until no further protein eluted. The column was then washed with 5 ml of buffer A minus Triton X-100 and bound material eluted in buffer A containing 20 mM reduced glutathione (Sigma). Eluted protein was concentrated by centrifugation through a centricon 30 membrane (Amicon) and stored frozen at −70°C.

The pGEX-H1Δ deletion construct was generated by excising a *Bam*HI–*Pst*I fragment from the pGEX-H1 plasmid and cloning it into

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the *Bam*HI–*Pst*I sites of plasmid pBluescript KS$^+$. This construct was then digested with *Bam*HI and the overhanging 5′ ends filled-in with dNTPs using the Klenow fragment of DNA polymerase, prior to digestion with *Eco*RI. To produce an in-frame fusion, the resulting fragment was cloned into the *Bam*HI (filled-in)–*Eco*RI sites of plasmid pGEX-3X generating plasmid pGEX-H1Δ. Sequencing of this plasmid confirmed that it contained cDNA sequence coding for 193 amino acids of p53BP2, starting at the same position within the cDNA as pGEX-H1, and did not encode the 98 amino acids normally found at the C-terminus of the protein. Expression and purification of the truncated protein was performed as for pGEX-H1 and pGEX-H8, except that IPTG was added to a final concentration of 0.1 mM and expression was at 37°C for 3 h. These conditions were used to minimise degradation of the fusion protein which occurred on overnight incubation at lower temperatures.

2.3. Construction and purification of epitope-tagged PP1γ_1

In order to add the epitope EFMPME(H)$_6$ to the C-terminus of human PP1γ_1, the complementary oligonucleotides 5′-GATCACAA-AGCAAGCAAAGAAAGAATTTATGCCGATGGAACACCAC-CACCACCACCACTAGA-3′ and 5′-AGCTTCTAGTGGTGGTG-GTGGTGGTGTTCCATCGGCATAAATTCTTTCTTTGCTTGCT-TTGT-3′ incorporating a *Bcl*I site (underlined) and a *Hin*dIII site (double underlined) were synthesized. They were phosphorylated with ATP at the 5′ ends using T4 kinase, hybridised by heating in equimolar amounts in 50 mM Tris-HCl pH 8.0 to 95°C, allowed to cool to 60°C, maintained at 60–65°C for 2 h and then allowed to cool to room temperature. PP1γ_1 cDNA in pBS vector [21,22] was cleaved with *Bcl*I and *Hin*dIII. The fragment containing the vector and the region coding for amino acids 1–317 of PP1γ_1 was purified by gel electrophoresis, eluted from the gel using a Spin-X column and then ligated to the hybridised oligonucleotides. The ligation mixture was used to transform *E. coli* JM109 and DNA from several transformants was examined after gel electrophoresis and Southern blotting for hybridisation to the [γ-^{32}P]ATP-labelled oligonucleotides. Positive transformants were selected and sequenced. A *Nde*I–*Hin*dIII fragment encoding PP1γ_1-EFMPME(H)$_6$ was cleaved from one transformant with the correct sequence and subcloned into the expression vector pCW [23] using the same restriction sites. The pCW-PP1γ_1-EFMPME(H)$_6$ construct was transformed into *E. coli* BL21(DE3) and expressed as described for PP1γ_1 in [22]. Purification of the His-tagged protein was by metal affinity chromotography. The *E. coli* extract containing the pCW- PP1γ_1-EFMPME(H)$_6$ protein was made 1 mM in imidazole-HCl and 0.5 M in NaCl and applied to a nickel-nitrilotriacetate-agarose column equilibrated in 50 mM Tris-HCl pH 7.5, 1 mM PMSF, 10 mM imidazole-HCl and 0.5 M NaCl. After washing in the same buffer until the absorbance of the eluate at 280 nm was <0.01, the column was washed with 50 mM Tris-HCl pH 7.5 containing 30 mM imidazole-HCl, 0.5 M NaCl, 0.03% (v/v) Brij 35 and PP1γ_1-EFMPME(H)$_6$ was eluted with 50 mM Tris-HCl pH 7.5 containing 300 mM imidazole-HCl, 0.15 M NaCl, 0.03% (v/v) Brij 35, 2 mM $MnCl_2$ and 5% (v/v) glycerol. Fractions were analysed by SDS-PAGE gel electrophoresis and those containing virtually pure protein were pooled and dialysed at 0°C against 25 mM Triethanolamine-HCl pH7.5, 1 mM $MnCl_2$, 0.1 mM EGTA, 0.2 M NaCl, 0.03% (w/v) Brij 35, 0.1% (v/v) 2-mercaptoethanol and 50% (v/v) glycerol and stored at −20°C.

2.4. Preparations of antibodies to the epitope EFMPME

Hybridoma cells expressing monoclonal antibodies against a polyoma medium T epitope, which recognise the sequence EFMPME, were a kind gift from Professor Gernot Walter, University of California, San Diego, USA [24]. The protein in the supernatant from the hybridoma cell culture was precipitated in 50% ammonium sulphate and the precipitate redissolved in phosphate buffered saline to one tenth of the original volume and dialysed against the same buffer. The partially purified monoclonal antibodies to the EFMPME epitope were covalently attached to protein G-Sepharose (Pharmacia) using dimethylpimelimidate (Sigma) [25] and the resulting matrix stored at 4°C.

2.5. Expression and purification of wild-type human p53 in E. coli

Full length wild-type human p53 expressed in *E. coli* was purified as in [26] with the exception that gel filtration was not performed. 0.5 mg of approximately 20% pure p53 was isolated from 2 L of bacterial culture.

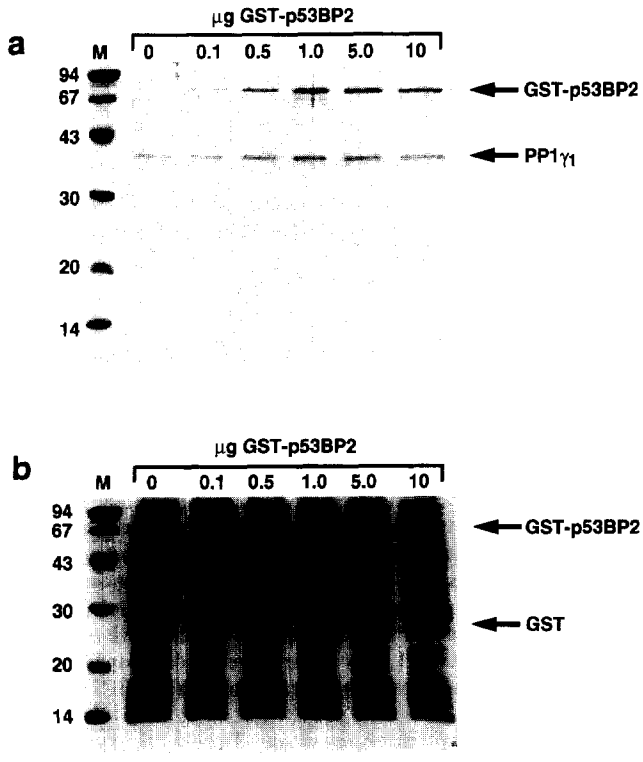

Fig. 1. Immunoprecipitation of p53BP2 from *E. coli* extracts with epitope-tagged PP1γ_1. 100 μl aliquots (0.3 mg protein) of *E. coli* extracts expressing GST at approximately 150 μg/ml were prepared. Varying amounts of pure GST-p53BP2 (expressed from the GST-H1 clone) and 2 μg of epitope-tagged PP1γ_1 were added and immunoprecipitations performed as described in section 2. Pellet and supernatant (10 μl aliquot) fractions were separated on a 12.5% SDS-PAGE gel and visualised by staining with Coomassie blue. Panel (a) shows the immunoprecipitated proteins and panel (b) shows the supernatants. Numbers above lanes indicate the amount (in μg) of GST-p53BP2 added to the extracts. Markers (M) are in kDa. Arrows in (a) indicate the immunoprecipitated GST-p53BP2 and PP1γ_1 and in (b) the GST-p53BP2 added to, and the GST expressed in, the *E. coli* extracts.

2.6. Immunoprecipitation and glutathione-affinity methods

Immunoprecipitation experiments were performed in 50 or 100 μl of 50 mM Tris-HCl pH7.5, containing 150 mM NaCl, 0.1% (v/v) 2-mercaptoethanol, 0.02% (v/v) Brij 35 and 0.1 mg/ml bovine serum albumin (BSA). Indicated amounts of interacting proteins were incubated in this buffer for 1 h at 4°C, then 10 μl of anti-EFMPME-Sepharose added and incubation at 4°C was continued with shaking for 1 h. After centrifugation, the supernatant was removed and the pellet was washed twice with 1 ml of buffer (lacking BSA), then denatured by heating in SDS-PAGE loading buffer at 95°C for 5 min. Glutathione-affinity precipitations were performed in an identical manner except that anti-EFMPME-Sepharose was replaced by glutathione-agarose (Sigma).

2.7. Protein phosphatase assays

^{32}P-labelled rabbit skeletal muscle glycogen phosphorylase was prepared as described in [27]. Chicken gizzard smooth muscle myosin P-light chains expressed in bacteria were a gift from Dr J. Kendrick-Jones (MRC Laboratory of Molecular Biology, Cambridge) and phosphorylated by Miss Deborah Johnson (University of Dundee) using myosin light chain kinase kindly provided by Dr M. Ikebe (Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, USA). The specific activity of the [γ-^{32}P]ATP used for all phosphorylations was 10^6 cpm/nmol. The native catalytic subunit of PP1 (PP1c) was purified from rabbit skeletal muscle by Dr G. Moorhead (University of Dundee). Protein phosphatase assays were performed in the absence of divalent cations [27] using the above substrates. One unit of phosphatase activity is that amount of enzyme

which catalyses release of 1 μmol [^{32}P]phosphate/min from ^{32}P-labelled substrate in the standard assay.

3. Results

3.1. Isolation of human p53BP2 cDNA using expressed human PP1γ_1 in the yeast two hybrid system

Proteins capable of interacting with PP1γ_1 were identified in yeast cells containing a pASPP1γ_1 construct in which PP1γ_1 was fused to the DNA binding domain of GAL4 [18]. These cells were transformed with DNA from a pACT library containing human peripheral lymphocyte cDNA sequences [28] fused to the GAL4 transactivation domain. After growth on the selection medium for twelve days, ten colonies were obtained, representing 0.005% of the cells transformed [18]. Of the colonies which were taken through another round of selection on medium containing 3-aminotriazole and also shown to possess β-galactosidase activity, two contained overlapping sequences coding for human p53BP2 [19]. One, termed PP1H1, encoded amino acids 239–529 and the second, termed PP1H8, amino acids 297–529. Both clones also possessed the entire 3' untranslated region of p53BP2 identical to that reported in [19].

3.2. Co-immunoprecipitation of human p53BP2 and human PP1

Since the signal in the two hybrid assay could have been mediated by artifactual interactions, we sought to confirm the binding of p53BP2 to PP1 by immunoprecipitation studies. For these experiments the coding region from the longest clone, PP1H1, was subcloned into pGEX-3X to produce a fusion protein of glutathione-S-transferase (GST) linked to p53BP2. To test the specificity of the interaction, varying amounts of the purified fusion protein were added to crude extracts of *E. coli* containing approximately 0.15 mg/ml GST, followed by addition of epitope-tagged PP1γ_1. Immunoprecipitation of the complexes with antibodies to the EFMPME epitope (coupled to protein G-Sepharose beads) is shown in Fig. 1. The data demonstrate that the interaction of p53BP2 and PP1 is extremely specific, in that p53BP2 is the only protein immunoprecipitated by the PP1-antibody complex from crude extracts containing as little as 100 ng of GST-p53BP2. No GST (26 kDa), which was present in a large (3–300 fold) excess over p53BP2, was observed in the immunoprecipitates. The approximately equal intensity of p53BP2 and PP1 in the immunoprecipitates indicates that the interaction is near stoichiometric. In order to investigate the stability of the p53BP2-PP1 complex, immunoprecipitations were performed at varying salt concentrations. Fig. 2 shows that the interaction of p53BP2 with PP1γ_1 is stable in concentrations of NaCl up to and including 1 M. In contrast, the complex of p53 with p53BP2 was present at 250 mM NaCl, but disrupted in concentrations of 0.5 M NaCl or higher (Fig. 2). No binding of p53 to PP1 was seen at any NaCl concentration used.

3.3. Identification of the site of interaction of p53BP2 with PP1

The fragment of p53BP2 comprising amino acids 297–529 (encoded by clone PP1H8) binds equally well to PP1 as the fragment of p53BP2 comprising amino acids 239–529 (encoded by clone PP1H1) (Fig. 3b), indicating that amino acids 239–297 are not required for the interaction. The region of p53BP2 encoded by both these clones contains two ankyrin repeats and a SH3 domain (Fig. 3a). In order to determine which of these

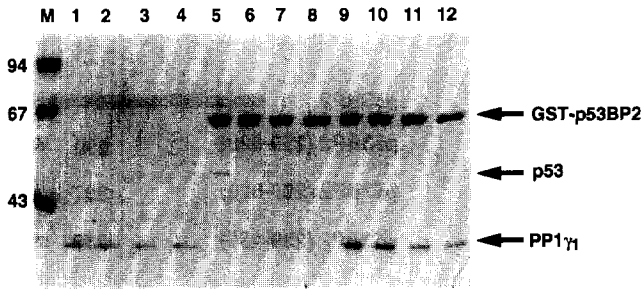

Fig. 2. Stability of the p53BP2-PP1 complex to NaCl. 50 μl reaction volumes containing 1μg of each test protein and the stated amount of NaCl were precipitated as described in section 2 using either anti-EFMPME antibodies (PP1γ_1 + p53) or glutathione-agarose (p53 + p53BP2 and PP1γ_1 + p53BP2). Wash buffers contained the same concentrations of NaCl as the incubation buffers. Pellet fractions were separated on a 10% SDS-PAGE gel and visualised by staining with Coomassie blue. Lanes are: (1–4), PP1γ_1 + p53; (5–8), p53 + p53BP2; (9–12), PP1γ_1 + p53BP2. NaCl concentrations were 250 mM in lanes (1,5,9), 500 mM in lanes (2,6,10), 750 mM in lanes (3,7,11) and 1 M in lanes (4,8,12). Markers (M) are in kDa. Arrows indicate the immunoprecipitated GST-p53BP2, p53 and PP1γ_1.

regions was necessary for binding to PP1, a construct, PP1H1Δ, was generated in which DNA encoding the SH3 domain was deleted from the PP1H1 clone (Fig. 3a). The resulting fragment of p53BP2 comprising amino acids 239–431 could also be co-immunoprecipitated with PP1 (Fig. 3b), indicating that the SH3 domain is not essential for binding. Interestingly, both p53BP2(239–529) and p53BP2(239–431) migrate anomonously on SDS-PAGE, with apparent molecular masses larger than predicted from their amino acid sequences. The proline-rich amino-terminal region of these fragments may underlie this effect because p53BP2(297–529), which does not contain this region, migrates on SDS-PAGE with an apparent molecular mass consistent with its predicted size.

3.4. p53BP2 modulates the specificity of PP1

Several regulatory subunits of PP1 are known to modulate its activity [10]. The effect of p53BP2 on the activity of the native PP1 catalytic subunit (PP1c) purified from rabbit skeletal muscle was therefore examined. Fig. 4a shows that nanomolar concentration of p53BP2 (239–529) virtually eliminate PP1 activity towards the standard substrate normally used to assay PP1, glycogen phosphorylase. This inhibition occurs in the same concentration range as other modulators of PP1 activity, such as inhibitor-2. In contrast, p53BP2 has virtually no effect on (or slightly enhances) the activity of PP1 towards myosin P-light chains, demonstrating that p53BP2 is not a non-specific inhibitor of PP1. There was no effect of p53BP2 at 1 μM on the phosphorylase phosphatase and myosin light chain phosphatase activities of PP2A (data not shown).

3.5. p53 and PP1 binding to p53BP2 is mutually exclusive

In order to investigate whether p53, p53BP2 (239–529) and PP1 can form a ternary complex, we added increasing concentrations of PP1 to a complex of p53 and p53BP2. As shown in Fig. 5, this resulted in loss of p53 binding to p53BP2. However, addition of p53 caused little dissociation of the p53BP2-PP1γ_1 complex over the concentration range used. This result is consistent with the relative stabilities of the two complexes to high concentrations of NaCl (Fig. 2).

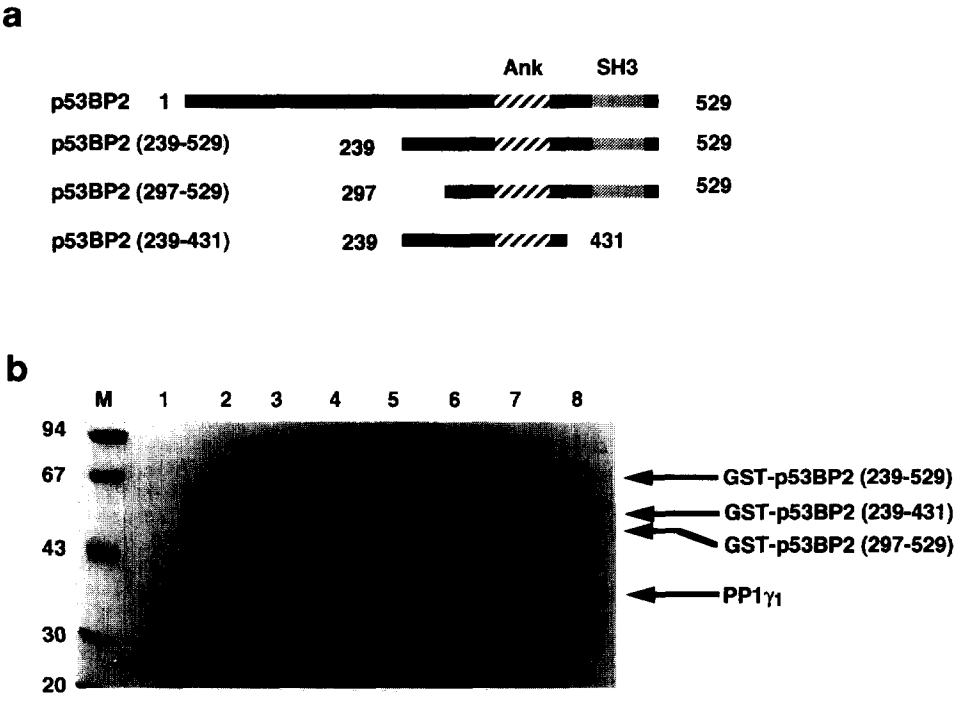

Fig. 3. Identification of the region of p53BP2 which binds to PP1. (a) Schematic diagram of the region of p53BP2 which interacts with PP1γ_1. p53BP2 fragments identified in the two hybrid screen (from clones PP1H1 and PP1H8) and p53BP2 truncated at the C-terminus (expressed from clone PP1H1Δ) are aligned with the longest known p53BP2 sequence. Numbers indicate amino acid positions with respect to the original p53BP2 sequence. Positions of the ankyrin repeats and SH3 domain are indicated. (b) Immunoprecipitation of GST-p53BP2 fusion proteins with epitope-tagged PP1γ_1. 50 μl reactions containing 2 μg of GST-p53BP2 fusion proteins plus or minus 2 μg of PP1γ_1 were precipitated as described in materials and methods using anti-EFMPME antibodies. Pellet fractions were separated on a 10% SDS-PAGE gel and visualised by staining with Coomassie blue. Lanes are: (1,5) GST; (2,6) GST-p53BP2 (239–529); (3,7) GST-p53BP2 (297–529); (4,8) GST-p53BP2 (239–431). Lanes (5–8) contained epitope-tagged PP1γ_1. Markers (M) are in kDa. Arrows indicate the immunoprecipitated GST-p53BP2 fragments and PP1γ_1.

4. Discussion

We have identified the p53 binding protein p53BP2 as a protein that interacts with PP1 in the yeast two hybrid system. The finding that two different clones both encoding p53BP2 were among the ten positive clones isolated using the two hybrid screen strengthened the likelihood that the interaction of p53BP2 with PP1 is not an artifact. The specificity of the inter-

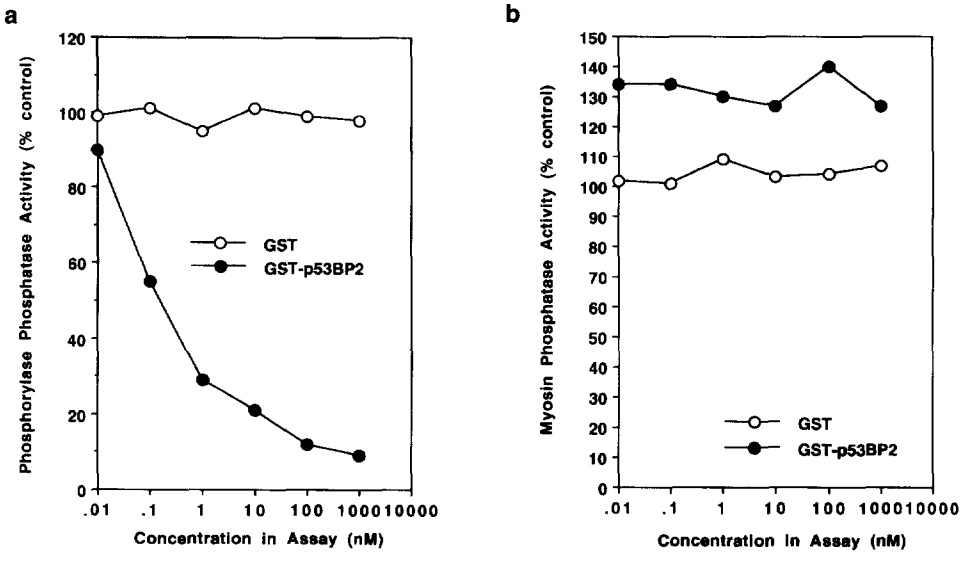

Fig. 4. Effect of p53BP2 on the phosphatase activity of PP1 catalytic subunit. Assays were performed with the catalytic subunit of PP1 (PP1c) at 0.16 mU/ml and included GST or GST-p53BP2 (239–529) in the concentration range (0–1 μM). Protein phosphatase activity is plotted as a percentage of the activity observed when no GST or GST-p53BP2 (239–529) is present. Panels show dephosphorylation of ^{32}P-labelled substrates, (a) phosphorylase and (b) myosin P-light chain.

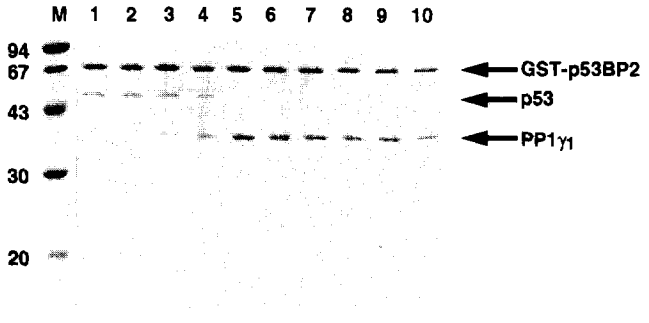

Fig. 5. Binding of PP1 and p53 to p53BP2 is mutually exclusive. 50 μl reactions containing 1 μg of GST-p53BP2 (239–529), 2 μg of either PP1γ_1 or p53 and varying amounts of competing protein were precipitated using glutathione agarose. Pellet fractions were separated on a 12.5% SDS-PAGE gel and visualised by staining with Coomassie blue. Lanes 1–5 contain GST-p53BP2 and p53 plus varying amounts of PP1γ_1, (lane 1) 0 μg, (lane 2) 0.1 μg, (lane 3) 0.5 μg, (lane 4) 1 μg and (lane 5) 5 μg; lanes 6–10 contain GST-p53BP2 and PP1γ_1 plus varying amounts of p53, (lane 1) 0 μg, (lane 2) 0.1 μg, (lane 3) 0.5 μg, (lane 4) 1 μg and (lane 5) 5 μg. Markers (M) are in kDa. Arrows indicate the immunoprecipitated GST-p53BP2, p53 and PP1γ_1.

action was confirmed by near stoichiometric co-precipitation of bacterially expressed p53BP2 and PP1 from low levels (<100 ng of p53BP2) in crude *E. coli* extracts. p53BP2 appears to bind more strongly to PP1 than to p53, since the p53BP2-PP1 complex was stable to 1M NaCl while the complex of p53BP2 and p53 was disrupted in 0.5M NaCl. Our data also show that PP1 and p53 cannot bind simultaneously to p53BP2, since no evidence for formation of a ternary complex was obtained. This suggests that p53BP2 is more likely to exist in a complex with PP1 in the cell than with p53. p53BP2 has been shown to interact with the central sequence-specific DNA binding domain of p53 and particular amino acid residues in p53 have been shown to be crucial to this interaction [19, 29]. It is therefore possible that PP1 binds to the region of p53BP2 that interacts with this domain of p53 or, alternatively, PP1 might bind elsewhere and alter the conformation of p53BP2 such that it cannot bind to p53.

The region of p53BP2 which binds to both p53 [19] and PP1 contains two ankyrin repeats and an SH3 domain. Deletion of the SH3 domain did not affect the interaction of p53BP2 with PP1, implicating the section (amino acids 297–431) containing the ankyrin repeats as being essential for PP1 binding. It is of interest that smooth muscle M_{110} myosin binding subunit that interacts with PP1 contains seven ankyrin repeats [13].

The strongest evidence that formation of a complex of p53BP2 and PP1 might be functionally important in vivo comes from the effect of p53BP2 on PP1 activity. The interaction of p53BP2 with PP1 almost abolished its activity towards glycogen phosphorylase, but intriguingly, does not abolish its activity towards myosin P-light chains. This indicates that p53BP2 is likely to have a selective effect on the substrate specificity of PP1, as has been noted for other regulatory subunits of PP1. For example, the M_{110} subunit decreases the phosphorylase phosphatase activity of PP1 and enhances its myosin P-light chain phosphatase activity [22]. In contrast, the cytosolic protein, inhibitor-2, inhibits both the phosphorylase phosphatase and myosin P-light chain phosphatase activity of PP1, but has little effect on the dephosphorylation of histone H1 [22]. Thus p53BP2 affects the specificity of PP1 in a different manner from other known regulatory subunits.

There is considerable evidence that phosphorylation plays a role in the regulation of p53. The p53 protein is phosphorylated by a large number of kinases in vitro, including protein kinase C and casein kinase II which activate the sequence specific binding of p53 to DNA [9]. However, the nature of the phosphatase responsible for the dephosphorylation of these sites is unclear. It is possible that, just as other regulatory subunits of PP1 enhance its activity towards certain substrates, the function of p53BP2 is to enhance PP1 dephosphorylation of p53 in the nucleus and inhibit PP1 activity against other substrates. The failure of p53, a fragment of p53BP2 and PP1 to form a ternary complex in the present work might argue against the idea that p53BP2 targets PP1 to p53. However, the bacterially expressed p53 used in these studies is not phosphorylated. It is also possible that covalent modification of p53BP2 may lead to formation of ternary complexes or that native full-length p53BP2 might interact with p53 in such a way that a ternary complex can be formed in vivo. However, many other alternatives still need to be explored. For example, SH3 domains play prominent roles in a number of cellular signalling processes, raising the possibility that the SH3 domain of p53BP2 may couple PP1 dephosphorylation of p53 to cell signalling events. Other possibilities that need to be considered are whether p53BP2 targets PP1-p53 to particular nuclear locations, or whether PP1 plays a role in regulating the function of p53BP2 itself.

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