Heat-Shock Protein hsp90 Governs the Activity of pp60v-src Kinase

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Notes:
Heat-shock protein hsp90 governs the activity of pp60v-src kinase
(protein-tyrosine kinase/cell cycle block)

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ABSTRACT During or immediately after synthesis in vertebrate cells, the oncogenic protein-tyrosine kinase pp60v-src associates with the ~90-kDa heat-shock protein (hsp90). In this complex, pp60v-src is not functional as a kinase. When pp60v-src is subsequently found inserted into the plasma membrane, it is active as a kinase and is no longer associated with hsp90. We have taken advantage of genetic manipulations possible in Saccharomyces cerevisiae to investigate the function and specificity of the association between hsp90 and pp60v-src. Expression of pp60v-src is known to be toxic to S. cerevisiae cells. We find that this toxicity is due to a very specific effect on growth, arrest at a particular point in the cell cycle. In cells expressing v-src, a mutation that lowers the level of hsp90 expression (i) relieves cell cycle arrest and rescue growth, (ii) reduces the level of tyrosine phosphorylation mediated by pp60v-src, (iii) changes the pattern of tyrosine phosphorylation, and (iv) reduces the concentration of pp60v-src. We conclude that hsp90 does not simply suppress pp60v-src kinase activity during transit to the plasma membrane, as previously suggested, but also stabilizes the protein and affects both its activity and specificity. This function of hsp90 is highly selective for pp60v-src; the same hsp90 mutation has no effect on the activity or specificity of the exogenous pp160v-abl tyrosine kinase; similarly, it does not affect the specificity and has only a very small effect on the activity of the exogenous pp60v-src kinase.

The transforming protein of Rous sarcoma virus, pp60v-src, is sufficient to initiate and maintain oncogenic transformation in a variety of cell types. Although the gene encoding this protein was the first genetically characterized oncogene, the precise mechanism by which it transforms cells is still unknown. pp60v-src and its cellular counterpart, pp60c-src, are nonreceptor tyrosine kinases. The majority of nonreceptor tyrosine kinase genes, including yes, lck, and fyn, are closely related to src and constitute a single family (1, 2). A few other nonreceptor tyrosine kinase genes, such as abl and fps, have very different structures and clearly do not belong to the src family (1, 2). Both the Src and Abl nonreceptor kinases are highly conserved in the vertebrate lineage and closely related proteins are also present in nonvertebrates such as Drosha- lita (3–6).

All members of the Src family, including pp60v-src and pp60c-src, are between 500 and 550 amino acids long and have a similar structural organization. They associate with the plasma membrane through a myristoylation modification on their N-terminal glycine residues. A short, highly variable region is then followed by 450 residues with 80% identity between members. This region contains at least three domains: a kinase domain and two Src homology domains (SH2 and SH3). The latter are shared, in various combinations, with a wide variety of other proteins, including other tyrosine kinases such as the Abl and Fps kinases, regulatory proteins, and cytoskeletal proteins (1, 2, 7).

Although the oncogenic protein pp60v-src and its cellular homolog pp60c-src are structurally homologous, the biochemical properties of the two proteins are distinct. pp60v-src has much lower kinase activity than pp60c-src and is not oncogenic, even when overexpressed (8). The two proteins also appear to differ in their interactions with one of the major heat-shock proteins, hsp90 (9).

Moreover, immediately after stimulation in vertebrate cells, pp60v-src can be coimmunoprecipitated with hsp90 (9–13). In this complex, pp60v-src is unphosphorylated, incapable of auto-phosphorylation, and inefficient in phosphorylating exogenous substrates (9). Shortly thereafter, when the kinase is inserted into the plasma membrane, it no longer coprecipitates with hsp90 and its tyrosine kinase activity is high (14). Several other oncogenic nonreceptor tyrosine kinases, such as v-Yes and v-Fps, behave in the same manner (9, 10, 12). Moreover, certain mutants of pp60v-src that are temperature-sensitive for transformation retain their association with hsp90, are inactive as kinases, and are not targeted to the membrane at the nonpermissive temperature; at the permissive temperature they dissociate from hsp90, are activated as kinases, and are targeted to the membrane (9, 15). These and other results have suggested that hsp90 serves to keep pp60v-src kinase inactive while it is being transported to the plasma membrane (9, 11).

In contrast, the cellular homolog pp60c-src either does not interact with hsp90 or interacts with it only very weakly during transit to the plasma membrane (8, 9). Thus, the above-mentioned results with temperature-sensitive pp60v-src mutants can also be interpreted to suggest that hsp90–pp60c-src complexes represent merely a side pathway for the disposal or salvage of misfolded proteins (1, 2). Currently, the function of hsp90 in the maturation of pp60v-src remains a mystery. Utilizing genetic methods available in Saccharomyces cerevisiae, we have reinvestigated the interaction between pp60v-src and hsp90.

MATERIALS AND METHODS

Plasmids, Strains, and Yeast Transformation. The wild-type v-src gene, the kinase-activated mutant v-src gene (Lys295 → Met), and the deletion mutant v-src gene (amino acids 169–264) were from pN4, pv-srcMet295, and p18-3, respectively (gifts from H. Hanafusa, Rockefeller University (8, 9, 16, 17)). The coding regions of these genes were inserted into the EcoRI–Cla I sites of the yeast galactose-regulated GAL10 promoter of a vector carrying the HIS3 gene, pRS303-Gal, and a vector carrying the TRP1 gene, pRS304-Gal, creating corresponding v-src expression vectors for yeast. To produce higher levels of v-src expression, a sequence designed to maximize translational activity in yeast was used to replace the region immediately upstream of the v-src coding sequence.

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sequence in these constructs, creating another set of v-src expression vectors. For transformation, the plasmids were digested with restriction enzymes ({	extit{Nde I}} for derivatives of pRS303-Gal and {	extit{HindIII}} for derivatives of pRS304-Gal) to increase integration efficiency. Also, wild-type and hsc82 yeast deletion strains were transformed with centromere-containing plasmids carrying the GAL10 promoter with (pPT-v-abl) or without (pPT) an insertion of the v-abl coding sequence at the EcoRI site of the GAL10 promoter (gift of Y. Maru and O. Witte, University of California, Los Angeles) or with a multicopy plasmid carrying the GAL10 promoter with an insertion of the c-src coding sequence at the HindIII site of the GAL10 promoter (pJH-c-src; gift of J. Broach, Princeton University).

To disrupt the HSC82 gene with LEU2, the Bgl II fragment of the LEU2 gene was inserted into the Bgl II site of hsc82 in pKAT7, creating plasmid pKAT12, and the BamHI fragment of this construct was employed for transformation. To disrupt the HSC82 gene with URA3, the BamHI–EcoRI fragment of Yip5 (containing the URA3 gene) was used to replace the Bcl I–EcoRI fragment of HSC82, creating plasmid pUTX212, and the HindIII fragment of this construct was employed for transformations. To disrupt the HSP82 gene, the Bgl II fragment of LEU2 was inserted into the Bgl II site of HSP82, creating pUTX123, and the Xba I fragment was used for transformation (18). To restore hsp90 levels, hsc82 deletion strains were transformed with centromere-containing plasmids carrying the GAL1 promoter with (pTCl) or without (pTTh) an insertion of HSP82 coding sequences at the BamHI site of the promoter (19).

The yeast strains used were W303 (MATa ade2 his3 leu2-3,112 trp1), Y294 (MATa his3 leu2 ura3 trp1), and Y653 (MATa leu2 ura3 trp1 HIS3::GAL10-c-src; gift of J. Broach, Princeton University). Similar results were obtained with both W303 and Y294 strains. The figures in this paper report data obtained from Y294 and its derivatives. Yeast cells were transformed by the lithium acetate method (20, 21) with the DNAs described above. Integrations of the src genes, deletions of the HSP82 and HSC82 genes, and other yeast transformations were confirmed by immunological analysis of electrophoretically separated proteins.

**Yeast Growth and Kinase Induction.** Strains were grown in the rich medium YPDA (1% yeast extract/2% Bacto-Peptone/2% glucose with adenine sulfate at 10 μg/ml) or in the minimal medium SD (0.67% yeast nitrogen base without amino acids, supplemented with all amino acids except the one used in selection, plus 2% glucose), with 2% agar for solid medium only. Raffinose- and galactose-containing media were prepared in the same manner, except that glucose was replaced by raffinose or galactose.

For growth on solid medium, colonies picked from the glucose plates were streaked directly onto galactose medium. For growth in liquid medium, cells were inoculated into glucose medium and incubated overnight, then shifted to raffinose medium to relieve glucose repression of the GAL10 promoter and thereby permit rapid induction by galactose. Cells were incubated in raffinose medium until they reached midlogarithmic phase (10^9 cells per ml) and were then transferred to galactose medium. Cells were counted with a hemacytometer (Baxter Scientific Products). Strains carrying integrated plasmids were grown in rich or minimal medium. Strains carrying extrachromosomal plasmids were grown in minimal medium to maintain selection for cells carrying the plasmids. All experiments reported in this paper were done at normal temperatures (25°–30°C).

**Immunological Analysis of Tyrosine-Phosphorylated Proteins and of the Kinases.** Cells were collected by centrifugation and lysed with glass beads in ethanol containing 1 mM phenylmethylsulfonyl fluoride. Samples were agitated on a vortex mixer for 10 min at 4°C and held at −20°C for 1 hr. Proteins were collected by centrifugation at 1000 × g for 5 min, vacuum-desiccated for 10 min, and suspended in SDS sample buffer. Proteins were separated by 7% SDS/PAGE and transferred to Immobilon-P filters (Millipore). The blots were stained with Coomassie blue to confirm equal loading (4 × 10^6 cells per lane) before reaction with antibodies.

All blots were preincubated with 5% milk in phosphate-buffered saline (NaCl, 8 mg/ml; KCl, 0.2 mg/ml; Na, 1.44 mg/ml; K, 0.24 mg/ml; pH adjusted to 7.4). To assay tyrosine phosphorylation, blots were incubated with the anti-phosphotyrosine monoclonal antibody 4G10 (UBI, Lake Placid, NY), followed by protein A-conjugated horseradish peroxidase (Boehringer Mannheim). Immune complexes were visualized by the ECL method (Amersham). To assay pp60-src, blots were reacted with anti-src monoclonal antibody 327 [gift of J. Brugge, University of Pennsylvania (22)] and the immune complexes were visualized as for antibody 4G10, except that rabbit anti-mouse IgG Fc was employed prior to reaction with protein A. hsp82 and hsc82 were detected with a polyclonal antisemir specific for yeast hsp82 and hsc82 (18) and the immune complexes were visualized as for antibody 4G10.

**RESULTS**

**v-src Expression Blocks Yeast Growth in a Cell Cycle-Specific Manner.** Src-related kinases have not been observed in {	extit{S. cerevisiae}}, and the endogenous tyrosine kinase activity of this organism is very low (23). When exogenous pp60^src, which is expressed in {	extit{S. cerevisiae}}, its biochemical properties are very similar to those of pp60^src produced in avian cells, but expression of pp60^src is toxic to yeast cells (24, 25). This toxicity may be due to the high tyrosine kinase activity of exogenous pp60^src or to some other property of the protein. Before examining the relationship between hsp90 and pp60^src, we investigated in greater detail the effects of src expression on wild-type yeast cells.

We transformed two different strains of yeast with v-src under the control of a galactose-inducible promoter. The constructs were integrated at different positions in the genome and carried different translation leader sequences, resulting in very different pp60^src expression levels. In all cases, when the cells were incubated in medium containing galactose, they were arrested at a specific point in the cell cycle, with large buds (Fig. 1A) and chromosomes at the neck (Fig. 1B).

Several lines of evidence indicate that this phenotype is not due to the titration of a limited quantity of hsp90, an essential protein, by high levels of the exogenous pp60^src. First, the same phenotype was observed with different levels of pp60^src expression. Moreover, even at its highest expression level, the concentration of pp60^src was still very low compared with the concentration of hsp90. Finally, as described below, reducing the concentration of hsp90 in these strains eliminates this phenotype.

To demonstrate that the cell cycle arrest was directly related to pp60^src kinase activity, we transformed cells with a v-src allele carrying a mutation in the catalytic domain (Lys^305 Æ Met) that inactivates the protein's tyrosine kinase activity. Expression of this protein did not cause a cell cycle arrest, nor did it perturb growth in any detectable fashion. We conclude that the growth inhibition produced in yeast cells by pp60^src is not due to gross metabolic derangement but rather to the specific, Src-mediated phosphorylation of some protein or proteins involved in cell division.

**Deletion of HSC82 Rescues Yeast Cells from the Arrest Caused by pp60^src.** To investigate the role of hsp90 in association with pp60^src, we compared the effects of pp60^src on yeast cells expressing normal levels of hsp90 with its effects on cells expressing very low levels of hsp90. S.
gene and a wild-type HSC82 gene did not grow on galactose medium (Fig. 1C). Disruption of HSC82 in this strain fully restored growth on galactose (Fig. 1C). Microscopic examination revealed a normal distribution of cells at all stages of the cell cycle (data not shown). As expected, disruption of HSC82 had no effect on the growth of isogenic strains lacking v-src.

Similar experiments were performed by deleting HSP82 rather than HSC82 (data not shown). The deletion of HSP82, which accounts for only a small fraction of the hsp90 expressed at normal temperatures, did not rescue cells from v-src-mediated death. These results suggest that the rescue of pp60v-src-expressing cells by the hsc82 deletion is due to the dramatic reduction in hsp90 levels associated with this mutation. To confirm that this is the case, hsp90 levels were restored by transforming the pp60v-src-expressing hsp82 deletion strain with a vector carrying the functionally equivalent HSP82 gene under the control of a GAL10 promoter. When these cells were grown on galactose, hsp82 accumulated to a much higher level than when expressed from its own promoter. This accumulation had no effect on the growth of the parental hsc82 deletion strain (Fig. 1D). However, in cells expressing v-src, it restored the cell cycle block, preventing growth on galactose (Fig. 1D).

**Deletion of HSC82 Affects the Activity of pp60v-src.** To investigate pp60v-src kinase activity in these strains, electrophoretically separated proteins were reacted with a monoclonal antibody specific for phosphorytosine (Fig. 2A). To maximize resolution of proteins in the 60-kDa range, 7% polyacrylamide gels were employed. In the parental strain, only faint bands of reaction with the antibody were observed. Deletion of HSC82 had no effect on this pattern. In cells expressing pp60v-src, phosphorytosine levels were much higher. Several bands that were faintly visible in the parental strain were intensified, suggesting that pp60v-src might phosphorylate some of the same substrates as endogenous yeast tyrosine kinase(s). Several new bands of phosphorylated protein also appeared. It is unclear whether these were novel phosphorylation substrates or substrates whose phosphorylation was previously too low to detect. A substantial increase in phosphorytosine was also observed in a 60-kDa protein migrating at the position of pp60v-src. These results are consistent with reports that (i) endogenous tyrosine kinase activity is very low in yeast cells, (ii) pp60v-src has very high kinase activity, and (iii) pp60v-src is capable of autophosphorylation (1, 2).

**FIG. 1.** An hsc82 deletion can rescue yeast cells from cell cycle arrest caused by pp60v-src. (A) Phase-contrast micrograph of cells carrying the GAL10-v-src gene. Logarithmic-phase cells were grown to 10^6 cells per ml in rich raffinose medium (YPR) and then transferred to rich galactose medium (YPGal) for 6 hr. As in A, except that cells were fixed with ethanol and stained with 4',6-diamidino-2-phenylindole (DAPI) as previously described (33) and were visualized by epifluorescence. (C) Cells were streaked on rich galactose plates and grown at 30°C for 48 hr. P, parental strain; V, cells carrying the v-src gene; Δ, cells carrying the hsc82 deletion. (D) Cells designated as in C were transformed with a galactose-regulated HSP82 gene (GalHSP82) or the vector alone (Gal–). The transformants were streaked on minimal (selective) galactose-containing medium in order to retain the plasmids and were grown for 48 hr. S. cerevisiae produces two proteins in the hsp90 family (18). These proteins are functionally equivalent to one another, as well as to the hsp90 proteins of vertebrate cells. [Expression of the human hsp90 gene rescues yeast cells from the lethality associated with deletion of both yeast genes (ref. 19; L. Arwood, B. Khursheed, J. Taulien, and S.I., unpublished work).] The protein produced by one of the two yeast genes, HSC82, is among the most abundant proteins in S. cerevisiae cells at normal growth temperatures (25–30°C). The protein encoded by the other gene, HSP82, is strongly induced by heat but is expressed at a low level at 25°C. When the HSC82 gene is deleted, expression of hsp82 remains low as long as cells are maintained at normal temperatures. Although the deletion of HSC82 dramatically decreases the level of hsp90, growth rates are unaffected at 25–30°C (18). Thus, a small fraction of the usual complement of hsp90 (=1/15th the wild-type level) is sufficient for growth at normal temperatures.

Strains expressing low levels of hsp90 were created by disrupting HSC82 in cells carrying v-src, as well as in wild-type cells. All of the resulting strains grew equally well on glucose-containing medium (data not shown). As observed earlier, cells carrying the galactose-regulated v-src

**FIG. 2.** Effects of hsc82 deletion on the tyrosine kinase activity, specificity, and accumulation of pp60v-src. (A) Electrophoretically separated total cellular proteins reacted with anti-phosphotyrosine antibody 4G10. Lane 1, parental cells; lane 2, parental cells with hsc82 deletion; lane 3, cells carrying the GAL10-v-src gene; lane 4, cells with the GAL10-v-src gene and the hsc82 deletion; lane 5, one-fourth of sample in lane 3. Molecular size markers (kDa) are at left. (B) A duplicate of the blot in A reacted with anti-Src antibody 327. Cells were grown as in Fig. 1A but incubated for 8 hr in galactose-containing medium. Due to the high total protein concentration in lanes 1–4, migration is slightly faster than in lane 5—a common gel artifact.
Deletion of HSC82 in cells expressing pp60v-src greatly reduced the intensity of most phosphoprotein bands. More significantly, the deletion changed the pattern of phosphorylation. To reveal this change more clearly, a smaller quantity of protein from src-expressing cells with a wild-type HSC82 gene was layered next to protein from src-expressing cells with the hsc82 deletion (Fig. 2A, lanes 4 and 5), so that the total levels of phosphorylated protein were roughly equivalent. The most prominent phosphorylated proteins observed in the former sample were barely detectable in the latter.

**Deletion of HSC82 Affects the Concentration of pp60v-src.** Since hsp90 and pp60v-src form complexes in vertebrate cells, the changes in Src kinase activity we have observed likely result from a reduction of the direct interaction between the two proteins in the hsc82 deletion strain. Indeed, by using v-Src mutants that form unusually stable complexes with hsp90 in vertebrate cells, pp60v-Src complexes can be detected in yeast by communoprecipitation and by affinity matrix chromatography (H. J. Chang, Y. X., and S. L., unpublished data). Reduction in the level of one of the proteins in a complex often leads to increased proteolysis of other proteins in the complex. We therefore inquired whether lowering the concentration of hsc82 would reduce the stability of pp60v-src. As may be seen in Fig. 2B, the level of pp60v-src was reduced by a factor of 5 by the hsc82 deletion.

**Function of hsp90 in Kinase Maturation Is Very Specific.** We next investigated the specificity of hsp90 for pp60v-src. In particular, we examined the effects of the hsc82 deletion on the activity of two other exogenous kinases, pp160v-abl and pp60v-src. The v-abl gene was originally discovered as the transforming gene of the Abelson murine leukemia virus. Like v-src, v-abl encodes a nonreceptor tyrosine kinase. However, it is not a member of the src family and has little sequence homology with v-src except in the region encoding two Src homology domains (SH2 and SH3). It does not cause growth arrest in yeast cells (Y. Maru and O. Witte, personal communication, confirmed in the present study). There is no evidence that pp160v-abl interacts with hsp90 in vertebrate cells.

Yeast cells transformed with a galactose-inducible v-abl gene exhibit a large increase in phosphotyrosine-modified proteins when shifted to medium containing galactose. The pattern of phosphorylation was very different from that obtained with pp60v-src. Deletion of HSC82 altered neither the level nor the specificity of tyrosine phosphorylation mediated by the v-abl product (Fig. 3A). In addition to demonstrating the specificity of hsp90 for pp60v-src, this result indicates that the influence of hsp90 on pp60v-src-mediated phosphorylation does not occur simply through an indirect, general effect on tyrosine phosphatases.

Finally, we examined the effect of the hsc82 deletion on the activity of pp60v-src, the cellular homolog of pp60v-src. In vertebrate cells, little or no pp60v-src can be communoprecipitated with hsp90 (8, 9, 26). In *in vitro* reconstitution experiments, however, pp60v-src does show some capacity to interact with hsp90 (26). When expressed in yeast cells, pp60v-src phosphorylates a wide variety of proteins (Fig. 3A). As expected, a side-by-side comparison of proteins from yeast cells expressing pp60v-src and pp60v-src (data not shown) revealed a very similar pattern of phosphotyrosine-modified proteins. However, the level of phosphorylation was much lower with pp60v-src than with pp60v-src, and longer exposures were required to detect it. The levels of Src protein, detected with a monoclonal antibody that recognizes both proteins, were much higher in cells expressing pp60v-src than in cells expressing pp60v-src. The difference in phosphorylation was therefore probably due to a difference in the specific activity of the two proteins, as reported previously (2, 24).

![Fig. 3. hsc82 deletions have no or little effect on pp160v-abl and pp60v-src activity, specificity, and accumulation. (A) Proteins reacted with anti-phosphotyrosine antibody 4G10. Lane 1, parental cells carrying the galactose-regulated vector, GAL--; lane 2, cells carrying GAL-v-abl and the hsc82 deletion; lane 3, parental cells carrying the galactose-regulated v-abl gene, GAL-v-abl; lane 4, cells carrying GAL-v-abl and the hsc82 deletion; lane 5, parental cells carrying the galactose-regulated c-src gene, GAL-c-src; lane 6, cells carrying GAL-c-src and the hsc82 deletion. (B) Proteins reacted with anti-Src antibody 327. Lanes 1 and 2, same as lanes 5 and 6 in A. Proteins were separated by electrophoresis and analyzed as in Fig. 2.](https://example.com/fig3)

Deletion of HSC82 caused only a small change in pp60v-src kinase activity, and no change in specificity, even when c-src was expressed from a high-copy-number vector (Fig. 3A). Further, the deletion had no significant impact on the concentration of pp60v-src (Fig. 3B). Although staining was less intense, similar results were obtained with a single-copy c-src plasmid (data not shown). This result suggests that earlier attempts to communoprecipitate hsp90 and pp60v-src failed because the proteins do not interact strongly in vivo. Moreover, because pp60v-src and pp60v-src phosphorylate the same proteins, this result confirms that the profound impact of the hsp90 mutation on phosphotyrosine levels in pp60v-src-expressing cells is not a consequence of an effect of the mutation on tyrosine phosphatases.

**DISCUSSION**

Our results demonstrate that hsp90 plays a critical role in determining the nature of tyrosine phosphorylation mediated by pp60v-src in yeast. We believe for several reasons this role will extend to vertebrate cells. First, previous studies have established a direct physical interaction between pp60v-src and hsp90 in vertebrate cells (9). Second, interactions between hsp90 and a wide array of other cellular proteins (including other cellular kinases, cytokskeletal proteins, prolyl isomerases, and steroid hormone receptors) have been highly conserved in evolution. This conservation has been demonstrated by a number of communoprecipitation, reconstitution, and colocalization studies (27-30) and has also been demonstrated genetically. The human hsp90 protein not only rescues mutant yeast cells from lethality but also supports normal rates of growth. Furthermore, the yeast hsp90 protein is fully capable of activating mammalian steroid hormone receptors (19). Thus, it is highly likely that hsp90 plays a key role in modulating the activity of pp60v-src in vertebrates.

Since the phosphorylation activity of pp60v-src is closely correlated with its transforming potential in vertebrate cells, we further suggest that the interaction of pp60v-src with hsp90 will prove to be a crucial feature of pp60v-src-mediated...
oncogenesis. Indeed, it is striking that several different oncogenic tyrosine kinases of the nonreceptor class associate with hsp90 (9). This association with hsp90 may be a common feature in determining the oncogenic activity of these proteins.

The precise mechanism by which hsp90 exerts its effect on pp60-src levels and activity is not clear. Interaction with hsp90 might inform the structure of the kinase, which could, in turn, both protect pp60-src from proteases and change the nature of its interaction with substrates. An alternative, though not mutually exclusive, possibility is that hsp90 promotes the insertion of pp60-src into the plasma membrane, thereby stabilizing the kinase and changing its access to substrates. Our attempts to analyze the interaction between hsp90 and pp60-src at a biochemical level have been hindered by the low concentration of v-src, even in our highest-expressing strains, and the instability of the protein in yeast lysates. These difficulties may be surmounted through the design of new vectors and the transfer of this system to pro tease-deficient strains. Alternatively, it may be possible to reconstruct the interaction in vitro with highly purified proteins.

Our results have two important implications concerning the nature of hsp90 functions. First, they demonstrate that hsp90’s functions are highly selective. Reducing the cellular concentration of hsp90 profoundly diminishes the activity of pp60-src. However, it only weakly affects the activity of its cellular counterpart, pp60- src, and has no effect at all on the activity of another nonreceptor tyrosine kinase, pp160-cyt. On the basis of in vitro experiments involving the reactivation of denatured proteins, it was recently proposed that hsp90 might serve as a general protein chaperone (31). We suggest that hsp90 is not a general chaperone in vivo but rather that its functions are highly specific for certain target proteins.

Second, we have demonstrated that hsp90 has a strong positive effect on pp60-src kinase activity. This result was somewhat surprising in the context of earlier work suggesting that hsp90 simply represses the kinase activity of pp60-src (9, 30). The present findings show a striking parallel to recent work investigating the function of hsp90 when associated with certain steroid hormone receptors. It was initially thought that hsp90 simply repressed receptor function, because receptors are unable to bind DNA until hsp90 has dissociated (29, 30). However, genetic analysis in yeast (19) and reconstitution experiments in vitro (32) have demonstrated that hsp90 also has a strong positive effect on receptor activity.

The symmetry of these findings suggests that the same mechanism may be operating in both systems: the association of hsp90 with these target proteins stabilizes them and helps them to assume a conformation from which they can be readily activated upon receipt of the proper signal. For the receptors, this signal would be hormone binding. For the Src-related oncogenic tyrosine kinases, it might be phosphorylation or insertion into the plasma membrane. As long as the association with hsp90 is maintained, however, the target protein remains quiescent. Thus, with at least two very different proteins, the chaperone function of hsp90 has apparently been subsumed into regulatory functions.

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