Published in final edited form as:

Mol Cell Endocrinol. 2007 March 30; 268(1-2): 67–74.

Uncoupling of Hormone-dependence from chaperone-dependence in the L701H mutation of the androgen receptor

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Abstract

The mechanisms underlying androgen receptor (AR)-mediated progression of prostate cancer following androgen ablation have yet to be fully determined. On this basis we screened naturally occurring mutants of human AR for hormone independent activity using a yeast model system. An initial screen of 43 different mutants revealed that ARs having a Leu701His mutation (AR^{L701H}) exhibited hormone-independent activation of a lacZ reporter gene. The AR^{L701H} mutant bound dihydrotestosterone to a similar extent as did wild type AR, although its ability to be induced by hormone for transactivation was reduced substantially. Subsequent studies focused on the dependence of AR^{L701H} on molecular chaperones for folding to the active state. We found that AR^{L701H} was highly dependent on Hsp90 for its hormone independent activation, suggesting that this chaperone functions in AR^{L701H} folding. However, the mutant did not respond specifically to increased levels of FKBP52, suggesting that this chaperone functions at the hormone-dependent activation stage in the folding process. Further studies of AR^{L701H} in PC3 cells suggested that this mutant is prohibited from hormone-independent transactivation in mammalian cells. However, basal expression of a reporter gene by AR^{L701H} was not impaired by the presence of 17-allylamino-17-demethoxygeldanamycin as was wild type AR, suggesting differential interactions of these receptors with molecular chaperones in animal cells.

Introduction

Human androgen receptor (AR) signaling axis is a major target of drugs used to treat prostate cancer. Competitive hormone antagonists are used to inhibit transactivation by AR and are effective to a point, although it is common for tumors to eventually become resistant to these drugs [1]. It is now thought that hormone-independence for tumor growth occurs via the AR itself which is still present and functional in the transformed cells. Two AR-dependent mechanisms for this effect involves amplification of the AR locus (which occurs in approximately 30% of tumors) and somatic mutation of the X-linked AR gene [2]. Other factors that may contribute to hormone-independent AR activation include phosphorylation (indirectly

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resulting from autocrine or paracrine mechanisms) [1]. Furthermore, alterations in ligand-preference for transactivation may accompany somatic mutation of AR [3,4], and these effects may be compounded by changes in co-activator or co-repressor expression patterns [5].

In the past few years, a new class of drug has begun to emerge as a promising candidate for chemotherapy. These drugs, such as geldanamycin and 17-AAG, all inhibit Hsp90 and belong to the ansamycin class of antibiotics [6]. Hsp90 is an abundant molecular chaperone that is known to facilitate folding and maturation of proteins involved in signal transduction – including members of the nuclear receptor family including the AR [7]. The drugs work by competitively inhibiting Hsp90's ATPase. Ansamycin antibiotics induce rapid proteasome-dependent degradation of the AR as a consequence of their ability to inhibit Hsp90 [8]. Since many protein kinases are similarly susceptible to Hsp90 inhibition, this class of drug may be able to target several growth promoting signaling pathways simultaneously. This action is promoted by the interesting finding that geldanamycin and its analogues preferentially accumulate in tumors over non-transformed cells. This is possibly due to changes in Hsp90 itself, resulting in the chaperone having a higher affinity for both the drug and ATP [9].

Results from previous studies showed that AR depends on Hsp90 and other molecular chaperones to bind hormone with high affinity [10]. The ligand binding domain of AR is the site of Hsp90 binding, although little is known of the mechanism underlying its ability to fold AR. Like other type I nuclear receptors, Hsp90 interacts with unliganded AR and this interaction is maintained until hormone binding stimulates chaperone complex dissociation. Hormone bound AR is then able to relocate from the cytosol to the nucleus where it dimerizes, interacts with co-activators and chromatin prior to transcriptional activation of its target genes. One important question that has not been answered is whether Hsp90 is required for activation of the AR by hormone-independent mechanisms. If Hsp90 is not required for hormone-independent activation of AR, then drugs like 17-AAG might be ineffective toward prostate tumor growth. To this end, we screened a panel of AR mutants, many originally characterized in prostate tumors, for hormone-independent transactivation using a yeast model system. One mutant, AR^{L701H}, was found to transactivate a reporter gene in a hormone independent manner. AR^{L701H} was subjected to a panel of tests to assess its dependence on molecular chaperones for its functional properties. Our results are consistent with AR^{L701H} being dependent on Hsp90 even for its hormone-independent transactivation function.

Results

Identification of L701H as a hormone independent AR mutant

Our initial approach was to screen 43 different AR mutants by functional assays using a yeast model system. These mutants, many of which were characterized originally from prostate tumors (see methods), were cloned into a yeast expression vector by homologous recombination. This strategy avoided separate cloning and transformation steps. Initial studies focused on whether any of the mutants exhibited hormone independent AR activity. This was assessed by testing whether an ARE-lacZ reporter could be transactivated in the absence of added DHT using a plate-based assay. This assay involved plating cells expressing wild type and different AR mutants on media containing X-gal. As shown in Fig 1, two different colonies turned blue in the presence of X-gal on media lacking hormone: AR^{T877A} and AR^{L701H}. None of the other colonies turned blue, not even the naturally occurring double mutant AR^{L701H,T877A} [3]. In the presence of 5 nM DHT, all of the colonies turned blue on X-gal plates but to differing extents (not shown). Leucine 701 sits at the bottom of the ligand binding domain in close proximity to bound ligands (Figure 1B); indeed Histidine in this position interacts directly with bound fluorocortisol and contributes to the ability of the AR^{L701H,T877A} double mutant to be activated by glucocorticoids [11].

Subsequent studies focused on AR^{L701H} and two other AR mutants for their ability to induce a LacZ reporter gene under a variety of conditions. The two other mutants included in this study were AR^{M780T}, which is androgen insensitive [12], and AR^{T877A} which is the AR mutation found in LNCaP cells and has also been observed as a naturally occurring double mutant with AR^{L701H} [12]. Initial studies investigated DHT dose-dependence for lacZ gene expression. Cells expressing wild type AR, AR^{L701H}, AR^{M780T} and AR^{T877A} were grown to mid-log in selective media and incubated with DHT at 0-10nM for 2 hours. Extracts from these cells were then prepared and quantitative assays for AR-dependent lacZ gene expression performed as described in materials and methods. The results of these experiments showed that AR^{L701H} mutant displayed a 6.5-fold increase in hormone-independent lacZ gene expression compared to the wild type (Fig. 2). Hormone inducible lacZ gene expression was not observed in AR^{L701H} except at the highest DHT concentration used in the experiment. As expected, the AR^{M780T} mutant displayed reduced androgen sensitivity [13]. The AR^{T877A} mutant, found in many prostate tumors and LNCaP cells, had a similar androgen sensitivity compared to the wild type. Further studies were performed using estradiol as the ligand which has previously been shown to induce AR activity and to stimulate AR^{L701H} over and above wild type AR [14]. The results of these experiments, shown in Fig. 2B, were similar to those presented for DHT, except that the AR^{M780T} mutant was completely insensitive to estradiol. The AR^{L701H} mutant showed a small increase in sensitivity to estradiol compared to the wild type.

The hormone independent activity of the AR^{L701H} mutant could potentially be due to increased levels of AR protein. However, this was not the case as demonstrated by a Western blot analysis of yeast whole cell lysates (Fig. 3A) with antisera specific to AR. Indeed, the WT and mutant ARs all accumulated to a similar extent. We also analyzed the ability of the AR^{L701H} mutant to bind to the synthetic androgen, R1881. Specific binding of ³H-labeled R1881 was calculated by incubation of cells with the labeled hormone with and without a 100-fold excess of cold R1881 [10]. Non-specific binding was determined by subtracting the counts retained by the cells in the presence of excess cold hormone. As expected the hormone insensitive AR^{M780T} mutant displayed a reduced amount of hormone binding, especially at 0.1 and 1 nM R1881. However, both the AR^{L701H} and AR^{T877A} mutants displayed a similar ability to bind the synthetic androgen as the wild type AR (Fig. 3).

Molecular chaperone dependence of the AR^{L701H} mutant

Previous studies have shown that AR requires Hsp90 for high affinity hormone binding and for transactivation [10]. We therefore wanted to investigate whether the hormone-independent activity of AR^{L701H} still required Hsp90 and other molecular chaperones. We utilized a temperature sensitive mutant of Hsp90, glycine 170 to aspartate (G170D) which has been reported to have a wild type phenotype at 30°C and a loss of function phenotype above 34°C [15]. Previous studies have shown that wild type AR hormone binding and transactivation are reduced in this mutant at the non-permissive temperature [10]. Accordingly, we introduced the AR mutants and the lacZ reporter into a yeast strain expressing *hsp82*^{G170D} as the sole source of Hsp90. This strain is isogenic to the wild type strain used for the experiments shown in Figure 2. LacZ reporter gene expression was monitored after growing the strains at 30°C and after a shift for two hours to 37°C without or with different concentrations of DHT. As shown in Fig. 4, the AR^{L701H} mutant lost its hormone independent AR activity even when the cells were grown at the permissive temperature of 30°C, or even at 25°C (Fig. 4A and data not shown). However, the cells still retained their ability for hormone-dependent activation, albeit at reduced levels. At the restrictive temperature of 37°C, however, we observed a complete loss of hormone-inducible AR activity for both the wild type and mutants. Previous studies showed that levels of AR are quite stable in this mutant even at 37°C [10]. These data show that the hormone independent activity of AR^{L701H} is exquisitely sensitive to Hsp90 chaperone

function. Similar experiments were carried out to test the role of the chaperone Cdc37, which also affects the activity of wild type AR [16,17]. In this case, we found that mutation of Cdc37 led to small reduction of the hormone-independent signaling by AR^{L701H}, although not its elimination as was found for mutation of Hsp90 (Fig. 5).

Previous studies have shown that FKBP52 immunophilins can activate the GR and AR while FKBP51 immunophilins are inhibitory or stimulate to a lesser degree. This effect is due to FKBP52 stimulating AR hormone binding affinity in a manner that is dependent on its peptidyl-prolyl isomerase activity [18]. We tested whether either immunophilin affected the hormone-independent activity of the AR^{L701H} mutant. We prepared yeast strains that expressed AR^{L701H} and the lacZ reporter in addition to either FKBP51, FKBP52 or vector alone. DHT-dependent and DHT-independent signaling was then determined after incubation of the cells with or without exogenous hormone. As shown in Fig. 6A, hormone-independent signaling by AR^{L701H} was higher than for the wild type AR, consistent with previously shown results (Fig. 2A and 2B). Interestingly, overexpression of FKBP51 and FKBP52 both enhanced hormone-independent signaling by AR^{L701H}, although this effect was less than 2-fold. Since there is no specificity for these effects, because both FKBP proteins acted similarly, the increase in basal signaling by AR^{L701H} may result from a non-specific chaperoning effect. By contrast, specificity in the actions of FKBP51 and FKBP52 were observed in wild type AR treated with DHT (Fig. 6B). In this case, lacZ was induced to a greater extent by wild type AR when co-expressed with FKBP52 compared with FKBP51, consistent with previous results [18]. However, the ability of AR^{L701H} to promote hormone-dependent activity of the reporter gene was not influenced at all by co-expression of either FKBP protein. These results suggest that AR^{L701H} has an altered interaction with these immunophilins compared with wild type AR, as the specific effect of FKBP52 on AR signaling was lost for the AR^{L701H} mutant.

Analysis of AR^{L701H} in human prostate cancer cells

The ansamycin antibiotic derivative, 17-allylamino-17-demethoxygeldanamycin (17-AAG), disrupts Hsp90 function in mammalian cells, preventing Hsp90-mediated maturation and chaperoning of client proteins including the AR. We utilized this property of 17-AAG to examine the relative contribution of Hsp90 to basal and ligand-induced activity of wild type AR and AR^{L701H} in human prostate cancer PC-3 cells. In these cells wild type AR is induced by DHT to a much greater extent than the AR^{L701H} mutant (Fig. 7A). As expected, the transactivation capacity of both wild type AR and AR^{L701H} in the presence of DHT was reduced in a dose-dependent manner by increasing concentrations of 17-AAG (Figure 7A). However, in an important distinction from studies in yeast, basal activity of wild type AR (i.e. in the absence of ligand) was significantly greater than that of AR^{L701H} (Fig. 7B, in which the scale of the graph shown in Fig. 7A has been expanded to reveal low levels of luciferase activity). Moreover, increasing 17-AAG decreased basal activity of wild type AR in a dose-dependent manner to the level exhibited by AR^{L701H} under all conditions (Figure 7B). The degree to which AR^{L701H} could transactivate in the absence of hormone, however, was insensitive to 17-AAG. Thus, AR^{L701H} maintains an altered relationship to molecular chaperones even in animal cells since it is insensitive to the effects of 17-AAG, which inhibits Hsp90.

Discussion

Our approach was to test whether Hsp90 would be required for maturation of a mutant form of the AR that was hormone-independent for its transactivation activity. The rationale for this approach was based on the previous findings that Hsp90 was required for hormone binding [10], and whether ansamycin antibiotics would be likely to inhibit such mutants. The results of our study show quite clearly that Hsp90 is indeed required for the ability of the AR^{L701H}

mutant to signal in a hormone independent manner in yeast although it does not appear to do so in prostate cancer cells (Fig. 2 and 7).

The results of the genetic screen of 43 AR mutants yielded only one that was hormone-independent, AR^{L701H}. Previous studies have characterized this mutant as being responsive to other steroids including estradiol and glucocorticoids in the context of a double mutant with AR^{T877A} [3,14]. In contrast to the studies shown here, however, others have shown that the AR^{L701H} mutant did not have hormone-independent activity in yeast [14], nor was the mutant found to have substantial hormone binding in animal cells [3]. For the yeast studies performed by Shi et al [14], it is possible that the hormone-independence of AR^{L701H} in yeast was mild compared with the AR^{K508R} mutant that displayed this phenotype. And, since their assays were of a qualitative nature it is possible that the hormone-independent activity of AR^{L701H} was obscured. Interestingly, the first characterization of AR^{L701H} as a double mutant with AR^{T877A} in PCA 2A prostate cancer cells, noted a 15-fold higher PSA expression compared to LNCaP cells in androgen-deprived media [12]. This supports the finding that AR^{L701H} supports ligand-independent activation of AR responsive genes. On the other hand, AR^{L701H} in animal cells also fails to bind DHT with high affinity [3], in contrast to the studies using R1881 in the yeast system (Figure 3). Why this should be is unclear, although AR^{L701H} displays reduced hormone responsiveness in both yeast (Figure 2) and animal cells [3]. One possibility is that the AR^{L701H} binds normally to R1881 (Fig. 3) but fails to interact properly with DHT, as described by Zhao et al [3]. There is precedent for such distinctions in ligand binding specificity for several AR mutants [19], suggesting this is a plausible explanation of our results.

As shown in Figure 3, AR^{L701H} appears to be quite stable in yeast. Interestingly, AR with both L701H and T877A mutations was reported to be quite stable in the absence of ligand when overexpressed in *E. coli*, in contrast to wild type AR [11]. This suggests that L701H contributes to the stability of the AR hormone-binding domain in the absence of ligand, and supports the findings shown here that this mutant can transactivate a target gene in the absence of hormone. The lack of hormone-independent activity of AR^{L701H} in animal cells probably reflects its association with specific co-regulatory proteins (co-repressors) that do not exist in yeast.

The results of our chaperone dependence studies showed quite clearly that AR^{L701H} needs Hsp90 for proper folding and for hormone-independent activation. On the other hand, basal activation of AR^{L701H} in the absence of hormone in PC-3 cells was insensitive to 17-AAG. These data further indicate that Hsp90 and its co-chaperones function early in the folding process and not just to maintain AR in a hormone-binding competent conformation. Furthermore, since there were similar levels of wild type and AR^{L701H} in yeast, it is likely that AR^{L701H} somehow becomes disengaged from the chaperone machinery without the inducement of a ligand. One way this may occur is that replacement of Leu701 by His helps in the folding of the ligand-binding domain to an extent that occludes the chaperone-binding site. This might alleviate the anchoring function of the chaperone complex in the cytosol and permit translocation to the nucleus. In animal cells the mutant would have interactions with specific co-regulatory proteins (co-activators and co-repressors) that do not exist in yeast suggesting that further complexities in the function of AR^{L701H} would occur. However, it is clear that this mutant has the capacity to signal in a hormone-independent manner given the right conditions.

In contrast to Hsp90, FKBP52 does not appear to influence hormone-dependent signaling in AR^{L701H} as it did with wild type AR. Since both FKBP51 and FKBP52 can stimulate hormone-independent signaling of AR^{L701H}, it seems likely that both can bind to the mutant receptor and have some chaperone function. Conversely, FKBP52 seems incapable of supporting in AR^{L701H} those structural changes that potentiate greater hormone responsiveness in wild type AR. It is quite possible that the effects we see on FKBP51 promoting the activity of

AR^{L701H} could affect the etiology of any tumors that depend on AR^{L701H} for their growth. The rationale here is that FKBP51 is a positive regulator of AR when overexpressed in LNCaP prostate cancer cells. Furthermore, FKBP51 is an AR-responsive gene in prostate [20]. Since we find that FKBP51 stimulates the hormone-independent activity of AR^{L701H}, this could create a positive feedback loop wherein increased AR stabilization leads to increased FKBP51 expression and further AR stabilization.

Materials and Methods

Yeast strains

Wild type yeast strain S288c (Mat α *ura3*, *his3*, *leu2 ade2::KanMX*) was used for initial screening. Functional studies of AR were performed using strains P82a (wild type *HSP82*) and G170Da (*hsp82*^{G170D}) which were a gift from Dr. S. Lindquist [15]. Both P82a and G170Da have endogenous *HSC82* and *HSP82* deleted and replaced by wild type or mutant *HSP82* on a plasmid.

Construction of AR Variant Expression Plasmids

Base substitutions required to generate AR variants were introduced into an expression vector containing the complete human AR gene coding sequence under the control of the cytomegalovirus promoter (ie. pCMV-AR)[21] by PCR-based megaprimer *in vitro* mutagenesis [22] using pCMV-AR as the template as previously described [23,24]. Megaprimers were created using a downstream 21-base oligonucleotide primer which spans a restriction site unique to pCMV-AR and an upstream primer which contains the requisite AR gene base substitution as per the AR gene mutations data base [25]. Megaprimers were purified and used in a second PCR reaction with a common upstream primer spanning a second unique restriction site in pCMV-AR. PCR amplified products were digested with the appropriate restriction enzymes and cloned into pCMV-AR digested with the same enzymes. The presence of the required base substitution and the integrity of each AR variant plasmid construct were confirmed by DNA sequencing of both strands of the recreated expression plasmid including the entire region amplified in the PCR-based mutagenesis procedure. Mutant AR plasmids were based on pARH [10]. This plasmid was digested with SacI to create a gap in the coding region. The coding region was removed by subsequent transformation of the gapped plasmid with a short DNA fragment created by annealing the following oligonucleotides and filling in the ends with Pfu polymerase. This oligonucleotide recombined with the gapped pARH and in doing so replaced the AR coding sequence from codon 50 to the stop codon with a polylinker sequence. This plasmid, pHAR-PL was rescued from yeast and used in subsequent cloning experiments. This was done by gapping pHAR-PL with EcoRV and NotI and co-transforming into yeast with DNA fragments encoding 43 different AR mutants excised from pCMV-AR vectors using XhoI and XbaI. Transformants were selected on media lacking histidine and by a functional assay for AR activity. In this assay, the cells contained a lacZ reporter plasmid (pPGKarelacZC; [26]) and turned blue on plates containing X-gal and DHT (20nM) if functional AR was present.

Hormone independence was screened for on plates containing selective media buffered with 100 mM sodium phosphate pH 7 and X-gal (160 μ g/ml final concentration, stored as 20 mg/ml in dimethylformamide).

β -galactosidase assays—For quantitative assays, cells were grown to mid-log phase in selective media at 30°C and DHT was added (as shown in figure legends) in ethanol. After two hours, the cells were harvested and assayed for β -galactosidase activity using the Galacto-Star system (Applied Biosystems) according to instructions provided. β -galactosidase activity was measured using a Turner Biosystems luminometer. For experiments with G170Da, the

cells were grown at 30°C to mid-log phase and then shifted to 37°C for an additional 2 hours prior to hormone administration.

Hormone binding assay—The procedure used for binding of ³H-R1881 to AR in intact yeast cells was performed exactly as described previously [10].

Western blot analysis—Cell extracts were prepared according to Robzyk et al [27], resolved by denaturing electrophoresis and transferred to PVDF membrane. AR was detected using a mouse monoclonal anti-AR from BD biosciences (554225).

Transactivation assays in mammalian cell—Luciferase reporter gene assays were conducted using 10,000 AR negative PC-3 cells/well seeded in 96-well plates for 24 hours as previously described (Buchanan et al., 2004). Briefly, cells were transiently transfected for four hours with an AR expression vector (pCMV-AR3.1; 0.5ng) and the minimal androgen responsive luciferase probasin promoter (tk81-PB3; 100ng). After transfection, media was replaced with RPMI-1640 containing 5% dextran coated-charcoal treated FCS and supplemented with vehicle, DHT and/or increasing concentrations of 17-allylamino-17-demethoxygeldanamycin (17-AAG). Six to eight individually transfected wells received identical treatment. After 24 hours, cells were lysed and assayed for luciferase activity using a plate-reading luminometer.

Acknowledgements

The authors thank Dr. David Smith for plasmids encoding FKBP51 and FKBP52 and for helpful discussions. This work was supported by grants from NIH (DK60596 and GM70596 to AJC), the National Health and Medical Research Council of Australia (#299048 to WT), The United States Department of Defense (W81XWH-04-1-0017 to WT) and Prostate Cancer Foundation (to NR).

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Figure 1. Selection of AR^{L701H} as a hormone independent activator

A. Schematic of the human androgen receptor showing the amino-terminal transactivation domain (NTD), DNA binding domain (DBD), ligand binding domain (LBD) and hinge regions (H), the three major activation functions (AFs), and glutamine (polyQ), glycine (polyG) and proline (polyP) repeat sequences. The position of naturally-occurring and synthetically derived AR variants analyzed in this study are indicated by arrows. B. Wild type yeast strains expressing different AR mutants were plated on selective media plus X-gal for three days. Cells exhibiting androgen independent β -galactosidase activity were scored as blue colonies (arrow). The key for each strain is shown at right giving the identity of each mutant or the wild type (WT). *I* = insertion of two lysine residues into the polyglutamine tract as described [23]. C. Structure of AR ligand binding domain with R1881. Residues Leu701, Met780 and Thr877 are highlighted. Structure was rendered in Cn3D using PDB 1XQ3 from [28].

Figure 2. Transactivation of wild type and mutant AR in yeast

A. Cultures of yeast strains expressing wild type and mutant AR proteins were treated with DHT for two hours at the concentrations indicated. β -galactosidase activity was measured in cell extracts as described in materials and methods. All strains contain the lacZ reporter plasmid and in addition; V, cells transformed with vector only without AR; WT, wild type AR; 701, AR^{L701H}; 780, AR^{M780T}; 877, AR^{T877A}. B. As in A. except that the cells were treated with estradiol at the concentrations indicated. N = 3 for all experiments. Bars indicate SEM.

Figure 3. Characterization of wild type and mutant AR stability and ligand binding

A. Western blot analysis of AR in yeast whole cell extracts. V, cells transformed with vector only; WT, wild type AR; 701, AR^{L701H}; 780, AR^{M780T}; 877, AR^{T877A}. Molecular size markers at left in kDa. B. Hormone binding by wild type and mutant AR in intact live cells using ³H-R1881. At each concentration, specific binding calculated by subtracting non-specific counts determined by the counts retained from incubation with a 100-fold excess of non-labeled R1881. Key as in A. N = 3, bars indicate SEM.

Figure 4. AR^{L701H} activity in an *hsp82* mutant strain

A. Wild type and AR mutants were expressed in *hsp82*^{G170D} yeast. Cells were grown at 30°C and incubated with DHT at the concentrations indicated for 2 hours. β-galactosidase activity in assayed in whole cell extracts. N = 3, bars indicate SEM. B. As in A. except that the cells were shifted to 37°C for 2 hours prior to hormone administration. V, cells transformed with vector only; WT, wild type AR; 701, AR^{L701H}; 780, AR^{M780T}; 877, AR^{T877A}.

Figure 5. Role of Cdc37 on hormone-dependent and hormone-independent signaling by AR^{L701H}

Yeast strains containing wild type *CDC37* or a mutant form (*cdc37*^{S14A}) were transformed with plasmids expressing the lacZ reporter and either wild type AR or AR^{L701H}. Cultures of both strains were grown to mid-log and treated with ethanol alone (-) or DHT to a concentration of 1 nM (+). Whole cell extracts were prepared and β-galactosidase activity determined. N = 3, bars represent SEM.

Figure 6. Effect of FKBP51 and FKBP52 on the activity of AR^{L701H}

A. Wild type yeast expressing FKBP51 and FKBP52 or vector alone were analyzed for hormone independent β-galactosidase activity. N = 3, bars represent SEM. B. Hormone-dependent induction was analyzed using 1 nM DHT. The data are expressed as fold induction above levels of β-galactosidase in the absence of hormone. N= 3, bars represent SEM.

Figure 7. Functional requirement of Hsp90 for AR and AR^{L701H} transactivation capacity in mammalian cells. A

Assay of AR and AR^{L701H} transactivation capacity performed in human PC-3 cells in the presence of DHT (1nM) and/or increasing concentrations of 17-AAG (1-250nM) as indicated. Luciferase activity, as a surrogate of AR transactivation capacity, was measured in lysates from each well and is presented as relative light units (RLU) representing mean ($\pm$ sem) activity determined from 6–8 independently transfected wells. B. Data from part A. showing greater detail of the effect of increasing amounts of 17-AAG on basal activity of the AR constructs in the absence of DHT.