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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**INTRODUCTION**

Heat-shock proteins (hsp90s) are major targets for the immune response to several bacterial, fungal and parasitic infections (Young, 1992). Several microbial hsp90s 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., 1991, 1995). 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 infections in which Hsp90 was a major antigenic fragment.

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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 YCp111 (Gietz & Sugino, 1988). First, a 414 bp ADH1 terminator PsI/HindIII fragment from pGBT9 (Clontech) was ligated into PsI/HindIII-cleaved YCp111 (Gietz & Sugino, 1988). Next, the HSC82 promoter was inserted as a BamHI/SalI-cleaved PCR-generated fragment, to give vector pHSCpom. This promoter fragment had previously been amplified from pHSC82 (Panaretou et al., 1998) using the primers CGAGGCTCGGATCCCGG and GTTTTACCGTCGACCTTATTCAAGATGATTTC (BamHI and SalI sites, respectively, underlined). Finally, the complete C. albicans HSP90 gene coding region was inserted into pHSCprom as a SalI/PstI-cleaved PCR product, producing pCA90. This had been PCR-amplified from C. albicans strain 3153A genomic DNA using PCR primers CCTACAAATGTCGACCTATTAGGC-TGACGC and ATATGCTTATAGCGGCCGCTGAC GATTTCCTTTTAGC (SalI and PstI sites, respectively, underlined). The S. cerevisiae HSP82 gene coding region was also placed in pHSCpom as a SalI/PstI-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 AGCGAAGATGGCATGGCTATGGAAAC and AT- GATTGGTTACTACGGATACTACCTTCTCCTTC (SalI and PstI sites, respectively, underlined).

Strains. C. albicans Berkhour 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: BY30[pHSC82] (MATa trp1-289 leu2-3,112 his3-bio 200 ura3-52 ade2-101 lys2-801m hsc82::KANMX4 hsp82::KANMX4[pHSC82]) (Panaretou et al., 1998); E929-6C-20 (MATa ste11Δ α can1 leu2-3,112 trp1Δ1 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 (1.5 ± 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 then determined as described by Bohen (1998).

Antibody preparation and analysis of Hsp90s by Western blotting. As part of our investigation into the structural 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 antiserum. These antiserum 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 antisera raised against the 1–221 region of Hsp82 recognized an epitope at the extreme N-terminus of full-length Hsp82 and the antisera 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 ∆hsc82 ∆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 aURA3 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).

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Fig. 1. (a) Growth in the absence (left) and presence (right) of FOA of S. cerevisiae PP30(pHSC82) transformed to leucine
prototropy 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 µM 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.
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 Hsp90β 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
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 (±5%) units, respectively. In contrast, values in the control cultures without steroid addition were only 0.05 and 0.09 (±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

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**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 YPlg/lycerol 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).
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 antisera that recognizes the C-terminal domain of Hsp90 (Fig. 2a), but not with an antisera 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 [PP30(pCA90)]. With transfer from glucose to a respiratory carbon source (glycerol) the two cultures (Fig. 2a) 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)_n 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 septicemia. 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 subfragments 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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