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Mutant conformation of p53 translated *in vitro* or *in vivo* requires functional HSP90

(mutated p53/geldanamycin/chaperone/conformational recognition)

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ABSTRACT **The p53 mutant, 143^{ala}, was translated *in vitro* in either rabbit reticulocyte lysate (RRL) or wheat germ extract (WGE). In RRL, p53-143^{ala} protein of both mutant and wild-type conformation, as detected immunologically with conformation-specific antibodies, was translated. The chaperone protein HSP90, present in RRL, was found to coprecipitate only with the mutated conformation of p53. Geldanamycin, shown previously to bind to HSP90 and destabilize its association with other proteins, decreased the amount of immunologically detectable mutated p53 and increased the amount of detectable wild-type protein, without affecting the total translation of p53. When translated in WGE, known to contain functionally deficient HSP90, p53-143^{ala} produced p53 protein, which was not recognized by a mutated conformation-specific antibody. In contrast, the synthesis of conformationally detectable wild-type p53 in this system was not compromised. Reconstitution of HSP90 function in WGE permitted synthesis of conformationally detectable mutated p53, and this was abrogated by geldanamycin. Finally, when p53-143^{ala} was stably transfected into yeast engineered to be defective for HSP90 function, conformational recognition of mutated p53 was impaired. When stable transfectants of p53-143^{ala} were prepared in yeast expressing wild-type HSP90, conformational recognition of mutated p53 was antagonized by macbecin I, a geldanamycin analog also known to bind HSP90. Taken together, these data demonstrate a role for HSP90 in the achievement and/or stabilization of the mutated conformation of p53-143^{ala}. Furthermore, we show that the mutated conformation of p53 can be pharmacologically antagonized by drugs targeting HSP90.**

The tumor suppressor protein p53 is one of the most commonly mutated proteins found in tumor cells (1, 2). A large percentage of tumors retain expression of mutated p53 even if the wild-type allele is deleted, suggesting a dominant positive role for some forms of mutated p53 in tumorigenesis (3, 4). In addition, mutated p53 protein can interact with and inactivate the wild-type protein, thereby demonstrating a dominant negative function in situations in which one wild type allele still remains (5, 6). The vast majority of p53 mutations cluster in four highly conserved regions of the gene and commonly result in a protein of altered conformation and prolonged half-life (for review see ref. 7). Both mutated and wild-type conformations of the protein are immunologically distinct and can be recognized by conformation-specific monoclonal antibodies (8–11).

Wild-type p53 protein is conformationally flexible (12–14). Thus, growth stimulation of normal lymphocytes has been reported to convert wild-type p53 to a "mutated" conformation (15), while the addition of fresh serum results in loss of the wild-type conformation of murine p53 (16). To date, however, loss of p53's mutated conformation has only been reported during dimethyl sulfoxide-induced differentiation of erythroleukemia cells (17). Understanding the parameters that regulate p53's mutant conformation might allow one to interfere with this process, thereby inactivating the mutant protein and in some cases perhaps even restoring its normal function.

We reported recently that geldanamycin (GA), a benzoquinone ansamycin antibiotic, destabilized mutated p53 proteins in several breast, prostate, and leukemia cell lines (18). Without affecting p53 mRNA level or protein synthesis, GA reduced the half-life of the mutated proteins and, intriguingly, abrogated their recognition by mutated conformation-specific monoclonal antibodies. Neither the steady-state level nor DNA damage inducibility of the wild-type protein was affected by GA.

The heat shock protein HSP90 is required for achievement of the functional conformation of several transcription factors, including MyoD, members of the steroid receptor family, retinoid receptors, and the aryl hydrocarbon receptor (19–27). We demonstrated previously that a predominant intracellular target of GA and other benzoquinone ansamycins is HSP90 (28). By specifically binding to this chaperone, GA blocks assembly of an HSP90–p23 molecular complex implicated in HSP90-dependent protein folding (29, 30).

A role for HSP90 in achieving and/or stabilizing the mutated p53 conformation was suggested by the fact that only GA derivatives that bound to HSP90 were capable of affecting mutated p53 (18). However, we were unable to observe *in vivo* association of HSP90 with p53. Because HSP90 heterocomplexes are detergent-sensitive, unstable, and easily disrupted by standard cell lysing and immunoprecipitation procedures, we decided to utilize *in vitro* translation systems to further explore the possible involvement of HSP90 in regulating p53 conformation. Rabbit reticulocyte lysate (RRL) is rich in HSP90 and auxilliary proteins and has been used to demonstrate HSP90 association with, and folding of, several signal transduction proteins (20, 28, 30, 31). In contrast, wheat germ extract (WGE) is known to contain HSP90 but to be deficient in its function (24), requiring the addition of a novel protein designated p23 ito fully reconstitute HSP90 chaperone activity (32).

Yeast expressing mutant HSP90 have been used previously to obtain *in vivo* evidence of the importance of HSP90 in the proper function of several proteins whose conformational state is critical to activity (21, 22, 25, 26, 33). We also used this system to investigate the importance of HSP90 for p53 conformation *in vivo*.

Our *in vitro* and *in vivo* findings demonstrate a critical role for HSP90 in the proper folding of mutated p53. In contrast to

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Abbreviations: GA, geldanamycin; RRL, rabbit reticulocyte lysate; WGE, wheat germ extract.

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results obtained with RRL or yeast expressing wild-type HSP90, both WGE and yeast defective for HSP90 function produced p53-143^{ala} protein, which was conformationally unrecognizable. Furthermore, recognition of the mutant protein in RRL, in WGE supplemented with p23, and in yeast expressing wild-type HSP90 was abrogated by the HSP90 antagonists GA and macbecin I.

MATERIALS AND METHODS

Reagents. A TnT^{R}-coupled reticulocyte lysate system and a TnT^{R}-coupled wheat germ extract system were purchased from Promega and used as per manufacturer's instructions. The p53 plasmids, pC53-SN3 (wild type) and pC53-SCx3 (V143A mutant, p53-143^{ala}), in a pCMV-Neo-Bam vector were obtained as a kind gift from B. Vogelstein (Johns Hopkins University, Baltimore, MD). Wild-type p53 and p53-143^{ala} inserts were excised with *Bam*HI and cloned into pSP72 (Promega). Transcription/translation occurred from the T7 promoter using the Promega TnT kit. The bacterially expressed purified protein, p23, was obtained as a kind gift from D. Toft (Mayo Clinic, Rochester, MN). GA and macbecin I were obtained gratis from the Drug Synthesis and Chemistry Branch, National Cancer Institute (Rockville, MD). Translation grade [^{35}S]methionine (specific activity greater than 1000 Ci/mmol) was purchased from Amersham. Conformation-specific p53 monoclonal antibodies (PAb 240, mutated conformation specific, and PAb1620, wild-type conformation specific), and conformation nonspecific p53 monoclonal antibodies (PAb 421 and PAb 1801) were purchased from Oncogene Science. PAb 240 and PAb 1801 antibodies covalently coupled to agarose beads were purchased from Santa Cruz Biotechnology. The monoclonal antibody detecting HSP90 (clone MA3-011) was purchased from Affinity BioReagents (Neshanic Station, NJ).

***In Vitro* Translations and p53 Immunoprecipitations.** Four μg of either wild-type or mutated p53 plasmids were added to either RRL or WGE translation systems (1 μg/50 μl of final mixture), together with 350 μCi of [^{35}S]methionine, and translation was allowed to procede for 90 min at 30°C according to manufacturer's instructions. Under these conditions, wild-type p53 translated in RRL could be immunoprecipitated with PAb 1620, but not with PAb 240. Conversely, mutated p53 (p53-143^{ala}) translated in RRL was immunoprecipitated by PAb 240, but not by PAb 1620. To permit conformational flexibility, particularly in the case of p53-143^{ala}, a temperature-sensitive p53 mutant (34), ATP (10 mM), and dithiothreitol (1 mM) were added to the translation mixture (35). Because these additions dramatically inhibited protein synthesis in RRL and WGE (J.T., M.B., and L.N., unpublished observations), ATP, and dithiothreitol were routinely added at the end of 90 min, and samples were allowed to incubate an additional 15 min at 30°C. Under these conditions, p53-143^{ala}, and to a lesser extent wild-type p53, possesed both mutant and wild-type conformations in RRL, consistent with previous reports (5, 35).

GA (10 μM final concentration) or dimethyl sulfoxide (0.1% final concentration) were added where indicated. Although GA was usually added at the same time as ATP, in preliminary experiments GA was added to both RRL and WGE at the initiation of translation. In neither case did GA affect absolute translation efficiency, either of the p53 plasmids described above, or of a luciferase control plasmid (J.T., M.B., and L.N., unpublished observations). When indicated, purified p23 protein (2.5 μg per reaction) was added to WGE at the beginning of the incubation period as described (29, 32).

Following *in vitro* translations, 10 μl of reaction mixture were diluted in 300 μl of TNES buffer (50 mM Tris, pH 7.5/2 mM EDTA/100 mM NaCl/1% Nonidet P-40/20 μg/ml aprotinin/20 μg/ml leupeptin/1 mM phenylmethylsulfonyl fluoride) and immunoprecipitated with 1 μg of appropriate anti-p53 antibody, as described (18). ^{35}S-labeled immunoprecipitates were electrophoresed through 10% polyacrylamide gels, and the gels were fixed, enhanced (Enlightening, DuPont), dried, and exposed to Kodak XAR-5 film. ^{35}S-labeled p53 bands were semi-quantitatively analyzed by scanning densitometry (18).

In some experiments, gels containing immunoprecipitates were transferred to nitrocellulose membranes (18), which were used for chemiluminescent Western blot analysis of co-precipitated HSP90 (36). Anti-HSP90 monoclonal antibody was used at a final dilution of 1:500.

Yeast Strains, Plasmids, Transformations, and Immunoprecipitations. Yeast strains used are isogenic to UH1-GRGZ, which has both chromosomal HSP90 genes (*HSC82* and *HSP82*, respectively) disrupted by insertions of the *LEU2* gene. Strains stably express wild-type or G313N mutant HSP82 from a *HIS3*, low copy plasmid, as described (21). Wild-type p53 or p53-143^{ala} are expressed under control of the GAL1 galactose-inducible promoter from the TRP1, low copy plasmids pRS-wt and pRS-143, respectively, and all strains contain the *URA3*-marked, low copy p53-responsive β-galactosidase reporter plasmid WG2-lacZ. Yeast p53 expression and reporter plasmids are described in Pietenpol *et al.* (37) and were the generous gift of B. Vogelstein.

p53 expression was induced essentially as described (6). Briefly, overnight cultures were grown at 30°C in synthetic liquid medium containing 2% raffinose and 0.1% sucrose as carbon sources. These cultures were diluted 1:10 in fresh medium containing 1% galactose, 1% raffinose, and 0.1% sucrose and incubated for 18–24 h at 30°C. Cells were harvested, and extracts were prepared as described previously (26). Extracts were cleared by centrifugation for 30 min at 85,000 rpm in a 100.1 titanium rotor in a Beckman tabletop ultracentrifuge. Protein concentrations were normalized to 10 mg/ml with lysis buffer. Cells and extracts were maintained at 4°C throughout.

Immunoprecipitation of p53 from yeast extracts was carried out essentially as described (18). In brief, 50 μg of lysate from wild-type yeast and 500 μg of lysate from the G313N mutant were precleared with protein A-Sepharose beads and immunoprecipitated with PAb1801 or PAb240, covalently coupled to agarose beads. After gel electrophoresis, p53 was analyzed by chemiluminescent Western blotting with pAb1801 (18).

RESULTS

Conformational Recognition of Mutated, but Not Wild Type, p53 Translated in RRL Is Sensitive to GA. RRL is rich in HSP90 and auxilliary proteins and has been used to demonstrate the importance of this chaperone in the proper folding of several signaling proteins. GA has been used successfully in this system to block the chaperone activity of HSP90 (28, 29, 30). Inclusion of GA during the *in vitro* translation of wild type p53 affected neither its synthesis nor its conformation-specific detection (Fig. 1 lanes 1–4 and Fig. 3 lanes 1 and 2). In contrast, GA abrogated the conformation-specific detection of mutant p53-143^{ala}, without affecting either total synthesis of the protein or its conformation nonspecific recognition (Fig. 1 lanes 5–8 and Fig. 3 lanes 3 and 4).

Conformational Recognition of Mutated, but Not Wild-Type, p53 Translated in WGE Is Dependent on HSP90 and Exogenous Addition of p23. WGE contains functionally deficient HSP90 and therefore has been used in conjunction with RRL to demonstrate the importance of this chaperone in the productive folding of several proteins. Recently, it has been demonstrated that exogenous addition of the auxilliary protein p23 reconstituted HSP90 function in WGE (32). When wild-type p53 was translated in WGE, the protein was recognized by a wild-type conformation specific antibody, and the presence of either p23 or GA during translation had no effect on

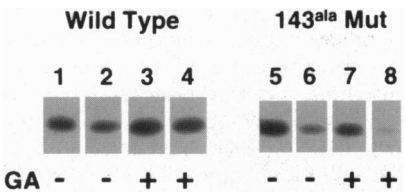

FIG. 1. Conformation of mutated, but not wild-type, p53 translated in RRL is sensitive to GA. Wild-type p53 (lanes 1–4) or p53-143^{ala} (lanes 5–8) were translated in RRL containing [^{35}S]methionine, as described in *Materials and Methods*. GA (10 μM) was present as indicated. ^{35}S-labeled p53 protein was immunoprecipitated with the conformation nonspecific antibody PAb 421 (lanes 1, 3, 5, and 7), the wild-type conformation-specific antibody PAb 1620 (lanes 2 and 4), or the mutated conformation specific antibody PAb 240 (lanes 6 and 8).

that recognition (Fig. 2 lanes 1–3). In contrast, mutant-specific conformational recognition of p53-143^{ala} translated in WGE required the presence of exogenous p23 (Fig. 2 lanes 4 and 5), implying the importance of both HSP90 and p23 in the proper folding of the mutated protein. Additionally, GA abrogated the restorative properties of p23, further supporting a role for HSP90 in this process (Fig. 2 lanes 5 and 6). Although p23 addition was required for conformational recognition of mutated p53, the overall translation of the mutated protein in WGE was not affected by either p23 or GA addition (Fig. 3 lanes 5–8).

HSP90 Is Co-precipitated in RRL with the Mutated, but Not the Wild-Type, Conformation of p53. p53-143^{ala} is temperature-sensitive for conformation. *In vivo*, at 37°C it exists primarily in a mutated conformation, but at 32.5°C a significant portion reverts to wild-type p53 conformation (34). When translated *in vitro* in RRL using the conditions described in *Materials and Methods*, p53-143^{ala} could be detected in both wild-type and mutated conformations.

We determined whether RRL HSP90 could be co-precipitated with either or both p53 conformations translated from the identical p53-143^{ala} transcript. As can be seen in Fig. 4, RRL HSP90, as detected by Western blotting, co-precipitated with p53 in the mutated conformation, but not with p53 in the wild-type conformation. The presence of GA in the reaction mixture markedly reduced both the amount of mutated p53 detectable and the amount of co-precipitated HSP90. Direct nonspecific binding of RRL HSP90 to the mutated conformation specific p53 antibody was ruled out by using this antibody in lysates containing only wild-ype p53. Under these conditions, no HSP90 was co-precipitated (data not shown). The presence of GA consistently shifted the p53-143^{ala} conformational ratio in favor of the wild-type conformation (Fig. 4*B* compare lanes 2 and 3 to lanes 5 and 6, and data not shown). This was due not only to diminution of the mutated form, but also to an increase in the wild-type conformation (Fig. 4*B* compare lanes 3 and 6, and data not

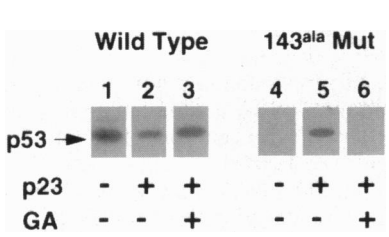

FIG. 2. Conformation of mutated, but not wild-type, p53 translated in WGE is dependent on exogenous p23. Wild-type p53 (lanes 1–3) or p53-143^{ala} (lanes 4–6) were translated in WGE containing [^{35}S]methionine, as described in *Materials and Methods*. Bacterially expressed, purified p23 protein (2.5 μg) and GA (10 μM) were added as indicated. ^{35}S-labeled p53 protein was immunoprecipitated with either the wild-type conformation-specific antibody PAb 1620 (lanes 1–3) or the mutated conformation-specific antibody PAb 240 (lanes 4–6).

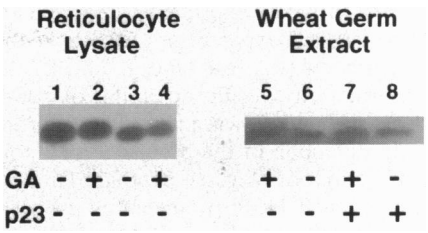

FIG. 3. Neither GA nor p23 affect the *in vitro* translation of p53 protein. Wild-type p53 (lanes 1–2) or p53-143^{ala} (lanes 3–8) were translated in RRL (1–4) or WGE (5–8) as described in the legends to Figs. 1 and 2, respectively. At the end of the translation period, 5 μl of reaction mixture were analyzed by polyacrylamide gel electrophoresis. The ^{35}S-labeled bands corresponding to *in vitro* translated p53 protein are displayed.

shown). These data are consistent with an essential role for HSP90 in achieving and/or stabilizing the mutated conformation of p53-143^{ala}.

Interestingly, the conformation nonspecific antibody Ab421 was unable to co-precipitate HSP90 (Fig. 4, *A* and *C*, lane 1). However, another conformation nonspecific antibody, Ab1801, did co-precipitate HSP90 in RRL (Fig. 4*C* lane 2).

Conformational Recognition of Mutated p53 Synthesized in Yeast Defective for HSP90 Function Is Impaired. Yeast strains with partially inactivating mutations in *HSP82* (the yeast homolog of *HSP90*) or strains in which *HSP82* can be down-regulated, have been utilized to study chaperone requirements for various signal transducers (21, 25, 26, 33, 38). The *HSP82* mutant used in these experiments, G313N, has been shown previously to be severely compromised in HSP90 function (21, 26). The yeast strains used in the following experiments have been engineered to stably express either wild-type or mutant HSP82 from a *HIS3* low copy plasmid (21). Both wild-type yeast and the G313N mutant were stably transfected with a p53-143^{ala} expression plasmid under control of a GAL1 galactose-inducible promoter. Native p53-143^{ala} could be immu-

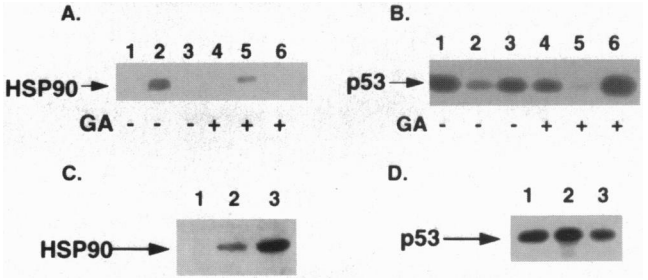

FIG. 4. HSP90 from RRL is co-precipitated with the mutated, but not wild-type, conformation of p53-143^{ala}. p53-143^{ala} was translated in RRL as described in the legend to Fig. 1. p53 protein was immunoprecipitated with either the non-conformation-specific antibody PAb 421 (lanes 1 and 4), the mutated conformation-specific antibody PAb 240 (lanes 2 and 5), or the wild-type conformation-specific antibody PAb 1620 (lanes 3 and 6). Gels containing electrophoresed immunoprecipitates were transferred to nitrocellulose membranes. The membranes were cut horizontally at the level of the 68 kDa standard, and the upper portion was used for chemiluminescent Western blot analysis of co-precipitated HSP90 (*A*), while the lower portion was exposed directly to XAR-5 film to detect labeled p53 protein (*B*). Data shown in *C* and *D* were obtained identically to that shown in *A* and *B*, except that GA was not added to the translation mix. p53-143^{ala} was translated in RRL and immunoprecipitated with either of two conformation nonspecific antibodies, Ab421 (lane 1) or Ab1801 (lane 2), or Ab240 (lane 3). After gel electrophoresis and transfer to nitrocellulose membranes, the membranes were cut horizontally at the level of the 68-kDa standard. The upper portion was used for chemiluminescent Western blot analysis of co-precipitated HSP90 (*C*), and the lower portion was exposed directly to XAR-5 film for detection of radiolabeled p53 (*D*).

noprecipitated from yeast expressing wild-type HSP90 with either a conformation nonspecific or a mutated conformation-specific p53 antibody (Fig. 5*A* lanes 1 and 2). In contrast, mutated conformation specific detection of p53-143^{ala} produced in the G313N mutant was markedly compromised when compared with detection of the protein with a conformation nonspecific p53 antibody (Fig. 5*A* compare lanes 4 and 5). In this characteristic, p53-143^{ala} produced *in vivo* in the *HSP82* yeast mutant is similar to the protein synthesized *in vitro* in RRL containing GA or in WGE lacking exogenous p23.

Finally, treatment of yeast expressing wild-type HSP90 with macbecin I, a GA analog with good anti-HSP90 activity in yeast (S.B., unpublished observations), abrogated conformational recognition of mutated p53 without affecting conformation nonspecific recognition (Fig. 5*B* lanes 1–4).

DISCUSSION

We recently reported that GA, an antagonist of HSP90 chaperone activity, destabilizes several mutated p53 proteins *in vivo* and abrogates their mutated conformation-specific recognition without altering either their synthesis or non-conformation-specific recognition (18). Evidence for the participation of HSP90 in the proper folding of mutated p53 was, however, only circumstantial, in that only those GA derivatives that bound to HSP90 affected mutated p53, while HSP90-mutated p53 heterocomplexes could not be directly demonstrated.

In this study we sought direct proof that HSP90 is required for the proper folding of mutated p53 *in vitro* and *in vivo*. First, we compared the *in vitro* translation of both wild-type and mutated p53 in RRL and WGE. Because HSP90 chaperone activity is present in RRL but lacking in WGE, a comparison of conformations obtained with the two extracts has been used previously to assign a role for HSP90 in the functional folding of specific proteins (24).

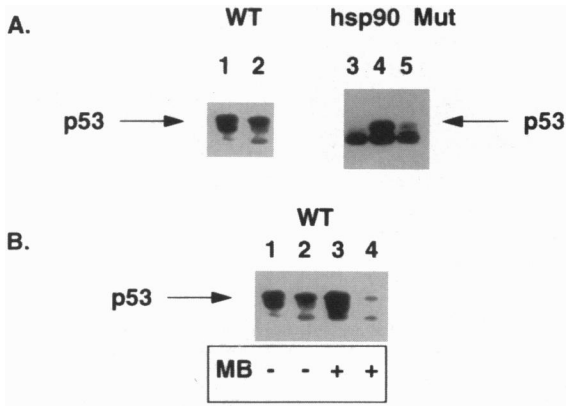

FIG. 5. Functional HSP90 is required for conformational recognition of mutated p53 produced in yeast. *A*, p53-143^{ala} was expressed in yeast containing either wild-type HSP90 (lanes 1 and 2) or mutated HSP90 (G313N; lanes 4 and 5). Endogenous p53 protein was immunoprecipitated from yeast extracts using either the conformation nonspecific p53 antibody PAb 1801 (lanes 1 and 4) or the mutated conformation specific p53 antibody PAb 240 (lanes 2 and 5). Lane 3 represents yeast extract from cells not expressing p53, immunoprecipitated with PAb 240, ito identify the signal arising from immunoglobulin heavy chain cross-reactivity. Both immunoprecipitating antibodies were covalently coupled to agarose beads ito reduce the cross-reactive signal. p53 was detected by chemiluminescent Western blotting with PAb 1801 (not coupled to beads). *B*, p53-143^{ala} was expressed in yeast containing wild-type HSP90 in the presence or absence of macbecin I (MB, 10 μM) as indicated. Endogenous p53 protein was immunoprecipitated with agarose-bound PAb 1801 (lanes 1 and 3) or PAb 240 (lanes 2 and 4).

Our data clearly demonstrate that p53-143^{ala} achieved the mutated conformation in RRL but failed to do so in WGE. In contrast, wild-type p53 achieved its correct (i.e., wild-type) conformation in both *in vitro* translation systems. In agreement with previous reports on other proteins requiring HSP90 chaperone activity (29, 30), the addition of GA to RRL markedly reduced the ability of p53-143^{ala} to achieve its mutated conformation. Similar effects were not observed when wild-type p53 was translated in the presence of GA.

When translated in RRL, p53-143^{ala} appeared in both the mutated and wild-type conformation, probably due to its temperature-dependent structural flexibility (34). Our data show that RRL HSP90 was co-precipitated with the p53-143^{ala} translation product exclusively in the mutated conformation and not with that portion of p53-143^{ala} possessing a wild-type conformation. The presence of GA appeared to direct more of the translation product into the wild-type conformation, while reducing the amount detectable in the mutated conformation.

Although one conformation nonspecific p53 antibody, Ab421, was unable to co-precipitate HSP90 in RRL containing mutated p53, another conformation nonspecific antibody, Ab1801, was able to do so. These antibodies recognize epitopes on opposite ends of the p53 molecule, raising the possibility that HSP90 binding to mutated p53 masks the epitope recognized by Ab421. Alternatively, all mutated p53 molecules may not express the 421 epitope, as has been shown for the wild-type protein (for review, see refs. 11 and 14).

Recently, Hutchison *et al.* (32) were able to restore HSP90 chaperone activity to WGE by adding a novel protein, termed p23, which is present in eukaryotic cells and RRL (32). Johnson and Toft (29,31) previously demonstrated p23 to be a necessary component of the RRL HSP90 chaperone protein complex required for *in vitro* reconstitution of the ligand-binding conformation of the progesterone receptor. Likewise, we found that when p53-143^{ala} was translated in WGE to which exogenous p23 and ATP had been added, conformational recognition of the mutated protein was restored.

Toft and coworkers (29) and Smith and coworkers (30) have reported that GA interferes with establishment of the HSP90–p23 chaperone heterocomplex (29, 30). Our current data support this view, in that GA added to WGE blocked the restorative effects of p23, abrogating conformational recognition of the newly synthesized p53-143^{ala} by mutated conformation specific anti-p53 antibodies. Taken together, these findings are consistent with a role for HSP90 in achieving and/or stabilizing the mutated, but not the wild-type, conformation of p53 *in vitro*.

Dependence of p53's mutated conformation on functional HSP90 is strengthened by the *in vivo* data reported here. The p53-143^{ala} protein produced in a yeast strain expressing mutant HSP90 was poorly recognized by a mutated conformation-specific p53 antibody, although a non-conformation-specific antibody clearly demonstrated the presence of the mutated protein. In contrast, when the same yeast strain expressed wild-type HSP90, the p53-143^{ala} protein produced in these cells was recognized with equal efficiency by mutated conformation-specific and conformation nonspecific p53 antibodies. Finally, macbecin I, a GA analog active in yeast, abrogated conformation-specific recognition of mutated p53 in yeast expressing wild-type HSP90.

Our *in vitro* and *in vivo* findings clearly support a requirement for HSP90 in achieving the mutated p53 conformation. Mutated p53 can thus be added to a list of several other, structurally diverse transcription factors, including steroid receptors, retinoid receptors, aryl hydrocarbon receptors, and the basic helix-loop-helix proteins E12 and MyoD, whose functional conformation requires either transient or stable interaction with HSP90 (19–27). Most transforming p53 mutants form complexes with HSP70 both *in vitro* and *in vivo*, and this chaperone has been implicated in regulating p53 confor-

mation, at least *in vitro* (35, 39–41). Because HSP90 and HSP70 are known to participate jointly in several of their chaperone activities (30, 32), our data may have identified another of the cast of players of a single multiprotein chaperone complex involved in folding p53 structural mutants. Alternatively, HSP90 may be acting independent of HSP70. In either case, the critical importance of HSP90 in this process is underscored by the following findings: (*i*) association of HSP90 with mutant, but not wild type, p53 translated *in vitro* from a single transcript; (*ii*) failure of p53-143^{ala} to fold properly in WGE in the absence of p23; (*iii*) loss of p53 mutated conformation in RRL, and WGE supplemented with p23, in the presence of GA [GA does not interact with HSP70 (28)]; (*iv*) failure of p53 to attain mutated conformation in yeast expressing mutant HSP90, or in wild-type HSP90-expressing yeast treated with the GA analog macbecin I.

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