THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 18, Issue of May 4, pp. 15164–15173, 2001

Printed in U.S.A.

Physical Interaction with Yes-associated Protein Enhances p73 Transcriptional Activity*

Received for publication, November 20, 2000, and in revised form, January 16, 2001
Published, JBC Papers in Press, January 24, 2001, DOI 10.1074/jbc.M010484200

Sabrina Strano‡§¶, Eliana Munarriz‡§, Mario Rossi‖, Luisa Castagnoli‖, Yosef Shaul, Ada Sacchi‡, Moshe Oren‡‡, Marius Sudol§§, Gianni Cesareni‖, and Giovanni Blandino‡¶¶**

*From the ‡Molecular Oncogenesis Laboratory, Regina Elena Cancer Institute, Rome 00158, Italy, ‖Department of Biology, University of Tor Vergata, Rome 00133, Italy, the Departments of **Molecular Genetics and ‡‡Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel, and the §§Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029-6574*

Specific protein-protein interactions are involved in a large number of cellular processes and are mainly mediated by structurally and functionally defined domains. Here we report that the nuclear phosphoprotein p73 can engage in a physical association with the Yes-associated protein (YAP). This association occurs under physiological conditions as shown by reciprocal co-immunoprecipitation of complexes from lysates of P19 cells. The WW domain of YAP and the PPPPY motif of p73 are directly involved in the association. Furthermore, as required for ligands to group I WW domains, the terminal tyrosine (Y) of the PPPPY motif of p73 was shown to be essential for the association with YAP. Unlike p73α, p73β, and p63α, which bind to YAP, the endogenous as well as exogenously expressed wild-type p53 (wt-p53) and the p73γ isoform do not interact with YAP. Indeed, we documented that YAP interacts only with those members of the p53 family that have a well conserved PPXY motif, a target sequence for WW domains. Overexpression of YAP causes an increase of p73α transcriptional activity. Differential interaction of YAP with members of the p53 family may provide a molecular explanation for their functional divergence in signaling.

The p53 tumor suppressor gene is the most frequent target for genetic alterations in human cancer (1). The wild type (WT)[1] p53 protein is apparently latent under normal conditions but becomes activated in cells exposed to DNA damage as well as to other types of stress (2). As a consequence of such stimuli, the level of p53 protein increases suddenly and correlates a variety of antiproliferative effects, including cell cycle arrest, apoptosis, and differentiation (3–5). These biological effects are achieved mainly through the activation of a plethora of specific target genes. For instance, $p21^{waf1}$, a cyclin-dependent kinase inhibitor, is mainly responsible for p53-induced G_1 arrest (6, 7). Furthermore, several apoptotic proteins including Bax, are involved in the p53-induced apoptosis (8, 9). Thus, the transcriptional activity of p53 is crucial for p53-induced cell cycle arrest as well as programmed cell death.

Two p53 homologues, p73 and p63, that share a remarkable homology in DNA sequence as well as in protein structure have recently been identified (11–14). As expected for p53-like proteins, p73 and p63 are nuclear proteins that can bind to canonical p53 DNA binding sites and can activate transcription from p53-responsive promoters in transiently transfected cells. Furthermore, overproduction of p73 as well as p63 can induce apoptosis, growth arrest, and differentiation in p53+/+ and p53−/− tumor cells (10, 14, 15, 16). In contrast to p53, p73 and p63 are alternatively spliced, giving rise to a family of different isoforms whose individual physiological functions are still unknown (10, 14, 17–20).

Viral oncoproteins such as large T antigen, E6, and E1Bp55, which are known as inactivators of p53, are unable to promote the inactivation of p73 (21–24). Similarly, Mdm2, a key regulator of p53 stability does not induce degradation of p73α, although it binds to it and interferes with its transcriptional activity (25–29). Taken together, these results suggest that the mechanisms underlying p73α stability are distinct from those known to regulate p53 stability. Recently, a growing number of proteins have been identified as p73 binding partners (30, 31). It was originally reported that human tumor-derived p53 mutants (m-p53) bind to p73 and markedly decrease its ability to transactivate target genes as well as to induce apoptosis (32). It was subsequently reported that m-p53 can associate *in vivo* with all of the isoforms of p73 (33, 34). Different types of DNA damage cause different p73-mediated responses. Unlike UV irradiation, cisplatin as well as γ-radiation cause stabilization and tyrosine phosphorylation of p73 (35–37). These post-translational modifications of p73 occur through the physical interaction with kinase-active c-Abl and result in the enhancement of the apoptotic activity of p73 (35–37). The proline-rich region of p73α and p73β mediates the association through the interaction with the SH3 domain of c-Abl (36, 37).

In an attempt to identify new proteins interacting with p73, we looked at the ability of various SH3 as well as WW domains to bind to p73α in pull-down assays. Although structurally distinct, SH3 and WW domains are functionally related due to their ability to bind to proline-rich ligands. WW domains are small protein modules composed of 38–40 amino acids and characterized by two conserved tryptophan residues that are

* This work was supported by Telethon-Italy Grant 369/bi (to G. B.), Associazione Italiana per la Ricerca sul Cancro Telethon and the Consiglio Nazionale delle Ricerche target project in Biotechnology (to G. C.), and by Human Frontier Science Program Organization Grant RG0234 and National Institutes of Health Grants AR45626 and CA45757 (to M. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ These authors contributed equally to this work.

¶ Supported by European Community Grant QLG1-1999-00273.

¶¶ To whom correspondence should be addressed: Molecular Oncogenesis Laboratory, Regina Elena Cancer Institute, Via delle Messi d'Oro 156, Rome 00158, Italy. Tel.: 39-06-49852563; Fax: 39-06-49852505; E-mail: blandino@ifo.it.

[1] The abbreviations used are: WT, wild type; BES, *N*,*N*-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid; YAP, Yes-associated protein; GST, glutathione *S*-transferase; PCR, polymerase chain reaction; HA, hemagglutinin; mAb, monoclonal antibody; PAGE, polyacrylamide gel electrophoresis; IB, immunoblot.

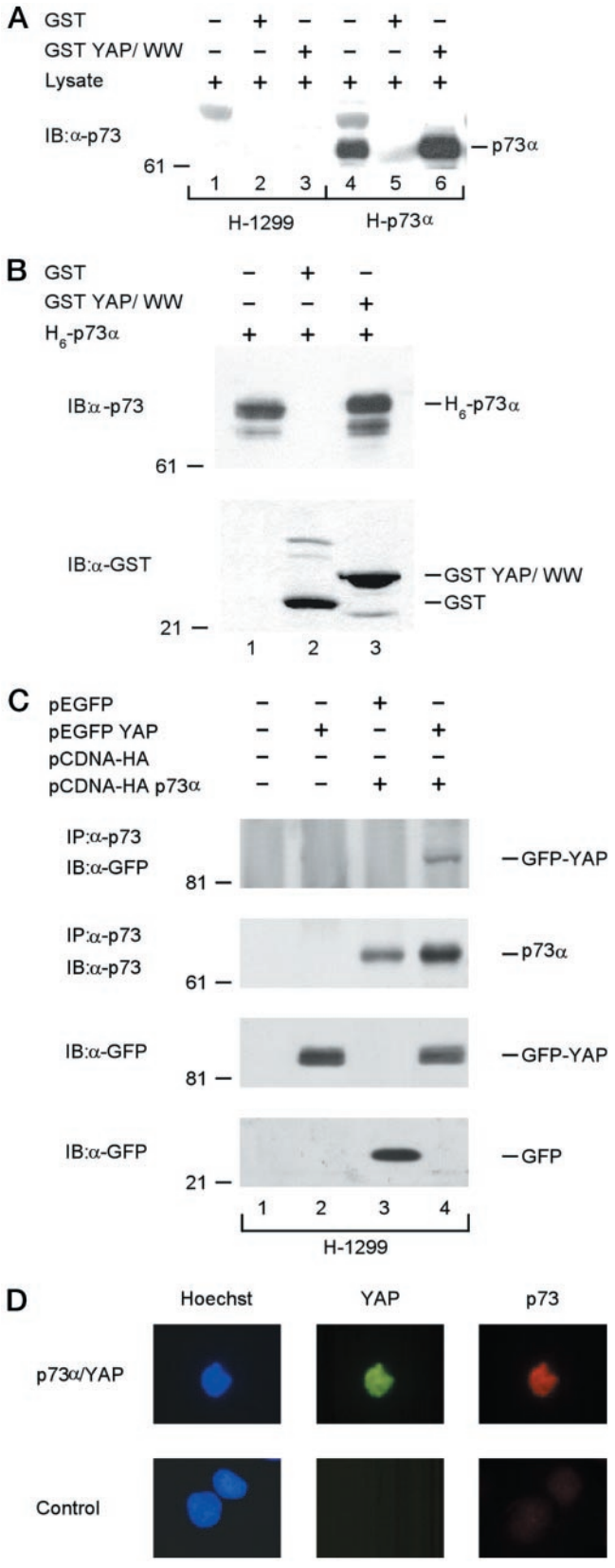


FIG. 1. **p73α associates *in vitro* and *in vivo* with YAP.** *A*, H1299 cells were transiently transfected either with a vector encoding p73α-HA or with an empty vector. Cell lysates (2 mg) were first precleared with glutathione-agarose beads and then incubated with the indicated GST fusion proteins for 2 h at 4 °C. Specifically bound p73α was detected by immunoblotting with anti-p73 polyclonal serum. *Lanes 1* and *4* contain aliquots of unprocessed lysates (100 μg/lane), loaded directly on the gel. *B*, bacterial purified H6-p73α protein was incubated either with GST-YAP/WW (*lane 3*) or with GST alone (*lane 2*) and transferred to nitrocellulose membrane. The blot was probed with anti-p73 polyclonal serum (*upper panel*). The same blot was reprobed with anti-GST polyclonal serum (*lower panel*). *C*, p73α as well as GFP-YAP or GFP alone were overexpressed in H1299 cells by transient transfection. Cell extracts were precleared with protein G-agarose and followed by immunoprecipitation (*IP*) with anti-p73 antibody. Immunoprecipitates were subjected to immunoblot with anti-GFP polyclonal serum (*upper panel*). The same blot was reprobed with anti-p73 polyclonal serum (*middle upper panel*). Aliquots of total cell extracts from unprocessed cells (100 μg/lane) were directly subjected to immunoblot analysis (*lower panels*). *D*, subcellular localization of GFP-YAP and p73α is shown. Cells were stained with Hoechst to visualize nuclei and anti-p73 polyclonal serum to visualize p73. Protein molecular sizes markers are indicated on the *left*.

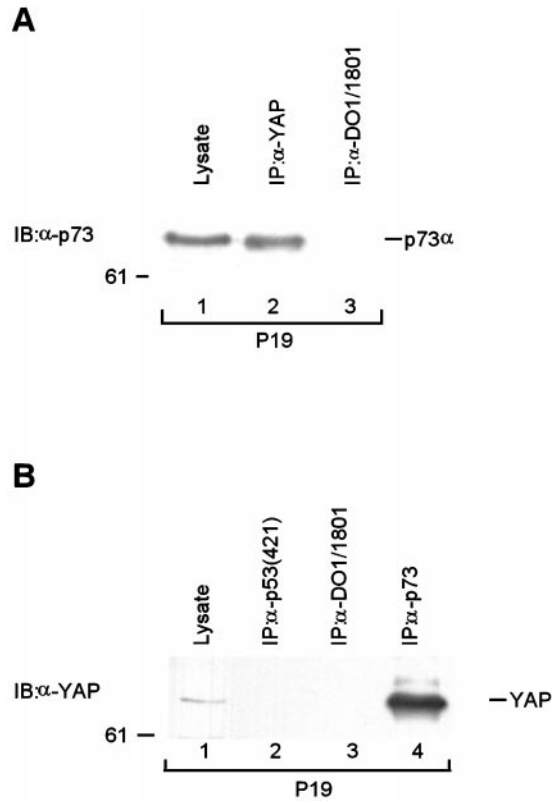


FIG. 2. **The association between p73 and YAP occurs under physiological conditions.** P19 mouse embryo-carcinoma cells were extracted and subjected to reciprocal co-immunoprecipitation as described under "Experimental Procedures." *A*, *lanes 2* and *3* represent immunoprecipitates (*IP*) corresponding to 5 mg of total cell protein performed with indicated antibodies. *Lane 1* contains an aliquot (100 μg/lane) of unprocessed extract of the above indicated cells applied directly on the gel. The blot was probed with anti-p73 polyclonal serum. *B*, an identical amount of cell lysate employed in *A* was immunoprecipitated with the indicated antibodies. An aliquot (50 μg/ml) of unprocessed extract was run in *lane 1*. The blot was probed with anti-YAP polyclonal serum.

20 residues apart (38–40). WW domains can be grouped into four classes according to their ligand binding preference (39, 40). Class I includes WW domains binding to the core sequence PP*X*Y (41–44). Class II WW domains prefer ligands containing a stretch of prolines interrupted by a leucine (45, 46). Class III includes WW domains interacting with proline-rich sequence that contains arginines or lysines (47–49). WW domains binding phosphoserine or phosphothreonine followed by a proline residue are grouped in class IV (50–52).

Here we report a novel interaction between p73 and YAP. YAP was originally identified as a protein binding to the SH3 domain of the Yes proto-oncogene product that belongs to the Src family of protein-tyrosine kinases. The interaction between p73 and YAP occurs *in vitro* as well as under physiological

conditions in the cell. It involves the WW domain of YAP and the PPPPY proline-rich region of p73α and p73β. In contrast to p73α and p73β and p63α, p53 does not associate with YAP. The binding with YAP enhances transcriptional activity of p73α.

EXPERIMENTAL PROCEDURES

Library Construction—Phage library construction was according to Santi *et al.* (53) with minor modification. The p73α monkey gene was amplified from plasmid pCDNA3-HA p73α (15) using a primer annealing to region encoding the first 6 amino acids of the protein (ATCGCGGATCCATGGCCCAGTCCACCACCAC), and a second primer annealing to the region encoding the last 6 amino acids (GACCGGATATCTCAGTGGATCTCGGCCTCCG). The PCR product was electrophoresed on an agarose gel (1%) and purified using a NucleoSpin column (Clontech). To generate first-strand cDNA copies, 1 μg of this purified fragment was mixed with an *Spe*I-tagged random primer (ACGCGGACTAGTN$_6$), 5 μl of dNTP (25 mM each) and 7 μl of Klenow buffer (New England Biolabs) in a final volume of 70 μl, boiled for 5 min, and immediately chilled on ice. Afterward, 10 units of Klenow (New England Biolabs) were added and incubated for 2 h at 37 °C. This mixture was purified through a Quiaquick column (Qiagen) and used to generate the second-strand cDNA with a *Not*I tagged random primer (TCGGCGGCCGCN$_6$) in the same way as described above. The resulting double-stranded inserts were amplified using *Spe*I (CTGCTGACGCGGACTAGT) and *Not*I primers (TGGATCTCGGCGGCCGC) in order to generate fragments in the three possible reading frames. Products were purified and digested with *Spe*I and *Not*I (Promega).

The λ display 3 vector was digested by *Spe*I and *Not*I, purified, and concentrated by isopropanol precipitation. The ligation of the vector (2 μg) and the inserts (15 ng) was performed by adding 2000 units of T4 DNA ligase (New England Biolabs) and incubating overnight at 16 °C. The ligation mixture was *in vitro* packaged by using a λ packaging kit, Gigapack (Stratagene), and plated by infection of BB4 cells onto 10 plates (15-cm diameter). Phage elution was achieved with the standard method (54).

The complexity of the libraries calculated as total independent clones obtained after plating was 1×10^5 plaque-forming units (the average length of the insert was 150 base pairs).

Affinity Selection—Affinity selection was performed using the glutathione *S*-transferase (GST)-YAP/WW bound to glutathione-agarose beads (Sigma). Approximately 10 μg of protein bound to resin were mixed with 400 μl of the p73α phage library (1×10^{10} plaque-forming units) with 1% bovine serum albumin and incubated overnight at 4 °C. The resin was washed five times with 1 ml of TBST and then once with 1 ml of λ buffer and resuspended in 100 μl of the same buffer. Selected phages were recovered by adding 200 μl of BB4 cells in 10 mM $MgSO_4$ at $A_{600} = 2$ and incubating for 20 min at 37 °C. Infected cells were collected and plated onto one plate (15 cm). After overnight incubation at 37 °C, plaque-forming phage were collected with the standard method (54). The phages that specifically bound to YAP/WW were evidenced with an immunoscreening assay performed as described by Zucconi *et al.*[2]

λ plaques that were found to be positive in a plaque-screening assay were isolated and eluted into 50 μl of λ buffer, and 1 μl of the suspension was PCR-amplified for sequencing of the DNA insert.

Cell Lines—The H1299 cell line is derived from a human large cell lung carcinoma. H1299 cells were maintained in RPMI medium, supplemented with 10% fetal calf serum (Life Technologies, Inc.). Before transfection, the culture medium was changed to Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. H1299 cells stably overexpressing inducible WT p53 were produced as previously reported (34, 55). Induction of WT p53 was achieved by the addition of ponasterone A (2.5 μM/ml). P19 mouse embryo-carcinoma cells were maintained in α-minimal essential medium (Life Technologies, Inc.) without ribonucleotides and deoxyribonucleotides supplemented with 7.5% newborn calf serum (Life Technologies, Inc.) and 2.5% fetal calf serum.

Plasmids and Transient Transfections—Overexpression of p73 was achieved by transfection of pcDNA3-HA-p73α (kindly provided by Dr. W. Kaelin) and pcDNA3-HA-p73γ (kindly provided by Dr. G Melino). pCDNA3-HA-p73α,Y^{99}-F, pCDNA3-HA-p73α,Y^{121}-F, pCDNA3-HA-3α, Y$^{99/121}$-F, pcDNA3-HA-p73α,P338A, and pcDNA3-HA-p73α,Y487P were obtained by site-directed PCR mutagenesis followed by subcloning into pCDNA3-HA vector. Sequences of the oligonucleotides and primers are available on request. Overexpression of GFP-YAP and GFP was

[2] A. Zucconi, G. Cesareni, manuscript in preparation.

TABLE I
Interactions between SH3 and WW domain-containing proteins and p73α.

The binding of the listed GST fusion proteins to p73α was tested as reported in Fig. 1*a*.

SH3 or WW domains	Binding to p73α
Nedd 1	−
Yap 1	+
Yap 2	+
Fe65	−
Abl	+
EPS8	−
P85	−
PLCγ	−
SRC	−

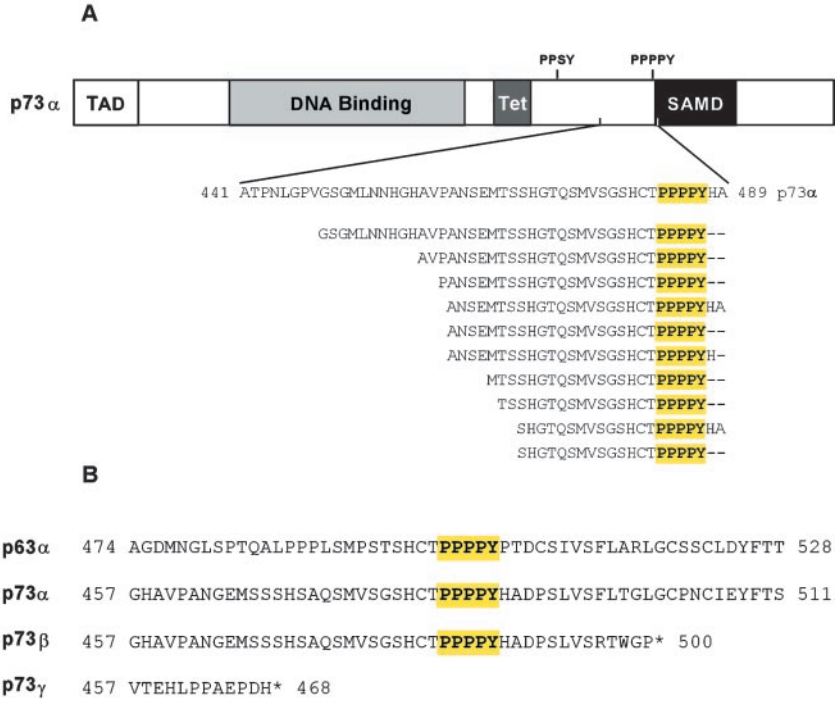


FIG. 3. **The canonical PPPPY motif of p73 is directly involved in the association with YAP.** *A*, the two ligand binding consensus sequences for YAP are depicted along the schematic representation of p73 modular structure. The clones selected by phage display analysis performed as reported under "Experimental Procedures," and the PPPPY motif of each clone is marked in *gray*. *B*, schematic representation of YAP ligand binding consensus on the sequences of p73α, p73β, p73γ, and p63α. The PPPPY motif of each sequence is marked in *gray*.

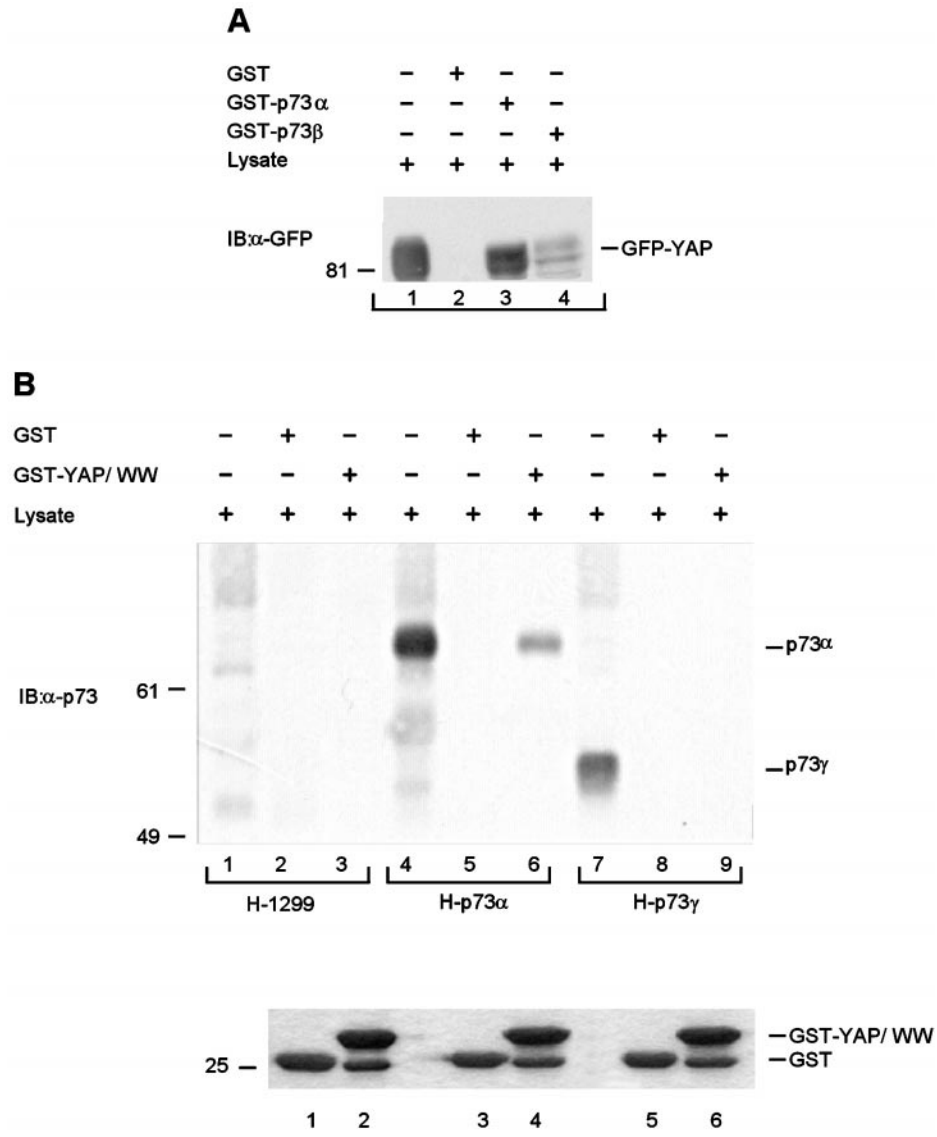


FIG. 4. **Ligand binding specificity governs the association between YAP and the p73*α*, p73*β*, and p73*γ* isoforms.** *A*, H1299 cells transiently transfected with GFP-YAP were extracted and, following a preclearing with glutathione-agarose beads, were incubated with GST-p73*α*, GST-p73*β*, or GST alone. The blot was probed with an anti-GFP polyclonal serum. *B*, cell lysates of H1299 cells transiently transfected with vectors encoding either p73*α* (*lanes 4–6*) or p73*γ* (*lanes 7–9*) or with an empty vector (*lanes 1–3*) were processed as reported above and incubated with the indicated GST fusion proteins. The blot was probed with anti-p73 polyclonal serum. Coomassie staining of replica gel showing the GST fusion proteins (*lower panel*).

achieved by transfection of the plasmids pEGFP-YAP and pEGFP-C2. The parental vectors pCMVneo or pEGFP were used to keep the amount of the transfected DNA constant among samples. Transient transfections were done by the calcium phosphate method in the presence of BES (Sigma). The precipitates were left for 12 h, after which the medium was changed again to RPMI plus 10% fetal calf serum. The cells were harvested 36 h posttransfection.

Coprecipitation and Western Blot Analysis—H1299 cells were transfected in 60-mm plates with 10 μg of total DNA and harvested at 36 h after the transfection. Cells were lysed in 900 μl of lysis buffer (50 mM Tris (pH 8), 100 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EDTA, 100 mM NaF, 1 mM $MgCl_2$, 2 mM phenylmethylsulfonyl fluoride, protease, and phosphatase inhibitors), and the extracts were sonicated for 10 s and centrifuged at 14,000 rpm for 10 min to remove cell debris. Protein concentrations were determined by a colorimetric assay (Bio-Rad) assay. After preclearing for 1 h at 4 °C, immunoprecipitations were performed by incubating 2 mg of whole-cell extract with 1.5 μg/sample of anti-p73 mAb (Ab4) (Neomarkers Inc.) with rocking at 4 °C for 1 h. Immunocomplexes were precipitated with protein G-agarose beads (KPL). The immunoprecipitates were washed three times with 1 ml of wash NET-gel buffer (50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.25% gelatin, 0.1% Nonidet P-40). The excess liquid was aspirated, and 40 μl of 5× sample buffer was added. Immunoprecipitates as well as 1% of each extract were resolved by SDS-9% PAGE. Protein gels were transferred to nitrocellulose membranes (Sartorius). For GFP-YAP detection, a polyclonal anti-GFP antibody (Invitrogen) was used at 1:5000 dilution; for p73 detection, an anti-p73 polyclonal serum was used at 1:3000. For Bax detection, a polyclonal anti-Bax antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used at 1:500 dilution.

P19 cells were lysed as previously described. Aliquots of cell extracts containing 5 mg of total proteins were immunoprecipitated with anti-YAP affinity-purified polyclonal IgG (56), with anti-p73 polyclonal serum, with anti-p53 mAb 421, or with a mixture of anti-p53 mAbs DO1 and 1801. For p73 detection, an anti-p73 polyclonal serum was used at 1:3000 dilution; for YAP detection, an anti-YAP polyclonal serum was used at 1:3000 dilution.

Western blot analysis was performed with the aid of the enhanced chemiluminescence Supersignal West Pico Stable Peroxidase Solution (Pierce).

Production of Recombinant Proteins—GST fusion proteins containing lengths p73*α*, p73*β*, p63*α*, and p53 were obtained by PCR amplification of the appropriate fragment from monkey p73, mouse p63, and mouse p53 followed by cloning in the pGEX-2KT expression vector in frame with the GST moiety. The p73*α* mutant Y487F was obtained by site-directed PCR mutagenesis followed by cloning in the pGEX-6P1 (Amersham Pharmacia Biotech). YAP/WW was obtained by PCR amplification followed by cloning in the pGEX-2KT. Sequences of the oligonucleotides and primers used are available on request. Purification of the GST fusion proteins by glutathione-agarose beads (Sigma) was performed following standard procedures (34). H6-p73*α* was obtained by PCR amplification followed by cloning in the pQE30 vector (Qiagen). Purification and elution of H6-p73*α* protein were performed following standard procedures.

Pull-down Assays—Pull-down assays were performed using 20 μg of immobilized purified GST fusion proteins or wild-type GST that was incubated with 2.5 mg of total cellular proteins prepared from H1299 cells transiently transfected with the following plasmids encoding p73*α*-HA, p73*α*-HA (Y99F), p73*α*-HA (Y121F), p73*α*-HA (Y99F/Y121F), p73*α*-HA (P338A), p73*γ*-HA, GFP-YAP, or GFP. The lysates were first

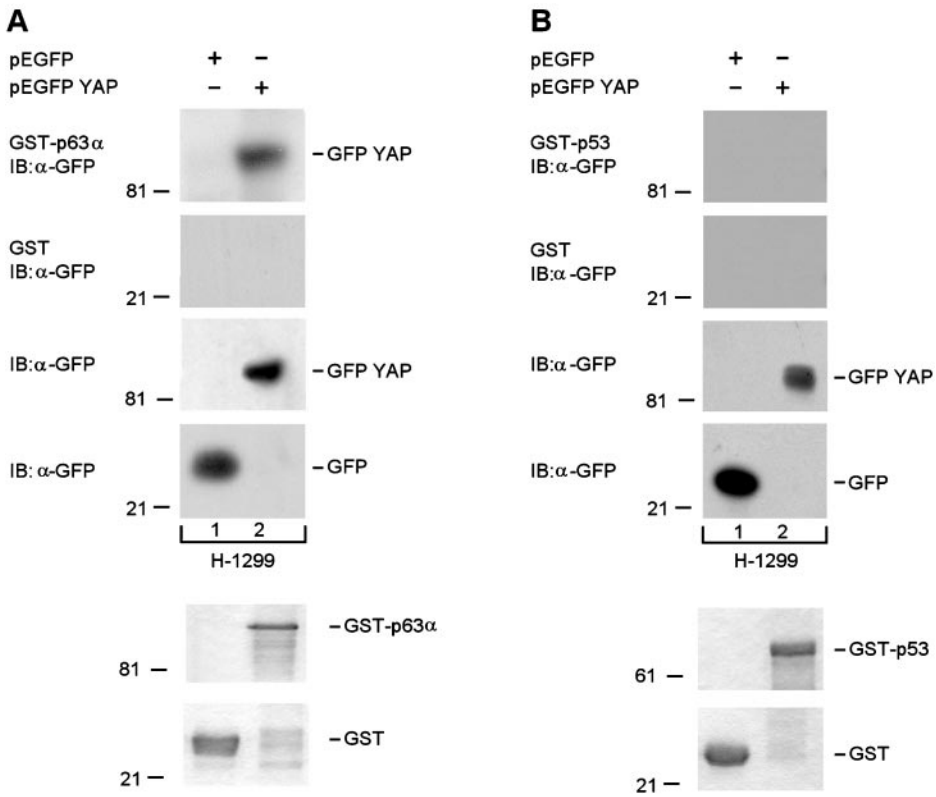


FIG. 5. **Differential binding of YAP to p63α and p53.** *A*, cell extracts of H1299 cells transfected with the indicated plasmids were incubated with GST-p63α (*upper panel*) or GST alone (*upper middle panels*). The blot was probed with anti-GFP polyclonal serum. The *lower panels* show protein levels of GFP-YAP and GFP employed in the transient transfections. *B*, identical cell lysates processed as above reported were incubated with GST-p53 or GST alone. The blot was probed with anti-GFP polyclonal serum. Coomassie staining of a replica gel showing the p63α and p53-GST fusion proteins as well as GST alone are reported in the *last panels* of *A* and *B*. *C*, cell extracts derived from P19 cells were processed as reported in *A* and incubated with GST-YAP/WW (*lane 3*) or GST alone (*lane 2*). The blot was probed with anti-p53 mAb 421. *D*, H1299 cells stably overexpressing inducible human WT p53 were extracted and, following a preclearing, were incubated with GST-YAP/WW (*lanes 3* and *6*) or GST alone (*lanes 2* and *4*). The blot was probed with a mixture of anti-p53 mAbs DO1 and 1801. Coomassie staining of replica gel showing the GST fusion proteins (*lower panel*).

precleared with glutathione-agarose beads and then incubated for 2 h at 4 °C. Following three consecutive washes with HNTG buffer (20 mM Hepes (pH 7.5), 150 mM NaCl, 0.1% Triton X-100, 10% glycerol), excess liquid was aspirated, and 40 μl of 5× sample buffer was added. Immunoprecipitates and 1% of each extract were resolved by SDS-9% PAGE. The immunoblots were probed with anti-p73, anti-GFP, or anti-YAP polyclonal serum. Detection was performed with the aid of the enhanced chemiluminescence Supersignal West Pico Stable Peroxidase Solution. (Pierce).

Indirect Immunofluorescence—H1299 cells transiently transfected with the indicated combination of plasmids were first incubated for 5 min at room temperature with a solution containing 10% bovine serum albumin, 0.5% Tween 20 in 1× PBS (PAT solution) and then fixed in 3.75% PBS-paraformaldeyde plus 0.1% Triton-100 for 10 min on ice. After rehydration with PAT for 5 min, the cells were incubated in 50% PAT and then stained for 1 h with anti-p73 polyclonal serum used at 1:100 dilution. Staining with the secondary antibody and with Hoechst was performed as described before (57), followed by visualization under a fluorescence microscope.

Luciferase Assays—H1299 cells were transfected with reporter plasmid together with the indicated expression plasmid combinations. 36 h later, cells were rinsed with cold phosphate-buffered saline, resuspended in cell lysis buffer (Promega), and incubated for 10 min at room temperature (34). Insoluble material was spun down, and luciferase activity was quantified using a commercially available kit (Promega) with the aid of a TD-20E luminometer (Turner).

RESULTS

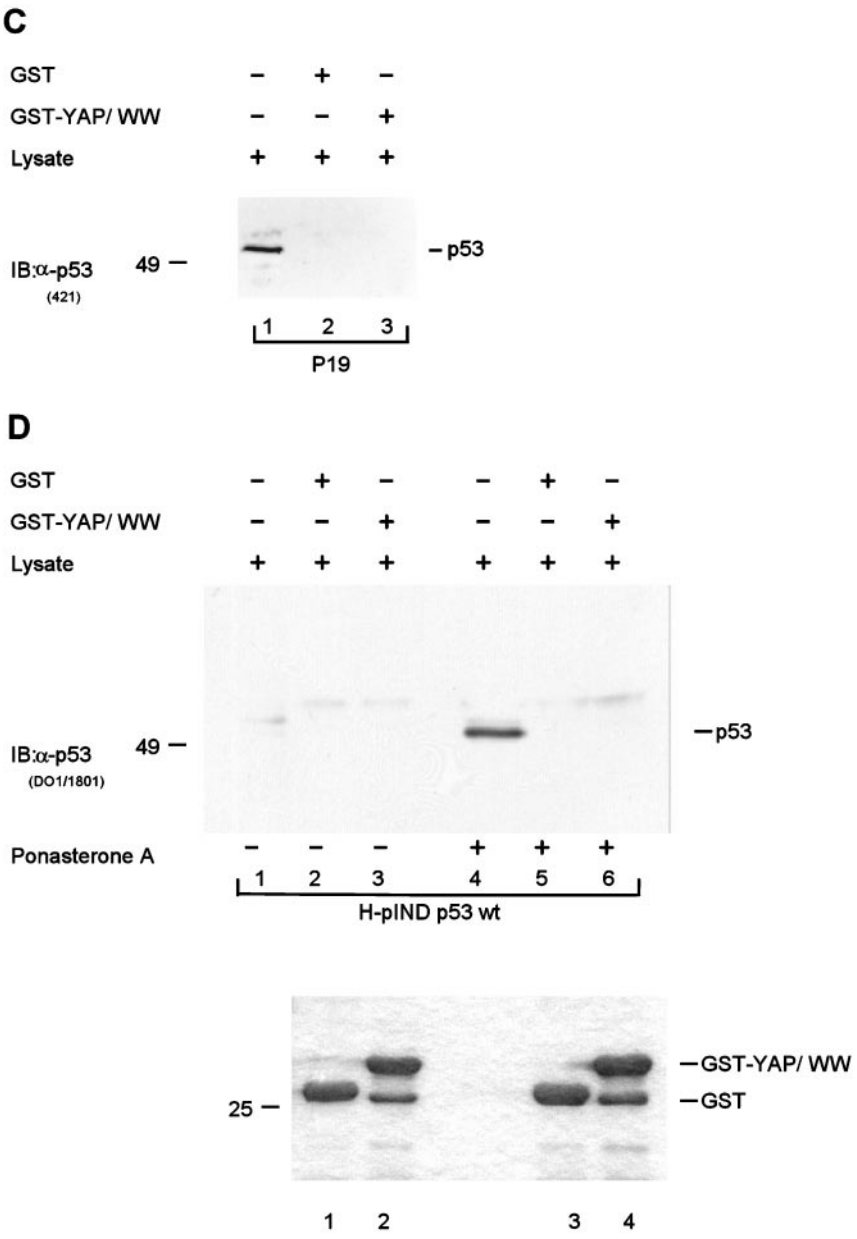
p73α Associates in Vitro and in Vivo with YAP—In a search for new p73-binding proteins, we checked whether various SH3 as well as WW domains associate with p73α in pull-down assays. To this end, H1299 cells were transiently transfected either with an empty vector (H1299) or with a vector encoding a hemagglutinin (HA)-tagged version of p73α (H-p73α) (15). Equal amounts of protein lysates were first precleared with glutathione-agarose beads and then incubated with GST fusions of SH3 or WW domains (listed in Table I). The pulled down proteins were separated by gel electrophoresis on an SDS-9% PAGE, transferred to nitrocellulose membranes, and probed with anti-p73 polyclonal serum (34). As shown in Fig. 1*A*, p73α was pulled down by human YAP WW domain (*lanes 5* and *6*) only when incubated with protein lysates of H-p73α cells. No p73α protein was pulled down when lysates of H1299 cells transfected with an empty vector were incubated with GST-YAP/WW or with GST alone (*lanes 2* and *3*). 1% of unprocessed cell lysates derived from either H1299 or H-p73α cells were concomitantly run on the gel (*lanes 1* and *4*).

To explore the possibility that the association between p73 and YAP is direct, we performed a pull-down assay incubating GST-YAP/WW or GST fusion proteins with purified His-tagged p73α (H_6-p73α) protein expressed in bacteria. As shown in Fig. 1*B*, purified H_6-p73α protein efficiently binds to GST-YAP/WW (*upper panel*, *lanes 2* and *3*). In order to show the presence of GST fusion proteins, the lower part of the same blot was probed with anti-GST polyclonal serum (Fig. 1*B*, *lower panel*).

To test whether the association between p73α and YAP would occur in a cell, H1299 cells were transiently transfected with a combination of vectors encoding p73α-HA and a GFP-tagged version of human YAP. Cell lysates derived from the above mentioned cells were first precleared with protein G-agarose beads and then subjected to immunoprecipitation with a mixture of anti-p73 polyclonal sera (34). The immunoprecipitates were separated on SDS-9% PAGE, transferred to nitrocellulose membranes, and probed with anti-GFP polyclonal serum. As shown in Fig. 1*C*, a coprecipitated GFP-tagged YAP was detected only when both p73α and YAP were co-expressed (*upper panel*, *lane 3*). For control, the same blot was reprobed with anti-p73 polyclonal serum (Fig. 1*C*, *middle upper panel*, *lanes 2* and *3*). The protein levels of GFP-YAP (*lane 3*) and GFP alone (*lane 2*) are shown in the *lower panels* of Fig. 1*C*. Immunostaining analysis revealed a predominant nuclear localization for both p73α and GFP-YAP when transiently co-expressed in H1299 cells (Fig. 1*D*).

These results demonstrate that p73α associates with YAP *in vitro* and *in vivo* and that this association might occur directly.

The Association between p73 and YAP Occurs under Physiological Conditions—To verify whether the association of p73 and YAP occurs under physiological conditions, lysates of P19 cells



FIG; 5—*continued*

were immunoprecipitated with anti-YAP and as an internal control for p53 family members, with a mixture of anti-human p53 mAbs DO1 and 1801. As shown in Fig. 2*A*, endogenous p73 was co-immunoprecipitated by anti-YAP (*lane 2*) but not by anti-p53 antibodies (*lane 3*). In a reciprocal experiment, the same cell lysates were first precleared with protein G-agarose beads and then subjected to immunoprecipitation with an anti-p73 polyclonal serum or, as controls, with anti-p53 mAb 421 or with a mixture of anti-p53 mAbs DO1 and 1801. Again the endogenous YAP was brought down by anti-p73 antibody but not by anti-p53 mAbs DO1/1801 and 421 (Fig. 2*B*, *lanes 1–4*).

Taken together, these results suggest that the association between p73 and YAP occurs under physiological conditions. Furthermore, they show that, at least under our experimental conditions, p53 does not associate with YAP.

Differential Binding of YAP to the Various Members of the p53 Family—The p73α sequence contains two peptides whose sequences match the PP*X*Y binding consensus for the YAP-WW domain (40, 41). The first peptide, ^{403}QPPSYG^{409} is at the carboxy-side of the oligomerization domain, while the second, ^{482}PPPPY^{488}, immediately precedes the SAM domain. To distinguish between the two candidate target sites, we have generated fragments of the p73 coding sequence and expressed them as part of the gene for the lambda D capsid protein (see "Experimental Procedures"). The resulting chimeric phages displayed different fragments of the p73 protein on the capsid. By panning this repertoire with the YAP-WW fused to GST, we have selected the clones that display a peptide fragment that binds to YAP. Only those phage-displayed peptides that only contained the ^{482}PPPPY^{488} were selected, suggesting that this peptide could be sufficient for the formation of the complex with the WW domain of YAP (Fig. 3*A*). Since this consensus is conserved in p73α, p73β, and p63α but is not present in p53 and p73γ (Fig. 3*B*), this result would imply that YAP associates selectively with the different protein products of the p53 gene family. To investigate this possibility, H1299 cells were transiently transfected either with a vector encoding a GFP-tagged version of human YAP or with an empty vector. Cell lysates were first precleared with glutathione-agarose beads and then incubated with p73α, p73β, p63α, and p53 GST fusion proteins as well as with GST alone. After separation on SDS-9% PAGE, the pulled down proteins were transferred to nitrocellulose

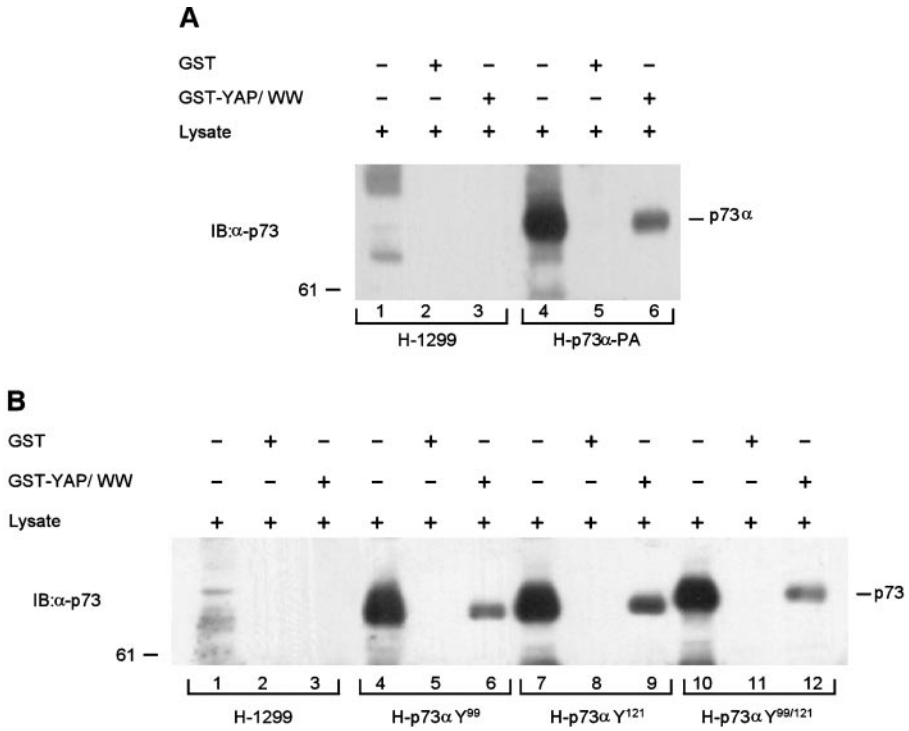


FIG. 6. **Binding to as well as tyrosine-phosphorylation of p73α by c-Abl is dispensable for the association with YAP.** H1299 cells were transiently transfected with the indicated plasmids. Cell extracts (2 mg) were first precleared with glutathione-agarose beads and then incubated with GST-YAP/WW or GST fusion proteins. The blot was probed with anti-p73 polyclonal serum. *A*, binding of p73α P338A mutant to the above mentioned GST fusion proteins (*lanes 2* and *3* and *lanes 5* and *6*). *B*, binding of p73α Y99F, p73α Y121F, p73α Y99F/Y121F mutants to the indicated GST fusion proteins (*lanes 2* and *3*, *5* and *6*, *8* and *9*, and *11* and *12*). Aliquots containing 100 μg of total protein from unprocessed lysates were subjected to immunoblot as previously reported: *lanes 1* and *4* (*A*); *lanes 1*, *4*, *7*, and *10* (*B*).

membranes and probed with anti-GFP polyclonal serum. In agreement with the results reported above, YAP binds to p73α, p73β, and p63α but not p53 (Figs. 4*A* and 5, *A* and *B*). The absence of the association between p53 and YAP was further investigated by pull-down assays in P19 as well as in H1299 cells stably overexpressing ponasterone-induced human WT p53 (H-p53) (55). As reported in Fig. 5, *C* and *D*, incubation of cell lysates of either P19 or H-p53 cells with GST-YAP/WW or with GST alone did not reveal any specific binding with p53. Aliquots of unprocessed cell lysates (100 μg/lane) derived from both types of cells were concomitantly run to show endogenous p53 in P19 (Fig. 5*C*, *lane 1*) and exogenous p53 in H1299 cells (Fig. 5*D*, *lane 4*). To verify whether YAP would bind to the differentially spliced variants of p73, H1299 cells were transiently transfected with vectors encoding p73α or p73γ or with empty vector. Precleared cell lysates were incubated with GST-YAP/WW or with GST alone. As shown in Fig. 4*B*, in contrast to p73α, p73γ does not associate with YAP. Coomassie Blue staining of replica gels showed equal amounts of recombinants proteins (Figs. 4*B* and 5*D*, *lower panels*). Taken together, these data suggest that binding to the PPPPY motif governs the specificity in the association between YAP and the members of the p53 family.

Binding to and Tyrosine Phosphorylation of p73α by c-Abl Are Dispensable for the Association with YAP—It has recently been reported that c-Abl interacts physically with p73 (35–37). As a consequence, p73 is tyrosine-phosphorylated, and its apoptotic activity is increased (36, 37). In order to investigate whether the binding of c-Abl to p73 influences the association of p73 with YAP, we employed pull-down assays.

First, we checked whether the p73α mutant, which has an alanine instead of proline at residue 338 (P338A) and is unable to bind to c-Abl, associated with YAP (36). To this end, H1299 cells were transiently transfected with a vector encoding p73α-P338A-HA (H-p73α-PA) or with an empty vector (H1299). As shown in Fig. 6*A*, p73α-P338A-HA was pulled down only by GST-YAP/WW.

Second, by a similar approach, we proved that p73α mutants carrying substitutions of tyrosine to phenyalanine (Y99F, Y121F, and Y99F/Y121F) could still associate with YAP (Fig. 6*B*).

Thus, we conclude that both the binding of p73α to c-Abl and the phosphorylation of p73α by c-Abl do not influence the association of p73 with YAP.

YAP Functions as a Transcriptional Co-activator of p73α—It has previously been reported that YAP itself contains a potent transactivation domain and stimulates the activity of the transcription factor, polyomavirus enhancer binding protein 2 (61).

To assess whether the association with YAP influences p73α transcriptional activity, we co-transfected H1299 cells with p73α together with GFP-YAP or GFP alone and a luciferase reporter gene driven by the p73-responsive *Bax* promoter or by the p73-responsive *mdm2* promoter. We report that while a limiting amount of transfected p73α stimulated the *mdm2* promoter as well as the *Bax* promoter activity 1.5–2-fold, respectively, over the control vector, the stimulation became much more evident when YAP was also added (GFP-YAP/p73α) (Fig. 7, *A* and *B*).

To determine whether the enhancement of transcriptional activity mediated by p73α operated not only on artificial promoter constructs but also on endogenous p73-responsive chromosomal genes, we analyzed the levels of Bax protein in cells overexpressing p73α alone or together with a plasmid encoding human YAP. As shown in Fig. 7*C*, only in the presence of co-transfected YAP, p73α increases steady state levels of the Bax protein. Thus, YAP can interact with p73α not only physically but also functionally.

A Single Point Mutation Tyr → Phe in the PPPPY Motif of p73α Abolishes the Association with, and Is Not Co-activated by, YAP—The structure of a WW domain of human YAP with its cognate ligand was originally solved by NMR spectroscopy (58). The hallmarks of the binding pocket of the WW domain of human YAP include three hydrophobic residues, leucine, tyrosine, the second conserved tryptophan, and histidine (58). Two prolines of the ligand (PP*X*Y) form van der Waals contacts with the second tryptophan, whereas the terminal tyrosine of the ligand fits into a hydrophobic pocket. Extensive mutagenesis of the WW domain of YAP and its cognate ligand and the recent determination of a high resolution structure of the WW domain of dystrophin in complex with β-dystroglycan also confirmed the requirement of Y in the PP*X*Y motif for complex formation (44, 59, 60).

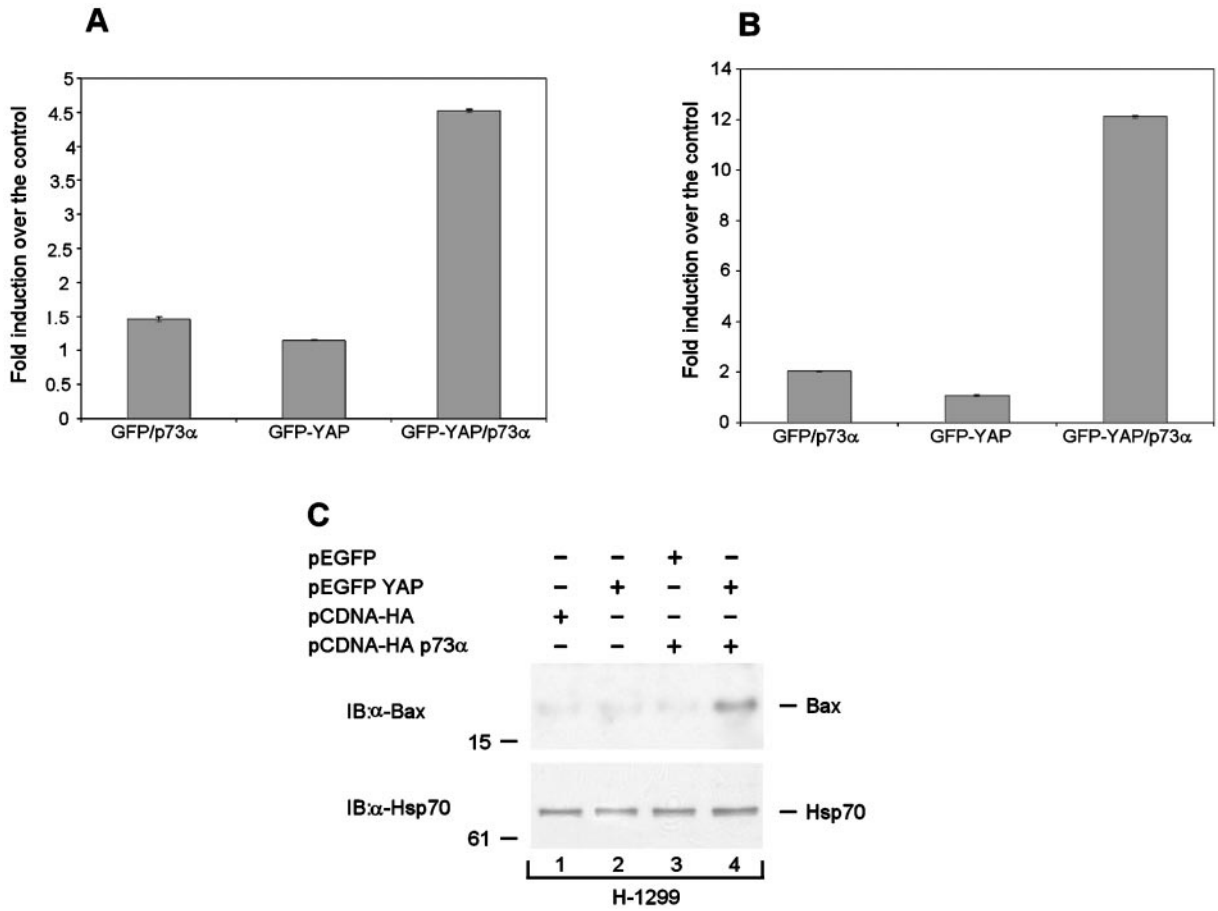


FIG. 7. **YAP enhances the transcriptional activity as well as the induction of Bax protein by p73α.** H1299 cells were transiently transfected with the indicated combinations of plasmids encoding p73α (25 ng/60-mm dish) or YAP (100 ng/dish) or pEGFP vector control together with *mdm2* (*A*) and *Bax* (*B*) luciferase reporter plasmids (50 ng/dish). The total amount of transfected DNA in each dish was keep constant by the addition of empty vector wherever necessary. Cell extracts were prepared 36 h later and subjected to determination of luciferase activity. Results are represented as -fold induction of luciferase activity compared with the control cells transfected with an empty pEGFP expression vector. Histograms show the mean of a typical experiment of three performed in triplicate; *bars* indicate S.D. *C*, H1299 cells were transiently transfected with the indicated combinations of plasmids encoding p73α (2 μg/60-mm dish) or YAP (4 μg/60-mm dish) or pEGFP vector control. Cell extracts (100 μg/lane) were prepared 36 h later, subjected to SDS-12.5% PAGE, and immunoblotted with anti-Bax polyclonal serum or with anti-Hsp70 antibody for equal loading.

In order to investigate the precise role of the PPPPY motif of p73α in the association with YAP, we generated a GST-p73α mutant in which the tyrosine in the YAP-WW ligand binding consensus was mutated to phenyalanine (59). The ability of this mutant to bind to YAP was tested by pull-down assay. As shown in Fig. 8*A* (*lanes 2–4*), endogenous YAP is pulled down by GST-p73α but not by GST-p73α, Y487P, or GST alone.

We next investigated whether binding to YAP is required for the co-activation of p73α. To this end, we assessed the effects of YAP on the transcriptional activity of the p73α Y487P mutant. As reported in Fig. 8*C*, co-expression of YAP, unlike for p73α, does not promote the transcriptional activity of the p73α mutant measured as ability to stimulate the p73-responsive *Bax* promoter.

The reported results demonstrate that the integrity of the PPPPY motif of p73α is crucial for the association between p73α and YAP, and the terminal tyrosine of that motif is required for the association of p73α with YAP. Furthermore binding to YAP is required for co-activation of p73α.

DISCUSSION

In the present study, we report that the WW domain of YAP binds to a region of p73 immediately preceding the SAM domain. Furthermore, we show that this same interaction mediates the association between YAP and p73, under physiological conditions, as shown in P19 cells by reciprocal co-immunoprecipitation experiments. YAP is a ubiquitously expressed phosphoprotein interacting with the SH3 domain of the proto-oncogene protein c-Yes, a nonreceptor tyrosine kinase that belongs to the Src family. Its WW domain preferentially binds to ligands containing a PP*X*Y motif and belongs to the first class (class I) of the current classification of the diverse WW domains (40, 41).

Specific protein-protein interactions are often mediated by functionally and structurally defined families of small protein modules (39, 62). We have shown that the association between p73 and YAP involves the binding of the WW domain of YAP to a p73 region containing a PPPPY motif. Unlike p73α, p73β, and p63α, YAP does not bind to p53, highlighting the principle of sequence-specific protein-protein interaction as a determinant of precise biological output(s). A further level of specificity for the association between p73 and YAP exists among the different isoforms of p73. Our findings demonstrate that in contrast to isoforms α and β, p73γ does not bind to YAP. Although a complete comprehension of the functional implications of these differential interactions requires further analysis, this observation provides the first step in the understanding of the fine tuning of the biological output of the p53 family interaction network.

Because of its modular structure and the absence of an enzymatic activity, YAP can be classified as an adapter protein. *In vitro* YAP was characterized as a ligand of cytoplasmic kinases like c-Yes, c-Src, and Crk, but *in vivo* it is found preferentially in

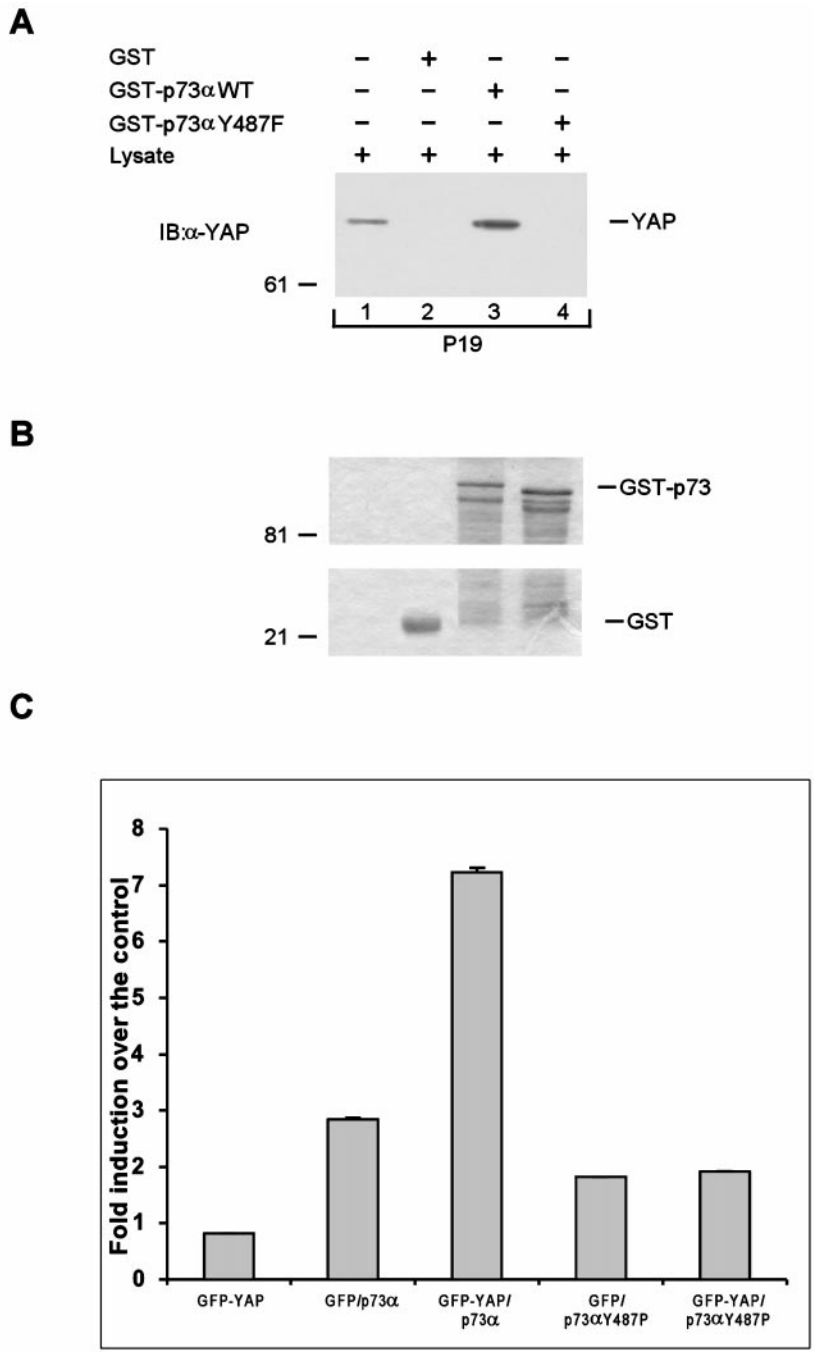


FIG. 8. **Point mutation of the terminal tyrosine of the PPPPY motif of p73α abolishes the binding with, and is not co-activated by, YAP.** *A*, cell extracts derived from P19 cells were first precleared with glutathione-agarose beads and then incubated with the indicated GST fusion proteins (*lanes 2–4*). The blot was probed with anti-YAP polyclonal serum. *B*, Coomassie staining of a replica gel showing the p73α and p73α Y487F GST fusion proteins. *C*, H1299 cells were transiently transfected with the indicated combinations of plasmids encoding p73α or p73α Y487P (25 ng/60-mm dish), YAP (100 ng/dish), or pEGFP vector control together with Bax luciferase reporter plasmid (50 ng/dish) The total amount of transfected DNA in each dish was kept constant by the addition of empty vector wherever necessary. Cell extracts were prepared 36 h later and subjected to determination of luciferase activity. Results are represented as -fold induction of luciferase activity compared with the control cells transfected with an empty pEGFP expression vector. Histograms show the mean of a typical experiment performed in triplicate; *bars* indicate S.D.

the nucleus, suggesting that it might contribute to transmit signals from the cytoplasm to the nuclear compartment (56, 61). For instance, c-Src is triggered by c-Fms/macrophage colony stimulatory factor-1 receptor for macrophage colony stimulation factor (63). p73-deficient mice show several defects including inflammatory processes that might result from a deficiency in appropriate differentiation along the white cell lineage. We and others have observed that p73 protein is strongly induced upon macrophage differentiation of HL-60 cells.[3] Although these considerations are, at the moment, rather speculative, they permit us to logically connect extracellular signals to a nuclear output through an interaction chain that includes YAP and p73. Thus, YAP might integrate signals incoming from c-Src to activate p73. The similarity between YAP and other signaling proteins that sense external signals near the plasma membrane and transduce them to the nucleus is reinforced by the recent observation that YAP localizes to the apical plasma membrane in polarized airway epithelial cells by binding to the second PDZ domain of EBP50 (64). YAP, in this respect, mimics the behavior of Smad, Stat, or JAB1 proteins that, upon activation of the tyrosine kinase activity associated with growth factor receptors or by integrin-mediated signals, translocate into the nucleus to form functional complexes with transcription factors (65, 66). These considerations, taken together with the recent findings that YAP associates with the transcription factor polyomavirus enhancer binding protein 2 and the reported data showing the interaction with p73, strongly indicate that the nucleus is the important site of YAP activity (61). Thus, the PPPPY motif of p73, by binding to YAP, might contribute to transmission of cytoplasmic signals to the nucleus. Here we report that the binding to YAP enhances p73α transcriptional activity. To some extent, YAP is reminiscent of β-catenin, which takes part in both cell-cell contact mediated by

[3] B. Cristofawelli and G. Blandino, unpublished observations.

cadherin and in transcriptional co-activation downstream of Wnt family members as well as of p53 (67–69). It is reasonable to depict a scenario in which proline-rich regions of p73 can be recruited by proteins containing SH3 as well as WW domains following the activation of specific signaling pathways. Our results suggest that the interactions of p73 with Abl and YAP are independent events, since binding to c-Abl and tyrosine phosphorylation of p73 are not required for the association with YAP.

A biological read-out for the complex formed by p73 and YAP is likely to emerge from the study of muscle differentiation. YAP is expressed at considerable levels in muscle cells, and p73 transcript is strongly up-regulated along muscle differentiation (70).[4] An additional facet of YAP function in modulating transcriptional activity of p53 family members was recently revealed by the characterization of a functional complex between YAP and p53-binding protein 2 (71). It appears that the interaction between p53-binding protein 2 and YAP renders p53-binding protein 2 inactive in terms of complex formation with p53. Thus YAP could influence the activity of the p53 family members either by direct or indirect means.

Further analysis is required to verify whether differential binding of YAP to the p53 family members might provide a molecular explanation to their functional divergence in signaling.

Acknowledgments—We thank W.G. Jr Kaelin, G. Melino and X. Espanel for expression plasmids, D. Lane for DO1 antibody and A.M. Salvatori for P19 cells. We are particularly grateful to O. Segatto and O. Monti for helpful suggestions and collaboration.

REFERENCES

1. Hollstein, M., Soussi, T., Thomas, G., von-Brevern, M., and Bartsch, H. (1997) *Recent Res. Cancer Res.* **143,** 369–389
2. Oren, M. (1999) *J. Biol. Chem.* **274,** 36031–36034
3. Levine, A. J. (1997) *Cell* **88,** 323–331
4. Hansen, R., and Oren, M. (1997) *Curr. Op. Genet. Dev.* **7,** 46–51
5. Almog, N., and Rotter, V. (1997) *Biochim. Biophys. Acta* **1333,** F1–F27
6. Dulic, V., Kaufmann, W. K., Lees, S. J., Tisty, T. D., Lees, E., Harper, J. W., Elldge, S. J., and Reed, S. (1994) *Cell* **76,** 1013–1023
7. El-Deiry, W. S., Tokino, S., Velculescu, V. E., Levy, D. B., Parson, R., Trent, J. M., Lin, D., Mercer, W. E., Kinzler, K. W., and Vogelstein, B. (1993) *Cell* **75,** 817–825
8. Miyashita, T., and Reed, J. C. (1995) *Cell* **80,** 293–299
9. Polyak, K., Xia, Y., Zweier, J. L., Kinzler, K. W., and Vogelstein, B. (1997) *Nature* **389,** 300–305
10. Kaghad, M., Bonnet, H., Yang, A., Creancier, L., Biscan, J., Valent, A., Minty, A., Chalon, P., Lelias, J., Dumont, X., Ferrara, P., McKeon, F., and Caput, D. (1997) *Cell* **90,** 809–819
11. Oren, M. (1997) *Cell* **90,** 829–832
12. Osada, M., Ohba, M., Kawahara, C., Ishioka, C., Kanamaru, R., Katoh, I., Ikawa, Y., Nimura, Y., Nakagawara, A., Obinata, M., and Ikawa, S. (1998) *Nat. Med.* **4,** 839–843
13. Trink, B., Okami, K., Wu, L., Sriuranpong, V., Jen, J., and Sidransky, D. (1998) *Nat. Med.* **4,** 747
14. Yang, A., Kaghad, M., Wang, Y., Gillett, E., Fleming, M. D., Dostch, V., Andrews, N. C., Caput, D., and McKeon, F. (1998) *Mol. Cell* **2,** 305–316
15. Jost, C. A., Marin, C. M., and Kaelin, W. J. (1997) *Nature* **389,** 191–194
16. De Laurenzi, V., Raschellà, G., Barcaroli, D., Annichiarico-Petruzzelli, M., Ranalli, M., Catani, M. V., Tanno, B., Costanzo, A., Levrero, M., and Melino, G. (2000) *J. Biol. Chem.* **275,** 15226–15231
17. De Laurenzi, V., Costanzo, A., Barcaroli, D., Terrinoni, A., Falco, M., Annichiarico-Petruzzelli, M., Levrero, M., and Melino, G. (1998) *J. Exp. Med.* **188,** 1763–1768
18. De Laurenzi, V., Catani, M. V., Costanzo, A., Terrinoni, A., Corazzari, M., Levrero, M., Knight, R. A., and Melino, G. (1999) *Cell Death Differ.* **6,** 389–390
19. Yang, A., Walker, N., Bronson, R., Kaghad, M., Oosterwegel, M., Bonnin, J., Vagner, C., Bonnet, H., Dikkes, P., Sharpe, A., McKeon, F., and Caput, D. (2000) *Nature* **404,** 99–103
20. Marin, M. C., and Kaelin, W. G., Jr. (2000) *Biochim. Biophys. Acta* **1470,** 93–100
21. Marin, M. C., Jost, C. A., Irwin, M. S., DeCaprio, J. A., Caput, D., and Kaelin, W. G. (1998) *Mol. Cell. Biol.* **18,** 6316–6324
22. Dobbelstein, M., and Roth, J. (1998) *J. Gen. Virol.* **79,** 3079–3083
23. Roth, J., Konig, C., Wienzek, S., Weigel, S., Ristea, S., and Dobbelstein, M. (1998) *J. Virol.* **72,** 8510–8516
24. Steegenga, W. T., Shvarts, A., Riteco, N., Bos, J. L., and Jochemsen, A. G. (1999) *Mol. Cell. Biol.* **9,** 3885–3894
25. Bottger, A., Bottger, V., Sparks, A., Liu, W. L., Howard, S. F., and Lane, D. P. (1997) *Curr. Biol.* **7,** 860–869
26. Haupt, Y., Maya, R., Kazaz, A., and Oren, M. (1997) *Nature* **387,** 296–299
27. Kubbutat, M. H. G., Jones, S. N., and Voudsen, K. H. (1997) *Nature* **387,** 299–303
28. Zeng, X. Y., Chen, L. H., Jost, C. A., Maya, R., Keller, D., Wang, X. J., Kaelin, W. G. J., Oren, M., Chen, J. D., and Lu, H. (1999) *Mol. Cell. Biol.* **19,** 3257–3266
29. Balint, E., Bates, S., and Voudsen, K. H. (1999) *Oncogene* **18,** 3923–3929
30. Scharnhorst, V., Dekker, P., von der EB, A. J., and Jochemsen, A. G. (2000) *J. Biol. Chem.* **275,** 10202–10211
31. Minty, A., Dumont, X., Kaghad, M., Caput, D. (2000) *J. Biol. Chem.* **275,** 36316–36323
32. Di Como, C. J., Gaiddon, C., and Prives, C. (1999) *Mol. Cell. Biol.* **19,** 1438–1449
33. Marin, M. C., Jost, C. A., Brooks, L. A., Irwin, M. S., O'Nions, J., Tidy, J. A., James, N., McGregor, J. M., Harwood, C. A., Yulug, I. G., Voudsen, K. H., Allday, M. J., Gusterson, B., Ikawa, S., Hinds, P. W., Crook, T., and Kaelin, W. G. (2000) *Nat. Genet.* **25,** 47–55
34. Strano, S., Munarriz, E., Rossi, M., Cristofanelli, B., Shaul, Y., Castagnoli, L., Levine, A. J., Sacchi, A., Cesareni, G., Oren, M., and Blandino, G. (2000) *J. Biol. Chem.* **275,** 29503–29512
35. Gong, J. G., Costanzo, A., Yang, H. Q., Melino, G., Kaelin, W. G. J., Levrero, M., and Wang, J. Y. J. (1999) *Nature* **399,** 806–809
36. Agami, R., Blandino, G., Oren, M., and Shaul, Y. (1999) *Nature* **399,** 809–813
37. Yuan, Z. M., Shioya, H., Ishiko, T., Sun, X. G., Gu, J. J., Huang, Y. Y., Lu, H., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1999) *Nature* **399,** 814–817
38. Bork, P., and Sudol, M. (1994) *Trends Biochem. Sci.* **19,** 531–533
39. Sudol, M. (1998) *Oncogene* **17,** 1469–1474
40. Sudol, M., and Hunter, T. (2000) *Cell* **103,** 1001–1004
41. Chen, H. I., and Sudol, M. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 7819–7823
42. Schild, L., Lu, Y., Gautschi, I., Schneeberger, E., Lifton, R. P., and Rossier, B. C. (1996) *EMBO J.* **15,** 2381–2387
43. Rentschler, S., Lim, H., Deiminger, K., Bedford, M. T., Espanel, X., and Sudol, M. (1999) *Biol. Chem.* **380,** 431–442
44. Huang, X., Poy, F., Zhang, R., Joachimiak, A., Sudol, M., and Eck, M. J. (2000) *Nat. Struct. Biol.* **8,** 634–638
45. Ermekova, K. S., Zambrano, N., Linn, H., Minopoli, G., Gertler, F., Russo, T., and Sudol, M. (1997) *J. Biol. Chem.* **272,** 32869–32877
46. Bedford, M. T., Chan, D. C., and Leder, P. (1997) *EMBO J.* **16,** 2376–2383
47. Bedford, M. T., Reed, R., and Leder, P. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 10602–10607
48. Komuro, A., Saeki, M., Kato, J. (1999) *J. Biol. Chem.* **274,** 36513–36519
49. Waragi, M., Lammers, C. H., Takeuchi, S., Imafuku, I., Udagawa, Y., Kanazawa, I., Kawabata, M., Mouradian, M. M., and Okazawa, H. (1999) *Hum. Mol. Genet.* **8,** 977–987
50. Ranganathan, R., Lu, K. P., Hunter, T., and Noel, J. P. (1997) *Cell* **89,** 875–886
51. Lu, P. J., Zhou, X. Z., Shen, M., and Lu, K. P. (1999) *Science* **283,** 1325–1328
52. Verdecia, M. A., Bowman, M. E., Lu, K. P., Hunter, T., and Noel, J. P. (2000) *Nat. Struct. Biol.* **8,** 639–643
53. Santi, E., Capone, S., Mennuni, C., Lahm, A., Tramontano, A., Luzzago, A., Nicosia, A. (2000) *J. Mol. Biol.* **296,** 497–508
54. Sambrook, J., Fritsch, E. F., Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., pp. 2.64–2.66, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
55. Wang, Y., Blandino, G., Oren, M., and Givol, D. (1998) *Oncogene* **17,** 1923–1930
56. Sudol, M. (1994) *Oncogene* **9,** 2145–2152
57. Blandino, G., Levine, A. J., and Oren, M. (1999) *Oncogene* **18,** 477–485
58. Macias, M. J., Hyvonen, M., Baraldi, E., Schultz, J., Sudol, M., Saraste, M., and Oschkinat, H. (1996) *Nature* **382,** 646–649
59. Chen, H. I., Einbond, A., Kwak, S. J., Linn, H., Koepf, E., Paterson, S., Kelly, J. W., and Sudol, M. (1997) *J. Biol. Chem.* **272,** 17070–17077
60. Linn, H., Ermekova, K. S., Rentschler, S., Sparks, A. B., Kay, B. K., and Sudol, M. (1997) *Biol. Chem.* **378,** 531–537
61. Yagi, R., Chen, L. F., Shigesada, K., Murakami, Y., and Ito, Y. (1999) *EMBO J.* **18,** 2551–2562
62. Pawson, T., and Nash, P. (2000) *Genes Dev.* **14,** 1027–1047
63. Courtneidge, S. A., Dhand, R., Pilat, D., Twamley, G. M., Waterfield, M. D., and Roussel, M. F. (1993) *EMBO J.* **12,** 943–950
64. Mohler, P. J., Kreda, S. M., Boucher, R. C., Sudol, M., Stuttus, M. J., and Milgram, S. L. (1999) *J. Cell Biol.* **147,** 879–890
65. Darnell, J. E., Jr. (1997) *Science* **277,** 1630–1635
66. Bianchi, E., Denti, S., Granata, A., Bossi, G., Geginat, J., Villa, A., Rogge, L., Pardi, R. (2000) *Nature* **404,** 617–621
67. Wodarz, A., and Nusse, R. (1998) *Annu. Rev. Cell Dev. Biol.* **14,** 59–88
68. Damalas, A., Ben-ze'ev, A., Simcha, I., Shtutman, M., Martinez Leal, J. F., Zhurinsky, J., Geiger, B., and Oren, M. (1998) *EMBO J.* **18,** 3054–3063
69. Peifer, M., and Polakis, P. (2000) *Science* **287,** 1606–1609
70. Levrero, M., De Laurenzi, V., Costanzo, A., Sabatini, S., Gong, J., Wang, J. W. J., and Melino, G. (2000) *J. Cell Sci.* **113,** 1661–1670
71. Espanel, X., and Sudol, M. (2001) *J. Biol. Chem.*, in press

[4] G. Fontemaggi and G. Blandino, unpublished observations.

Physical Interaction with Yes-associated Protein Enhances p73 Transcriptional Activity

Sabrina Strano, Eliana Munarriz, Mario Rossi, Luisa Castagnoli, Yosef Shaul, Ada Sacchi, Moshe Oren, Marius Sudol, Gianni Cesareni and Giovanni Blandino

J. Biol. Chem. 2001, 276:15164-15173.
doi: 10.1074/jbc.M010484200 originally published online January 24, 2001
