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Purification of Complexes of Nuclear Oncogene p53 with Rat and *Escherichia coli* Heat Shock Proteins: In Vitro Dissociation of hsc70 and dnaK from Murine p53 by ATP

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Oligomeric protein complexes containing the nuclear oncogene p53 and the simian virus 40 large tumor antigen (D. I. H. Linzer and A. J. Levine, Cell 17:43–51, 1979), the adenovirus E1B 55-kilodalton (kDa) tumor antigen, and the heat shock protein hsc70 (P. Hinds, C. Finlay, A. Frey, and A. J. Levine, Mol. Cell. Biol. 7:2863–2869, 1987) have all been previously described. To begin isolating, purifying, and testing these complexes for functional activities, we have developed a rapid immunoaffinity column purification. p53-protein complexes are eluted from the immunoaffinity column by using a molar excess of a peptide comprising the epitope recognized by the p53 monoclonal antibody. This mild and specific elution condition allows p53-protein interactions to be maintained. The hsc70-p53 complex from rat cells is heterogeneous in size, with some forms of this complex associated with a 110-kDa protein. The maximum apparent molecular mass of such complexes is 660,000 daltons. Incubation with micromolar levels of ATP dissociates this complex in vitro into p53 and hsc70 110-kDa components. Nonhydrolyzable substrates of ATP fail to promote this dissociation of the complex. Murine p53 synthesized in *Escherichia coli* has been purified 660-fold on the same antibody affinity column and was found to be associated with an *E. coli* protein of 70 kDa. Immunoblot analysis with specific antisera demonstrated that this *E. coli* protein was the heat shock protein dnaK, which has extensive sequence homology with the rat hsc70 protein. Incubation of the immunopurified p53-dnaK complex with ATP resulted in the dissociation of the p53-dnaK complex as it did with the p53-hsc70 complex. This remarkable conservation of p53-heat shock protein interactions and the specificity of dissociation reactions suggest a functionally important role for heat shock proteins in their interactions with oncogene proteins.

p53 was originally identified in simian virus 40 (SV40)-transformed cells because of its association with the SV40 large T antigen in an oligomeric protein complex (22, 26). In such transformed cells the levels of p53 are greatly increased owing to the stabilization of p53, which has a very short half-life in nontransformed cells (29). Elevated levels of p53 have also been observed in human tumors and in cells transformed by a variety of agents including chemicals and viruses (for a review, see reference 19). Transfection of p53 into primary cells leads to immortalization of these cells in culture (18), and cotransformation of p53 plus an activated *ras* gene generates clonable foci that give rise to tumors in animals (10, 11, 30). Accordingly, p53 is now classified, along with *myc*, *myb*, and *fos*, as a nuclear oncogene. In p53-plus-*ras*-transformed cells, p53 is expressed at levels much higher than normal and is associated with one or both of the heat shock responsive cellular proteins hsp70 and hsc70 (15, 32). The interaction of p53 with hsc70 appears to be analogous to the p53-SV40 T-antigen complex in that the half-life of p53 is greatly extended (11). The possible significance of the p53-hsc70 interactions in contributing to the transformed state is supported by the observation that mutations which activate p53 cDNA clones in a focus-forming assay also result in the synthesis of mutant p53 protein that preferentially forms hsc70-p53 complexes (11, 15).

hsc70 and hsp70 are members of a diverse family of proteins that are induced by stress such as heat, DNA damage, ethanol, or developmental signals (25, 42). In mammalian cells, hsp70 is highly induced by heat shock, whereas hsc70 is present constitutively and is induced only about twofold (31). hsc70 is found at higher concentrations in dividing cells than in resting cells (31). Some activities carried out by these proteins have been identified. For example, hsc70 functions as a clathrin-uncoating ATPase (34), and hsp70 is directed to the nucleolus upon heat shock, where it appears to colocalize with preribosomes (45), and it can be released from the nucleus by ATP (24). However, other functions of these proteins may be more primordial. The *Escherichia coli* homolog dnaK shows an overall homology of 50% with the hsp-hsc70 family of proteins (2), also possesses an ATPase activity (48), and was initially discovered because it is required for the DNA replication of bacteriophage λ (12). The λ DNA replication activity of dnaK may have implications about the function of hsc70 with p53. p53 may function in the pathway for the entry of cells into the S phase of the cell cycle (33).

To begin testing the p53 protein and its oligomeric protein complexes for functional activities, we used an immunoaffinity column with the PAb421 antibody. The procedure is shown to permit rapid purification of p53-hsc70 complexes from p53-plus-*ras*-transformed rat cell lines. A 660,000-dalton (Da) protein complex composed of p53-hsc70 and a third protein of 110 kDa was isolated and shown to dissociate into free p53 and a hsc70 110- kDa product upon incubation with micromolar levels of ATP. Nonhydrolysable forms of

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ATP analogs failed to promote this dissociation. Murine p53 synthesized in *E. coli* was also rapidly purified over 600-fold by this antibody affinity procedure. When this was done, some of the p53 isolated was found in a complex with a 70-kDa protein which was identified as dnaK, a heat shock protein of *E. coli*. The p53-dnaK complex was also dissociated by incubation with ATP. The complexes formed between hsc70-p53 and dnaK-p53 share the same specificity of interaction (resistance to high salt concentrations and washing conditions), as well as the specificity of dissociation conditions. This extensive conservation of protein recognition signals suggests a conservation in function.

MATERIALS AND METHODS

Antisera and other reagents. The rabbit antipeptide antiserum to hsc70/hsp70 was prepared as described by Hinds et al. (15). Rabbit antiserum to dnaK was a gift from C. Georgopoulos (47). PAb421 hybridoma cells producing monoclonal antibodies against p53 were provided by E. Harlow (14). ATP analogs (ADP-NP and ATPγS), other nucleotides, yeast hexokinase, and Sephacryl S-300 were obtained from Pharmacia, Inc.; ^{125}I-protein A was purchased from New England Nuclear Corp.

Production of the synthetic peptide. A peptide, NH_2-LKTKKGQSTSRHKK-COOH, containing the epitope recognized by the p53 monoclonal antibody PAb421 (43), was synthesized on an Applied Biosystems model 403A peptide synthesizer. The peptide was isolated and purified as described previously (15).

Protein assays. Proteins were assayed by the method of Bradford (4). Samples were solubilized in 50 μl of 1.0 N NaOH–0.05% sodium dodecyl sulfate (SDS) and analyzed in a total volume of 5.5 ml. Bovine plasma IgG (Bio-Rad Laboratories) was used as a standard.

Cell lines and extract preparation. A1 cells (15) were maintained in Dulbecco modified Eagle medium plus 10% fetal calf serum, and cell lysates were prepared from cell cultures grown on 15-cm plates. Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and collected into ice-cold PBS by scraping. Cell pellets obtained from five 15-cm plates were stored frozen at −70°C. Pellets were allowed to thaw on ice in 11 ml of lysis buffer (50 mM Tris hydrochloride [pH 8.0], 5 mM EDTA, 150 mM NaCl, and 0.5% Nonidet P-40 [NP-40] containing 1 mM phenylmethylsulfonyl fluoride added just before use from a 200 mM stock in ethanol). The suspension was subjected to five rounds of sonication, 10 s each on channel 5 of a Branson sonicator. Unlysed cells and nuclei were sedimented at 500 × *g* for 10 min at 4°C. The supernatant was centrifuged at 100,000 × *g* for 1 h at 4°C. The protein concentration of the supernatants ranged between 5 and 7 mg/ml.

Plasmid construction. The plasmids pU11-4 and pUKH215 were constructed to place murine p53 coding sequences under the control of the *E. coli* β-galactosidase promoter. pU11-4 was constructed by isolating the 2.4-kilobase *Bam*HI fragment of p11-4 (39) and inserting it in the *Bam*HI site of pUC8 (Bethesda Research Laboratories, Inc.). This procedure was also used to generate pUKH215, except that the p53 coding sequences were derived from SVKH215, a derivative of p11-4 containing an in-frame *Hin*dIII linker at the *Kpn*I site (39). The plasmids were maintained in *E. coli* HB101 (*recA13 hsdR lacY1*). Since some 5′ noncoding sequence is present, these constructs do not place p53 in frame with the β-galactosidase translation initiation codon. Nevertheless, a stop codon at position +2 allows reinitiation of translation to occur and accounts for the observed expression of p53 from these constructs.

Preparation of the immunoaffinity column. Tissue culture supernatant (300 to 500 ml) from PAb421 hybridoma cells (14) was circulated overnight through a 2-ml protein A-Sepharose (Sigma Chemical Co.) column at a flow rate of 60 ml/h as described by Simanis and Lane (36). The column was washed as described previously (36), and immunoglobulin Gs were eluted with 0.1 M sodium citrate (pH 4.0) and collected in an equal volume of 1.0 M Tris (pH 8.0). The eluate was dialyzed against a total of 10^8 volumes of 150 mM NaCl–100 mM $NaHCO_3$ (pH 8.3). Generally, 15 to 20 mg of immunoglobulin G was recovered from 500 ml of tissue culture supernatant. After dialysis, the immunoglobulin Gs were coupled to Affi-Gel 10 (Bio-Rad) at a ratio of 1 to 2 volumes of resin to 4 volumes of antibody (at a concentration of 0.5 to 1 mg/ml) by following the protocol described by the manufacturer. Approximately 1 mg of immunoglobulin Gs was coupled per ml of Affi-Gel 10.

Purification of p53. Cultures of *E. coli* HB101 carrying either pU11-4 or pUKH215 were grown at 37°C in Luria-Bertani medium to an optical density at 600 nm of 0.2 to 0.3. The expression of p53 was induced by adding isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 2 mM. Cultures were incubated at 37°C with aeration for another 4 to 6 h, and cells were collected by centrifugation, washed once in ice-cold PBS, and stored as frozen cell pellets at −20°C. Cell pellets from 2 liters of culture were suspended in 70 ml of ice-cold buffer A (50 mM Tris [pH 8.0], 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). Cells were lysed in a French press (AMINCO) by using two passes at 900 lb/in^2, and the lysate was centrifuged at 500 × *g* for 5 min at 4°C to sediment any remaining intact cells. The supernatant (containing 8 to 10 mg of protein per ml) was diluted with an equal volume of buffer A containing 2% NP-40 and 20 mM 3[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS; Sigma) and stirred for 2 to 3 h at 4°C. The extract was centrifuged (100,000 × *g* for 1 h at 4°C), and the supernatant was diluted with an equal volume of buffer A and circulated overnight at a flow rate of 60 ml/h on a column containing 1 ml of PAb421 immunoaffinity resin. The lysate was cycled through the column one last time, and the flowthrough material was collected. Gel electrophoresis and immunoblotting analysis of the p53 in the column flowthrough fraction indicated that the column was efficiently depleting the lysate of p53. The column was washed at 4°C with 10 to 15 column volumes of the following buffers: buffer A plus 5 mM CHAPS and 0.5% NP-40; buffer A plus 350 mM NaCl and 0.5% NP-40; buffer A plus 0.5% NP-40 and 500 mM LiCl (in place of the NaCl); and, finally, 0.12 M sodium thiocyanate (pH 7.5) plus 0.5% NP-40. These washes removed nonspecific *E. coli* proteins from the resin. The resin was reequilibrated with buffer A plus 0.5% NP-40, removed from the column, and suspended in 5 volumes of buffer A plus 0.5% NP-40 and 100 μg of the peptide containing the PAb421 epitope per ml. After a 1-h incubation at room temperature, the resin was sedimented, the supernatant containing the eluted p53 was collected, and the procedure was repeated one more time. After collection of the second eluate, the column was regenerated by eluting the PAb421 epitope peptide with 100 mM glycine (pH 2.2).

The protocol used to purify p53 from A1 cell extracts was essentially the same as described for the *E. coli* synthesized murine p53. A1 cell lysates (the 100,000 × *g* supernatant

containing 5 to 7 mg of protein per ml) were incubated with PAb421 immunoaffinity resin overnight at 4°C with rotation, at a ratio of 10:1 (vol/vol) lysate to resin. Following incubation, the resin was sedimented (1,000 × *g* for 2 min at 4°C), and then washed in 13–14 volumes of lysis buffer. This washing step was repeated four times. Elution of p53 from the immunoaffinity resin was effected as described above.

Gel electrophoresis and immunoblotting analysis. SDS–10% polyacrylamide gels were prepared and proteins were analyzed as described by Laemmli (21). Following electrophoresis, proteins were transferred to Imobilon PVDF membranes (Millipore Corp.) by using a semidry electroblotter (Sartorius Balances) as specified by the manufacturers. After transfer, membranes were analyzed by immunoblotting in one of two ways. For analysis of p53, the membranes were incubated in blocking buffer (10 mM Tris [pH 7.4], 0.9% NaCl, 5% bovine serum albumin) as described by Burnette (6). These membranes were then incubated for 1 h at 37°C or overnight at 4°C in a 1:1 (vol/vol) solution of hybridoma PAb421 tissue culture supernatant and blocking buffer. Membranes were washed and incubated with 1 μCi of ^{125}I-protein A (Amersham Corp.) as described by Burnette (6). Alternatively, for analysis of the dnaK polypeptide, membranes were incubated for 1 h at room temperature in PBS au lait (10 mM potassium phosphate, 0.2 M NaCl [pH 7.4], 5% Carnation nonfat dry powdered milk). Membranes were then incubated for 1 h at 37°C or overnight at 4°C in PBS–0.2% nonfat powdered milk–rabbit dnaK antiserum at a 1:1,000 (vol/vol) dilution (47). Membranes were then washed in PBS au lait, incubated for 1 h at room temperature in PBS au lait–0.1 μCi of ^{125}I-protein A per ml, and then washed again in PBS au lait. Membranes were air dried and exposed to Kodak XAR-5 film with Cronex Lightning-Plus enhancing screens (E. I. du Pont de Nemours & Co., Inc.) (38).

Densitometric scanning was used to quantitate relative amounts of polypeptides. For silver-stained gels, a picture of the gel on EDP paper (Kodak) was scanned by using reflectance detection. Autoradiograph films of immunoblots were scanned by using transmittance. Twofold serial dilutions of samples were tested to verify the linearity of the detection system.

To stain directly for total proteins transferred to the Imobilon membranes, we used a general protein stain. Following electroblot transfer, membranes were incubated for 10 min at room temperature in 50% methanol–10% acetic acid–0.1% (wt/vol) Coomassie brilliant blue R-250. Membranes were then destained for 10 to 15 min in 50% methanol–10% acetic acid. The last traces of stain were removed by a brief incubation (1 to 2 min) in 90% methanol.

Dissociation of the p53-hsc70 and p53-dnaK complex by ATP. ATP and nucleotide elution conditions were as described by Lewis and Pelham (24). Immunoaffinity resin containing bound p53 and hsc70 was incubated with 4 volumes of TBS (15 mM Tris hydrochloride [pH 7.4], 140 mM NaCl, 5 mM KCl, 3 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) containing designated nucleotides. When indicated, nucleotides were preincubated for 5 min at room temperature with 50 mM glucose and 100 U of yeast hexokinase per ml as described by Schlossman et al. (35). Incubations of resin in TBS plus nucleotides were carried out at either 4°C for 10 min to obtain low levels of elution in the absence of nucleotide or at room temperature for 30 min to obtain maximum yield of nucleotide eluted material.

Immunoaffinity resin containing bound p53 and dnaK was prepared as described above. Elutions with 1 mM ATP and peptide were carried out as described for the hsc70-p53 complex.

Gel filtration chromatography. PAb421 peptide eluates containing p53 and hsc70 or ATP eluates containing hsc70 were applied to a column of Sephacryl S-300 (0.9 cm by 27 cm) equilibrated at 4°C in lysis buffer. Fractions (0.3 ml) were collected at a flow rate of 4.2 ml/h. Molecular weight markers were detected by a Bradford protein assay (4) or by enzymatic activity (alcohol dehydrogenase). The total volume of the column was calculated from the elution position of ^{14}C-labeled amino acids. The void volume was determined from the elution position of blue dextran.

RESULTS

Purification of a p53-hsc70 complex. The A1 cell line is a transformed, tumorigenic cell line derived from transfection of a primary rat embryo fibroblast with pLTRp53cG (a murine cDNA genomic hybrid under the control of the Harvey murine sarcoma virus long terminal repeat) and an activated Ha-*ras* gene (15). An A1 cell lysate was prepared, and a 100,000 × *g* supernatant was cycled over an immunoaffinity column composed of the anti-p53 monoclonal antibody PAb421 (see Materials and Methods). All steps of the purification were monitored by SDS-gel electrophoresis and immunoblotting. The percent yield and the fold purification achieved with the immunoaffinity column were determined by quantitating autoradiographs of immunoblot analyses by densitometric scanning. The activity of p53 (or hsc70) was expressed as the area of the densitometric peak divided by the amount of protein analyzed in the corresponding gel lane. Figure 1A shows the immunoblotting analysis during the cell lysate preparation and adsorption to and elution from the immunoaffinity column. Based on densitometry of the autoradiograph in Fig. 1A and determination of total protein recovered during each step, more than 90% of the p53 and hsc70 proteins were retained in the 100,000 × *g* supernatant. Material not retained by the p53 immunoaffinity column is shown in lane 5. Approximately 15% of the total p53 is present in the flowthrough fraction, and approximately 90% of the hsc70 is in the flowthrough fraction. In other experiments all the p53 in the lysate 100,000 × *g* supernatant could be adsorbed by increasing the amount of immunoaffinity resin to 1 volume of resin per 11 ml of A1 cell lysate (see Materials and Methods). The column was washed with a total of 70 volumes of lysis buffer, and the p53-hsc70 complex was eluted by incubating the column resin with 3 volumes of lysis buffer containing the PAb421 epitope peptide at 100 μg/ml. A peptide-mediated elution of p53 from PAb421 has been described by Wade-Evans and Jenkins (43). Figure 1A, lanes 11 and 12, show two consecutive peptide elutions. The p53 and hsc70 polypeptides are the two major proteins eluted by the PAb421 epitope peptide, as demonstrated by silver staining of SDS-polyacrylamide gels (Fig. 1B). Small amounts of other proteins can be detected when more sample is analyzed. One such species, a polypeptide of 110 kDa, appears to be associated with the p53-hsc70 complex and is discussed below. Other lower-molecular-mass species are likely to be due to proteolytic breakdown products of p53 as determined by Western immunoblotting (data not shown).

The percent yield and fold purification achieved by the immunoaffinity purification are shown in Table 1. The p53 recovered in the two peptide elutions was purified 300-fold with a yield of 45%. hsc70 was copurified along with p53; it was purified approximately 80-fold with a yield of 12%. The

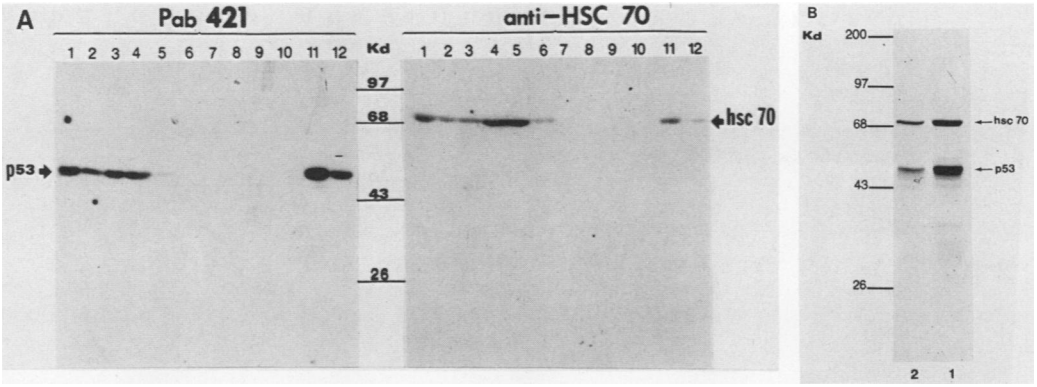

FIG. 1. Copurification of p53 and hsc70 on a PAb421 immunoaffinity column. Fifteen plates of A1 cells (15-cm plates) were lysed, and a soluble extract was incubated with 2.2 ml of PAb421 immunoaffinity resin as described in Materials and Methods. The resin was washed extensively, and bound material was eluted with 100 μg of the PAb421 epitope peptide per ml. (A) Portions of extract at various points during the purification were analyzed by SDS-polyacrylamide gel electrophoresis (10% polyacrylamide) and immunoblotting with either PAb421 or rabbit antiserum to hsc70. Arrows indicate the hsc70 and p53 polypeptides. Samples applied to each lane are as follows: lane 1, 95 μg of 500 × *g* supernatant; lane 2, 57 μg of 500 × *g* pellet; lane 3, 135 μg of 100,000 × *g* pellet; lane 4, 84.5 μg of 100,000 × *g* supernatant; lane 5, 84 μg of nonbound flowthrough material; lanes 6 to 10, 60 μl from each of washes 1 to 5 (each wash contained 14 ml); lane 11, 20 μl of the first 6.25-ml peptide eluate; lane 12, 20 μl of the second 6.25-ml peptide eluate. Molecular mass standards are indicated in kilodaltons (Kd). (B) Aliquots (100 μl) of the first and second peptide elutions were subjected to SDS-polyacrylamide gel electrophoresis and silver staining. Arrows identify the p53 and hsc70 polypeptides. Molecular mass standards are indicated in kilodaltons (Kd).

relatively low fold purification of hsc70 and the lower yield indicate that, as in a variety of other cell types, hsc70 is present at high levels (35, 44). Extracts that contain hsc70 protein but not p53 protein (from HeLa cells) were used as a control to demonstrate that hsc70 does not bind to the p53 antibody column or elute with the p53 epitope peptide.

Dissociation of hsc70 from p53 by ATP. The ATPase activity of hsc70 powers the dissociation of clathrin triskelions from coated vesicles (5) and is required for the release of hsc70 (and hsp70) from both heat-shocked and normal nuclei in vitro (24). To test the effect of ATP on the p53-hsc70 complex, immunoaffinity resin containing bound p53 (and associated hsc70) was treated with various concentrations of ATP. Maximal release of hsc70 from the p53-immunoaffinity resin was attained at 10 μM ATP (Fig. 2). About 60% of the hsc70 can be dissociated from the resin under these conditions (10-min incubation at 4°C). Elution with 0.1 μM ATP released about 30% of the hsc70, whereas buffer alone released approximately 15%. Elutions carried out at room temperature for 30 min resulted in 80% release at 1 μM ATP and greater than 95% release at 1 mM ATP. However, a 40% elution of hsc70 in the absence of nucleotide was observed under these conditions. The concentration of ATP effective at dissociating the hsc70-p53 complex was very close to the K_m for ATP (0.9 μM) measured by Schlossman et al. (35) for the hsc70 clathrin-uncoating reaction. This is also the concentration effective at eluting hsc70 and hsp70 from heat-shocked nuclei (24).

Nonhydrolyzable analogs of ATP (ATPγS and ADP-NP) did not cause any release of hsc70 over background levels (Fig. 2). Other forms of adenine nucleotides, dATP and ADP, were also inactive. Of the other nucleotides tested, only CTP and GTP had any activity over background, and these nucleotides at 1 mM were not as effective as 1 μM ATP. The high specificity for ATP and the apparent requirement for ATP hydrolysis has also been observed for the clathrin disassembly and nuclear disassociation activities of hsc70 (5, 24).

Determination of the size of the p53-hsc70 complex. p53-hsc70 complex eluted by the PAb421 epitope peptide was fractionated on a Sephacryl S-300 gel filtration column. The elution position of p53 coincided with the major peak of hsc70 (Fig. 3A). Also eluting within one fraction of p53 was a protein of 110 kDa. The p53–hsc70–110-Kd protein complex eluted from this column with an apparent molecular weight of 660,000. The size estimate is dependent on these proteins having the same globular shape as the marker proteins. Also, interaction of the complex with a large

TABLE 1. Purification of p53-hsc70 complex[a]

Purification step	Total protein (mg)	p53				hsc70			
		Sp act (U/μg)[b]	Total activity (10^6 U)	Purification (fold)	% Yield	Sp act (U/μg)[b]	Total activity (10^6 U)	Purification (fold)	% Yield
100,000 × *g* supernatant	192	120	23.1	(1)	(100)	101	19.4	(1)	(100)
1st peptide eluate	0.19[c]	36,400	6.83	303	29.6	9,310	1.75	92	9.0
2nd peptide eluate	0.09[c]	38,400	3.60	320	15.6	6,570	0.617	65	3.2
Combined peptide elutions	0.28[c]	37,100	10.5	309	45.5	8,400	2.37	83	12.2

[a] The p53 and hsc70 signals in the Fig. 1 autoradiographs were quantitated by densitometric scanning (see Materials and Methods). Units (fold, percent yield) are arbitrary and are based upon starting material.

[b] Specific activity was calculated by dividing the peak area by the amount of protein applied to the designated gel lane.

[c] The amount of p53 and hsc70 protein in the peptide eluted material was estimated from Coomassie blue and silver stained gels with bovine serum albumin as a standard. Numbers represent the amount of either p53 or hsc70, individually.

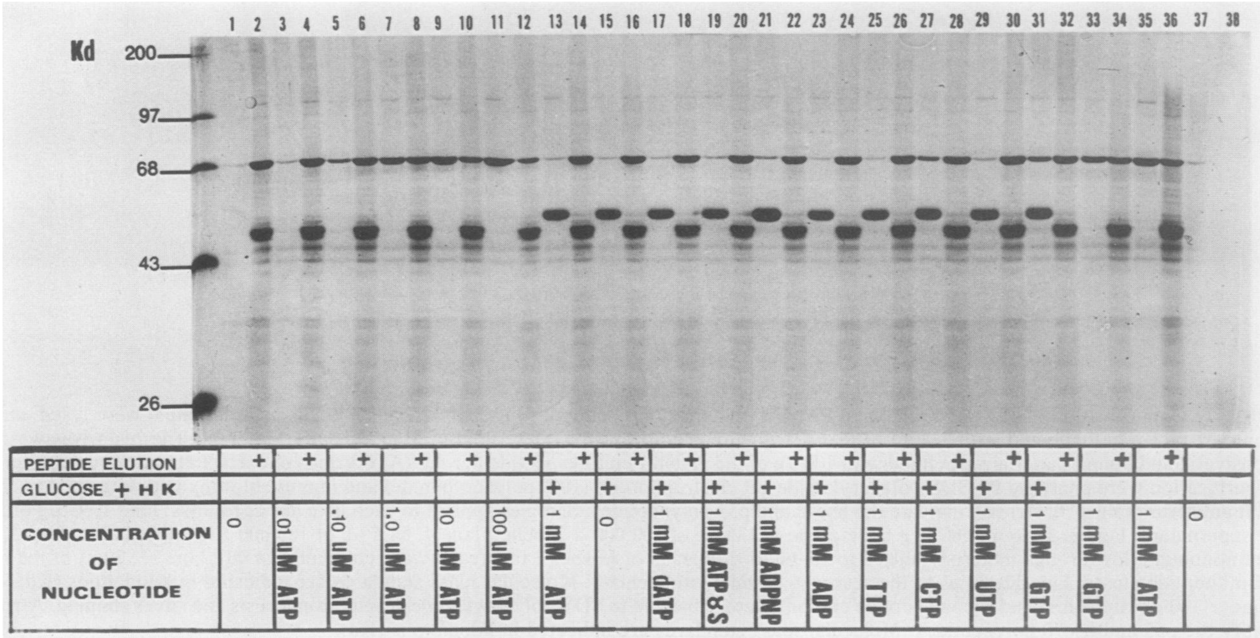

FIG. 2. Dissociation of the p53-hsc70 complex by ATP. PAb421 immunoaffinity resin was incubated with an A1 cell lysate. Nonspecific proteins were removed by a series of five washes as described in the legend to Fig. 1. Aliquots (40 μl) of packed resin (containing p53 and hsc70) were incubated with 160 μl of TBS containing the designated final concentration of nucleotide for 10 min at 4°C. Aliquots (50 μl) of supernatants containing nucleotide and any eluted material were analyzed (odd-numbered lanes). The resin was washed once with 1 ml of lysis buffer and then incubated for 1 h at room temperature in 160 μl of lysis buffer containing 250 μg of PAb421 epitope peptide per ml. Aliquots (50 μl) of supernatants containing peptide and eluted material were analyzed (even-numbered lanes). Lanes 1 and 37 are TBS buffer controls (without any nucleotide); lane 38 is a lysis buffer control (without peptide). +, Pretreatment of various nucleotides with glucose plus hexokinase (HK) (see Materials and Methods). These lanes contain the hexokinase polypeptide of 56 kDa. p53 and hsc70 are identified by arrows. Molecular mass standards are identicated in kilodaltons (Kd).

number of NP-40 monomers of micelles has not been taken into account and would influence the elution position of this complex (8). The second peak of hsc70 has an apparent molecular weight of 125,000 and probably consists of a mixture of monomers and dimers (35). This second, lower-molecular-weight peak of hsc70 is probably due to dissociation of hsc70 from the complex during the chromatography. This is supported by the observation that an ATP-dissociated hsc70 eluted from the column with an apparent molecular weight of 160,000 (Fig. 3B). This elution position is actually within the second peak of hsc70 in Fig. 3A and provides evidence that the largest peak in Fig. 3A represents a complex between p53 and hsc70. The 110-kDA polypeptide coelutes with hsc70 in Fig. 3B. Thus, although ATP dissociates hsc70 from p53, it does not dissociate hsc70 from the 110-kDa species.

The relative molar amounts of hsc70, p53, and the 110-kDa polypeptide in Fig. 3 can be estimated by normalizing the integrated density to molecular weights and assuming that the staining per unit mass is the same. The ratio of p53 to hsc70 in fractions 25 to 31 in Fig. 3A ranges from three to five p53 monomers to one hsc70 monomer. The ratio of hsc70 to 110-kDa protein in the peak fractions in Fig. 3A and B is about 1 to 0.2. Since the overall molecular weight is not large enough to accommodate this stoichiometry, the complexes are very probably heterogeneous.

Plasmid construction and expression of p53 in *E. coli*. A source of naive p53 (that is, p53 free of other cellular or viral proteins) would be useful in studying the properties of p53 alone and in complexes. As an aid in the production of high levels of p53 and to enable its purification free from other cellular or viral proteins, plasmids were constructed (pU11-4 and pUKH215) that placed murine p53-coding sequences under the direction of the *E. coli* β-galactosidase promoter (see Materials and Methods). The p53 polypeptides encoded by these two constructs differ at amino acid position 215 such that the pU11-4 sequence is identical with the sequence of a mouse genomic clone at this position (3) but the pUKH215 sequence contains a linker insertion mutation that replaces Val-Pro with Pro-Ser-Leu-Ala (39). As determined by immunoblotting analysis, *E. coli* HB101 harboring pU11-4 or pUKH215 synthesizes a protein that is recognized by the p53 monoclonal antibody PAb421 (Fig. 4, lane 2; data not shown). This polypeptide comigrates with murine p53, and its synthesis is induced by IPTG (data not shown). Immunoblotting analysis of *E. coli* HB101 in the absence of any plasmid gave no signal (data not shown).

Purification of p53 synthesized in *E. coli*. *E. coli* harboring the pU11-4 or pUKH215 plasmid was grown in Luria-Bertani medium, and p53 synthesis was induced by IPTG as described in Materials and Methods. All steps of the purification were analyzed by gel electrophoresis and immunoblotting. Cells were suspended in buffer A and lysed in a French press, and the detergents CHAPS and NP-40 were added. This detergent combination was required to solubilize p53, since in the absence of detergents the majority of p53 was associated with bacterial membrane fragments. The combination of CHAPS and NP-40 proved to be as effective as 4 M urea in solubilizing p53. By this procedure, approximately 50% of the total p53 in *E. coli* was soluble under conditions of centrifugation for 1 h at 100,000 × *g*. The detergent extract was applied to a PAb421 immunoaffinity column, and the column was washed with several buffers which did not elute p53 but which did elute other *E. coli*

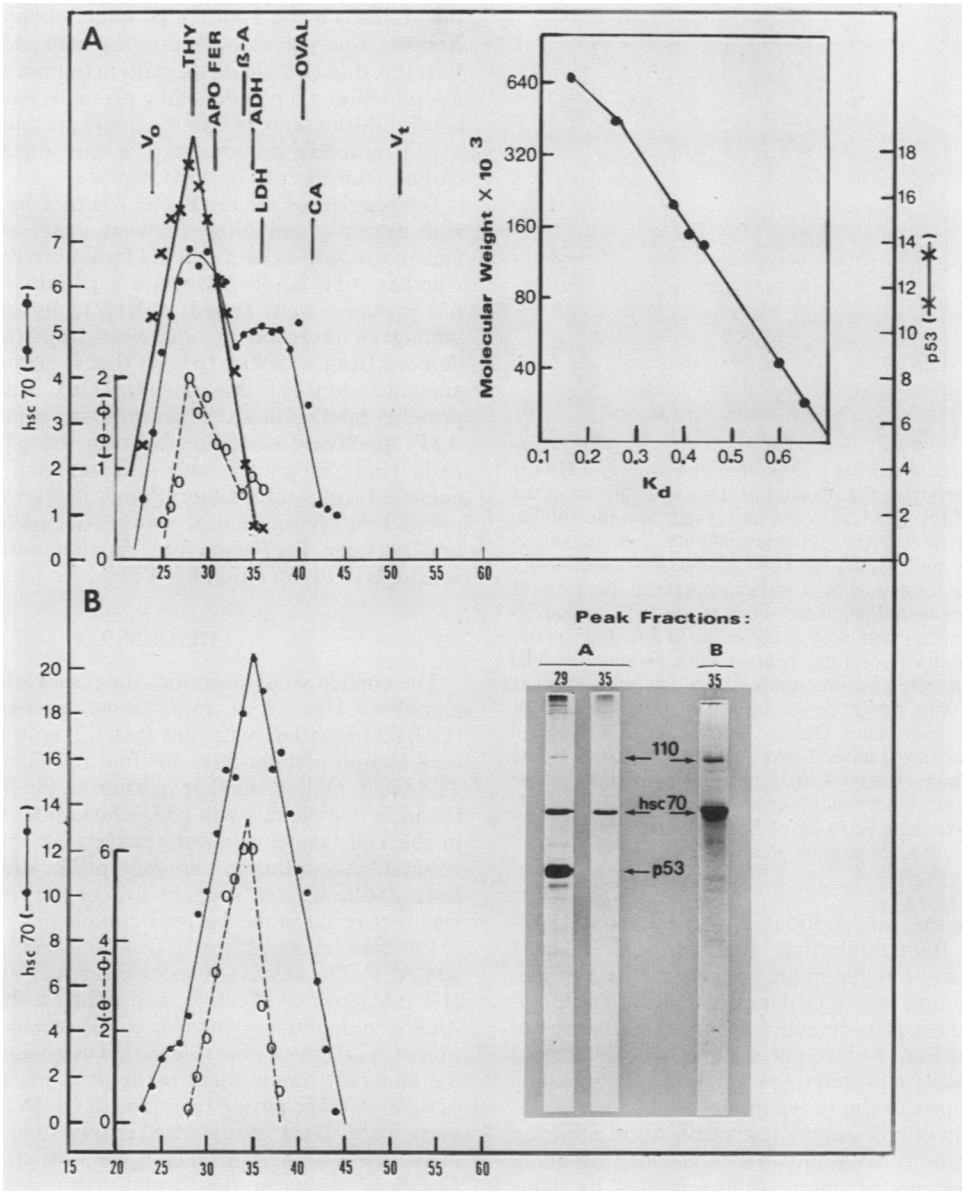

FIG. 3. Gel filtration chromatography of the hsc70-p53 complex on Sephacryl S-300. PAb421 immunoaffinity resin containing p53 and hsc70 was prepared as described in the legend to Fig. 2. (A) PAb421 epitope peptide-eluted material from 0.30 ml of packed resin (approximately 50 μg of complex) was fractionated as described in Materials and Methods. Relative amounts of p53 (×) hsc70 (●), and the 110-kDa protein (○) were quantitated in each fraction by densitometric scanning of a silver-stained SDS-polyacrylamide gel. The scales represent integrated density; the units are arbitrary but comparable within each panel. Marker proteins are indicated by vertical arrows and were assayed as described in Materials and Methods. The distribution coefficients K_d [(peak volume − void volume)/(total volume − void volume)] of the marker proteins are shown in the inset in panel A. Molecular weight values used were as follows: thyroglubulin, 669,000; apoferritin, 443,000; β-amylase, 200,000; alcohol dehydrogenase, 150,000; lactate dehydrogenase, 136,000; ovalbumin, 43,000; and carbonic anhydrase, 29,000. The peak containing p53, hsc70, and the 100-kDa protein has a K_d of 0.167, and the peak containing hsc70 has a K_d of 0.444. Approximate molecular weights obtained from these data are 660,000 and 125,000 for the complex and hsc70, respectively. (B) ATP-eluted material from 0.30 ml of packed immunoaffinity resin (approximately 25 μg) was fractionated, and relative amounts of hsc70 (●) and the 110-kDa protein (○) were quantitated as in panel A. The peak in panel B has a K_d of 0.407, indicating an approximate molecular weight of 160,000. The inset in panel B shows a silver-stained SDS-polyacrylamide gel containing peak fractions from both peptide-eluted fractions (fraction 29 and 35) from panel A and the ATP-eluted fraction (fraction 35) from panel B material. The 110-kDa polypeptide, hsc70, and p53 are indicated by arrows.

proteins (see Materials and Methods). The PAb421 immunoaffinity column effectively bound p53; there was no detectable p53 by immunoblot analysis in the flowthrough material or in any of the washes (data not shown). p53 was gently and efficiently eluted by incubating the column resin in buffer A supplemented with 0.5% NP-40 and 100 μg of the peptide containing the PAb421 epitope per ml. The elution ability of the peptide at 100 μg/ml was equal in efficiency to that of highly chaotropic buffers, including 4 M sodium thiocyanate, 2.5 M $MgCl_2$, 100 mM triethylamine (pH 11.5), and 100 mM glycine (pH 2.2). Figure 4, lane 2, shows the proteins present in the *E. coli* lysate applied to the column,

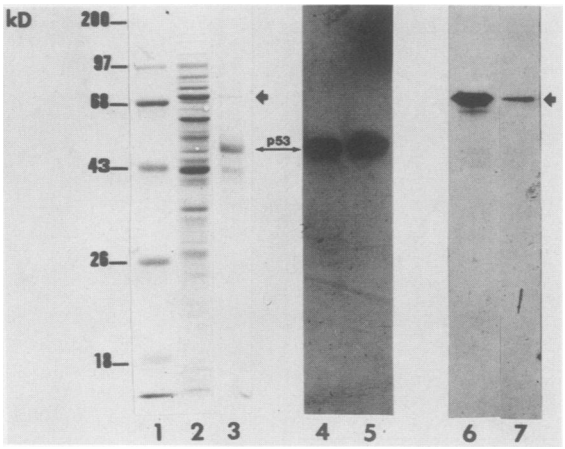

FIG. 4. Association of murine p53 synthesized in *E. coli* HB101 and dnaK. *E. coli* harboring the plasmid pU11-4 were grown in the presence of IPTG. Cells were collected and lysed, and the soluble extract was applied to a PAb421 immunoaffinity column as described in Materials and Methods. The column was extensively washed and p53 was eluted with a molar excess of the PAb421 epitope peptide. Protein samples were subjected to SDS-polyacrylamide gel electrophoresis and then transferred to Imobilon membranes and either stained for total protein with Coomassie blue (lanes 1 to 3) or subjected to immunoblot analysis with PAb421 (lanes 4 and 5) or with rabbit dnaK antiserum (lanes 6 and 7). Samples applied to each lane are as follows: lane 1, protein molecular weight standards; lanes 2 and 5, 100 μg of *E. coli* lysate (100,000 × *g* supernatant); lane 3, 0.25 ml of the PAb421 epitope peptide eluate concentrated by lyophilization and analyzed; lane 4, 20 μl of peptide eluate; lane 6, 10 μg of *E. coli* lysate (100,000 × *g* supernatant); lane 7, 60 μl of peptide eluate.

and lane 3 shows the polypeptides eluted by the PAb421 epitope peptide. Immunoblotting analysis with PAb421 showed that the major polypeptide species in the peptide-eluted column fraction was p53 (lanes 4 and 5). The polypeptide at 43,000 D is also detected in longer exposures of the immunoblot in Fig. 4 (data not shown), thus indicating that it is very probably a protelytic product of p53 containing the carboxyl terminus of the polypeptide.

The percent yield of p53 and the fold purification achieved with the immunoaffinity column were determined by quantitating autoradiographs of immunoblot analyses by using densitometric scanning as described in Materials and Methods. The preparation of p53 shown in Fig. 4 was purified 660-fold with a yield of 26%; 240 μg of p53 was recovered from 638 mg of total *E. coli* proteins. This purification protocol generally resulted in yields of p53 between 20 and 30% and in purifications ranging between 500- and 900-fold.

Complex formation between p53 and *E. coli* dnaK. Another polypeptide, of 70,000 Da, was consistently observed to coelute with p53 (Fig. 4, lane 3). The 70,000-Da polypeptide was not removed by washing conditions that failed to elute p53. Since the 70,000-Da polypeptide did not bind directly to the PAb421 antibody (Fig. 4, lanes 4 and 5), it was concluded that this species was forming a complex with p53. Previous studies of the hsc70-p53 complex in transformed rat cells led us to postulate that this polypeptide might be an *E. coli* heat shock protein. *E. coli* possesses a remarkably conserved counterpart to hsc70: a 70,000-Da heat shock protein, dnaK (2). Thus, it seemed likely that this persistent contaminant of 70,000 Da might be dnaK. This was investigated directly by using a rabbit dnaK antiserum (47). Immunoblot analysis of the PAb421 epitope peptide-eluted column fraction shown in Fig. 4, lanes 6 and 7, provided immunological proof that the 70,000-Da polypeptide copurifying with p53 is indeed dnaK. Extracts that contain dnaK protein but not p53 protein (from *E. coli* without a p53-encoding plasmid) have been used as a control. More than 98% of the dnaK protein does not bind to a p53 antibody column, nor is any dnaK detected after elution with the p53 epitope peptide.

Dissociation of the dnaK-p53 complex by ATP. Given the high degree of homology between dnaK and hsc70 and the fact that dnaK exhibits an ATPase activity (46), we tested whether ATP could dissociate a p53-dnaK complex. Two p53 proteins were tested: a KH215 linker insertion (containing an insertion at amino acid position 215) and p53 derived from a cDNA (p11-4) that is identical to a murine genomic clone at this position (39). Both of these p53 proteins bind to dnaK when they are expressed in *E. coli*. ATP effectively elutes dnaK from the p53-immunoaffinity resin (Fig. 5, lanes 3 and 4) compared with the elution achieved with buffer alone (Fig. 5, lanes 5 and 6). Thus, the interaction between dnaK and p53 is similar to the interaction between hsc70 and p53, in that both complexes are sensitive to dissociation by ATP.

DISCUSSION

The current study describes the purification of a p53-hsc70 complex. Use of a monoclonal antibody murine p53 (PAb421) together with the PAb421 epitope peptide-mediated elution resulted in a 300-fold purification of p53 and a 45% yield. Only a small proportion of the hsc70 present was found in complexes with p53, since about 90% of the hsc70 in the cell lysate was not retained by the immunoaffinity column. Accordingly, the fold purification of hsc70 was lower (80-fold), and roughly 10% of the total hsc70 present was recovered in the purified complex.

Efficient release of hsc70 from p53 can be effected by 1.0 μM ATP. The release is specific for ATP; other nucleotides at 1 mM are not effective, and nonhydrolyzable analogs of ATP were inactive in mediating this release. The concentration of ATP that is effective in this dissociation reaction, and the apparent requirement for hydrolysis, are characteristic of other ATP-requiring reactions in which hsc70 is known to participate. This study also shows that ATP dissociates dnaK from a dnaK-p53 complex. Most of the sequence homology between dnaK and hsc70 is localized to the amino-terminal portion (16), the domain which is thought to contain ATP hydrolysis activity (7, 24).

The significance of the p53-hsc70 complex remains to be elucidated, but the ATP-mediated dissociation of hsc70 from p53 may prove to be analogous to the other ATP-driven reactions performed by hsc70. The ATPase activity of hsc70 has been demonstrated to drive the dissociation of clathrin triskelions from coated vesicles (34) and to be required for effective release of hsc70 from heat-shocked nucleoli (24). Although the function of the dnaK ATPase is still uncertain, dnaK is required for primosome assembly on the λ origin in in vitro λ DNA replication systems, and participates in a localized λ origin-unwinding reaction (9). Since the dnaK ATPase activity can be modulated by λ-encoded DNA replication proteins (46), ATP hydrolysis by dnaK may be involved in the λ DNA replication process. It is tempting to speculate that ATP hydrolysis by the hsc70-p53 complex is mediating a similar type of reaction.

Alternatively, hsc70 could be mediating a p53-disaggregating type of reaction, similar to the clathrin-uncoating activity. In this regard, it is interesting that the p53-hsc70

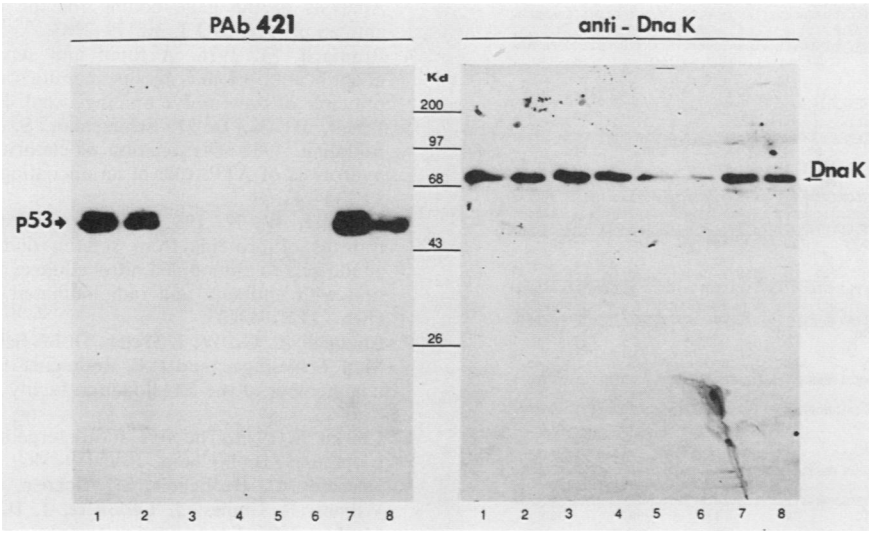

FIG. 5. Dissociation of the dnaK-p53 complex by ATP. Murine p53 synthesized in *E. coli* HB101 from the plasmids pU11-4 or pUKH215 was purified by immunoaffinity chromatography as described in Materials and Methods. The resin was washed as described, and aliquots of resin were incubated with 5 volumes of one of the following elution buffers: 100 μg of PAb421 epitope peptide per ml at room temperature for 30 min in lysis buffer (lanes 1 and 2); 1 mM ATP in TBS at room temperature for 10 min (lanes 3 and 4); TBS at room temperature for 10 min (lanes 5 and 6); or, following ATP elution, 100 μg of PAb421 epitope peptide per ml as described for lanes 1 and 2 (lanes 7 and 8). p53 is derived either from the plasmid pU11-4 (lanes 1, 3, 5, and 7) or from pUKH215 (lanes 2, 4, 6, and 8). Eluted material (100 μl) was analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with either PAb421 or rabbit antiserum to *E. coli* dnaK. Arrows identify the dnaK and p53 polypeptides. Molecular mass standards are indicated in kildaltons (kD).

complex is very large, with an apparent molecular weight of 660,000. The ratio of p53 and hsc70 monomers ranges from about 3:1 to 5:1. Perhaps hsc70 is responsible for keeping p53 in a disaggregated or soluble form. Another component of at least some of the p53-hsc70 complexes is a 110,000-Da protein. This polypeptide is present in substoichiometric amounts, indicating that it is present in only a subpopulation of the complexes or that it is readily dissociated from the complex. The 110,000-dalton polypeptide appears to be associated with hsc70, since ATP causes the hsc70–110,000-Da polypeptide complex to dissociate from p53. It is interesting that a 110,000-Da heat shock protein has been described that is localized to nucleoli (37). The identity of the 110,000-Da species is currently under investigation.

Although one of the reasons for using a bacterial expression system was to avoid purifying complexes of p53 with cellular or viral proteins, such a complex was in fact observed. The complex formed between *E. coli* dnaK and murine p53 demonstrates the same specificity of interaction as the complexes formed by p53 and rat hsc70 (15) or p53 and the large T antigen of SV40 (26). There is a 50% overall homology between dnaK and the hsp70 family of proteins (16). The evolutionary conservation of sequence between dnaK and hsc70 is now illustrated in another way, by the current observation that these two proteins bind to the same ligand, p53. The high degree of homology of dnaK and rat hsc70 is shown in Fig. 6. There are eight regions designated by boxes that show between 82 and 92% identity. Since identity in sequence implies identity in function, it seems likely that one or more of these regions is involved in binding to p53.

dnaK and hsc70 show other intriguing similarities. Both proteins hydrolyze ATP with similar kinetics (one ATP hydrolyzed per min per protein monomer) (5, 46). The ATPase activity of hsc70 is required for the disassembly of clathrin triskelions from clathrin-coated vesicles (5). There is some evidence that the ATPase activity resides in the amino-terminal fragment of hsc70; such a fragment hydrolyzes ATP in a clathrin-independent manner, suggesting that at least part of the clathrin-binding site maps to the carboxyl terminus (7). The ATPase activity of dnaK may be modulated in vitro (up and down, respectively) by the λO and λP proteins (46). These proteins are responsible for directing the DNA replication enzymes of *E. coli* to the λ origin of replication (23). DNA replication of λ is dependent on dnaK; in vitro DNA replication systems indicate that dnaK is required for the formation of a preprimosome complex and is involved in a localized unwinding reaction at the λ origin (9, 46). Pelham (31) has speculated that the functions of dnaK and hsc70 are similar in that they both mediate the assembly and disassembly of large protein-protein or protein-nucleic acid complexes.

Although there is no requirement for dnaK in the replication of the *E. coli* chromosome (20), all of the dnaK mutants obtained are temperature sensitive for growth (12). At the restrictive temperature these mutants exhibit a loss of cell division control; DNA and RNA synthesis is inhibited, and the cells form long filaments, indicating the failure to divide (17, 41). It is interesting that in p53-plus-*ras*-transformed cells, which express abnormally high levels of p53 and hsc70, the cells have abnormal growth patterns and an altered control of cell division (15). In such cells the p53 in a hsc70-p53 complex has a longer half-life than free p53 (11), in a manner analogous to the observed stabilization of p53 in T-antigen–p53 complexes from SV40-transformed cells (29).

Yet another parallel may be drawn between the ability of hsc70 to regulate levels of p53 and the ability of dnaK to regulate levels of σ^{32} in *E. coli*. σ^{32} levels mediate the induction of the *E. coli* heat shock response (13). dnaK mutants are unable to bring about the normal down regulation of the heat shock response (40). dnaK has been shown to alter σ^{32} levels by a mechanism that is posttranscriptional (13), and it seems likely that dnaK is controlling levels of σ^{32} by controlling σ^{32} stability (1, 27). Thus, these two proteins,

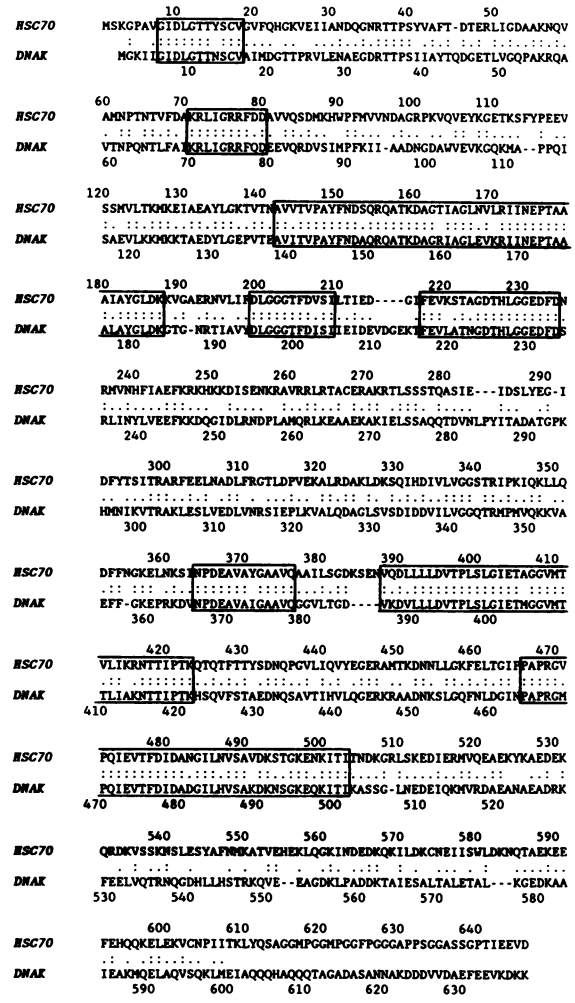

FIG. 6. Comparison of the predicted amino acid sequences of *E. coli* dnaK and rat hsc70. The amino acids identical in each sequence are shown by two dots. Conservative substitutions are designated by one dot. The eight boxed sequences delineate regions of at least 10 residues which show greater than 80% homology. The sequence of dnaK is obtained from Bardwell and Craig (2). The sequence of hsc70 is from O'Malley et al. (28).

dnaK and hsc70, may also serve to mediate the levels of proteins involved in controlling growth.

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LITERATURE CITED

1. **Bahl, H., H. Echols, D. B. Straus, D. Court, R. Crowl, and C. P. Georgopoulos.** 1987. Induction of the heat shock response of *E. coli* through stabilization of σ^{32} by the phage λ cIII protein. Gene Dev. **1**:57–64.
2. **Bardwell, J. C. A., and E. A. Craig.** 1984. Major heat shock gene of Drosphila and the *Escherichia coli* heat-inducible dnaK gene are homologous. Proc. Natl. Acad. Sci. USA **81**:848–852.
3. **Bienz, B., R. Zakut-Houri, D. Givol, and M. Oren.** 1984. Analysis of the gene coding for the murine cellular tumor antigen p53. EMBO J. **3**:2179–2183.
4. **Bradford, M.** 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
5. **Braell, W. A., D. M. Schlossman, S. L. Schmid, and J. E. Rothman.** 1984. Dissociation of clathrin coats coupled to the hydrolysis of ATP: role of an uncoating ATPase. J. Cell Biol. **99**:734–741.
6. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112**:195–203.
7. **Chappell, T. G., W. J. Welch, D. M. Schlossman, K. B. Palter, M. J. Schlesinger, and J. E. Rothman.** 1986. Uncoating ATPase is a member of the 70 kilodalton family of stress proteins. Cell **45**:3–13.
8. **Clarke, S.** 1975. The size and detergent binding of membrane proteins. J. Biol. Chem. **250**:5459–5469.
9. **Dodson, M., H. Echols, S. Wickner, C. Alfano, K. Mensa-Wilmot, B. Gomes, J. LeBowitz, J. D. Roberts, and R. McMacken.** 1986. Specialized nucleoprotein structures at the origin of replication of bacteriophage λ: localized unwinding of duplex DNA by a six protein reaction. Proc. Natl. Acad. Sci. USA **83**:7638–7642.
10. **Eliyahu, D., A. Raz, P. Gross, D. Givol, and M. Oren.** 1984. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature (London) **312**:646–649.
11. **Finlay, C., P. Hinds, A. B. Frey, and A. J. Levine.** 1988. Mutations which activate p53 transformation with *ras* produce an altered p53 protein that preferentially binds to a heat shock protein hsc70. Mol. Cell. Biol. **8**:531–539.
12. **Friedman, D. I., E. R. Olson, C. Georgopoulos, K. Tilly, I. Herskowitz, and F. Benvett.** 1984. Interactions of bacteriophage and host macromolecules in the growth of bacteriophage λ. Microbiol. Rev. **48**:299–325.
13. **Grossman, A. D., D. B. Straus, W. A. Walter, and C. A. Gross.** 1987. σ^{32} synthesis can regulate the synthesis of heat shock proteins in *Escherichia coli*. Gene Dev. **1**:179–184.
14. **Harlow, E., L. V. Crawford, D. C. Pim, and N. M. Williamson.** 1981. Monoclonal antibodies specific for simian virus 40 tumor antigen. J. Virol. **39**:861–869.
15. **Hinds, P., C. Finlay, A. Frey, and A. J. Levine.** 1987. Immunological evidence for the association of p53 with a heat shock protein, hsc70, in p53-plus-*ras*-transformed cell lines. Mol. Cell. Biol. **7**:2863–2869.
16. **Hunt, C., and R. I. Morimoto.** 1985. Conserved features of eukaryotic hsp70 genes revealed by comparison with the nucleotide sequence of human hsp70. Proc. Natl. Acad. Sci. USA **82**:6455–6459.
17. **Itikawa, H., and J.-I. Ryu.** 1979. Isolation and characterization of a temperature-sensitive *dnaK* mutant of *Escherichia coli* B. J. Bacteriol. **138**:339–344.
18. **Jenkins, J. R., K. Rudge, and G. A. Currie.** 1984. Cellular immortalization by a cDNA clone encoding the transformation associated phosphoprotein p53. Nature (London) **312**:651–654.
19. **Klein, G.** 1982. Advances in viral oncology, vol. 2. Raven Press, Publishers, New York.
20. **Kornberg, A.** 1980. DNA replication. W. H. Freeman, San Francisco.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
22. **Lane, D. P., and L. V. Crawford.** 1980. The complex between simian virus 40 T antigen and a specific host protein. Proc. R. Soc. Lond. Sect. B. **210**:451–463.
23. **LeBowitz, J. H., M. Zylicz, C. Georgopoulos, and R. McMacken.** 1985. Initiation of DNA replication on single stranded DNA templates catalyzed by purified replication proteins of bacteriophage λ and *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:3988–3992.
24. **Lewis, M. J., and H. R. B. Pelham.** 1985. Involvement of ATP in

the nuclear and nucleolar functions of the 70Kd heat shock protein. EMBO J. 4:3137–3143.
25. **Lindquist, S.** 1986. The heat shock response. Annu. Rev. Biochem. 55:1151–1191.
26. **Linzer, D. I. H., and A. J. Levine.** 1979. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells. Cell 17:43–51.
27. **Neidhardt, F. C.** 1987. What bacteriologists have learned about heat shock. Gene Dev. 1:109–110.
28. **O'Malley, K., A. Mauron, J. D. Barchas, and L. Kedes.** 1985. Constitutively expressed rat mRNA encoding a 70-kilodalton heat-shock-like protein. Mol. Cell. Biol. 5:3476–3483.
29. **Oren, M., W. Maltzman, and A. J. Levine.** 1981. Post-translational regulation of the 54-kilodalton cellular tumor antigen in normal and transformed cells. Mol. Cell. Biol. 1:101–110.
30. **Parada, L. F., H. Land, R. A. Weinberg, D. Wolf, and V. Rotter.** 1984. Cooperation between gene encoding p53 tumour antigen and *ras* in cellular transformation. Nature (London) 312:649–651.
31. **Pelham, H. R. B.** 1986. Speculations on the functions of the major heat shock and glucose-regulated proteins. Cell 46:959–961.
32. **Pinhasi-Kimhi, O., D. Michalovitz, A. Ben-Zeev, and M. Oren.** 1986. Specific interaction between the p53 cellular tumour antigen and major heat shock proteins. Nature (London) 320: 182–185.
33. **Reich, N. C., and A. J. Levine.** 1984. Growth regulation of a cellular tumor antigen, p53, in nontransformed cells. Nature (London) 308:199–210.
34. **Rothman, J. E., and S. L. Schmid.** 1986. Enzymatic recycling of clathrin from coated vesicles. Cell 46:5–9.
35. **Schlossman, D. M., S. L. Schmid, W. A. Braell, and J. E. Rothman.** 1984. An enzyme that removes clathrin coats: purification of an uncoating ATPase. J. Cell Biol. 99:723–733.
36. **Simanis, V., and D. P. Lane.** 1985. An immunoaffinity purification procedure for SV40 large T antigen. Virology 144:88–100.
37. **Subjeck, J. R., T. Shyy, J. Shen, and R. J. Johnson.** 1983. Association between the mammalian 110,000-dalton heat-shock protein and nucleoli. J. Cell Biol. 97:1389–1395.
38. **Swanstrom, R., and P. R. Shank.** 1978. X-ray intensifying screens greatly enhance the detection by autoradiography of the radioactive isotopes ^{32}P and ^{125}I. Anal. Biochem. 86:184–192.
39. **Tan, T.-H., J. Wallis, and A. J. Levine.** 1986. Identification of the p53 protein domain involved in formation of the simian virus 40 large T antigen-p53 protein complex. J. Virol. 59:574–583.
40. **Tilly, K., N. McKittrick, M. Zylicz, and C. Georgopoulos.** 1983. The dnaK protein modulates the heat shock response of *Escherichia coli*. Cell 34:641–646.
41. **Tsuchido, T., R. A. Van Bogelen, and F. C. Neidhardt.** 1986. Heat shock response in *Escherichia coli* influences cell division. Proc. Natl. Acad. Sci. USA 83:6959–6963.
42. **Van Bogelen, R. A., P. M. Kelley, and F. C. Neidhardt.** 1987. Differential induction of heat shock, SOS, and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli*. J. Bacteriol. 169:26–32.
43. **Wade-Evans, A., and J. R. Jenkins.** 1985. Precise epitope mapping of the murine transformation-associated protein p53. EMBO J. 4:699–706.
44. **Welch, W. J., and J. R. Feramisco.** 1985. Rapid purification of mammalian 70,000-dalton stress proteins: affinity of the proteins for nucleotides. Mol. Cell. Biol. 5:1229–1237.
45. **Welch, W. J., and J. P. Suhan.** 1986. Cellular and biochemical events in mammalian cells during and after recovery from physiological stress. J. Cell Biol. 103:2035–2052.
46. **Yamamoto, T., J. McIntyre, S. M. Sell, C. Georgopoulos, D. Skowyra, and M. Zylicz.** 1987. Enzymology of the pre-priming steps in λdv DNA replication *in vitro*. Proc. Natl. Acad. Sci. USA 84:7996–7999.
47. **Zylicz, M., and C. Georgopoulos.** 1984. Purification and properties of the *Escherichia coli* dnaK replication protein. J. Biol. Chem. 259:8820–8825.
48. **Zylicz, M., J. D. LeBowitz, R. McMacken, and C. Georgopoulos.** 1983. The dnaK protein of *Escherichia coli* possesses an AT Pase and autophosphorylation activity and is essential in an *in vitro* DNA replication system. Proc. Natl. Acad. Sci. USA 80:6431–6435.