Modular Control of Cross-oligomerization

ANALYSIS OF SUPERSTABILIZED Hsp90 HOMODIMERS IN VIVO

Natalie Wayne, YuShuan Lai, Les Pullen, and Daniel N. Bolon

Received for publication, August 26, 2009, and in revised form, October 19, 2009. Published, JBC Papers in Press, November 11, 2009, DOI 10.1074/jbc.M109.060129

Homo-oligomeric proteins fulfill numerous functions in all cells. The ability to co-express subunits of these proteins that preferentially self-assemble without cross-oligomerizing provides for controlled experiments to analyze the function of mutant homo-oligomers in vivo. Hsp90 is a dimeric chaperone involved in the maturation of many kinases and steroid hormone receptors. We observed that co-expression of different Hsp90 subunits in Saccharomyces cerevisiae caused unpredictable synthetic growth defects due to cross-dimerization. We engineered superstabilized Hsp90 dimers that resisted cross-dimerization with endogenous Hsp90 and alleviated the synthetic growth defect. Superstabilized Hsp90 dimers supported robust growth of S. cerevisiae, indicating that dissociation of Hsp90 dimers could be hindered without compromising essential function. We utilized superstabilized dimers to analyze the activity of ATPase mutant homodimers in a temperature-sensitive yeast background where elevated temperature inactivated all other Hsp90 species. We found that ATP binding and hydrolysis by Hsp90 are both required for the efficient maturation of glucocorticoid receptor and v-Src, confirming the critical role of ATP hydrolysis in the maturation of steroid hormone receptors and kinases in vivo.

The advent of modern genetic technology, including synthetic biology, enables many powerful approaches for both medical intervention and scientific exploration. Unfortunately, genetic manipulations frequently have unanticipated and hard-to-predict biochemical and physiological consequences. The analysis and manipulation of homo-oligomeric systems are particularly challenging because co-expression often leads to cross-oligomers with unanticipated activity (1). The ability to control cross-oligomerization is important for the investigation of numerous essential oligomeric proteins in vivo. Here, we present a general and modular approach to restrict cross-oligomerization and apply it to examine the role of ATP hydrolysis in the essential Hsp90 chaperone in vivo.

Hsp90 is essential in eukaryotes where it assists in the maturation of a limited set of medically important substrate proteins collectively referred to as clients. Hsp90 clients are frequently involved in signal transduction and include more than 40 kinases (2) and many steroid hormone receptors (3). Because mutated forms of these Hsp90 clients are frequently causative agents in cancer, Hsp90 has emerged as a promising pharmacological target (4). Many small molecules have been identified that competitively bind to the ATP site in Hsp90, some of which are in clinical trials as anticancer agents (5).

Hsp90 forms a thermodynamically stable dimer (6) that has a dissociation constant of about 60 nM (7), well below the estimated concentration of Hsp90 (about 15 μM) in Saccharomyces cerevisiae cells (8). Of note, Hsp90 subunits undergo subunit exchange with a half-life of less than 1 min (7), whereas Hsp90 hydrolyzes ATP with a turnover rate of about 1 min⁻¹ (7). Because subunit exchange requires dimer dissociation, these results indicate that Hsp90 monomers can form during the Hsp90 ATPase cycle.

In vitro studies have identified point mutations in Hsp90 that disrupt ATP binding and/or hydrolysis. Hsp90 contains three domains: N-terminal (N), middle (M), and C-terminal (C). ATP binds to the N-domain of Hsp90 (9). In the N-domain, the D79N point mutation disrupts hydrogen bonding with the adenine base and prevents nucleotide binding (10). Although Hsp90 does not contain signature Walker motifs, it does contain catalytic groups common to many ATPases, including glutamate 33, which is positioned to activate a water molecule for nucleophilic attack on the scissile phosphodiester bond (10). The E33A point mutation results in Hsp90 competent to bind but not hydrolyze ATP. Both the E33A ATP hydrolysis mutant and the D79N ATP-binding mutant fail to support yeast viability, demonstrating the critical role of ATP hydrolysis by Hsp90 in vivo (10, 11).

The function of Hsp90 is tied into many different biochemical pathways. Roughly 10% of the yeast proteome binds to Hsp90 (2), emphasizing the importance of studying Hsp90 in its cellular environment where all binding partners are present. Many of these Hsp90-binding partners are co-chaperones required for the maturation of clients (2, 3, 12, 13).

Investigating ATPase-deficient Hsp90 mutants in vivo has been challenging for two reasons: first, ATPase mutants are inviable; and second, co-expression of different Hsp90 variants leads to cross-dimerization. Temperature-sensitive (ts) Hsp90 mutants (14) provide a potential means to grow cells co-ex-
pressing ATPase mutants that can then be studied at elevated temperatures where Hsp90 is inactivated. However, co-expression of different Hsp90 subunits leads to the formation of cross-dimers whose biochemical activity is unpredictable. To overcome this challenge, we developed a strategy to thwart cross-dimerization. Using this system combined with inducible client expression systems, we investigated the maturation of clients in yeast where the only potential Hsp90 activity was from ATPase-deficient homodimers.

EXPERIMENTAL PROCEDURES

Construction of Hsp90 Variants—All Hsp90 variants analyzed contained a His6 sequence fused to the N terminus. The yeast Hsp90 expression vectors used are driven by a strong constitutive glyceraldehyde 3-phosphate dehydrogenase promoter as described previously (15). Standard PCR techniques were used to introduce the ATPase mutants (E33A, D79N). To generate superstabilized Hsp90 dimers, a GCN4-based coiled-coil computationally optimized for stability (16) was inserted after amino acid 678 of the HSP82 gene, resulting in the following protein sequence (I678GGGTSSVELEDKNELSEIAHKLNEVALKLKVGERTDG679). We refer to constructs containing this coiled-coil with the suffix coil. In addition, we refer to full-length Hsp90 containing the N-, M-, and C-domain as NMC to distinguish it from truncations containing only the C-domain. Thus, Ccoil refers to the C-domain appendixed with the coiled coil, and NMCcoil represents full-length constructs with the coiled-coil appended.

Yeast Strains—The haploid S. cerevisiae strain iG170Da (17) is a derivative of W303 with both endogenous Hsp90 genes, HSP82 and HSC82, knocked out and the ts G170D mutant of HSP82 chromosomally integrated. Previously described procedures were followed for yeast transformations (15). For the iG170Da strain transformed with NMC1579N, it took 6 days for colonies to grow (for all other strains, colonies were apparent after 2 days). To analyze glucocorticoid receptor (GR) function in yeast, the P2A/GRGZ plasmid was utilized as described previously (14, 15). To analyze v-Src activity, we used p316Galvscv5 (15), a URA3 plasmid containing v-src with a C-terminal v5 epitope tag expressed from a galactose-inducible promoter. To analyze galactose-induced expression of a non-Hsp90 client, the gene encoding the SspB protein (18) from Escherichia coli was cloned in place of v-src to create p316GalSspBv5.

Protein Production—Hsp90 purification was performed as described previously (15). Briefly, Hsp90 genes with N-terminal His6 tags were expressed from T7 promoters in E. coli and purified by metal affinity and anion exchange chromatography. Purified wild-type human cystic fibrosis transmembrane regulator (CFTR) nucleotide-binding domain 1 (NBD1) encompassing amino acids 388–673 was a kind gift from the laboratory of Phil Thomas (University of Texas Southwestern CFTR Folding Consortium).

Biophysical Analyses—Unfolded transitions were monitored by circular dichroism as described previously (15). Equilibrium analytical ultracentrifugation was performed as described previously (15), except the buffer consisted of 50 mM Tris, pH 7.5, 50 mM potassium chloride, 10 mM magnesium chloride, and the protein concentration was 12 μM.

Subunit Mixing Analysis—Rates of ATP hydrolysis were determined utilizing an NADH-coupled system as described previously (15). Before measuring ATPase activity, mixtures of full-length and truncated Hsp90 proteins were equilibrated in a volume of 90 μl for 24 h in 20 mM potassium phosphate, pH 6.8, 1 mM EDTA at 30 °C. After equilibration, 60 μl of ATPase components were added and ATPase measurements made over a 10-min time course at 37 °C. Final concentrations were 5 μM full-length Hsp90 and 0–30 μM truncated Hsp90. To determine the kinetics of NMCcoil and Ccoil subunit exchange, the equimolar experiment was repeated and ATPase activity measured as a function of preequilibration time. The resulting data were fit to an exponential decay model: \( V = A + Be^{-kt} \).

In Vitro Aggregation Assays—CFTR NBD1 experiments were performed essentially as described previously (19), except that native protein was used as the starting material. Right-angle light scattering was measured in a PTI QM-4SE spectrofluorometer in a temperature-controlled 0.3-cm pathlength cuvette. Protein samples were degassed for 20 min at room temperature, and aggregation was initiated by transfer to a cuvette prewarmed at 37 °C. Measurements were made as a function of time at a wavelength of 400 nm (excitation and emission) in CFTR buffer (100 mM Tris-HCl, pH 7.4, 0.385 M l-arginine, 10 mM dithiothreitol, 200 mM potassium chloride, 20 mM magnesium chloride) (17). Protein concentrations were 5 μM Hsp90 and 1 μM CFTR NBD1. To analyze aggregation of Hsp90 variants on their own, samples of 5 μM protein in buffer A (20 mM potassium phosphate, pH 7, 1 mM EDTA) were heated in a PCR machine for 5 min, cooled to room temperature, run on native PAGE (6% acrylamide, 30 mM Heps, 30 mM imidazole), and Coomassie stained.

Glucocorticoid Activity Assays—Yeast strain iG170Da with P2A/GRGZ and Hsp90 constructs were grown in synthetic dextrose medium lacking tryptophan and adenine at 25 °C to a cell density of about 5 × 10^6 cells/ml. Cells were collected by centrifugation and resuspended in medium prewarmed to 39 °C (to inactivate G170D Hsp90). Cultures were grown in a shaking incubator at 39 °C for 15 min and then split in half. We found that rapid heat inactivation of G170D Hsp90 required heating the medium to 37.5 °C (measuring medium temperature with a thermocoupled microthermometer) and that this could be readily accomplished by preheating the medium to a slightly elevated temperature. Special care was taken to ensure that all samples were heat-inactivated equivalently. To one culture, deoxycoartecosterone dissolved in ethanol was added to a final concentration of 10 μM, and ethanol alone was added to the uninduced half (final ethanol concentration was 0.1% in all cases). Cells were grown for a further 60 min at 37 °C and collected by centrifugation. Galactosidase levels were determined as described previously (15). GR assays were repeated three times starting from fresh yeast colonies. One-tailed Student’s t tests were used to compare the no-insert strain with the ATPase-defi-
Superstabilized Hsp90 Homodimers

![Diagram of Hsp90 dimers](image)

**FIGURE 1.** Co-expression of different NMC Hsp90 variants in vivo leads to the formation of cross-dimers with unpredictable function. A, left, growth of yeast expressing different NMC variants: ts G170D alone (upper left), G170D with the ATP-binding-deficient D79N point mutant (upper right), wild-type (WT) Hsp90 alone (lower left), and wild-type with D79N (lower right). Expression of NMC<sup>G170D</sup> caused an unanticipated growth defect when expressed with NMC<sup>D79N</sup>. Right, dimer exchange model of probable NMC dimers species present in the co-expression cells. B, strategy to hinder cross-dimerization. Appending a coiled-coil dimerization domain superstabilizes NMC<sub>coil</sub> homodimer species and shifts the dimer exchange equilibrium to disfavor NMC<sub>coil</sub>/NMC cross-dimers.

Cross-dimerization of Hsp90 Have Unpredictable Functions—Co-expression of ts NMC<sup>G170D</sup> (NMC refers to full-length Hsp90 containing the N-, M-, and C-domains) with the ATP-binding deficient NMC<sup>D79N</sup> caused a synthetic growth defect in yeast (Fig. 1A). In contrast, healthy growth was observed when the same NMC<sup>D79N</sup> construct was introduced into yeast with wild-type NMC. The G170D and D79N mutations are unlikely to affect dimer association because they are both located in the N-domain, which is physically distant from the constitutive dimer interface in the C-domain (20). In the co-expression strains, dimers should equilibrate to form a distribution of three possible dimer species (Fig. 1A). The synthetic growth defect from co-expression of G170D and D79N point mutants could be caused by G170D/D79N point mutants.

Superstabilization Strategy to Hinder Cross-dimerization—Cross-dimerization is governed by a subunit exchange reaction where the free energy of association for each dimer species dictates the equilibrium distribution (21). The difference in free energy of dimerization between the heterodimer and the average of both homodimers controls the equilibrium population of all species. To disfavor cross-dimerization, we fused NMC to an additional dimerization domain consisting of a 33-amino acid coiled-coil peptide to create superstabilized Hsp90 dimers that we refer to as NMC<sub>coil</sub> (Fig. 1B). In theory, the superstabilization of one homodimer is sufficient to shift the equilibrium away from cross-dimers.

Coiled-coil Superstabilizes Hsp90 Dimers—Because dimerization and folding of the C-domain are coupled (15), dimer strength can be determined from unfolding studies of this domain. We compared the thermodynamic unfolding stability of the isolated C-domain with a construct with the coiled-coil that we refer to as Ccoil (C-domain plus coiled-coil). Addition of the coiled-coil enables it to remain folded at concentrations of urea where the isolated C-domain itself unfolds (Fig. 2A). Fits of the denaturation curves indicate that the coiled-coil increases stability by 12 kJ/mol/M subunit. In a dimer exchange model, this energetic difference reduces cross-dimer accumulation to less than 1% of the total population when each subunit is at equal abundance.

**RESULTS**

**Cross-dimers of Hsp90 Have Unpredictable Functions**—Co-expression of ts NMC<sup>G170D</sup> (NMC refers to full-length Hsp90 containing the N-, M-, and C-domains) with the ATP-binding deficient NMC<sup>D79N</sup> caused a synthetic growth defect in yeast (Fig. 1A). In contrast, healthy growth was observed when the same NMC<sup>D79N</sup> construct was introduced into yeast with wild-type NMC. The G170D and D79N mutations are unlikely to affect dimer association because they are both located in the N-domain, which is physically distant from the constitutive dimer interface in the C-domain (20). In the co-expression strains, dimers should equilibrate to form a distribution of three possible dimer species (Fig. 1A). The synthetic growth defect from co-expression of G170D and D79N point mutants could be caused by G170D/D79N point mutants.

Superstabilization Strategy to Hinder Cross-dimerization—Cross-dimerization is governed by a subunit exchange reaction where the free energy of association for each dimer species dictates the equilibrium distribution (21). The difference in free energy of dimerization between the heterodimer and the average of both homodimers controls the equilibrium population of all species. To disfavor cross-dimerization, we fused NMC to an additional dimerization domain consisting of a 33-amino acid coiled-coil peptide to create superstabilized Hsp90 dimers that we refer to as NMCcoil (Fig. 1B). In theory, the superstabilization of one homodimer is sufficient to shift the equilibrium away from cross-dimers.
Superstabilization Leads to Preferential Homodimer Assembly—To analyze dimer assembly experimentally, we took advantage of the observation that NMC and truncated C-domain form cross-dimers with distinct ATPase activity (7, 15). Increasing C-domain concentration caused a marked decrease in ATPase activity from NMC (Fig. 3A). In contrast, the strengthened self-association of NMCcoil prevented cross-dimerization with C-domain and resulted in NMCcoil ATPase activity that was refractory to inhibition by C-domain. In principle, the observed lack of ATPase inhibition of NMCcoil by the C-domain could also be caused by either slow kinetics of subunit exchange (and failure to reach equilibrium dimer distributions) or NMCcoil/C-domain heterodimers having robust ATPase activity. In either of these cases, mixtures of NMCcoil and Ccoil would exhibit uninhibited ATPase levels. We found that mixtures of NMCcoil and Ccoil displayed essentially the same equilibrium ATPase inhibition response as NMC and C mixtures (Fig. 3B). Thus, NMCcoil dimer exchange equilibrates in the experiment, and NMCcoil ATPase activity can be reduced through cross-dimerizing with a truncated Ccoil. We conclude that NMCcoil dimers are refractory to cross-dimerization with Hsp90 subunits lacking the coiled-coil.

NMCcoilD79N Rescues Growth When Co-expressed with ts NMCG170D—If the synthetic growth defect of cells co-expressing G170D and D79N point mutants was caused by cross-dimerization, then preventing cross-dimerization should enable healthy growth of cells expressing both Hsp90 variants. Indeed, we observed that co-expressing NMCG170D and NMCcoilD79N resulted in healthy growth (Fig. 3C). This yeast strain is virtually identical to the strain in Fig. 1A (upper), with the exception that cross-dimers are suppressed. The phenotypic effect of this small change demonstrates the sensitive nature of the complex Hsp90 interactome in vivo and underscores the importance of robust regulation of cross-dimers.

ATPase-deficient Hsp90 Mutants Function at Elevated Temperature—The yeast system that we developed to study ATPase-deficient Hsp90 mutants in vivo requires the inactivation of ts NMCG170D at elevated temperature; therefore, we first sought to ensure that the ATPase mutants are refractory to heat inactivation at the required temperature of these experiments (37 °C). Temperature scans of both E33A and D79N ATPase-deficient mutants and wild-type NMC, monitored by circular dichroism, reproducibly show a small amplitude transition at about 55 °C (Fig. 4A). However, because of the small amplitude of this change, we sought a more robust metric of temperature induced Hsp90 misfolding. Higher order aggregation was the most pronounced physical affect that we observed for misfolded Hsp90. Using native PAGE, we observed temperature-dependent formation of higher order aggregates for wild-type Hsp90 (Fig. 4B) that occurred at the same transition temperature observed by circular dichroism. Using the native gel approach, we found that both of the ATPase mutants are natively structured at 37 °C (Fig. 4C). Next, we analyzed the ability of the ATPase mutants to serve as antiaggregation chaperones, an ATP-independent function of wild-type Hsp90 (19, 22). We performed light-scattering experiments to analyze aggregation of the hard-to-fold NBD1 of CFTR. As observed previously (19), NBD1 aggregation at elevated temperatures can be

FIGURE 2. Superstabilization strategy is compatible with Hsp90 function. A, appending a coiled-coil super-stabilizes the C-domain of Hsp90 as determined by urea denaturation monitored by circular dichroism. B, full-length NMC behaves as a dimer during analytical ultracentrifugation. Single-species fit indicates that the molecular mass is 181 kDa (similar to the theoretical molecular mass of a dimer: 172 kDa). C, NMCcoil is compatible with efficient ATP hydrolysis. ATP hydrolysis can be blocked with the Hsp90-specific inhibitor geldanamycin (GA). wt, wild-type. D, NMC supports yeast viability as the sole Hsp90 and expressed to levels similar to NMC based on Western blotting of yeast lysates. Both NMC and NMCcoil samples were analyzed on the same SDS-polyacrylamide gel and blot, and intervening lanes were removed for presentation clarity. FOA, 5-fluoroorotic acid.
reduced in vitro by Hsp90 in the absence of ATP (Fig. 4D). Both E33A and D79N NMC are capable of suppressing NBD1 aggregation at 37 °C (Fig. 4D). From these results, we conclude that the ATPase mutants of Hsp90 are stable at 37 °C.

Maturation of v-Src—Co-expression of ts NMCG170D with ATPase-deficient NMCcoil mutants enabled healthy growth at permissive temperature, providing a large reservoir of cells that could be heat-inactivated prior to inducing the expression of a client (Fig. 5A). When v-Src expression was induced at elevated temperature in ts NMCG170D yeast, v-Src accumulated at relatively low levels and failed to phosphorylate the tyrosines of endogenous yeast proteins (Fig. 5B). Because yeast have extremely low amounts of endogenous tyrosine kinases, background phosphotyrosine levels were minute. Importantly, both v-Src accumulation and kinase function could be rescued by co-expression with wild-type NMC or NMCcoil, indicating that the superstabilizing coiled-coil is compatible with efficient v-Src maturation. The E33A and D79N ATPase-deficient NMCcoil mutants both resulted in decreased v-Src accumulation and background levels of phosphotyrosine. Accumulation of SspB, a non-Hsp90-dependent protein, is not dramatically impacted by the ATPase mutants, indicating that general aspects of transcriptional activation (23) are functional in these cells (Fig. 5C).

GR Activation—GR transcriptional activation in response to the hormone activator deoxycorticosterone was monitored using a β-galactosidase reporter (24). Yeast harboring wild-type NMC showed a robust increase in hormone-stimulated reporter activity compared with yeast whose sole source of Hsp90 was heat-inactivated (Fig. 6A). We observed similar hormone-stimulated GR activity with both wild-type NMC and NMCcoil, indicating that the coiled-coil fusion is compatible with efficient hormone receptor activation. Both the ATP-binding mutant (D79N) and the ATP hydrolysis mutant (E33A) NMCcoil variants showed markedly reduced GR activity, indicating that both ATP binding and hydrolysis by Hsp90 are important for efficient hormone receptor activation. The accumulation of GR was impacted by the ATPase mutants with E33A and D79N, resulting in increased accumulation of GR (Fig. 6B).

Hsp90 Dimers Constitute the Essential Biological Oligomer—Hsp90 dimers transiently dissociate to monomers on the same time scale as ATP hydrolysis (7), leading to the possibility that both monomers and dimers are part of the chaperone cycle. In contrast to the rapid dimer exchange of wild-type subunits, we observed that superstabilized NMCcoil dimers have a half-life of 8 h to equilibrate (Fig. 7A). NMCdimers equilibrate with a half-life of about 30 s (supplemental Fig. S3). These results indicate that dimer dissociation is the rate-limiting step in the dimer exchange reaction and that NMCcoil Hsp90 dimers dissociate on a time scale that is too slow to be relevant for cellular processes such as signal transduction. Our observation that NMCcoil hydrolyzes ATP at a rate similar to NMC indicates that dissociation of Hsp90 subunits is not required for ATP hydrolysis. In addition, our observations that NMCcoil Hsp90 function is indis-
DISCUSSION

The ability of NMCcoil to chaperone clients efficiently and to support viability shows that superstabilized Hsp90 dimers are functional in vivo. The strength of NMCcoil subunit association in vivo may be influenced by interactions with co-chaperones. However, the 1000-fold reduced rate of subunit exchange observed in vitro for NMCcoil (Fig. 7A) makes it probable that subunit association is also strengthened in vivo. In addition, appending the coiled-coil to D79N rescues robust growth of yeast with the ts G170D (Fig. 3C), consistent with the coiled-coil strengthening dimerization and preventing subunit mixing in vivo. Together, these results indicate that strengthened subunit association is compatible with function, and we reported previously that preventing association of Hsp90 subunits abrogates chaperone function (15). Of note, the C-domain dimer interface is composed of a large patch of hydrophobic amino acids rich in Ile, Val, and Leu (supplemental Fig. S2). Protein folding studies indicate that clusters of these three aliphatic amino acids form strong associations capable of directing folding pathways (25, 26). The aliphatic patch at the C-domain dimer interface is consistent with the critical role of subunit association in the function of the Hsp90 and our observations that subunit dissociation does not appear to be required for essential Hsp90 function.

The mechanistic details of Hsp90 ATPase activity have been well examined in its purified form (7, 31, 32); however, until now it has not been possible to extend these analyses to monitoring client activation in vivo. Our findings that E33A and D79N NMCcoil fail to mature GR in vivo (Fig. 7B) are consistent with previous observations that analogous mutations prevent Hsp90 from stimulating the binding of progesterone receptor to its hormone ligand in vitro (33). Many studies demonstrate that the primary role of Hsp90 in stimulating
hormone receptors is opening of the receptor to enable access of hormone to the otherwise sequestered binding site (34). Our results indicate that in vivo this process requires both ATP binding and hydrolysis.

We have presented a modular strategy to control cross-oligomerization and utilized it to analyze ATPase mutants of Hsp90 in a ts yeast background. These studies confirm the critical role of ATP binding and hydrolysis by Hsp90 in the maturation of kinases and steroid hormone receptors in vivo. The superstabilized Hsp90 tools that we developed provide a foundation to explore other nonviable Hsp90 mutants in vivo and to extend our analysis to other clients. Because our superstabilization strategy is based on coiled-coil peptides whose characteristics are extremely modular, it is directly applicable to many homodimeric systems. In addition, the existence of α-helical peptides that specifically self-assemble with different orders, including dimers, trimers, tetramers, and pentamers (35, 36), makes the approach described here...

FIGURE 5. ATPase-deficient NMCCoil variants are unable to mature v-Src kinase in yeast. A, experimental setup to analyze ATPase-deficient Hsp90 mutants in yeast is shown. B, in yeast, NMCCoil mutations that disrupt ATP binding (D79N) or hydrolysis (E33A) both lead to reduced v-Src accumulation and background levels of tyrosine kinase activity. C, induction of general protein expression is only modestly impacted by the NMCCoil ATPase mutants as evidenced by the accumulation of a non-Hsp90 client, the SspB protein from E. coli.

FIGURE 6. Hsp90 ATPase mutants do not mature GR in yeast. A, GR activation monitored in yeast. Yeast with the ts NMCCoil as well as ATPase-deficient NMCCoil variants were heated to inactivate NMCCoil prior to stimulation with saturating levels of deoxycorticosterone (10 μM). Neither D79N nor E33A NMCCoil resulted in reporter activity levels significantly greater than background using a single-tailed Student’s t test. Error bars represent S.E. B, expression level of GR analyzed by Western blots of yeast lysates. wt, wild-type.

FIGURE 7. Dimers of Hsp90 constitute the essential biological oligomer whose ATPase function is required for maturation of clients. A, NMCCoil reduces the rate of subunit exchange to time scales that are irrelevant to the biochemical function of Hsp90 in vivo. Truncated and full-length protein were mixed and allowed to exchange subunits prior to assessing the amount of full-length/full-length dimers by monitoring specific ATPase activity. B, model of the ATPase-dependent function of Hsp90 dimers in vivo. ATP hydrolysis by Hsp90 dimers leads to the maturation of clients.
widely applicable to the investigation of homo-oligomeric systems.

Acknowledgments—We thank W. Kobertz and P. Zamore for fruitful discussions, P. Thomas (University of Texas Southwestern CFTR Folding Consortium) for purified CFTR NBD1 protein, C. R. Matthews for providing CD instrument time, and Kim Crowley and the University of Massachusetts Ultracentrifugation Facility for assistance in collecting analytical ultracentrifugation data.

REFERENCES

1. Bolon, D. N., Wah, D. A., Hersch, G. L., Baker, T. A., and Sauer, R. T. (2004) Mol. Cell 13, 443–449
2. Zhao, R., Davey, M., Hsu, Y. C., Kaplanek, P., Tong, A., Parsons, A. B., Krogan, N., Cagnye, G., Mai, D., Greenblatt, J., Boone, C., Emili, A., and Houri, W. A. (2005) Cell 120, 715–727
3. Pratt, W. B., and Toft, D. O. (2003) Exp. Biol. Med. 228, 111–133
4. Whitesell, L., and Lindquist, S. L. (2005) Nat. Rev. Cancer 5, 761–772
5. Taldone, T., Sun, W., and Chiosis, G. (2009) Bioorg. Med. Chem. 17, 2225–2235
6. Nemoto, T., Ohara-Nemoto, Y., Ota, M., Takagi, T., and Yokoyama, K. (1995) Eur. J. Biochem. 233, 1–8
7. Richter, K., Muschler, P., Hainzl, O., and Buchner, J. (2001) J. Biol. Chem. 276, 33689–33696
8. Ghaemmaghami, S., Huh, W. K., Bower, K., Howson, R. W., Belle, A., Dephoure, N., O’Shea, E. K., and Weissman, J. S. (2003) Nature 425, 737–741
9. Prodrumou, C., Roe, S. M., O’Brien, R., Ladbury, J. E., Piper, P. W., andPearl, L. H. (1997) Cell 90, 65–75
10. Panaretou, B., Prodrumou, C., Roe, S. M., O’Brien, R., Ladbury, J. E., Piper, P. W., and Pearl, L. H. (1998) EMBO J. 17, 4829–4836
11. Obermann, W. M., Sondermann, H., Russo, A. A., Pavletich, N. P., and Hartl, F. U. (1998) J. Cell Biol. 143, 901–910
12. Cox, M. B., and Miller, C. A., 3rd. (2002) Toxicol. Lett. 129, 13–21
13. Wandinger, S. K., Richter, K., and Buchner, J. (2008) J. Biol. Chem. 283, 18473–18477
14. Nathan, D. F., and Lindquist, S. (1995) Mol. Cell. Biol. 15, 3917–3925
15. Wayne, N., and Bolon, D. N. (2007) J. Biol. Chem. 282, 35386–35395
16. Havranek, J. J., and Harbury, P. B. (2003) Nat. Struct. Biol. 10, 45–52
17. Nathan, D. F., Vos, M. H., and Lindquist, S. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 12949–12956
18. Levchenko, I., Seidel, M., Sauer, R. T., and Baker, T. A. (2000) Science 289, 2354–2356
19. Youker, R. T., Walsh, P., Bellharz, T., Lithgow, T., and Brodsky, J. L. (2004) Mol. Biol. Cell 15, 4787–4797
20. Ali, M. M., Roe, S. M., Vaughan, C. K., Meyer, P., Panaretou, B., Piper, P. W., Prodrumou, C., and Pearl, L. H. (2006) Nature 440, 1013–1017
21. Bolon, D. N., Grant, R. A., Baker, T. A., and Sauer, R. T. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 12724–12729
22. Yonehara, M., Minami, Y., Kawata, Y., Nagai, J., and Yahara, I. (1996) J. Biol. Chem. 271, 2641–2645
23. Floer, M., Bryant, G. O., and Ptashne, M. (2008) Proc. Natl. Acad. Sci. U.S.A. 105, 2975–2980
24. Picard, D., Khursheed, B., Garabedian, M. J., Fortin, M. G., Lindquist, S., and Yamamoto, K. R. (1990) Nature 348, 166–168
25. Wu, Y., Vadrevu, R., Kathuria, S., Yang, X., and Matthews, C. R. (2007) J. Mol. Biol. 366, 467–484
26. Kathuria, S. V., Day, I. J., Wallace, L. A., and Matthews, C. R. (2008) J. Mol. Biol. 382, 467–484
27. Krukenberg, K. A., Förster, F., Rice, L. M., Sali, A., and Agard, D. A. (2008) Structure 16, 755–765
28. Prodrumou, C., Roe, S. M., Piper, P. W., and Pear, L. H. (1997) Nat. Struct. Mol. Biol. 4, 477–482
29. Hessling, M., Richter, K., and Buchner, J. (2009) Nat. Struct. Mol. Biol. 16, 287–293
30. Mickler, M., Hessling, M., Ratzke, C., Buchner, J., and Hugel, T. (2009) Nat. Struct. Mol. Biol. 16, 281–286
31. Richter, K., Soroka, J., Skalniak, L., Leskovar, A., Hessling, M., Reinstein, J., and Buchner, J. (2008) J. Biol. Chem. 283, 17757–17765
32. McLaughlin, S. H., Ventouras, L. A., Lobbezoo, B., and Jackson, S. E. (2004) J. Mol. Biol. 344, 813–826
33. Grenert, J. P., Johnson, B. D., and Toft, D. O. (1999) J. Biol. Chem. 274, 17525–17533
34. Pratt, W. B., Morishima, Y., Murphy, M., and Harrell, M. (2006) Handb. Exp. Pharmacol. 172, 111–138
35. DeLano, W. L., and Brünger, A. T. (1994) Proteins 20, 105–123
36. Ozbek, S., Engel, J., and Stetefeld, J. (2002) EMBO J. 21, 5960–5968