The Hsp90 of *Candida albicans* can confer Hsp90 functions in *Saccharomyces cerevisiae*: a potential model for the processes that generate immunogenic fragments of this molecular chaperone in *C. albicans* infections

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During infections with a number of important eukaryotic pathogens the Hsp90 molecular chaperone of the pathogen is recognized as an immunodominant antigen by the host immune system. Yeast molecular genetics should allow study of the extent of sequence variation within conserved immunodominant epitopes on pathogen Hsp90s that is compatible with essential Hsp90 functions, as well as the processes that generate antigenic subfragments of these Hsp90s. The Hsp90 of the fungal pathogen *Candida albicans* was shown in this study to provide both essential and nonessential (pheromone signalling and mammalian steroid receptor activation) Hsp90 functions in *Saccharomyces cerevisiae* cells. Much of the *C. albicans* Hsp90 expressed in respiratory *S. cerevisiae* cells was shown to undergo a partial degradation *in vivo*, a degradation that closely resembles that of the native Hsp82 (one isoform of the homologous Hsp90) in *S. cerevisiae*. Allowing for the differences in the length of the charged linker region between the N- and C-terminal domains of *C. albicans* Hsp90 and *S. cerevisiae* Hsp82, these two proteins expressed in *S. cerevisiae* appear to give the same major degradation products. These Hsp90 fragments are similar to the products of incomplete Hsp90 degradation found in *C. albicans* cultures.

Keywords: *Candida albicans*, *Saccharomyces cerevisiae*, Hsp90, partial protein degradation, candidiasis

INTRODUCTION

Heat-shock proteins (hsps) are major targets for the immune response to several bacterial, fungal and parasitic infections (Young, 1992). Several microbial hsps have been associated with protective immunity (Bonnefoy *et al.*, 1994; Gomez *et al.*, 1995; Lowrie *et al.*, 1994; Matthews *et al.*, 1991, 1995), the eukaryotic molecular chaperone Hsp90 being important in this respect. Surface-expressed parasite Hsp90 serves as an antigen in Chagas' disease (Dragon *et al.*, 1987), ascariasis (Kumari *et al.*, 1994), leishmaniasis (Salotra *et al.*, 1995; Skeiky *et al.*, 1995; Streit *et al.*, 1996) and

infections with *Schistosoma mansoni* (Johnson *et al.*, 1989). The successful treatment of children with malaria by the passive transfer of immunoglobulin from adults living where malaria is endemic may also be due to antibodies against the Hsp90 of *Plasmodium falciparum* (Bonnefoy *et al.*, 1994). Immunodominant antigens of *Candida albicans* are present on a 47 kDa subfragment of candidal Hsp90 that circulates in the serum of patients with candidiasis, a fragment that comprises the C-terminal domain of this abundant molecular chaperone (Matthews & Burnie, 1989; Matthews *et al.*, 1987). Both monoclonal and human recombinant antibodies to a highly conserved epitope on this fragment (LKVIRK) were protective in murine models of candidiasis (Matthews *et al.*, 1991, 1995). These antibodies are also always present in humans who have survived systemic

Abbreviations: 5-FOA, 5-fluoroorotic acid; GR, glucocorticoid receptor.

candidiasis, whereas their absence is invariably associated with fatality (Matthews, 1994; Matthews *et al.*, 1987). So highly conserved is the LKVIRK epitope on eukaryotic Hsp90s that antibodies against this epitope may cross-protect against a number of different microbial pathogens (Matthews, 1994). In addition, the essential functions of Hsp90 may not allow large mutational changes to this epitope, whereby microbes could become resistant to LKVIRK-targeting antibodies. There is therefore considerable interest in the potential diagnostic and therapeutic uses of Hsp90-targeting antibodies (Matthews & Burnie, 1996; Matthews, 1994).

The cellular functions of the abundant Hsp90 molecular chaperone have been most clearly identified in mammalian cells, where this protein is essential for the final activation step of several key proteins of signal transduction (Gething & Sambrook, 1992; Pratt & Dittmar, 1998; Rutherford & Lindquist, 1998; Toft, 1998). Hsp90 action in the ligand-dependent activation of mammalian steroid receptor proteins has been particularly well studied (Caplan, 1997; Nathan & Lindquist, 1995; Pratt & Dittmar, 1998). In contrast, although Hsp90 is an important antigen in candidal infections, virtually nothing is known about Hsp90 functions in *Candida*. Also there is no evidence to suggest how the 40/47 kDa subfragments of *C. albicans* Hsp90 found circulating in the serum of patients with candidiasis are generated during the pathology of this disease. We show here that the expression of the coding region of the *C. albicans* HSP90 gene in *Saccharomyces cerevisiae* can provide Hsp90 functions in the latter yeast. *S. cerevisiae* can therefore be used to show whether changes to conserved epitopes on *C. albicans* Hsp90 are compatible with essential Hsp90 functions. We also show that cells of both *C. albicans* and *S. cerevisiae* generate Hsp90 fragments, the incomplete degradation of *C. albicans* Hsp90 expressed in *S. cerevisiae* resembling partial degradation of this same protein in *C. albicans* cultures. A similar partial breakdown of the endogenous Hsp90 is also seen in *S. cerevisiae* cells adapting to respiratory growth. *S. cerevisiae* molecular genetics can therefore probably be applied to study the events of partial *in vivo* Hsp90 degradation, the process that may generate the antigenic fragments of this molecular chaperone that appear during candidal infections.

METHODS

Plasmid constructions. A vector (pCA90) for the expression of *C. albicans* Hsp90 in *S. cerevisiae* was constructed by sequential cloning of three DNA fragments into the *S. cerevisiae*-*Escherichia coli* shuttle vector YCplac111 (Gietz & Sugino, 1988). First, a 414 bp *ADH1* terminator *Pst*I/*Hind*III fragment from pGBT9 (Clontech) was ligated into *Pst*I/*Hind*III-cleaved YCplac111 (Gietz & Sugino, 1988). Next, the HSC82 promoter was inserted as a *Bam*HI/*Sal*I-cleaved PCR-generated fragment, to give vector pHSCprom. This promoter fragment had previously been amplified from pHSC82 (Panaretou *et al.*, 1998) using the primers CGAGCTCGGTACCCGG and GTTTCACCGTCTGACTT-ATTTCAGAATGATTC (*Bam*HI and *Sal*I sites, respectively, underlined). Finally, the complete *C. albicans* HSP90 gene

coding region was inserted into pHSCprom as a *Sal*I/*Pst*I-cleaved PCR product, producing pCA90. This had been PCR-amplified from *C. albicans* strain 3153A genomic DNA using PCR primers CCATACAAATGTCGACTTCATTATGGC-TGACGC and CTATGTCTAATAGCGGCCGCCTGCAGGATTTTCCTTTTAGC (*Sal*I and *Pst*I sites, respectively, underlined). The *S. cerevisiae* HSP82 gene coding region was also placed in pHSCprom as a *Sal*I/*Pst*I-cleaved PCR product, thereby producing a vector (pHSP82b) for expressing Hsp82 from the HSC82 promoter. This gene was PCR-amplified from pHSP82 (Panaretou *et al.*, 1998) using PCR primers ACGCAAAGATGTCGACATGGCTAGTGAAAC and ATGATGTTCTACCTGCAGCTAATCTACCTCTTC (*Sal*I and *Pst*I sites, respectively, underlined).

Strains. *C. albicans* Berkhout strain 3153A, from the London Mycology Reference Laboratory Collection, was kindly provided by Alistair Brown, University of Aberdeen, UK. The *S. cerevisiae* strains used were: PP30(pHSC82) [MATa *trp1*-289 *leu2*-3,112 *his3*-200 *ura3*-52 *ade2*-101^{oc} *lys2*-801^{am} *hsc82*::KANMX4 *hsp82*::KANMX4(pHSC82)] (Panaretou *et al.*, 1998); E929-6C-20 (MATα *ste11*-Δ6 *can1* *leu2*-3,112 *trp1*-Δ1 *ura3*-52) (Louvion *et al.*, 1998), SUB61 and SUB63 (Finley *et al.*, 1987). PP30(pHSC82) was transformed to leucine prototrophy with the *LEU2* vectors pHSP82b or pCA90, then cured of its pHSC82 *URA3* vector by growth on 5-fluoroorotic acid (FOA; Melford Laboratories) as described by Panaretou *et al.* (1998). This resulted in strains that expressed only the Hsp82 of *S. cerevisiae* [PP30(pHSP82b)] or only the Hsp90 of *C. albicans* [PP30(pCA90)].

Steroid hormone receptor assays. The ligand response of glucocorticoid receptor (GR) was determined by measuring β-galactosidase activity induced by dexamethasone. Yeast cells were grown overnight to exponential phase at 30 °C in selective medium containing tryptophan and adenine. To ensure that the ligand was added to cultures of equivalent volume and cell density, culture volumes were diluted with fresh medium to 100 ml (1.55 × 10⁶ cells ml⁻¹). Dexamethasone (10 μM final concentration, from a 20 mM stock in ethanol) was then added to half of the culture; an equivalent amount of ethanol (0.05%) being added to the control. Cultures were incubated for a further 2 h at 30 °C, before cells were collected by centrifugation (3000 g, 5 min). Cell pellets were resuspended in 500 ml 2 × Z buffer (60 mM Na₂HPO₄ · 7H₂O, 60 mM NaH₂PO₄ · H₂O, 5 mM KCl, 0.5 mM MgSO₄, 0.05% β-mercaptoethanol, pH 7). β-Galactosidase activity was determined as described by Bohen (1998).

Antibody preparation and analysis of Hsp90s by Western blotting. As part of our investigations into the structure of yeast Hsp82 we have expressed several regions of this protein in *E. coli* so that the fragments could then be used for crystallization trials (Prodromou *et al.*, 1997). Two of these fragments (corresponding to regions 1–221 and 300–704 of *S. cerevisiae* Hsp82) were also employed as antigens in raising polyclonal rabbit antisera. These antisera were shown to be Hsp90-specific (proteins in yeast total cell extracts showing no appreciable cross-reaction to the pre-immune sera). Also, investigations of their cross-reactivity to our different regions of Hsp82 expressed in *E. coli* revealed they are also domain-specific, in that the antiserum raised against the 1–221 region of Hsp82 recognized an epitope at the extreme N-terminus of full-length Hsp82 and the antiserum raised against the 300–704 region recognized epitopes exclusively within the C-terminal domain of full-length Hsp82 (Sinclair, 1998). Preparation of total yeast cell protein extracts was as described earlier (Cheng *et al.*, 1992; Panaretou *et al.*, 1998). For the analysis of Hsp90s

by Western blotting, these protein extracts were gel-fractionated, immobilized on nitrocellulose and probed at room temperature with the above Hsp82 N-domain-specific or C-domain-specific antisera. Antibody binding was visualized by incubating the blot with peroxidase-conjugated anti-rabbit IgG and then use of the Amersham enhanced chemiluminescence (ECL) kit according to the manufacturer's instructions.

RESULTS

C. albicans Hsp90 can confer essential Hsp90 functions in S. cerevisiae

C. albicans appears to have just one gene for Hsp90 (Swoboda *et al.*, 1996), whereas *S. cerevisiae* possesses two, *HSC82* and *HSP82*. In the latter yeast, *HSC82* is constitutively expressed at high levels and is only slightly heat-inducible, whereas *HSP82* is normally expressed at tenfold lower levels but is strongly induced by heat shock (Borkovich *et al.*, 1989). Hsp90 is thought to be an essential protein in eukaryotic organisms, its essential nature having been proven in *Drosophila* (Rutherford & Lindquist, 1998) and *S. cerevisiae* (a $\Delta hsc82 \Delta hsp82$ *S. cerevisiae* strain being inviable; Borkovich *et al.*, 1989). We recently constructed a *S. cerevisiae* strain [PP30(pHSC82)] that has the chromosomal coding

regions of both *HSC82* and *HSP82* totally deleted, but which is viable since a copy of the *S. cerevisiae* *HSC82* gene on a *URA3* plasmid provides the essential Hsp90 functions (Panaretou *et al.*, 1998). By introducing the genes for mutant or heterologous Hsp90s into this strain on a *LEU2* vector, then selecting for loss of the *URA3* vector on FOA plates, it is possible to determine whether introduced Hsp90 genes can confer the essential Hsp90 functions in *S. cerevisiae* (Panaretou *et al.*, 1998). Absence of growth on FOA shows that the introduced gene is nonfunctional, whereas growth on FOA reveals that it is functional.

The *C. albicans* *HSP90* ORF (Swoboda *et al.*, 1996) is devoid of CTG codons, which code for serine in *C. albicans* but leucine in most other organisms (Santos *et al.*, 1993). It also encodes a product with 84 and 78% amino acid sequence identity, respectively, to the Hsc82 and Hsp82 proteins of *S. cerevisiae*. We therefore thought it probable that expression of the *C. albicans* *HSP90* ORF in *S. cerevisiae* would generate functional Hsp90 in the latter yeast. To show that this was indeed the case, we constructed *LEU2* vectors (pCA90 and pHSP82b) in which the coding regions of either *C. albicans* *HSP90* or *S. cerevisiae* *HSP82* are expressed from the *S. cerevisiae* *HSC82* promoter (see Methods).

Fig. 1. (a) Growth in the absence (left) and presence (right) of FOA of *S. cerevisiae* PP30(pHSC82) transformed to leucine prototrophy with an empty vector (pHSC82prom) or vectors for expression of either the *C. albicans* Hsp90 (pCA90) or the native *S. cerevisiae* Hsp82 (pHSP82b). Plates were photographed after 3 d growth at 30 °C. (b) Growth in the absence (left) and presence (right) of 5 μ M α -factor of an *S. cerevisiae* *ste11* mutant strain (E929-6C-20) and *S. cerevisiae* cells expressing *C. albicans* Hsp90 or *S. cerevisiae* Hsp82 [(PP30(pCA90) and PP30(pHSP82b), respectively)]. Plates were photographed after 3 d growth at 30 °C.

Fig. 2. Detection of full-length Hsp90 and Hsp90 subfragments in cell extracts from *S. cerevisiae* strain SUB61 (S.c.) and *C. albicans* (C.a.). Cultures of both yeasts in exponential growth on YPD (glucose) medium at 30 °C were transferred to fresh YP/glycerol medium at time zero. Total protein extracts were then prepared after 0 (lane 1), 18 (lane 2), 24 (lane 3) or 42 (lane 4) h further aerobic maintenance at 30 °C. These extracts were gel-fractionated, blotted and probed with antisera that recognize epitopes either in the C-terminal domain (a) or at the N-terminus (b) of Hsp90. The latter antiserum does not detect the major Hsp90 degradation products of 72–76 and 47 kDa [arrowed in (a)]. Note that the shift to respiratory medium induces substantial Hsp90 partial degradation in *S. cerevisiae* but does not enhance this degradation in *C. albicans*.

These vectors were then used to transform *S. cerevisiae* PP30(pHSC82) to leucine prototrophy. When subsequently plated onto FOA plates, the cells that contained the pCA90 vector [PP30(pCA90)] grew as well as those expressing the native Hsp82 protein [PP30(pHSP82b)] (Fig. 1a). Since the only Hsp90 coding sequences in *S. cerevisiae* PP30(pCA90) growing on FOA are those corresponding to *C. albicans* Hsp90, this Hsp90 can clearly provide essential Hsp90 functions in *S. cerevisiae*.

C. albicans* Hsp90 can provide the Hsp90 requirement for pheromone signalling in *S. cerevisiae

Although Hsp90 is known to be essential for the activation of several important mammalian regulatory proteins, its only targets in *S. cerevisiae* identified to date are the Ste11 kinase needed for pheromone signalling (Louvion *et al.*, 1998) and heat shock transcription factor (Duina *et al.*, 1998). Pheromone signalling is defective in *S. cerevisiae* cells that either have very low Hsp90 levels or which express human Hsp90 β in place of the homologous Hsp90 (Louvion *et al.*, 1996). To determine if *S. cerevisiae* cells that express only the *C. albicans* Hsp90 are similarly defective in pheromone signalling, PP30(pCA90) cells were tested for their capacity to arrest growth in response to the *S. cerevisiae* mating pheromone α -factor. α -Factor arrested the growth of these cells (Fig. 1b), revealing that *C. albicans* Hsp90 can provide the Hsp90 function needed for pheromone signalling in *S. cerevisiae*.

C. albicans* Hsp90 can provide steroid-responsive activation of the mammalian GR expressed in *S. cerevisiae

There appears to be a physiological response of *C. albicans* to corticosteroids (Malloy *et al.*, 1993), although the molecular mechanisms of this response and the basis of the enhanced incidence of candidal infections in those patients administered corticosteroids remain unclear. We transformed both PP30(pCA90) and PP30(pHSP82b) with a *HIS3* vector (pHCA/rGR; Garabedian & Yamamoto, 1992) for *S. cerevisiae* expression of the F620S mutant form of mammalian GR. In this vector GR expression is under the control of the constitutively expressed glyceraldehyde-3-phosphate dehydrogenase promoter, whilst the F620S mutation seems to increase the affinity of GR for its steroid ligand (Garabedian & Yamamoto, 1992). We also transformed these cells with pAS26X, a *URA3* plasmid that contains a reporter gene for GR activity (Schena *et al.*, 1989). This reporter comprises three copies of the glucocorticoid response element in a modified *CYC1* promoter, elements that cause GR-responsive expression of an adjacent *lacZ* gene.

Transformation of PP30(pCA90) and PP30(pHSP82b) with both pHCA/rGR and pAS26X gave *HIS*⁺ *URA*⁺ transformants that turned blue when replicated onto X-Gal indicator plates containing the GR ligand deoxycorticosterone. The addition of deoxycorticosterone to liquid cultures of these transformants resulted in the

Fig. 3. Detection of full-length Hsp90 and Hsp90 subfragments in cell extracts from *S. cerevisiae* PP30(pHSP82b) and PP30(pCA90). Cultures of both strains, in exponential growth on YPD (glucose) medium at 30 °C were transferred to fresh YP/glycerol medium. Total protein extracts were then prepared after a further 0 (lane 1), 18 (lane 2), 24 (lane 3) or 42 (lane 4) h aerobic maintenance at 30 °C. These extracts were gel-fractionated, blotted and probed with antisera that recognize epitopes either in the C-terminal domain (a) or at the N-terminus (b) of Hsp90. Major Hsp90 degradation products of 72–76 and 47 kDa are arrowed in (a).

rapid induction of GR activity, induction levels being essentially identical irrespective of whether the cells were expressing the heterologous *C. albicans* Hsp90 or the native *S. cerevisiae* Hsp82. Two hours after dexamethasone addition to mid-exponential-phase cultures of *S. cerevisiae* PP30(pHSP82b) and PP30(pCA90) containing the GR receptor and GR-inducible *lacZ* reporter genes, β -galactosidase levels had increased above the basal level of 0.1 units to 17.9 and 18.0 ($\pm 5\%$) units, respectively. In contrast, values in the control cultures without steroid addition were only 0.05 and 0.09 ($\pm 5\%$) units β -galactosidase. *C. albicans* Hsp90 is therefore fully functional with regard to its ability to participate in steroid responsive activation of mammalian GR protein expressed in *S. cerevisiae*, an activation that is strictly dependent on Hsp90 function (Caplan, 1997; Nathan & Lindquist, 1995; Pratt & Dittmar, 1998).

***C. albicans* accumulates Hsp90 fragments, the major forms being of similar size to the N-terminally truncated forms of Hsp90 accumulated by respiratory *S. cerevisiae* cells**

When total soluble protein is extracted from exponentially growing *C. albicans* cultures not only full-length Hsp90 is detected, but also smaller Hsp90 subfragments (Fig. 2a). Prominent on the gels are fragments of 72–76 and 47 kDa, the latter most probably being the antigenic C-terminal domain subfragment of Hsp90 detected in earlier investigations (Matthews & Burnie, 1989; Matthews *et al.*, 1987). A similar series of Hsp90 fragments is also seen in *S. cerevisiae* cultures under certain conditions of growth (Figs 2a, 3a). Whilst

the cell extracts used for these experiments were prepared rapidly in the presence of a cocktail of protease inhibitors, it is still important to determine whether this partial Hsp90 degradation occurs within viable cells or is the result of cell lysis and the subsequent limited proteolysis of released cell contents. Several lines of evidence indicate that the former is the case. Firstly, plating of cells from the cultures used for protein extract preparation in these experiments showed that there had been no appreciable loss of cell viability. At the time of harvesting, practically 100% of the cells were able to form colonies on plates and stained with vital dyes. Secondly, a brief treatment of intact cells with chymotrypsin, prior to the addition of chymostatin, had no effect on these Hsp90 fragmentation patterns. Thirdly, this partial degradation of Hsp90, at least in *S. cerevisiae* cells, is *prevented* by a N-terminal (His)₆ tag on Hsp82 protein that still permits Hsp90 function (Chang & Lindquist, 1994), but is *unaffected* by a mutation (*pep4-3*) that largely inactivates the vacuolar proteases which constitute the major source of proteolysis in *S. cerevisiae* cell extracts (Jones, 1991) (see Discussion). If the partial degradation of Hsp90 was the result of appreciable cell lysis or incomplete protein degradation after cell breakage, these mutational changes would be expected to exert the converse effects from those actually observed.

We prepared total protein extracts of *S. cerevisiae* cultured under a number of different conditions and exposed to different stress regimes to investigate whether particular conditions induce the accumulation of relatively stable Hsp90 fragments. Stresses such as sublethal heat shock, osmotic stress or oxidant exposure did not

increase levels of Hsp90 fragments in glucose-grown *S. cerevisiae* (data not shown). Instead, we found that it was a transfer from fermentative to respiratory media that most effectively caused the appearance of 47 kDa Hsp90 forms in *S. cerevisiae* cultures (Figs 2a, 3a). With the *C. albicans* isolate used for this study (3153A) the pattern of these Hsp90 fragments was unaffected by growth at different temperatures and, in contrast to *S. cerevisiae*, levels of the 47 kDa fragment actually decreased with the transfer of *C. albicans* cells to a respiratory carbon source (Fig. 2a).

The Hsp90 subfragments from *C. albicans* cells cross-react with an antiserum that recognizes the C-terminal domain of Hsp90 (Fig. 2a), but not with an antiserum recognizing an epitope at the extreme N-terminus of Hsp90 (Fig. 2b). Therefore, they are probably N-terminally truncated forms of Hsp90, like the Hsp90 fragments that accumulate in respiratory *S. cerevisiae* cultures (see Discussion). It is noteworthy that the major Hsp90 fragments in cultures of *C. albicans* and in glycerol-grown *S. cerevisiae* cultures are remarkably similar in size (Fig. 2a), an indication that the events of incomplete Hsp90 degradation in these two organisms may be extremely similar.

Partial degradation of the heterologous *C. albicans* Hsp90 expressed in respiratory *S. cerevisiae* PP30(pCA90) closely resembles that of the homologous *S. cerevisiae* Hsp82 protein

We directly compared the accumulation of Hsp90 fragments in *S. cerevisiae* expressing either the homologous Hsp82 isoform of *S. cerevisiae* Hsp90 [PP30(pHSP82b)] or the *C. albicans* Hsp90 [PP30(pCA90)]. With transfer from glucose to a respiratory carbon source (glycerol) the two cultures accumulated an extremely similar, though not quite identical, series of Hsp90 subfragments (Fig. 3a). As also seen in another *S. cerevisiae* strain and in *C. albicans* itself (Fig. 2a), the major accumulated fragments were of 72–76 and 47 kDa (Fig. 3a) and lacked the Hsp90 N-terminus (Fig. 3b). Full-length *C. albicans* Hsp90 is actually a slightly smaller protein than *S. cerevisiae* Hsp82 (Figs 3a, b), due primarily to the highly charged linker region between the N-terminal and C-terminal domains of Hsp90 being some 7 amino acids shorter in the former (Swoboda *et al.*, 1996). The *S. cerevisiae* Hsp82 degradation products of 72–76 kDa arise from cleavages within the N-domain (see Discussion). Since a complete linker region is still present in these 72–76 kDa forms, the equivalent fragments derived from the *C. albicans* Hsp90 are also slightly smaller (Fig. 3a). In contrast, those Hsp90 degradation products in the 47 kDa size range arise from a cleavage at the beginning of the C-terminal domain (Matthews & Burnie, 1989) and lack the linker region. These 47 kDa fragments are of the same sizes when derived from the expression of either pHSP82b or pCA90 sequences (although there is a quantitative difference in that pHSP82b expression gave more of these 47 kDa forms than pCA90 expression;

Fig. 3a). The blot shown in Fig. 3(a) is therefore a strong indication that the *C. albicans* Hsp90 expressed in *S. cerevisiae* cells and the native Hsp82 protein are both being degraded by essentially similar processes. Furthermore, the major 72–76 and 47 kDa subfragments of *C. albicans* Hsp90 accumulated in respiratory *S. cerevisiae* PP30(pCA90) cells (Fig. 3a) are identical in size to the fragments isolated from *C. albicans* (Fig. 2a). It seems probable therefore that *S. cerevisiae* cells can reasonably faithfully reproduce the steps that generate the major products of incomplete Hsp90 degradation in *C. albicans* cultures (Fig. 2a). However, more work is needed to precisely define the nature, and also the ordering, of the proteolytic events that lead to these relatively stable chaperone subfragments.

DISCUSSION

We had two main objectives in constructing a *S. cerevisiae* strain that had all coding sequences for the native Hsp90s deleted and which expressed instead the *C. albicans* Hsp90. The first was to show that *C. albicans* Hsp90 is capable of providing Hsp90 functions in *S. cerevisiae*. This provides a system in which variants of *C. albicans* Hsp90 can be tested for functionality. Thus, by altering the sequences corresponding to conserved epitopes on Hsp90 [such as LKVIRK (Matthews, 1994)], then introducing these mutants into PP30(pHSC82) and plating transformants on FOA (see Methods), future studies will be able to determine the extent to which conserved pathogen Hsp90 epitopes can undergo mutational change. This in turn will indicate whether LKVIRK-targeting antibodies might protect against a number of eukaryotic pathogens (Matthews, 1994). Our second objective was to develop a system for investigating Hsp90 turnover, in particular to study whether partial Hsp90 degradation *in vivo* might be the origin of the antigenic C-domain subfragments of candidal Hsp90 found in the serum of patients with disseminated candidiasis (Matthews *et al.*, 1987). There had, prior to this work, been no attempt to address the origins of these pathogen-derived serum Hsp90 fragments.

In parallel with this study we have been investigating the mechanism of the incomplete degradation of Hsp82 protein in respiratory *S. cerevisiae* cells (unpublished). We found that this degradation is totally abolished by the presence of an N-terminal (His)₆ tag on Hsp82 protein. In contrast, an epitope tag placed at the C-terminus of Hsp82 does not abolish processing, but is retained on *both* the 72–76 and 47 kDa forms of Hsp82. The latter therefore comprise different forms of N-terminally truncated Hsp82 (which also accounts for their lack of detection by an antibody that detects the N-terminus of Hsp90s; Figs 2, 3). Whether the 72–76 kDa forms are precursors of the 47 kDa forms is at present unclear. However, the availability in *S. cerevisiae* of a wide range of mutants defective in either vacuolar proteolysis (Jones, 1991) or in the ubiquitination system for intracellular protein turnover (Hershko & Ciechanover, 1998) allowed us to probe the question of

how Hsp90 fragments arise. We have shown that their production in *S. cerevisiae* is unaffected by the loss of vacuolar proteases, but altered in cells lacking the Ubc4 ubiquitin-conjugating enzyme. We are therefore currently investigating *S. cerevisiae* ubiquitination system and proteosome mutants to try to identify other components involved in Hsp82 partial degradation. It should be noted that the vast majority of the proteins targeted for degradation by the ubiquitination system are rapidly and completely degraded (Hershko & Ciechanover, 1998). Only a few cases are known where a protein is *incompletely* degraded, most of them from mammalian systems. The partial degradation of Hsp90 (Figs 2, 3) appears to be the first identified case of an incomplete protein degradation event in yeast and, as such, should lend itself to genetic dissection. Whether loss of N-terminal sequences in a substantial fraction of the Hsp90 of respiratory *S. cerevisiae* serves any physiological purpose is also at present unclear. Removal of these sequences should lead to the loss of a domain essential for the Hsp90 chaperone function (Panaretou *et al.*, 1998).

There are indications that the release of Hsp90 or Hsp90 fragments from the cells of fungal pathogens causes appreciable toxicity to the host, rather analogous to the endotoxin release from Gram-negative bacteria that causes endotoxic shock in bacterial septicaemia. Even *S. cerevisiae*, a yeast that is normally considered non-pathogenic, becomes much more virulent in mice when its Hsp90 protein levels are elevated (Hodgetts *et al.*, 1996). The reasons for this enhancing effect of Hsp90 on yeast pathogenicity are still unclear, but it may involve the malfunctioning of serum proteins that bind Hsp90 fragments (Matthews, 1994). Suppression of this effect may be a major ameliorating influence of the presence of anti-Hsp90 antibodies, since there is a close correlation between a good antibody response to Hsp90 (47 kDa antigen) and recovery in candidiasis patients (Matthews & Burnie, 1996; Matthews *et al.*, 1987). In addition to Hsp90, other highly abundant cytoplasmic proteins are recognized by the immune system in candidal infections. These include the hsp70 molecular chaperone (Bromuro *et al.*, 1998) and the glycolytic enzyme enolase (Franklin *et al.*, 1990). These proteins are also present in the cell wall (Angiolella *et al.*, 1996; Lopez-Ribot *et al.*, 1996) even though, like Hsp90, they are initially made devoid of secretory signal sequences. It might therefore be assumed that candidal Hsp90 fragments arise in the serum through limited proteolysis after release of Hsp90 from lysed cells of the pathogen, and secretory proteinases are indeed found in the culture supernatants of clinically important *Candida* species [see Matthews (1994) for literature]. However, candidal secretory proteinases generally have a low pH optimum, unlike the tissues being invaded, which are mostly around neutral pH. Furthermore, our work indicates that the *primary* events leading to *generation* of the sub-fragments of *C. albicans* Hsp90 may be intracellular, not a process of limited degradation of Hsp90 after its release from lysed cells.

S. cerevisiae has become an extremely useful system for studying the ligand- and Hsp90-dependence of mammalian steroid receptors (Caplan, 1997; Pratt & Dittmar, 1998; Toft, 1998). Strains with conditional mutations in Hsp90 have been used to show that Hsp90 is required, not just for activation of some of these receptors, but also for maintaining them in their activated state (Nathan & Lindquist, 1995). In the oomycete water mould *Achlya ambisexualis*, binding of the fungal steroid antheridiol to its receptor/Hsp90 complex initiates hyphal branching (Brunt *et al.*, 1990). However, no steroid-dependent gene activators are known for *S. cerevisiae*, or indeed for any other ascomycete fungus. Nevertheless, mammalian steroid hormones do have effects on ascomycete yeasts. Oestradiol directly stimulates the dimorphic transition of *C. albicans* from the yeast to the hyphal form (Madani *et al.*, 1994), whilst corticosteroids and pregnancy predispose humans to candidiasis (Malloy *et al.*, 1993). The key targets whereby steroids produce such effects in *Candida* cells have still to be identified. Those *C. albicans* steroid-binding proteins that have been characterized to date are flavoproteins, with no obvious homology to the mammalian steroid receptors (Madani *et al.*, 1994; Malloy *et al.*, 1993). However, Hsp90-dependent steroid receptors may yet be uncovered in certain eukaryotic pathogens, most probably through the sequencing of their genomes. *S. cerevisiae* strains such as PP30(pCA90) offer the potential for co-expressing such receptors together with the appropriate pathogen Hsp90, a system which should make the ligand- and Hsp90-dependence of the receptors readily amenable to analysis.

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