finding that c-Abl induces the apoptotic function of p73 suggests that p73 may be an effector of a c-Abl-dependent apoptotic response to DNA damage. To determine whether p73 is involved in apoptosis induced by ionizing radiation, we transfected SAOS2 cells to make them overexpress GFP–p73β, p73β(Y^{99}-F) or p73β(Y^{121}-F), and then irradiated them. There was little apoptosis in irradiated cells expressing the empty vector, whereas cells expressing p73β or p73β(Y^{121}-F) showed an increase in the number of cells with a sub-G1 DNA content (Fig. 4c). By contrast, overexpression of p73β(Y^{99}-F) blocked in part the apoptotic response to ionizing radiation (Fig. 4c). These findings collectively support a model in which c-Abl-mediated phosphorylation of p73 contributes to DNA-damage-induced apoptosis.

The apoptotic response of irradiated cells has been associated with expression of wild-type p53 (refs 16–18). c-Abl interacts with p53 in irradiated cells, but does not phosphorylate it, and contributes to radiation-induced G1 arrest by a p53-dependent mechanism[11]. In contrast, c-Abl regulates DNA-damage-induced apoptosis predominantly by a p53-independent mechanism[4]. Our results support a model in which c-Abl regulates the p53-related p73 protein to induce DNA-damage-mediated apoptosis. Although it is known that p53 protein accumulates as a result of genotoxic stress[19] and p73 does not[1,2], our results show that p73 is phosphorylated by a c-Abl-dependent mechanism in the DNA-damage response. The functional significance of the c-Abl/p73 interaction is supported by our findings that active c-Abl, and not inactive c-Abl(K-R), induces both p73-mediated transactivation and apoptosis. From our demonstration that preventing interaction between c-Abl and p73 also prevents radiation-induced apoptosis, we conclude that p73 is regulated by c-Abl in the DNA-damage response. We have provided evidence that p73 is activated by c-Abl kinase and that it participates in the apoptotic response to DNA damage. □

Methods

Cell culture. COS7, 293, MCF-7/pSR and MCF-7/c-Abl(K-R)[4] cells were cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum (HI-FBS), 2 mM L-glutamine, 10 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. SAOS2 cells were grown in McCoy's 5a medium containing 10% HI-FBS, 2 mM L-glutamine, 10 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. Wild-type ($Abl^{+/+}$), c-Abl-deficient ($Abl^{-/-}$), human diploid (GM00637F) and ATM-deficient AT5BIVA fibroblasts were all grown as described[3,7,20]. Vectors were introduced into cells by using the Effectene transfection kit (Qiagen). Cells were treated with ionizing radiation at room temperature with a Gammacell 1000 (Atomic Energy of Canada) and a ^{137}Cs source emitting at a fixed rate of 0.21 Gy min^{-1}.

Plasmid construction. Vectors expressing GFP-p73α and GFP-p73β were generated by subcloning human p73α or p73β cDNAs[1] into pEGFP (Clontech). Vectors expressing Flag–p73α, Flag–p73β, Flag–p73β TAD (amino acids 1–125), Flag–p73β DBD (amino acids 128–313) and Flag–p73β OD (amino acids 311–499) were generated by subcloning full-length PCR-generated products from the p73α and p73β cDNAs[1] into Flag-tagged pcDNA3. Vectors expressing Flag–p73β(Y^{99}-F) and Flag-p73β(Y^{121}-F) were prepared by site-directed mutagenesis.

Immunoprecipitation and immunoblot analysis. Cell lysates were prepared in lysis buffer containing 0.5% Nonidet P-40 and immunoprecipitated as described[11] with anti-c-Abl (Ab-3; Oncogene Science), anti-RPA70 (Ab-1; Oncogene Science), anti-p73 (rabbit antiserum against p73; amino acids SAATPNLGPVGPGML) or anti-GFP (Clontech). Proteins were separated on SDS–polyacrylamide gels and western-blotted with anti-p73, anti-c-Abl, anti-RPA70, anti-P-Tyr (4G10; Upstate Biotechnology Inc.) or anti-p21 (Abl-1, Oncogene Science) antibodies.

Fusion-protein binding assays. Purified GST, GST–Abl SH3 (ref. 21) and GST–Abl SH2 (amino acids 115–213) proteins (5 μg) were incubated with ^{35}S-labelled Flag–p73α or Flag–p73β. GST–Abl SH3 was incubated with ^{35}S-labelled Flag–p73β TAD, Flag–p73β DBD or Flag–p73β OD and the adsorbates analysed by SDS–PAGE and autoradiography.

***In vitro* kinase assays.** Kinase-active c-Abl or kinase-inactive c-Abl(K-R) purified from baculovirus was incubated with immunoaffinity-purified Flag–p73α, Flag–p73β, Flag–p73β(Y^{99}-F), Flag–p73β(Y^{121}-F) or a GST–Crk control, and [γ-^{32}P]ATP in kinase buffer (20 mM HEPES, pH 7.5, 1 mM dithiothreitol, 10 mM $MgCl_2$) for 15 min at 30 °C. Phosphorylated proteins were separated by SDS–PAGE and analysed by autoradiography.

Luciferase assays. Transient transfections of SAOS2 cells with mdm2NA-Luc[14] were done with the Effectene transfection kit and cells were collected 24 h after transfection. Luciferase was assayed with an enhanced luciferase assay kit (1800K, Analytical Luminescence).

Apoptosis assays. DNA content was assessed by staining ethanol-fixed cells with propidium iodide and monitoring by FACScan (Becton-Dickinson). Numbers of GFP-positive cells with sub-G1 DNA content were determined with a MODFIT LT program.

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Correspondence and requests for materials should be addressed to D.K. (e-mail: donald_kufe@dfci.harvard.edu).

correction

p73 is a human p53-related protein that can induce apoptosis

Christine A. Jost, Maria C. Marin & William G. Kaelin Jr

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It has come to *Nature's* attention that the title of this Letter is misleading. In fact, the p73 cDNA sequence used in this work was of simian, not human, origin.

The GenBank database accession number for this African green monkey p73 sequence is Y11419. □

p73 is a human p53-related protein that can induce apoptosis

Christine A. Jost, Maria C. Marin & William G. Kaelin Jr

Dana-Farber Cancer Institute and Harvard Medical School, 44 Binney Street, Mayer 657, Boston, Massachusetts 02115, USA

The protein p53 is the most frequently mutated tumour suppressor to be identified so far in human cancers[1,2]. The ability of p53 to inhibit cell growth is due, at least in part, to its ability to bind to specific DNA sequences and activate the transcription of target genes such as that encoding the cell-cycle inhibitor p21$^{Waf1/Cip1}$ (ref. 3). A gene has recently been identified that is predicted to encode a protein with significant amino-acid sequence similarity to p53 (ref. 4). In particular, each of the p53 amino-acid residues implicated in direct sequence-specific DNA binding is conserved in this protein[5]. This gene, called *p73*, maps to the short arm of chromosome 1, and is found in a region that is frequently deleted in neuroblastomas[6]. Here we show that p73 can, at least when overproduced, activate the transcription of p53-responsive genes and inhibit cell growth in a p53-like manner by inducing apoptosis (programmed cell death).

To investigate the functions of p73, mammalian expression plasmids were made in which the complementary DNAs for two naturally occurring p73 isoforms were placed under the control of a cytomegalovirus (CMV) promoter. These isoforms, designated p73α and p73β, differ at their carboxy termini as a result of differential spicing of p73 messenger RNA[4]. Both isoforms contain the residues that, based on the p53 structure, are likely to participate in DNA recognition[5]. Site-directed mutagenesis was used to make plasmids encoding two mutant p73 isoforms in which one of these residues (Arg 292) was changed to histidine. The corresponding p53 mutant (Arg 273 → His) does not bind to canonical p53 DNA-binding sites and is defective for transcriptional activation and tumour-suppression functions[7]. All of these plasmids introduced an amino-terminal haemagglutinin (HA) epitope tag to facilitate the identification of its protein product and also contained a neomycin-resistance marker. Each plasmid gave rise to a comparably stable protein of the expected size, as determined by anti-HA steady-state western blot analysis of transiently transfected cells (Fig. 1a). Furthermore, each of the exogenous p73 species appeared to be located in the cell nucleus on the basis of anti-HA immunofluorescence staining (Fig. 1b, c).

In the next set of experiments, SAOS2 cells, which harbour a homozygous deletion at the *p53* gene locus and do not produce a

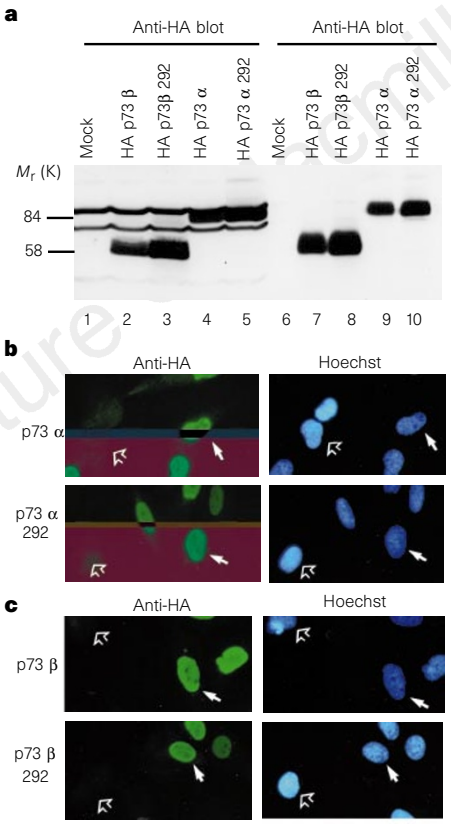

Figure 1 Expression and subcellular localization of p73. **a**, Anti-HA western blot of whole-cell extracts (lanes 1–5) or of anti-HA immunoprecipitates (lanes 6–10) following transfection of SAOS2 osteogenic sarcoma cells with plasmids encoding the indicated proteins. **b**, **c**, Anti-HA immunofluorescence (left) and Hoechst dye staining (right) of BHK cells producing the indicated proteins. Examples of transfected and untransfected cells are shown by solid and open arrows, respectively.

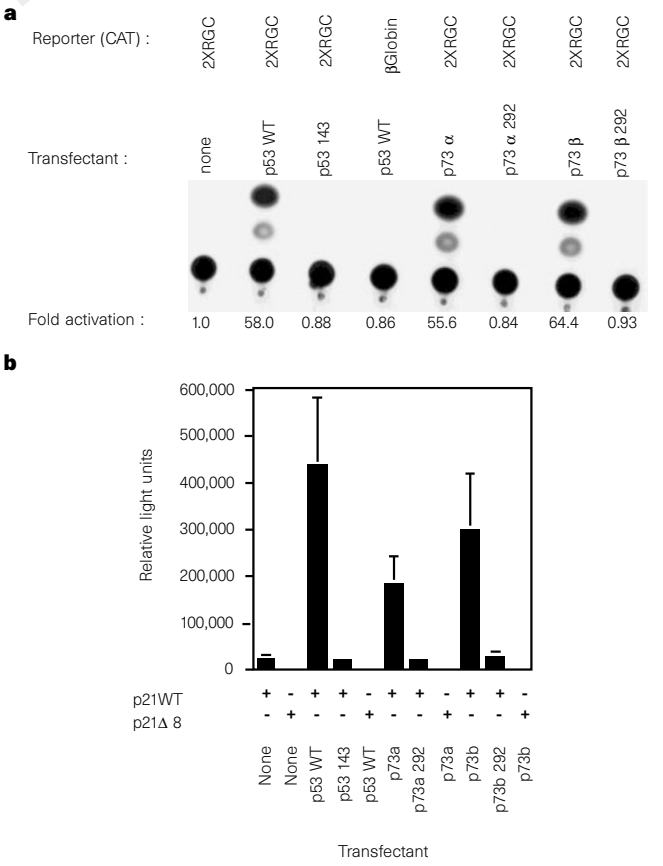

Figure 2 p73 activates p53-responsive promoters. **a**, CAT assay of SAOS2 cells transfected with reporter plasmids containing either the β-globin promoter (β-globin CAT) or a minimal promoter consisting of two p53 binding sites upstream of a TATA box (2 × RGC–CAT) together with p53 or p73 expression plasmids, as indicated. **b**, Luciferase assay of SAOS2 cells transfected with reporter plasmids containing an ~2.7-kb p21 genomic clone spanning the p21 promoter (p21 WT) or a deleted mutant lacking its p53 binding sites (del 8), together with p53 or p73 expression plasmids, as indicated. Shown are luciferase values corrected for transfection efficiency. Error bars indicate 1 standard error of the mean.

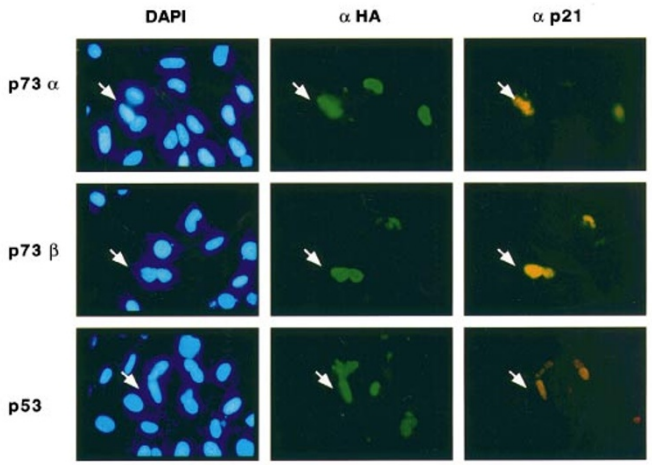

Figure 3 Induction of endogenous p21 by ectopically produced p73. SAOS-2 cells transfected with expression plasmids encoding HA-tagged p73α (top), p73β (centre) or p53 WT (bottom) were stained with either DAPI (5,6-diamino-2-phenyl indole; left), (centre), or anti-HA antibody p21 antibody (right). Bound antibody was detected by indirect immunofluorescence. Examples of transfected cells are indicated by the arrows.

p53 protein[8], were transfected with a chloramphenicol acetyltransferase (CAT) reporter plasmid containing a minimal promoter consisting of two p53 binding sites upstream of a TATA box (2XRGC-CAT). As expected, co-transfection of a plasmid encoding wild-type p53 produced a measurable CAT activity, whereas a tumour-derived p53 mutant (143Ala) did not (Fig. 2a). Likewise, co-transfection with either of the two wild-type p73 expression plasmids gave high levels of CAT activity, whereas the p73 292-His mutants did not. None of the expression plasmids activated a CAT reporter containing the β-globin promoter (Fig. 2, and data not shown). Similarly, wild-type p53, as well as the two wild-type p73 isoforms, could activate transcription from a reporter plasmid in which the *p21* promoter was placed upstream of a luciferase cDNA (p21 WT)[9] (Fig. 2b). Neither the p53 143-Ala nor the p73 292-His mutant was able to activate transcription from the p21 WT reporter. Furthermore, a p21 promoter mutant lacking functional p53 binding sites (p21Δ8) was unaffected by either p53 or p73.

To determine whether p73 could activate transcription of an endogenous p53-responsive gene, SAOS2 cells were transiently transfected with plasmids encoding either p73 or p53 and the induction of endogenous p21 was measured by anti-p21 immunofluorescence staining (Fig. 3). As expected, p21 was induced in cells ectopically producing wild-type but not mutant p53 (Fig. 3, and data not shown). Similarly, p21 was induced by wild-type but not mutant p73 α and β (Fig. 3, and data not shown). Together, these experiments indicate that p73 can activate transcription from promoters containing p53 DNA-binding sites.

Reintroduction of wild-type p53 suppresses the growth of SAOS2 cells[8]. To determine whether p73 might also have this effect, SAOS2 cells were transfected with the p73 expression plasmids and were placed under G418 selection. Approximately two weeks later, drug-resistant colonies were stained with crystal violet. Transfection with either of the two wild-type p73 expression plasmids did not give rise to any macroscopic colonies (Fig. 4a), in keeping with earlier results with wild-type p53. In contrast, many drug-resistant colonies formed following transfection with the backbone expression plasmid (pcDNA-3) or with the plasmids encoding the p73 mutants.

The suppression of SAOS2 cell growth by p53 is thought to be due largely to apoptosis[10]. To see whether p73 might have a similar effect, SAOS2 cells were transiently transfected with a plasmid encoding the cell-surface marker CD19 together with plasmids encoding either p53 or p73. The DNA content of CD19-positive cells was then analysed by fluorescence-activated cell sorting (FACS). Wild-type, but not mutant, p53 gave a significant increase in the number of cells undergoing apoptosis (cells with less than 2*N* DNA content) compared with cells transfected with the backbone expression plasmid (Fig. 4b). Effects were similar with the two wild-type p73 species, whereas the corresponding p73 mutants were virtually inert (Fig. 4b, and data shown). Because of the relatively high basal levels of apoptosis in SAOS2 cells, we next transiently transfected baby-hamster kidney (BHK) cells with these expression plasmids. Wild-type p53, as well as each of the two wild-type p73 species, induced apoptosis in these cells, as verified by changes in nuclear morphology and TUNEL assays performed 36 h after transfection (Fig. 4c). In contrast, no apoptosis was observed in cells transfected with the backbone expression plasmid and was greatly diminished when the corresponding p53 and p73 mutants were tested in parallel (data not shown).

These results suggest that p73 can, at least when overproduced, activate p53-responsive genes and act as a growth suppressor. The latter effect appears to be due, at least in part, to the induction of apoptosis. Whether p73 performs these functions under physiological conditions remains to be determined. Nonetheless, the striking amino-acid sequence similarity between p53 and p73 (ref. 4), together with our results, suggests that p73 and p53 are members of a protein family. Preliminary data suggest that p73, unlike p53, is not induced following exposure of cells to DNA-damaging agents such as ultraviolet irradiation (data not shown). Thus it is conceivable that p73 and p53, although similar to one another, are induced by different signals and play fundamentally different roles with respect to the maintenance of cell homeostasis.

Chromosome 1p36 is thought to harbour two or more neuroblastoma-suppressor genes and *p73* maps to the minimal region of chromosome 1p36 that is commonly deleted in neuroblastoma[4,6,11]. So far, however, no mutations have been identified in the remaining *p73* allele in neuroblastomas that are hemizygous at the *p73* locus[4]. One possibility is that p73 is not a neuroblastoma-suppressor gene; another is that hemizygosity at the *p73* locus contributes to the pathogenesis of neuroblastoma as a result of either a gene-dosage effect or of transcriptional silencing of the remaining allele. Earlier work had indicated that imprinting at 1p36 might contribute to the pathogenesis of neuroblastoma and it has been shown that p73 is monoallelically expressed[4,6,12]. This information, together with the apparent similarity of p73 to a known tumour suppressor, raises the possibility that *p73* encodes a neuroblastoma-suppressor protein. As p53 mutations are exceedingly rare in neuroblastomas[13,14], it may be that p73 acts as a p53-like protein in the precursor cells that give rise to neuroblastomas; consequently it is p73, rather than p53, that is inactivated in these tumours.

Allelic loss at 1p36 has been reported in a variety of human tumours, including melanoma, breast carcinoma and colon carcinoma[12]. What cell types normally produce p73, the significance of multiple p73 isoforms, and whether inactivation of p73 plays a role in human cancer, remain to be determined. Our results raise two possibilities, however: first, that there may be additional p53

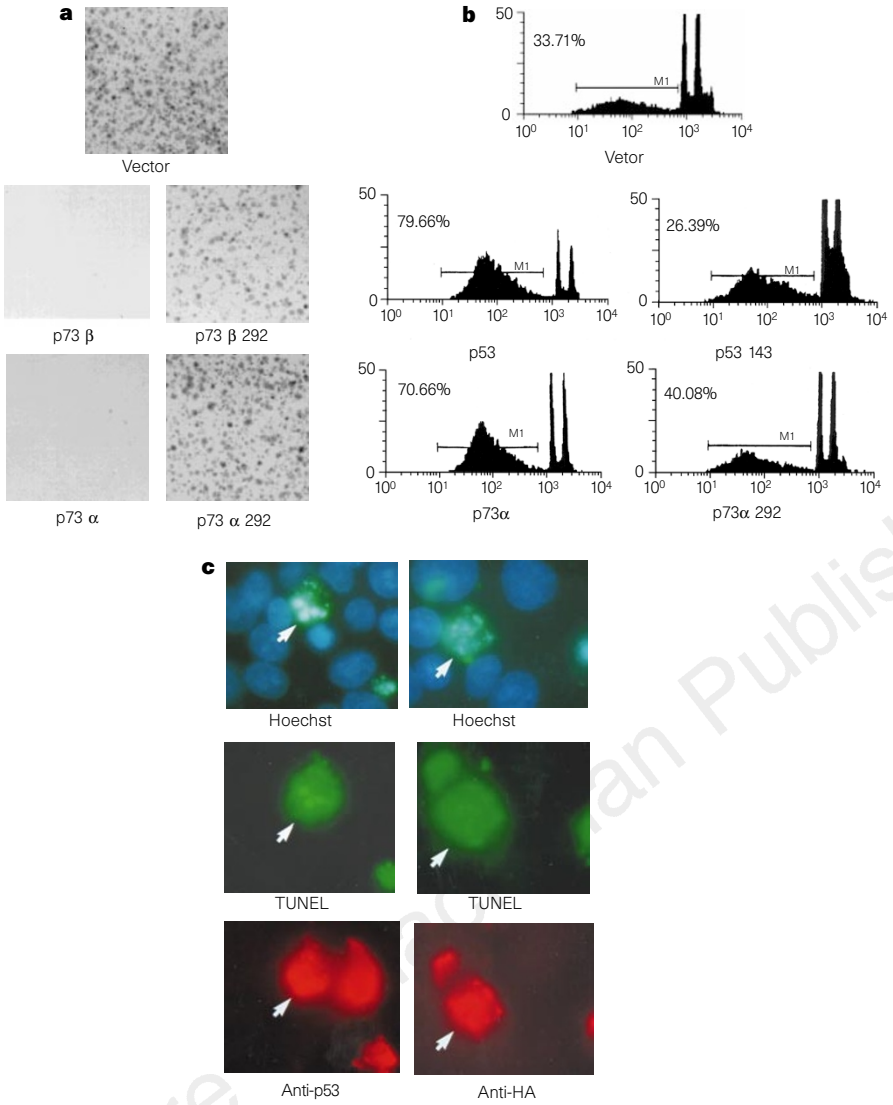

Figure 4 p73 suppresses tumour cell growth and induces apoptosis. **a**, SAOS2 colony suppression by wild-type p73 isoforms. **b**, DNA content of SAOS2 cells transiently transfected with plasmids encoding the indicated proteins, as determined by propidium iodide staining and FACS. **c**, Immunofluorescence and TUNEL analysis of BHK cells transfected with p53 (left) or p73α (right) expression plasmids. Examples of transfected cells are indicated by arrows.

family members; and second, that p53-function in $p53^{-/-}$ carcinomas might, in principle, be restored by activating p73. □

Methods

Plasmid construction. p73 cDNAs were ligated as *Xho*I/*Xba*I fragments into a pCMV1 vector containing a 200-bp lamin 5′ untranslated region and encoding an N-terminal HA tag[15]. The cDNAs were then excised as *Bgl*II–*Xba*I fragments and subcloned into pCDNA3 (Invitrogen).

2 × RGC–CAT and β-globin–CAT were a gift from E. Flemington. 2 × RGC–CAT was generated by introducing double-stranded oligonucleotides containing two RGC p53 sites[16] upstream from the β-globin minimal promoter in β-globin–CAT[17]. The p21 reporter plasmids were gifts from D. Cohen and K. Yu. A ~5.5-kb *Bam*HI–*Bam*HI p21 clone was isolated from a human placental genomic library (Clontech) using a PCR-generated 188-bp probe spanning the p21 transcriptional initiation site and subcloned into pBSK+ (Stratagene). The 3′ end of this p21 clone was digested with *Exo*III nuclease, followed by ligation to an *Xho*I linker so that it corresponded to −4.7 kb to +51 bp with respect to the transcriptional initiation site[18]. A 2.7-kb *Eco*RI–*Xho*I (− 2.7 kb ⟶ 51 bp) fragment was subcloned into pGL2-basic to generate p21-WTluc. This plasmid was restricted with *Kpn*I and *Mlu*I and digested with *Exo*III nuclease to generate a nested set of 5′ deletion mutants. p21Δ8 contains − 514 ⟶ +51 pb, includes two SP1 sites and the p21 TATA box[9,18], but lacks the two p53 binding sites present in p21-WTluc. pCMV-p53 and pCMV-p53V143A were gifts from B. Vogelstein[19].

Immunoprecipitation and western blot analysis. SAOS2 cells were transfected using the BBS/calcium phosphate method[20] and lysed 48 h later in EBC buffer (50 mM Tris, pH 8.0, 120 mM NaCl, 0.5% NP-40) supplemented with aprotonin (11.5 μg ml^{-1}), leupeptin (5 μg ml^{-1}), phenylmethylsulphonyl fluoride (50 μg ml^{-1}), 100 mM NaF, 0.2 mM Na_3VO_4. 150-μg aliquots of cell extract, as determined by the Bradford method, were resolved by electrophoresis in a 10% SDS–polyacylamide gel, transferred to a nylon membrane and probed with an anti-HA antibody (12CA5; Boehringer-Mannheim). Bound protein was detected colorimetrically using an alkaline-phosphatase-conjugated goat anti-mouse antibody (Fisher). Anti-HA (12CA5) immunoprecipitates recovered on protein A-Sepharose and eluted by boiling in SDS-containing sample buffer were similarly analysed.

Immunofluorescence staining. BHK cells were grown on coverslips and transfected using the calcium phosphate method[21]. Twelve hours after removal of the DNA precipitates, cells were processed for immunofluorescence as described[15]. The primary antibody used was anti-HA antibody (12CA5 hybridoma, supernatant diluted 1 : 50) and the secondary was FITC-conjugated anti-mouse antibody (diluted 1 : 500; Boehringer-Mannheim).

SAOS2 cells were grown on coverslips and transfected using BBS/calcium phosphate[22]; 24 h after removing the DNA precipitates, the cells were processed for immunofluorescence. The primary antibodies were anti-HA (12CA5 hybridoma, supernatant diluted 1 : 70) and rabbit polyclonal anti-p21 (C-19 (Santa Cruz) diluted 1 : 500). The secondary antibodies were FITC-conjugated anti-mouse and rhodamine-conjugated anti-rabbit (both diluted 1 : 200; ImmunoResearch).

TUNEL staining. BHK cells were transfected by the BBS method with 20 μg of either p53 or p73α expression plasmid. After removing the DNA precipitate, the cells were placed in serum-free medium; 16 h later, the cells were fixed in PBS + 4% formaldehyde, washed twice with PBS, and permeabilized in 70% ethanol (prechilled to −20 °C) for 30 min at room temperature. Following two washes with PBS, TUNEL staining was performed as described, except that FITC-dUTP (Boehringer-Mannheim) was used in place of biotinylated dUTP[23]. Cells were then processed for immunofluorescence using the anti-p53 antibody 1801 (diluted 1 : 500; Oncogene Science) or anti-HA antibody. The secondary antibody used was rhodamine-conjugated anti-mouse (diluted 1 : 500; Boehringer-Mannheim).

CAT assays. Cells were transfected using the BBS/calcium phosphate method with 5 μg of the indicated reporter plasmid, 2 μg pCMV-β gal, 1–2 μg of the indicated expression plasmids, and pRcCMV (Invitrogen) as a carrier plasmid, to a total of 20 μg. CAT assays were done as before, 24 h after the removal of the DNA precipitates[17].

FACS analysis. SAOS2 cells were transfected with 20 μg of the indicated expression plasmids together with 2 μg of a plasmid encoding the cell-surface marker CD19 (pCD19; a gift from T. Tedder). Anti-CD19 antibody B4 was provided by J. Gribben. CD19-positive cells were analysed for DNA content by FACS ~72 h later, as described[24].

Growth-suppression assay. SAOS2 cells were transfected by the BBS method with 20 μg of the indicated expression plasmids. 48 h later, the cells were placed under G418 selection (600 μg ml^{-1}), fixed, and stained with crystal violet[19,25] ~2 weeks later and photographed.

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Correspondence and requests for materials should be addressed to W.G.K.

Steroid receptor coactivator-1 is a histone acetyltransferase

Thomas E. Spencer*†, Guido Jenster*†‡, Mark M. Burcin*, C. David Allis§, Jianxin Zhou§, Craig A. Mizzen§, Neil J. McKenna*, Sergio A. Onate*, Sophia Y. Tsai*, Ming-Jer Tsai* & Bert W. O'Malley*

** Department of Cell Biology, One Baylor Plaza, Baylor College of Medicine, Houston, Texas 77030, USA*

§ Department of Biology, University of Rochester, Rochester, New York 14627, USA

† These authors contributed equally to this work.

Steroid receptors and coactivator proteins are thought to stimulate gene expression by facilitating the assembly of basal transcription factors into a stable preinitiation complex[1]. What is not clear, however, is how these transcription factors gain access to transcriptionally repressed chromatin to modulate the transactivation of specific gene networks *in vivo*. The available evidence indicates that acetylation of chromatin *in vivo* is coupled to transcription and that specific histone acetyltransferases (HATs) target histones bound to DNA and overcome the inhibitory effect of chromatin on gene expression[2–4]. The steroid-receptor coactivator SRC-1 is a coactivator for many members of the steroid-hormone receptor superfamily of ligand-inducible transcription factors[5]. Here we show that SRC-1 possesses intrinsic histone acetyltransferase activity and that it also interacts with another HAT, p300/CBP-associated factor (PCAF). The HAT activity of SRC-1 maps to its carboxy-terminal region and is primarily specific for histones H3 and H4. Acetylation by SRC-1 and PCAF of histones bound at specific promoters may result from ligand binding to steroid receptors and could be a mechanism by which the activation functions of steroid receptors and associated coactivators enhance formation of a stable preinitiation complex, thereby increasing transcription of specific genes from transcriptionally repressed chromatin templates.

In mammalian cells, a few proteins have been identified as nuclear HATs; these include PCAF[6], p300/CBP[7,8] and $TAF_{II}230/250$[9]. Steroid receptors and recruited cofactors, such as SRC-1 and p300/CBP, may facilitate specific gene transcription through targeted histone acetylation, resulting in localized chromatin remodelling and enhanced assembly of the basal transcription machinery into a stable preinitiation complex.

To determine whether SRC-1 contained histone acetyltransferase (HAT) activity, SRC-1 was immunoprecipitated from COS whole-cell extracts. Antibodies against both SRC-1 and CBP immunoprecipitated proteins with significant HAT activity in a filter-binding assay compared to the negative controls (Fig. 1a). In addition, SRC-1 immunoprecipitates from the T47D cell line also had significant HAT activity (data not shown). The HAT activity of SRC-1 was specific to histones, because BSA was not acetylated by anti-SRC-1 immunoprecipitates under similar conditions. As expected, western blot analysis confirmed that a protein of relative molecular mass (M_r) ~165K (SRC-1) and another of M_r ~265K (CBP) were present in their respective immunoprecipitates (Fig. 1b; lanes 1, 3 and 5). To estimate the HAT activity of SRC-1 relative to CBP, we used ^{35}S-Met and ^{35}S-Cys to label proteins in COS cells and immunoprecipitated SRC-1 and CBP from whole-cell extracts. One-half of the immunoprecipitate was analysed in a liquid HAT assay using free histones, and the other half was separated on a 7% SDS–PAGE gel and analysed by fluorography. Bands containing SRC-1 or CBP were excised and the amount of radioactivity

‡ Present address: Department of Urology, M.D. Anderson Cancer Center, University of Texas, Houston, Texas 77030, USA.