Molecular Biology of the Cell
Vol. 6, 1833–1842, December 1995

A Role for Hsp90 in Retinoid Receptor Signal Transduction

Sandra J. Holley* and Keith R. Yamamoto

Departments of Cellular and Molecular Pharmacology, and Biochemistry and Biophysics, Program in Biological Sciences, Biochemistry and Molecular Biology Program, University of California, San Francisco, San Francisco, California 94143-0450

Submitted August 8, 1995; Accepted September 12, 1995
Monitoring Editor: Guido Guidotti

The ubiquitous heat shock protein Hsp90 appears to participate directly in the function of a broad range of cellular signal transduction components, including steroid hormone receptors; however, an evolutionarily related subclass of intracellular receptors, exemplified by the retinoid receptors RAR and RXR, had been inferred from biochemical studies to function independently of Hsp90. To examine this issue genetically, we measured mammalian and avian retinoid receptor activity in a *Saccharomyces cerevisiae* strain in which the expression of the yeast Hsp90 homologue could be conditionally repressed ~20-fold relative to wild type. We tested transcriptional activation by RAR or RXR-RAR, from two types of retinoic acid response elements, triggered by three different agonist ligands. In every condition, we found that activation was severely compromised under conditions of low Hsp90 expression. We showed that the defect was in signal transduction rather than transcription activation per se, and that high affinity hormone binding was abolished in extracts of cells producing low levels of Hsp90. We suggest that Hsp90 may function in at least one step of signal transduction by all members of the intracellular receptor superfamily.

INTRODUCTION

The intracellular hormone receptor superfamily encompasses two major subfamilies: the steroid receptors, which include receptors for glucocorticoids (GR), mineralocorticoids (MR), progesterone (PR), estrogens (ER), and androgens (AR); and the vitamin receptors, which include receptors for retinoids (RAR and RXR), thyroid hormone (TR), and vitamin D (Evans, 1988; for review, see Tsai and O'Malley, 1994). The proteins within these subfamilies are similar in overall organization, each containing a characteristic zinc binding region, which binds to specific DNA sequences termed hormone response elements (HREs), contiguous with a C-terminal signaling domain, which interacts with the hormonal ligand (Evans, 1988; Tsai and O'Malley, 1994).

In the absence of ligand, the steroid receptors are recovered as transcriptionally inactive cytosolic aporeceptor complexes, comprised of a receptor monomer, a dimer of heat shock protein Hsp90, Hsp56, and several other proteins (Tai *et al.*, 1986; Smith *et al.*, 1990; Smith, 1993; Hutchison *et al.*, 1994). Upon hormone binding in vivo and in vitro, the nonreceptor proteins dissociate from the complex and the resultant activated receptor is fully competent to bind to cognate HREs and to regulate transcription from nearby promoters. In reticulocyte extracts, Hsp90 is required to reconstitute the hormone binding activity of GR (Scherrer *et al.*, 1990, 1992). Moreover, genetic studies in which steroid receptors are expressed in yeast, reveal that Hsp82 (yeast Hsp90 homologue) is essential for full activity of GR, MR, PR, and ER in that context; receptor activity from cognate response elements is compromised in yeast strains containing either *hsp82* point mutants or reduced levels of Hsp82 (Picard *et al.*, 1990; Bohen and Yamamoto, 1993).

Signaling by the vitamin receptors appears superficially to be remarkably different. In the absence of

* Present address: Department of Reproductive Medicine, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0674.

hormone, the vitamin aporeceptors are isolated in the nuclear rather than the cytosolic fraction; indeed, the thyroid aporeceptor can bind to its cognate response elements and repress transcription in the absence of hormone (Damm *et al.*, 1989; Graupner *et al.*, 1989). In addition, the vitamin aporeceptors, unlike their steroid counterparts, have not been recovered in complexes with heat shock proteins, either in cell extracts (Samuels *et al.*, 1974; Spindler *et al.*, 1975; Nervi *et al.*, 1989) or after synthesis in reticulocyte lysates (Dalman *et al.*, 1990, 1991). Finally, purified recombinant vitamin receptors are competent for hormone binding in the absence of associated factors (Ichikawa *et al.*, 1991; Lombardo *et al.*, 1994).

These findings seem to suggest that the vitamin receptors utilize a mechanism for signal transduction that is distinct from that of the steroid receptors, and specifically is independent of Hsp90. This divergence is unexpected in view of the evolutionary relatedness of the steroid and vitamin receptors. One finding that seems consistent with that relationship is that Hsp90 can be cross-linked to the *v-erbA* protein, a viral oncogene derivative of TR, in infected cell extracts (Privalsky, 1991). These results were taken to suggest that vitamin receptors harbor at least a potential to interact with Hsp90, perhaps forming transient rather than stable complexes.

Various studies have implicated Hsp90 (and other factors in the steroid aporeceptor complexes) in a broad range of cellular events associated with signal transduction (Bohen and Yamamoto, 1994; Bohen *et al.*, 1995; see DISCUSSION). Taken in this larger context, it was even more surprising that a role for Hsp90, whatever its mechanism, would have been bypassed by the vitamin receptor subfamily. Consequently, we decided to revisit this issue. As with steroid receptors (Metzger *et al.*, 1988; Schena and Yamamoto, 1988), retinoid receptors can function in yeast (Allegretto *et al.*, 1993; Hall *et al.*, 1993). Thus, using glucocorticoid receptor dependence on Hsp82 as a control, we set out to assess whether Hsp82 is required for retinoic acid receptor activity in a *Saccharomyces cerevisiae* strain whose Hsp82 levels are manipulable (Picard *et al.*, 1990). To do so, we tested human RARβ, with and without RXR as a dimer partner, in the context of different RAREs and agonist ligands.

MATERIALS AND METHODS

Yeast Strains, Plasmids, and Transformations

See Table 1 for a summary of the yeast strains used in this work. The strain GRLH was constructed from GRS4 to obtain an additional selective marker (TRP1), allowing co-expression of RAR and RXR proteins in the same cell. The GRLH yeast strain was derived in two steps from the GRS4 strain (originally derived from W303; Picard *et al.*, 1990), which has the following genotype: *hsp82::LEU2, hsc82::LEU2, ade2, ura3, his3, trp1* and contains the plasmid pTT8, a TRP1/Cen/ARS plasmid with the yeast *HSP82* gene under control of the GAL1 promoter (Picard *et al.*, 1990). First, GRS4 cells were transformed with a *URA3*/Cen/ARS plasmid containing a constitutively expressed human *HSP90* gene, and the pTT8 plasmid was segregated by growth on nonselective media using replica plating to monitor plasmid loss. Second, this intermediate strain was transformed with a *TRP1* plasmid (pG1RARβ or pG1RXRγ) along with pLCAgal82, which contains the yeast *HSP82* gene driven by the *GAL1* promoter inserted between the *Sal*I and *Sst*I sites of pRS315 (*LEU2*/Cen/ARS); transformants were selected for trp prototrophy and replica plated to 5-FOA plates to identify *ura*$^-$ colonies lacking the URA3/Hsp90 plasmid. The GRLH strain was confirmed by immunoblotting to express yeast Hsp82 at high levels in galactose and at low levels in glucose; repression of the *GAL1* promoter is leaky in GRS4 and GRLH, allowing sufficient Hsp82 expression in glucose for cell survival (Picard *et al.*, 1990).

Table 1. Strain key

Parental strains	Genotype
GRLH	*hsp82::LEU2, Hsc82::LEU2, ade2, ura3, trp1, his3,* with plasmid pLCAgal82
HILH	*hsp82::LEU2, hsc82::LEU2, ade2, ura3, trp1, his3,* with plasmid pLCAGPD82

Strain	Receptor 1	Receptor 2	Reporter
GRLH.R5	pG1RARβ	none	pδssDR5
GRLH.RX5	p2HGRARβ	pG1RXRγ	pΔssDR5
GRLH.RXT	p2HGRARβ	pG1RXRγ	pΔssTREpal
GRLH.G26	pG1N795	none	pΔ26x
GRLH.N26	pG1N525	none	pΔ26x
HILH.R5	pG1RARβ	none	pΔssDR5
HILH.RX5	p2HGRARβ	pG1RXRγ	pΔssDR5

The HILH strain was derived from HHIKAT6, the parent of GRS4, which is not leaky for GAL1 repression. HHIKAT6 has the genotype *hsp82::LEU2, hsc82::LEU2, ade2, ura3, his3, trp1* and carries pGalhhsp90, a *HIS*/Cen/ARS plasmid containing the human *HSP90* gene under control of the *GAL1* promoter. HILH was constructed by transforming HHIKAT6 with plasmid pLCAGPD82, which contains the yeast hsp82 gene under control of the constitutive GPD promoter inserted between the *Sal*I and *Sst*I sites of pRS315 (*LEU2*/Cen/ARS). Transformants were plated on glucose media, YEPD, which fully represses human Hsp90 expression; surviving cells, which must carry the pLCAGPD82 plasmid, were subsequently cured of the pGalhhsp90 plasmid.

The GR plasmids used were pG1N795, the full length (795 aa) rat GR under control of the constitutive GPD promoter, inserted into the *Bam*HI site of the pG1 vector (Schena and Yamamoto, 1988), and pHCAN525, which contains N525, a rat GR derivative truncated at amino acid 525 and therefore lacking the signaling region, under control of the GPD promoter inserted between the *Cla*I and *Eag*I sites of the pRS313 (*HIS3*/Cen/ARS) vector (Bohen and Yamamoto, 1993). The GRE reporter contains three tandem copies of the GRE from the tyrosine aminotransferase (TAT) gene inserted into the *Xho*I site of the pΔss reporter (*CYC1* promoter; Schena *et al.*, 1989). Retinoic acid receptor plasmids and reporters were gifts from Marty Privalsky (University of California, Davis, CA). pG1RARβ and p2HGRARβ contain human RARβ inserted, respectively, into plasmid pG1(*TRP1*, 2 μ) or p2HG(*HIS*, 2 μ), at the *Bam*HI site as described (Hall *et al.*, 1993). pG1RXRγ contains the chicken RXRγ gene inserted into the *Bam*HI site of pG1 (Hall *et al.*, 1993). The DR5 reporter plasmid pΔDR5 was constructed by inserting a single DR5 oligo (AGGTCAccgaaAGGTCA) into the *Xho*I site of the *URA3*/2μ Δss *LACZ* reporter (*CYC1* promoter; Schena *et al.*, 1989); the TRE

reporter pΔTREpal contains two tandem copies of the palindromic TRE element (TCGAGATCTCAGGTCATGACCTGAGATC) at the *Xho*I of the *URA3*/2 μ Δss vector (Privalsky *et al.*, 1990).

Transformations were performed using the lithium acetate method (Gietz *et al.*, 1992). Substrains of the GRLH and HILH strains (see Table 1) were constructed by transformation with the following combinations of plasmids: p2HGRARβ and pG1RXRγ with the pΔssDR5 β-gal reporter (GRLH.RX5, HILH.RX5); pG1RARβ with pΔssDR5 (GRLH.R5, HILH.R5); p2HGRARβ and pG1RXRγ with pΔssTREpal β-gal reporter (GRLH.RXT); pG1N795 with the pΔ26x β-gal reporter (GRLH.G26); and pG1N525 with pΔ26x (GRLH.N26).

β-Galactosidase Assays

GRLH strains were grown on 2% galactose, 0.1% sucrose minimal selective plates. Overnight cultures were inoculated into either galactose or glucose selective media and grown at room temperature for ~24 h (OD_{600} ~3.0). Galactose and glucose cultures were diluted to OD_{600} of 0.1 and 0.15, respectively. The cultures were allowed to recover for 5–6 h before addition of hormone (100× stocks of hormone were stored in 95% ethanol at −20°C) or, in controls, 95% ethanol alone (OD_{600} between 0.2 and 0.3 at hormone addition). All *trans*- and 13-*cis*-retinoic acid, and deoxycorticosterone were obtained from Sigma Chemical (St. Louis, MO); 9-*cis*-retinoic acid was a gift from Ligand Pharmaceuticals (La Jolla, CA). Cultures were grown at room temperature in the presence of hormone (or vehicle) for 12 h, and harvested at an OD_{600} of about 1.5; β-gal assays were performed on cell pellets from 1-ml cultures by permeabilization with chloroform and incubation for 2–20 min at 30°C with ONPG as described (Garabedian and Yamamoto, 1992). OD_{420} of incubations was normalized to OD_{600} of cell culture.

Immunoblotting

Yeast cultures were grown as described for β-gal assays. Ten milliliter cultures were centrifuged at 2000 rpm for 5 min, washed once with phosphate-buffered saline, and resuspended in 100 μl of cold extract buffer (10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], pH 7.4, 400 mM KCl, 1 mM EDTA, 2 mM dithiothreitol [DTT], 10% glycerol, 1 μg/ml each of pepstatin A [Boehringer Mannheim, Indianapolis, IN], leupeptin [Boehringer Mannheim], and aprotinin [Boehringer Mannheim], and 1 mM phenylmethylsulfonyl fluoride [Sigma]). Cells were lysed by vortex mixing with glass beads at 4°C for 30 min. Extracts were cleared by centrifugation for 2 min in a microfuge followed by 20 min at 90,000 rpm in a TLA 100 rotor. Fifty micrograms of extract (measured by Bradford assay) was loaded per lane for RAR blots and 12.5 μg of extract was loaded per lane for Hsp82 blots. Gels were blotted to nitrocellulose for 1 h using a Nova Blot dry blotting apparatus and then blocked in Tris-buffered saline, 0.5% Tween (TBST) with 2% bovine serum albumin. The Hsp82 blot was probed with a yeast-peptide–specific anti-Hsp82 rabbit anti-serum (a gift of S. Lindquist, University of Chicago, file #4–1-8) in TBST for 1 h. The RAR blot was probed with a rabbit anti-RARβ antibody from Affinity Bioreagents (Neshanik Station, NJ) in TBST for 1 h. Secondary antibodies were goat anti-rabbit alkaline phosphatase-conjugated from Bio-Rad (Richmond, CA). Blots were washed five times for 5 min in TBST between primary and secondary antibody incubations and before developing. The developing method was NBT/BCIP reagent for alkaline phosphatase (Kirekegard and Perry, Gaithersburg, MD). RAR protein levels were assayed before and after the high speed centrifugation and found to be the same.

In Vitro Hormone Binding Assays

Overnight cultures were grown as for β-gal assays, except no hormone or ethanol was added after dilution. Cultures were diluted to an OD_{600} of 0.2 in 50–100 ml of selective media and were grown at room temperature. At OD_{600} between 1.0 and 1.5, extracts were prepared as described for immunoblotting and adjusted to 10 mg/ml protein; 100 μg of extract protein was saved for immunoblotting. The hormone binding and hydroxyapatite (HAP) separation protocol was adapted from Allegretto *et al.* (1993): 20 μl of extract was added to 200 μl binding buffer to yield final concentrations of 10 mM HEPES, pH 7.4, 125 mM KCl, 1 mM EDTA, 10% glycerol, 10 mM DTT, 0.5% 3-([3-cholamidopropyl]dimethylammonio)-2-hydroxy-1-propanesulfonate. ^{3}H-*trans*-retinoic acid (NEN, Boston, MA; 50.3 Ci/mmol in 95% ethanol) was added at concentrations between 0.1 and 15 nM; for nonspecific binding, a 200-fold excess of unlabeled *trans*-retinoic acid (Sigma) was included. Extracts were incubated with hormone for 8–9 h on ice in the dark (optimal incubation time was shown in time course experiments to be 8–12 h). Immunoblotting on 100 μg of hormone-incubated and control extracts revealed no changes in RAR protein levels during the incubation. One hundred microliters of a 10% (v/v) HAP (Bio-Rad) slurry in wash buffer (same as binding buffer but with 1 mM DTT) was added and incubated with rotation in the dark for 1 h at 4°C. HAP was centrifuged at 2000 rpm for 2 min and washed three times with wash buffer. The pellet was resuspended in 100 μl of wash buffer and counted in 4 ml of Safety Solve scintillation fluid (Research Products International, Mt. Prospect, IL). Counter efficiency was determined to be 58%. Data from 3–5 experiments were pooled and analyzed using a weighted least-squares curve-fitting algorithm (MacLigand) that normalizes total binding and nonspecific binding between experiments (Munson and Rodbard, 1980).

RESULTS

Effects of Reduced Hsp82 on Transcriptional Activation by RAR

To assess the effects of Hsp82 levels on RAR activity, we monitored reporter gene expression in derivatives of a yeast strain GRLH, in which the HSP82 gene is under the control of the *GAL1* promoter (Table 1). In this strain, Hsp82 is expressed at about wild-type levels in galactose-containing media, whereas ~20-fold less Hsp82 protein is produced in glucose-containing media (Picard *et al.*, 1990; see Figure 2). Different combinations of receptor and reporter plasmids were stably introduced into GRLH to produce the strains described in Table 1. Hence, GRLH.RX5 expresses RAR and RXR, and harbors a reporter plasmid in which a β-galactosidase gene is linked to a DR5 RARE (composed of direct repeats of AGGTCA separated by 5 bp). In galactose, *trans*-retinoic acid (*trans*-RA) produced a >30-fold maximal induction of reporter gene expression (Figure 1A). In contrast, only ~fourfold β-gal induction by *trans*-RA was observed when this strain was propagated in glucose. At all hormone levels tested, β-gal activity was ~eightfold lower in conditions of reduced Hsp82 (glucose) compared with wild type (galactose).

As a positive control, we compared our findings with RAR-RXR to those with GR, which is known to interact with Hsp90 (Sanchez *et al.*, 1985; Howard and Distelhorst, 1988), and requires Hsp82 for normal activity in yeast (Picard *et al.*, 1990). In strain GRLH.G26, which expresses GR and a GRE-linked reporter, we found that β-gal induction at two concentrations of

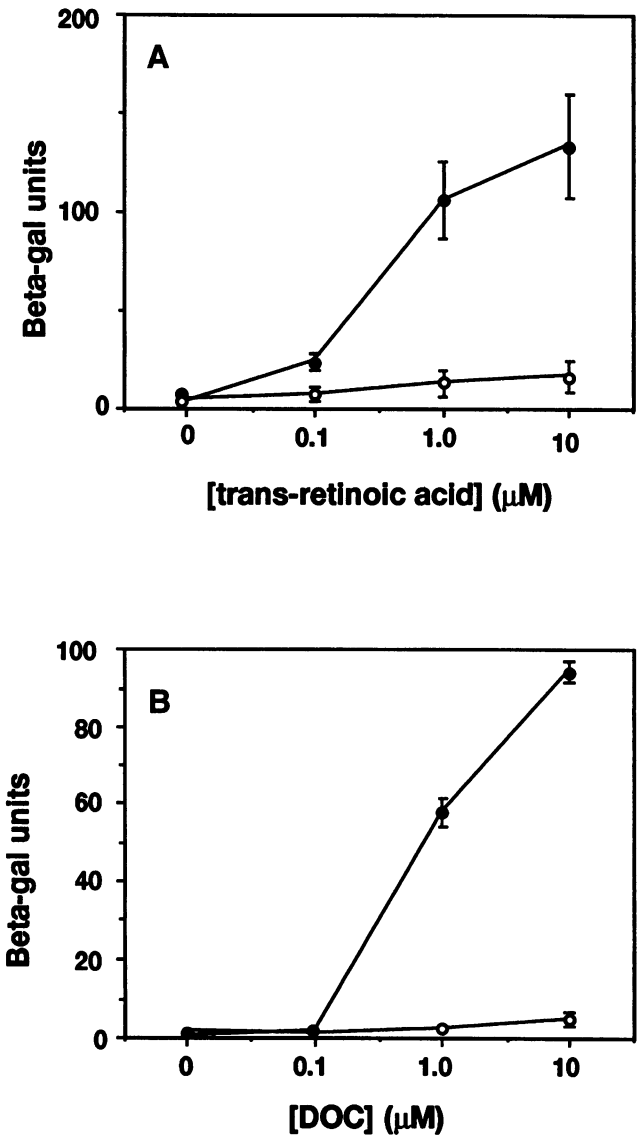

Figure 1. RAR and GR activities in yeast expressing normal or reduced levels of Hsp82. (A) Strain GRLH.RX5, expressing RAR and RXR and carrying a DR5 RARE-linked β-gal reporter, was cultured in galactose (closed circles; normal Hsp82 levels) or glucose (open circles; 20-fold reduced levels of Hsp82). Data represent the average of six experiments ± SD, using two independent transformants in each. β-gal units are normalized to OD_{600}. (B) Strain GRLH.G26, expressing GR and carrying a GRE-linked β-gal reporter, was cultured in galactose (closed circles; normal Hsp82 levels) or glucose (open circles; 20-fold reduced levels of Hsp82). Data represent the average of three experiments ± SD, using two independent transformants in each. β-gal units are normalized to OD_{600}. Cultures were treated with hormone (A, *trans*-RA; B, DOC, deoxycorticosterone) at concentrations shown.

deoxycorticosterone, a glucocorticoid agonist, was ~25-fold stronger in galactose-containing medium than in glucose (Figure 1B). Thus, although GR function is more strongly affected by the glucose growth conditions than is RAR-RXR function, there is clearly a substantial effect on both receptor activities, implying that Hsp82 participates in the actions of both.

Especially because our findings with RAR activity were unexpected, it was important to explore possible trivial explanations for the results. We first examined the intracellular accumulation of Hsp82 and RAR in glucose and galactose media in the GRLH.RX5 and GRLH.R5 strains. As expected, Hsp82 protein levels were dramatically higher in galactose than in glucose grown cells (Figure 2A). In contrast, RAR accumulation was unaffected by the carbon source (Figure 2B); in other experiments, we showed that hormone treatment did not alter RAR levels. Although we lacked a suitable antibody to assess RXRγ levels in these experiments, it is clear at least in the case of RAR (e.g., see Figure 4 below) that the differential receptor activities observed in glucose and galactose do not reflect altered receptor levels.

In a second series of controls, we examined retinoid receptor activities in yeast strains in which the choice of carbon source could be uncoupled from an effect on Hsp82 protein level. In the HILH strain, the *GAL*-inducible *HSP82* gene was replaced with constitutively expressed *HSP82*. In the strains HILH.R5 and HILH.RX5, which express RAR alone, and together with RXR, respectively (see Table 1), reporter gene induction by *trans*-RA was in every case 1.5- to 2-fold stronger in glucose than in galactose media, with the overall fold-induction at each hormone concentration being similar for galactose and glucose (Figure 3). We conclude that altered Hsp82 levels, and not the carbon source available to the cells, are responsible for the differential receptor activities observed in Figure 1.

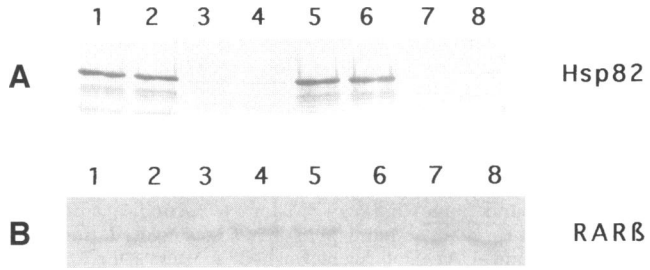

Figure 2. Intracellular levels of RAR and Hsp82. Cleared extracts from strain GRLH.RX5, which expresses RAR and RXR, or strain GRLH.R5, which expresses RAR, were fractionated by gel electrophoresis, blotted to nitrocellulose, and probed with antibodies as described in MATERIALS AND METHODS. Lanes 1–4, GRLH.RX5; lanes 5–8, GRLH.R5. Cultures were grown in galactose (lanes 1, 2, 5, and 6) or glucose (lanes 3, 4, 7, and 8). Paired lanes represent two individual transformants from the same strain. (A) 12.5 μg extract per lane, probed with anti-Hsp82 antibody. (B) 50 μg of extract per lane, probed with anti-RARβ antibody.

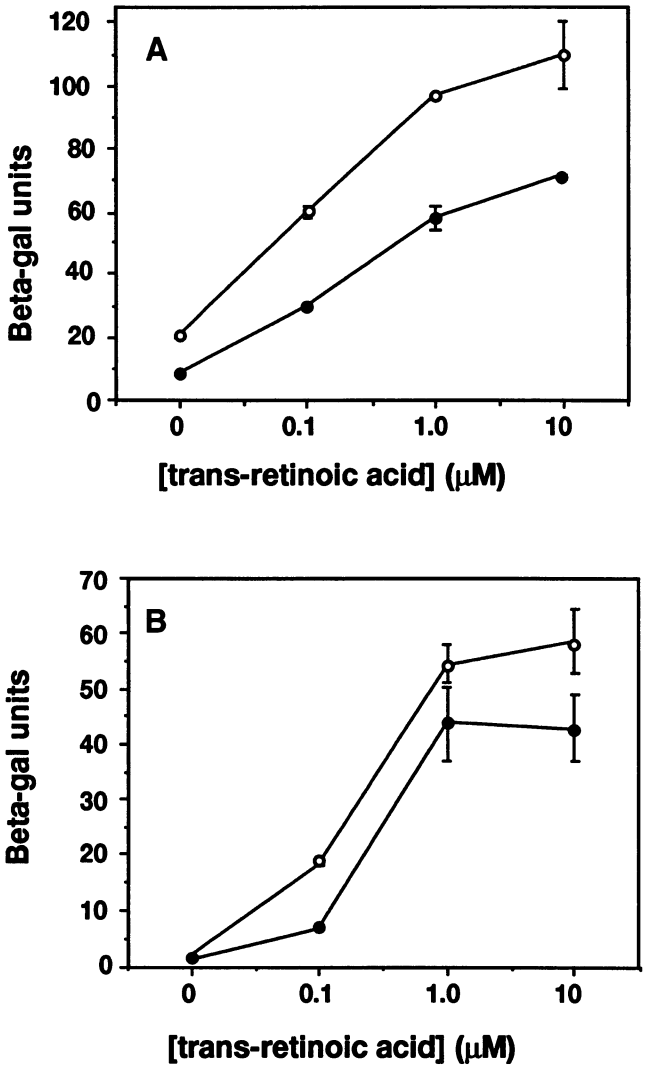

Figure 3. Effect of carbon source alone on retinoid receptor action. (A) Strain HILH.RX5, expressing RAR and RXR and constitutive Hsp82, and carrying a DR5 RARE-linked β-gal reporter, was cultured in galactose (closed circles) or glucose (open circles). (B) Strain HILH.R5, expressing RAR and constitutive Hsp82, and carrying a DR5 RARE-linked β-gal reporter, was cultured in galactose (closed circles) or glucose (open circles). Data are from one representative experiment, using two independent transformants for each strain; error bars represent SD.

Effects of RAR Context on Hsp82 Dependence

RAR is functional in diverse contexts, operating as a homodimer or heterodimer, at HREs with half-sites arranged as direct repeats or palindromes, and with several different biological ligands (Manglesdorf and Evans, 1992). Conceivably, the interaction of Hsp82 with RAR, or its functional significance, might differ in different receptor contexts. To examine this possibility, we tested the effects of impaired Hsp82 production on the activity of RAR in several of its modes of action.

We first compared the effects of Hsp82 reduction on RAR homodimers versus heterodimers with RXR, and on heterodimers at a direct repeat element versus a palindromic element. Figure 4 shows that in each case, receptor function is abrogated under conditions of reduced Hsp82 production. Thus, in strains expressing RAR-RXR, β-gal induction at both the DR5 and the palindromic TRE response elements was clearly dependent on normal levels of Hsp82 (Figure 4; RX5 and RXT). Similarly, in strains carrying the DR5 RARE-linked reporter, β-gal induction via RAR homodimers and RAR-RXR heterodimers was compromised in low Hsp82 (Figure 4; R5 and RX5). Although the overall efficacies differed over a two- to threefold range in these different contexts, fold-induction was reduced by 5- to 10-fold in each condition when Hsp82 production was reduced. We suggest from these results that Hsp82 is important for the ligand-responsive functions of RAR regardless of its dimerization partner or the response element at which it acts.

We next tested whether a role for Hsp82 could be detected under conditions in which RAR is activated by three distinct naturally occurring retinoid isomers–*trans*-RA, 9-*cis*-retinoic acid, and 13-*cis*-retinoic acid (Figure 5). In strains expressing the DR5-linked reporter and either RAR homodimers (Figure 5, R5) or RAR-RXR heterodimers (Figure 5, RX5), low Hsp82 production led to a pronounced reduction in β-gal induction with all three ligands. Consistent with previous findings (Allegretto *et al.*, 1993; Heery *et al.*,

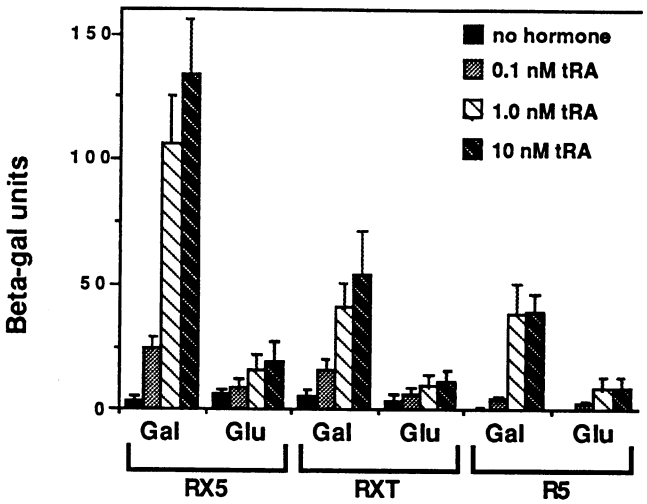

Figure 4. Retinoid receptor action in different contexts: effects of different dimerization partners and response element. Cultures were grown in galactose (normal Hsp82 levels) or glucose (20-fold reduced levels of Hsp82) media containing *trans*-RA at concentrations shown. RX5, GRLH.RX5 strain containing RAR, RXR, and DR5 reporter; RXT, GRLH.RXT strain containing RAR, RXR, and TRE reporter; R5, GRLH.R5 strain containing RAR and DR5 reporter. Data represent the average of at least three experiments ± SD, using two independent transformants in each.

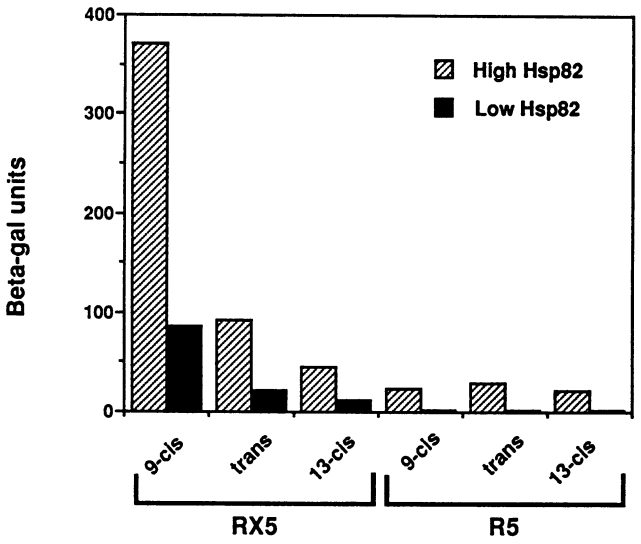

Figure 5. Retinoid receptor action in different contexts: effects of different retinoic acid isomers. Cultures were grown in galactose (hatched bars, normal Hsp82 levels) or glucose (solid bars, 20-fold reduced levels of Hsp82) media containing different isomers of retinoic acid at 10 μM. RX5, GRLH.RX5 strain containing RAR, RXR, and DR5 reporter; R5, GRLH.R5 strain containing RAR and DR5 reporter. 9-*cis*, 9-*cis*-retinoic acid; *trans*, all *trans*-RA; 13-*cis*, 13-*cis*-retinoic acid. Data represent the average from two experiments.

1994) the three isomers displayed different relative efficacy (9-*cis* > *trans* > 13-*cis*) in the presence of the RAR-RXR heterodimer. In each case, reduced Hsp82 expression evoked a four- to fivefold decrease in receptor activity. The ligands displayed greater similarity in efficacy in the presence of RAR homodimers than with heterodimers, and were similarly compromised by reduced Hsp82 levels. Thus, Hsp82 participates in RAR action irrespective of the specific agonist that converts it to a functional activator.

Signal Transduction, Not Transcription, Is Impaired by Reduced Hsp82 Levels

Because Hsp82 affects receptor activities triggered by all agonists tested, it was important to examine the possibility that Hsp82 might in fact directly alter the transcriptional regulatory functions of intracellular receptors, rather than modulating signal transduction. To assess this notion in a general way, we used N525, a constitutively active glucocorticoid receptor derivative that lacks the C-terminal signaling domain (Godowski *et al.*, 1987), and monitored its transcriptional activity under conditions of low and high Hsp82 production. The corresponding truncations of RAR are inactive and therefore uninformative, as the RAR transcriptional activation domain resides in the signaling region (Pratt *et al.*, 1990; Tsai *et al.*, 1992).

Relative to the <0.5 β-gal units produced from a GRE-linked reporter, pΔ26x (Schena *et al.*, 1989), in the absence of the receptor derivative, N525 GR elicited 980 ± 86 β-gal units in galactose and 1491 ± 32 units in glucose. Thus, N525 displayed very strong activity under both nutrient conditions; the ~1.5-fold stronger effect in glucose was consistent with previous comparisons of these carbon sources (see Figure 3). We infer from these results that the transcriptional regulatory activity of intracellular receptors is unaffected by alterations in Hsp82 level, and that receptor-mediated signal transduction must therefore be the target of Hsp82 action on the full length GR, and by extension, on RAR and RXR.

Low Hsp82 Levels Abrogate Hormone Binding

Previous studies have shown that dissociation of the complex of Hsp90 and other factors from the aporeceptor dramatically reduces the hormone binding affinities for several steroid receptors (Bresnick *et al.*, 1989; Caamano *et al.*, 1993; Smith, 1993). Although the biological significance of these biochemical findings has not been examined, they are consistent with the suggestion that Hsp82 affects the hormone binding step of signal transduction. Therefore, we tested whether hormone binding by RAR might depend upon Hsp82 in yeast.

GRLH.RX5 cultures were propagated in galactose or glucose, and cell extracts were prepared and incubated with various concentrations of ^{3}H-*trans*-RA. Hormone-receptor complexes were separated from unbound hormone by retention on hydroxylapatite (Allegretto *et al.*, 1993); no specific binding was detected in the absence of RAR expression. As shown in the Scatchard plot in Figure 6A, complex binding behavior was observed under conditions of normal Hsp82 expression; a weighted least-squares curve fitting analysis (Munson and Rodbard, 1980) indicated two sites with K_d of 0.3 nM and 60 nM. Thus, in the range of hormone concentrations tested (0.1–15 nM), the great majority of the bound hormone was associated with the high affinity complexes. The 0.3 nM (± 0.2 nM) K_d site agrees closely with the K_d of 0.36 nM for RARβ determined in simian cells (Allenby *et al.*, 1993), and 0.37 nM in baculovirus-infected insect cells (Allegretto *et al.*, 1993). Thus, we assume that the high affinity sites represent normal hormone-receptor interactions, and we calculate roughly 10^5 functional receptors per yeast cell. The nature of the low affinity binding sites is unknown, but they may be similar to the low affinity interactions observed in the low Hsp82 extracts, where a low level of binding was inferred at high concentrations of hormone (> 5 nM); over the range of hormone concentrations shown in Figure 6B, no specific binding was detected.

These findings establish the relevance in vivo of biochemical studies implicating Hsp82 as a key player in hormone binding by intracellular receptors, and

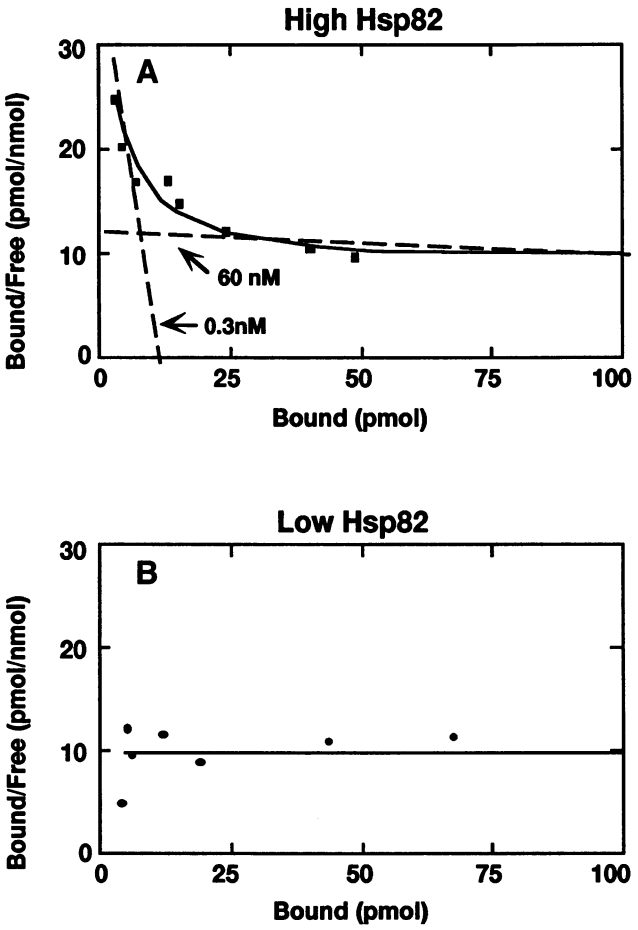

Figure 6. Scatchard analysis of hormone binding in vitro. Strain GRLH.RX5, expressing RAR and RXR and carrying a DR5 RARE-linked β-gal reporter, was cultured in galactose (A, normal Hsp82 levels) or glucose (B, 20-fold reduced levels of Hsp82) media. Extracts were incubated with various concentrations of ^{3}H-*trans*-RA, and hormone-receptor complexes were separated from free hormone on hydroxylapatite as described in MATERIALS AND METHODS. (A) Data are averaged from five separate experiments; (B) data are from three experiments; data are plotted on Scatchard plots and fitted using a weighted least-squares curve-fitting algorithm (see MATERIALS AND METHODS).

extend that conclusion from the steroid receptor subfamily to the vitamin receptor subfamily. We conclude that the signal transduction mechanisms for the steroid and vitamin receptor subfamilies are more similar than previously suggested.

DISCUSSION

We examined in yeast the functional relationship between the retinoic acid receptor and Hsp90. Using a strain with glucose-repressible Hsp82, we found that RAR activity is decreased ~eightfold at low levels of Hsp82. In controls, we showed that accumulation of RAR protein is unchanged by alterations in Hsp82 levels, and that carbon source has little intrinsic effect on RAR activity. We established that RAR activity is Hsp82 dependent in every context examined (dimer partner, response element, and agonist), and that high affinity hormone binding in particular is lost at low levels of Hsp82. We conclude that Hsp82 probably affects signal transduction by all intracellular receptors; whether Hsp82 modulates signaling functions in addition to hormone binding remains to be tested.

Our findings suggest that the signal transduction mechanisms across the intracellular receptor superfamily are more similar than previously thought. Two types of studies contributed to the earlier notion that RAR functions independently of Hsp90. First, Hsp90-containing aporeceptor complexes, such as those found for the steroid receptors, have not been observed in the case of RAR; similarly, we have so far failed to detect RAR-Hsp82 complexes in yeast extracts by co-immunoprecipitation. Second, Hsp90-free apoRAR, either salt extracted from nuclei or expressed in *Escherichia coli*, is competent for high affinity hormone binding. How might we account for the apparent discrepancy between those findings and our conclusions?

Conceivably, the Hsp82 requirement for RAR signaling in *S. cerevisiae* is an idiosyncrasy of expression in that heterologous species, and not a reflection of Hsp90 action elsewhere. Although we have not excluded this formal possibility, it seems highly unlikely that our results are merely artifactual, as they parallel the paradigm for steroid receptors established in mammals, amphibians, avians, and yeast. Hence, we favor the idea that RAR signaling requires a transient rather than a stable interaction with Hsp90, and that this requirement in bacteria may be satisfied by HtpG, the *E. coli* homologue of Hsp90 (Bardwell and Craig, 1987).

According to this view, different members of the intracellular receptor family might differ in the stability of their interactions with Hsp90, at least as measured in vitro, but signaling across the whole family may nevertheless require those interactions. Thus, there might exist a broad continuum of in vitro stabilities of various Hsp90-receptor complexes, and a continuum of requirements for that stability for subsequent ligand binding or other signaling functions. Along this spectrum, GR and MR reside at one end, requiring intact aporeceptor complexes for hormone binding; in contrast, hormone binding by PR requires intact aporeceptor complexes at 37°C (Smith, 1993) but not at 4°C (Bagchi *et al.*, 1990); ER and AR form stable aporeceptor complexes but are able to bind cognate hormones at 4°C after dissociation of the complexes (Wittliff *et al.*, 1990; Nemoto *et al.*, 1992); finally, RAR may define the other end of the continuum, in which the RAR-Hsp90 interaction is only transient but nev-

ertheless necessary for hormone binding. As RAR, PR, and ER produced in *E. coli* display hormone binding activity (Eul *et al.*, 1989; Wittliff *et al.*, 1990; Yang *et al.*, 1991) yet require Hsp82 in yeast (Picard *et al.*, 1990; Bohen and Yamamoto, 1993), it might be illuminating to test hormone binding by these receptors after synthesis in *E. coli* bearing *htpg* mutations.

Our suggestion that Hsp90 may interact only transiently with certain targets seems consistent with several previous studies. For example, it has been proposed that the MyoD1 protein undergoes a transient but functionally significant interaction with Hsp90 (Shaknovich *et al.*, 1992). Similarly, a v-ErbA-Hsp90 complex was detected reliably only after stabilization by chemical cross-linking (Privalsky, 1991). PR aporeceptor complexes are in a rapid ($t_{1/2}$ ~4–5 min) association-dissociation cycle, and hormone binding precludes re-entry of the dissociated complexes into the reassociation pathway (Smith, 1993). Finally, studies of various steroid receptors have established that disassembly of aporeceptor complexes can be rapid and apparently spontaneous, whereas reassembly requires Hsp70 and perhaps other molecular chaperones in an ATP-dependent process (Smith, 1993; Kimura *et al.*, 1995).

Of the proteins comprising the steroid aporeceptor complex, most attention has focused on Hsp90, as it was the first receptor-associated protein identified and the first shown to be functionally significant. Hsp90 has been shown to interact with multiple classes of signaling factors, such as the glucocorticoid and dioxin receptors (Sanchez *et al.*, 1985; Wilhelmsson *et al.*, 1990), tyrosine kinases, such as *src* (Oppermann *et al.*, 1981), and ser-thr kinases such as e-IF2α kinase, Raf, and cdc2 kinase (Rose *et al.*, 1987; Stancato *et al.*, 1993; Aligue *et al.*, 1994). In both budding and fission yeast and in *Drosophila*, Hsp90 is required for viability (Borkovich *et al.*, 1989; Aligue *et al.*, 1994; Cutforth and Rubin, 1994), and in *Drosophila*, it has been implicated in tyrosine kinase signaling by the *sevenless* receptor and in cell-cycle control (Cutforth and Rubin, 1994).

The Hsp90 interaction delivers to its targets a heterotypic protein complex that, in the case of the steroid receptors, is comprised of a dimer of Hsp90 and several other components including Hsp56, p60, and p23 (Tai *et al.*, 1986; Smith *et al.*, 1990; Smith, 1993; Hutchison *et al.*, 1994). This interaction in turn appears to be mediated by the Hsp70 molecular chaperone, which interacts with the receptors and requires ATP hydrolysis to drive aporeceptor assembly. Although it has been suggested that Hsp90 itself may harbor an ATP-independent chaperone-like function (Miyata and Yahara, 1992; Wiech *et al.*, 1992), it remains to be clearly established whether Hsp90 is more than simply an intermediary between its broad range of targets and the other factors in the complex. Bohen *et al.* (1995) suggested that the expenditure of energy to form the unliganded aporeceptor may yield a "poised" receptor conformation that is inactive but highly responsive to the triggering ligand, and that analogous interactions of Hsp90 chaperone complexes with other cellular signaling components may similarly produce molecular species well suited to sensing and transducing signals.

Our present findings imply a functional interaction between the retinoic acid receptor and Hsp90 that had gone undetected in biochemical studies. The suggestion that the RAR-Hsp90 interaction is transient, together with the notion that the interaction imparts on RAR a functional conformation, suggests in this case that the Hsp90 complex may be able to serve as a true chaperone, whereas in cases such as GR, it appears to be an active component of the aporeceptor complex. Nevertheless, in both cases, the Hsp90 complex may act by a similar mechanism to produce a signal-sensitive species. By this view, the great abundance and strong conservation of Hsp90 might be rationalized by assuming that it interacts with an even broader range of cell signaling proteins than previously suspected. It should prove interesting to investigate the selectivity of target selection by Hsp90, the significance of the transience or stability of its target contacts, and the molecular mechanisms of its actions.

ACKNOWLEDGMENTS

We thank Marty Privalsky for plasmids and plentiful advice, Sean Bohen and Natasha Kralli for coaching on yeast techniques and tricks, the National Institutes of Health Analytical Biostatistical Section for use of the Ligand computer program, Ligand Pharmaceuticals for 9-*cis*-retinoic acid and advice on hormone binding, and Sean Bohen, Peter Geiduschek, Natasha Kralli, Pam Mellon, Marty Privalsky, and Maria Vivanco for valuable comments on the manuscript. This research was supported by a grant from the National Science Foundation.

REFERENCES

Aligue, R., Akhavan, N.H., and Russell, P. (1994). A role for Hsp90 in cell cycle control: Wee1 tyrosine kinase activity requires interaction with Hsp90. EMBO J. *13*, 6099–6106.

Allegretto, E.A., McClurg, M.R., Lazarchik, S.B., Clemm, D.L., Kerner, S.A., Elgort, M.G., Boehm, M.F., White, S.K., Pike, J.W., and Heyman, R.A. (1993). Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: correlation with hormone binding and effects of metabolism. J. Biol. Chem. *268*, 26625–26633.

Allenby, G. *et al.* (1993). Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. Proc. Natl. Acad. Sci. USA *90*, 30–34.

Bagchi, M.K., Tsai, S.Y., Tsai, M.J., and O'Malley, B.W. (1990). Identification of a functional intermediate in receptor activation in progesterone-dependent cell-free transcription. Nature *345*, 547–550.

Bardwell, J.C., and Craig, E.A. (1987). Eukaryotic M_r 83,000 heat shock protein has a homologue in *Escherichia coli*. Proc. Natl. Acad. Sci. USA *84*, 5177–5181.

Bohen, S.P., Kralli, A., and Yamamoto, K.R. (1995). Hold'em and fold'em: chaperones and signal transduction. Science *268*, 1303–1304.

Bohen, S.P., and Yamamoto, K.R. (1993). Isolation of Hsp90 mutants by screening for decreased steroid receptor function. Proc. Natl. Acad. Sci. USA *90*, 11424–11428.

Bohen, S.P., and Yamamoto, K.R. (1994). Modulation of steroid receptor signal transduction by heat shock proteins. In: The Biology of Heat Shock Proteins and Molecular Chaperones, ed. R.J. Morimoto, A. Tissieres, and D. Georgopoulos, Plainview, NY: Cold Spring Harbor Laboratory Press, 313–334.

Borkovich, K.A., Farrelly, F.W., Finkelstein, D.B., Taulien, J., and Lindquist, S. (1989). hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol. Cell. Biol. *9*, 3919–3930.

Bresnick, E.H., Dalman, F.C., Sanchez, E.R., and Pratt, W.B. (1989). Evidence that the 90-kDa heat shock protein is necessary for the steroid binding conformation of the L cell glucocorticoid receptor. J. Biol. Chem. *264*, 4992–4997.

Caamano, C.A., Morano, M.I., Patel, P.D., Watson, S.J., and Akil, H. (1993). A bacterially expressed mineralocorticoid receptor is associated in vitro with the 90-kilodalton heat shock protein and shows typical hormone- and DNA-binding characteristics. Biochemistry *32*, 8589–8595.

Cutforth, T., and Rubin, G.M. (1994). Mutations in Hsp83 and cdc37 impair signaling by the sevenless receptor tyrosine kinase in *Drosophila*. Cell *77*, 1027–1036.

Dalman, F.C., Koenig, R.J., Perdew, G.H., Massa, E., and Pratt, W.B. (1990). In contrast to the glucocorticoid receptor, the thyroid hormone receptor is translated in the DNA binding state and is not associated with hsp90. J. Biol. Chem. *265*, 3615–3618.

Dalman, F.C., Sturzenbecker, L.J., Levin, A.A., Lucas, D.A., Perdew, G.H., Petkovitch, M., Chambon, P., Grippo, J.F., and Pratt, W.B. (1991). Retinoic acid receptor belongs to a subclass of nuclear receptors that do not form "docking" complexes with hsp90. Biochemistry *30*, 5605–5608.

Damm, K., Thompson, C.C., and Evans, R.M. (1989). Protein encoded by v-erbA functions as a thyroid-hormone receptor antagonist. Nature *339*, 593–597.

Eul, J., Meyer, M.E., Tora, L., Bocquel, M.T., Quirin, S.C., Chambon, P., and Gronemeyer, H. (1989). Expression of active hormone and DNA-binding domains of the chicken progesterone receptor in *E. coli*. EMBO J. *8*, 83–90.

Evans, R.M. (1988). The steroid and thyroid hormone receptor superfamily. Science *240*, 889–895.

Garabedian, M.J., and Yamamoto, K.R. (1992). Genetic dissection of the signaling domain of a mammalian steroid receptor in yeast. Mol. Biol. Cell *3*, 1245–1257.

Gietz, D., St. Jean, A., Woods, R.A., and Schiestl, R.H. (1992). Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. *20*, 1425.

Godowski, P.J., Rusconi, S., Miesfeld, R., and Yamamoto, K.R. (1987). Glucocorticoid receptor mutants that are constitutive activators of transcriptional enhancement. Nature *325*, 365–368.

Graupner, G., Wills, K.N., Tzukerman, M., Zhang, X.K., and Pfahl, M. (1989). Dual regulatory role for thyroid-hormone receptors allows control of retinoic-acid receptor activity. Nature *340*, 653–656.

Hall, B.L., Smit, M.Z., and Privalsky, M.L. (1993). Reconstitution of retinoid X receptor function and combinatorial regulation of other nuclear hormone receptors in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *90*, 6929–6933.

Heery, D.M., Pierrat, B., Gronemeyer, H., Chambon, P., and Losson, R. (1994). Homo- and heterodimers of the retinoid X receptor (RXR) activated transcription in yeast. Nucleic Acids Res. *22*, 726–731.

Howard, K.J., and Distelhorst, C.W. (1988). Evidence for intracellular association of the glucocorticoid receptor with the 90-kDa heat shock protein. J. Biol. Chem. *263*, 3474–3481.

Hutchison, K.A., Dittmar, K.D., Czar, M.J., and Pratt, W.B. (1994). Proof that hsp70 is required for assembly of the glucocorticoid receptor into a heterocomplex with hsp90. J. Biol. Chem. *269*, 5043–5049.

Ichikawa, K., Hashizume, K., Nishii, Y., Takeda, T., Kobayashi, M., Suzuki, S., and Yamada, T. (1991). Purification of human c-erb A beta protein. J. Mol. Endocrinol. *7*, 123–129.

Kimura, Y., Yahara, I., and Lindquist, S. (1995). Role of the protein chaperone YDJ1 in establishing Hsp90-mediated signal transduction pathways [see comments]. Science *268*, 1362–1365.

Lombardo, A., Costa, E., Chao, W.R., Toll, L., Hobbs, P.D., Jong, L., Lee, M.O., Pfahl, M., Ely, K.R., and Dawson, M.I. (1994). Recombinant human retinoic acid receptor beta: binding of synthetic retinoids and transcriptional activation. J. Biol. Chem. *269*, 7297–7303.

Manglesdorf, D.J., and Evans, R.M. (1992). Retinoid receptors as transcription factors. In: Transcriptional Regulation, ed. S.L. McKnight and K.R. Yamamoto, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1137–1168.

Metzger, D., White, J.H., and Chambon, P. (1988). The human oestrogen receptor functions in yeast. Nature *334*, 31–36.

Miyata, Y., and Yahara, I. (1992). The 90-kDa heat shock protein, HSP90, binds and protects casein kinase II from self-aggregation and enhances its kinase activity. J. Biol. Chem. *267*, 7042–7047.

Munson, P.J., and Rodbard, D. (1980). Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. *107*, 220–239.

Nemoto, T., Ohara, N.Y., and Ota, M. (1992). Association of the 90-kDa heat shock protein does not affect the ligand-binding ability of androgen receptor. J. Steroid Biochem. Mol. Biol. *42*, 803–812.

Nervi, C., Grippo, J.F., Sherman, M.I., George, M.D., and Jetten, A.M. (1989). Identification and characterization of nuclear retinoic acid-binding activity in human myeloblastic leukemia HL-60 cells. Proc. Natl. Acad. Sci. USA *86*, 5854–5858.

Oppermann, H., Levinson, W., and Bishop, J.M. (1981). A cellular protein that associates with the transforming protein of Rous sarcoma virus is also a heat-shock protein. Proc. Natl. Acad. Sci. USA *78*, 1067–1071.

Picard, D., Khursheed, B., Garabedian, M.J., Fortin, M.G., Lindquist, S., and Yamamoto, K.R. (1990). Reduced levels of hsp90 compromise steroid receptor action in vivo. Nature *348*, 166–168.

Pratt, M.A., Kralova, J., and McBurney, M.W. (1990). A dominant negative mutation of the alpha retinoic acid receptor gene in a retinoic acid-nonresponsive embryonal carcinoma cell. Mol. Cell. Biol. *10*, 6445–6453.

Privalsky, M.L. (1991). A subpopulation of the v-erb A oncogene protein, a derivative of a thyroid hormone receptor, associates with heat shock protein 90. J. Biol. Chem. *266*, 1456–1462.

Privalsky, M.L., Sharif, M., and Yamamoto, K.R. (1990). The viral erbA oncogene protein, a constitutive repressor in animal cells, is a hormone-regulated activator in yeast. Cell *63*, 1277–1286.

Rose, D.W., Wettenhall, R.E., Kudlicki, W., Kramer, G., and Hardesty, B. (1987). The 90-kilodalton peptide of the heme-regulated eIF-2 alpha kinase has sequence similarity with the 90-kilodalton heat shock protein. Biochemistry *26*, 6583–6587.

Samuels, H.H., Tsai, J.S., Casanova, J., and Stanley, F. (1974). Thyroid hormone action: in vitro characterization of solubilized nuclear receptors from rat liver and cultured GH1 cells. J. Clin. Invest. *54*, 853–865.

Sanchez, E.R., Toft, D.O., Schlesinger, M.J., and Pratt, W.B. (1985). Evidence that the 90-kDa phosphoprotein associated with the untransformed L-cell glucocorticoid receptor is a murine heat shock protein. J. Biol. Chem. *260*, 12398–12401.

Schena, M., Freedman, L.P., and Yamamoto, K.R. (1989). Mutations in the glucocorticoid receptor zinc finger region that distinguish interdigitated DNA binding and transcriptional enhancement activities. Genes Dev. *3*, 1590–1601.

Schena, M., and Yamamoto, K.R. (1988). Mammalian glucocorticoid receptor derivatives enhance transcription in yeast. Science *241*, 965–967.

Scherrer, L.C., Dalman, F.C., Massa, E., Meshinchi, S., and Pratt, W.B. (1990). Structural and functional reconstitution of the glucocorticoid receptor-hsp90 complex. J. Biol. Chem. *265*, 21397–21400.

Scherrer, L.C., Hutchison, K.A., Sanchez, E.R., Randall, S.K., and Pratt, W.B. (1992). A heat shock protein complex isolated from rabbit reticulocyte lysate can reconstitute a functional glucocorticoid receptor-Hsp90 complex. Biochemistry *31*, 7325–7329.

Shaknovich, R., Shue, G., and Kohtz, D.S. (1992). Conformational activation of a basic helix-loop-helix protein (MyoD1) by the C-terminal region of murine HSP90 (HSP84). Mol. Cell. Biol. *12*, 5059–5068.

Smith, D.F. (1993). Dynamics of heat shock protein 90-progesterone receptor binding and the disactivation loop model for steroid receptor complexes. Mol. Endocrinol. *7*, 1418–1429.

Smith, D.F., Schowalter, D.B., Kost, S.L., and Toft, D.O. (1990). Reconstitution of progesterone receptor with heat shock proteins. Mol. Endocrinol. *4*, 1704–1711.

Spindler, B.J., MacLeod, K.M., Ring, J., and Baxter, J.D. (1975). Thyroid hormone receptors: binding characteristics and lack of hormonal dependency for nuclear localization. J. Biol. Chem. *250*, 4113–4119.

Stancato, L.F., Chow, Y.H., Hutchison, K.A., Perdew, G.H., Jove, R., and Pratt, W.B. (1993). Raf exists in a native heterocomplex with hsp90 and p50 that can be reconstituted in a cell-free system. J. Biol. Chem. *268*, 21711–21716.

Tai, P.K., Maeda, Y., Nakao, K., Wakim, N.G., Duhring, J.L., and Faber, L.E. (1986). A 59-kilodalton protein associated with progestin, estrogen, androgen, and glucocorticoid receptors. Biochemistry *25*, 5269–5275.

Tsai, M.J., and O'Malley, B.W. (1994). Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu. Rev. Biochem. *63*, 451–486.

Tsai, S., Bartelmez, S., Heyman, R., Damm, K., Evans, R., and Collins, S.J. (1992). A mutated retinoic acid receptor-alpha exhibiting dominant-negative activity alters the lineage development of a multipotent hematopoietic cell line. Genes Dev. 2258–2269.

Wiech, H., Buchner, J., Zimmermann, R., and Jakob, U. (1992). Hsp90 chaperones protein folding in vitro. Nature *358*, 169–170.

Wilhelmsson, A., Cuthill, S., Denis, M., Wikstrom, A.C., Gustafsson, J.A., and Poellinger, L. (1990). The specific DNA binding activity of the dioxin receptor is modulated by the 90 kd heat shock protein. EMBO J. *9*, 69–76.

Wittliff, J.L., Wenz, L.L., Dong, J., Nawaz, Z., and Butt, T.R. (1990). Expression and characterization of an active human estrogen receptor as a ubiquitin fusion protein from *Escherichia coli*. J. Biol. Chem. *265*, 22016–22022.

Yang, N., Schule, R., Mangelsdorf, D.J., and Evans, R.M. (1991). Characterization of DNA binding and retinoic acid binding properties of retinoic acid receptor. Proc. Natl. Acad. Sci. USA *88*, 3559–3563.