Literature Lunch 5-1-13

Yichen –

J Mol Biol. 2013 May 13;425(9):1476-87. doi: 10.1016/j.jmb.2012.11.028. Epub 2012 Nov 24.

Structure and Allostery of the Chaperonin GroEL.

Saibil HR, Fenton WA, Clare DK, Horwich AL.

Source

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Abstract

Chaperonins are intricate allosteric machines formed of two back-to-back, stacked rings of subunits presenting end cavities lined with hydrophobic binding sites for nonnative polypeptides. Once bound, substrates are subjected to forceful, concerted movements that result in their ejection from the binding surface and simultaneous encapsulation inside a hydrophilic chamber that favors their folding. Here, we review the allosteric machine movements that are choreographed by ATP binding, which triggers concerted tilting and twisting of subunit domains. These movements distort the ring of hydrophobic binding sites and split it apart, potentially unfolding the multiply bound substrate. Then, GroES binding is accompanied by a 100° twist of the binding domains that removes the hydrophobic sites from the cavity lining and forms the folding chamber. ATP hydrolysis is not needed for a single round of binding and encapsulation but is necessary to allow the next round of ATP binding in the opposite ring. It is this remote ATP binding that triggers dismantling of the folding chamber and release of the encapsulated substrate, whether folded or not. The basis for these ordered actions is an elegant system of nested cooperativity of the ATPase machinery. ATP binds to a ring with positive cooperativity, and movements of the interlinked subunit domains are concerted. In contrast, there is negative cooperativity between the rings, so that they act in alternation. It is remarkable that a process as specific as protein folding can be guided by the chaperonin machine in a way largely independent of substrate protein structure or sequence.

J Mol Biol. 2013 May 13;425(9):1415-23. doi: 10.1016/j.jmb.2013.01.036. Epub 2013 Feb 8.

Allosteric Effects in the Regulation of 26S Proteasome Activities.

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Source

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Abstract

The 26S proteasome is the executive arm of the ubiquitin-proteasome system. This 2.5-MDa complex comprising the 20S core particle (CP) and the 19S regulatory particle (RP) is able to effectively execute its function due to a tightly regulated network of allosteric interactions. From this perspective, we summarize the current state of knowledge on these regulatory interdependencies. We classify them into the three functional layers-within the CP, within the RP, and at the CP-RP interface. In the CP, allosteric effects are thought to couple the gate opening and substrate proteolysis. Gate opening depends on events occurring in the RP-ATP hydrolysis and substrate binding. Finally, a number of processes occurring solely in the RP, like ATP hydrolysis or substrate deubiquitylation, are also proposed to be allosterically regulated. Recent advances in structural studies of 26S proteasome open up new avenues for dissecting and rationalizing the molecular basis of these regulatory networks.

Damian –

Du, Z.-Y., Chen, M.-X., Chen, Q.-F., Xiao, S., Chye, M.-L. (2013). Arabidopsis acyl-CoA-binding protein ACBP1 participates in the regulation of seed germination and seedling development. *Plant J.* 74: 294-309.

A family of six genes encoding acyl-CoA-binding proteins (ACBPs), ACBP1–ACBP6, has been characterized in *Arabidopsis thaliana*. In this study, we demonstrate that ACBP1 promotes abscisic acid (ABA) signaling during germination and seedling development. *ACBP1* was induced by ABA, and transgenic Arabidopsis ACBP1-over-expressors showed increased sensitivity to ABA during germination and seedling development, whereas the *acbp1* mutant showed decreased ABA sensitivity during these processes. Subsequent RNA assays showed that ACBP1 over-production in 12-day-old seedlings up-regulated the expression of *PHOSPHOLIPASE Dα1* (*PLDα1*) and three ABA/stress-responsive genes: *ABA-RESPONSIVE ELEMENT BINDING PROTEIN1* (*AREB1*), *RESPONSE TO DESICCATION29A* (*RD29A*) and *bHLH-TRANSCRIPTION FACTOR MYC2* (*MYC2*). The expression of *AREB1* and *PLDα1* was suppressed in the *acbp1* mutant in comparison with the wild type following ABA treatment. PLDα1 has been reported to promote ABA signal transduction by producing phosphatidic acid, an important lipid messenger in ABA signaling. Using lipid profiling, seeds and 12-day-old seedlings of ACBP1-over-expressing lines were shown to accumulate more phosphatidic acid after ABA treatment, in contrast to lower phosphatidic acid in the *acbp1* mutant. Bimolecular fluorescence complementation assays indicated that ACBP1 interacts with PLDα1 at the plasma membrane. Their interaction was further confirmed by yeast two-hybrid analysis. As recombinant ACBP1 binds phosphatidic acid and phosphatidylcholine, ACBP1 probably promotes PLDα1 action. Taken together, these results suggest that ACBP1 participates in ABA-mediated seed germination and seedling development.

Gusarov, I., Gautier, L., Smolentseva, O., Shamovsky, I., Eremina, S., Mironov, A., Nudler, E. (2013). Bacterial nitric oxide extends the lifespan of *C. elegans*. *Cell* 152: 818-830.

Nitric oxide (NO) is an important signaling molecule in multicellular organisms. Most animals produce NO from L-arginine via a family of dedicated enzymes known as NO synthases (NOSes). A rare exception is the roundworm *Caenorhabditis elegans*, which lacks its own NOS. However, in its natural environment, *C. elegans* feeds on *Bacilli* that possess functional NOS. Here, we demonstrate that bacterially derived NO enhances *C. elegans* longevity and stress resistance via a defined group of genes that function under the dual control of HSF-1 and DAF-16 transcription factors. Our work provides an example of interspecies signaling by a small molecule and illustrates the lifelong value of commensal bacteria to their host.

Indu –

1. Science. 2013 Apr 5;340(6128):25-7. doi: 10.1126/science.340.6128.25.

Molecular biology. 'Dead' enzymes show signs of life.

Leslie M.

PMID: 23559232 [PubMed - indexed for MEDLINE]

2. Science. 2013 Apr 5;340(6128):91-5. doi: 10.1126/science.1231965.

Transposition-driven genomic heterogeneity in the Drosophila brain.

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Recent studies in mammals have documented the neural expression and mobility of retrotransposons and have suggested that neural genomes are diverse mosaics. We found that transposition occurs among memory-relevant neurons in the Drosophila brain. Cell type-specific gene expression profiling revealed that transposon expression is more abundant in mushroom body (MB) ?? neurons than in neighboring MB neurons. The Piwi-interacting RNA (piRNA) proteins Aubergine and Argonaute 3, known to suppress transposons in the fly germline, are expressed in the brain and appear less abundant in ?? MB neurons. Loss of piRNA proteins correlates with elevated transposon expression in the brain. Paired-end deep sequencing identified more than 200 de novo transposon insertions in ?? neurons, including insertions into memory-relevant loci. Our observations indicate that genomic heterogeneity is a conserved feature of the brain.

PMID: 23559253 [PubMed - indexed for MEDLINE]

3. Science. 2013 Apr 5;340(6128):82-5. doi: 10.1126/science.1231197.

Translational repression and eIF4A2 activity are critical for microRNA-mediated

gene regulation.

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MicroRNAs (miRNAs) control gene expression through both translational repression and degradation of target messenger RNAs (mRNAs). However, the interplay between these processes and the precise molecular mechanisms involved remain unclear. Here, we show that translational inhibition is the primary event required for mRNA degradation. Translational inhibition depends on miRNAs impairing the function of the eIF4F initiation complex. We define the RNA helicase eIF4A2 as the key factor of eIF4F through which miRNAs function. We uncover a correlation between the presence of miRNA target sites in the 3' untranslated region (3'UTR) of mRNAs and secondary structure in the 5'UTR and show that mRNAs with unstructured 5'UTRs are refractory to miRNA repression. These data support a linear model for miRNA-mediated gene regulation in which translational repression via eIF4A2 is required first, followed by mRNA destabilization.

PMID: 23559250 [PubMed - indexed for MEDLINE]

Stephanie –

1) From Molecular Cell: **Hsp90 Regulates Nongenetic Variation in Response to Environmental Stress**

Abstract: Nongenetic cell-to-cell variability often plays an important role for the survival of a clonal population in the face of fluctuating environments. However, the underlying mechanisms regulating such nongenetic heterogeneity remain elusive in most organisms. We report here that a clonal yeast population exhibits morphological heterogeneity when the level of Hsp90, a molecular chaperone, is reduced. The morphological heterogeneity is driven by the dosage of Cdc28 and Cla4, a key regulator of septin formation. Low Hsp90 levels reduce Cla4 protein stability and cause a subpopulation of cells to switch to a filamentous form that has been previously suggested to be beneficial under certain hostile environments. Moreover, Hsp90-dependent morphological heterogeneity can be induced by environmental stress and is conserved across diverse yeast species. Our results suggest that Hsp90 provides an evolutionarily conserved mechanism that links environmental stress to the induction of morphological diversity.

2) From TIBS: **The chaperone Hsp90: changing partners for demanding clients**

Review: The heat shock protein (Hsp)90 chaperone machinery regulates the activity of hundreds of client proteins in the eukaryotic cytosol. It undergoes large conformational changes between states that are similar in energy. These transitions are rate-limiting for the ATPase cycle. It has become evident that several of the many Hsp90 cochaperones affect the conformational equilibrium by stabilizing specific intermediate states. Consequently, there is an ordered progression of different co-chaperones during the conformational cycle. Asymmetric complexes containing two different co-chaperones may be important for the processing of the client protein, although our understanding of this aspect, as well as the details of the interaction of Hsp90 with client proteins, is still in its infancy.

Keith –

Journal of Biological Chemistry

Small Heat Shock Protein IbpB Acts as a Robust Chaperone in Living Cells by Hierarchically Activating Its Multi-type Substrate-binding Residues

April 26, 2013 The Journal of Biological Chemistry, 288, 11897-11906.

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As ubiquitous molecular chaperones, small heat shock proteins (sHSPs) are crucial for protein homeostasis. It is not clear why sHSPs are able to bind a wide spectrum of non-native substrate proteins and how such binding is enhanced by heat shock. Here, by utilizing a genetically incorporated photo-cross-linker (p-benzoyl-L-phenylalanine), we systematically characterized the substrate-binding residues in IbpB (a sHSP from Escherichia coli) in living cells over a wide spectrum of temperatures (from 20 to 50 °C). A total of 20 and 48 residues were identified at normal and heat shock temperatures, respectively. They are not necessarily hydrophobic and can be classified into three types: types I and II were activated at low and normal temperatures, respectively, and type III mediated oligomerization at low temperature but switched to substrate binding at heat shock temperature. In addition, substrate binding of IbpB in

living cells began at temperatures as low as 25 °C and was further enhanced upon temperature elevation. Together, these in vivo data provide novel structural insights into the wide substrate spectrum of sHSPs and suggest that sHSP is able to hierarchically activate its multi-type substrate-binding residues and thus act as a robust chaperone in cells under

Crystal Structure of an Insect Antifreeze Protein and Its Implications for Ice Binding

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Antifreeze proteins (AFPs) help some organisms resist freezing by binding to ice crystals and inhibiting their growth. The molecular basis for how these proteins recognize and bind ice is not well understood. The longhorn beetle Rhagium inquisitor can supercool to below −25 °C, in part by synthesizing the most potent antifreeze protein studied thus far (RiAFP). We report the crystal structure of the 13-kDa RiAFP, determined at 1.21 Å resolution using direct methods. The structure, which contains 1,914 nonhydrogen protein atoms in the asymmetric unit, is the largest determined ab initio without heavy atoms. It reveals a compressed β-solenoid fold in which the top and bottom sheets are held together by a silk-like interdigitation of short side chains. RiAFP is perhaps the most regular structure yet observed. It is a second independently evolved AFP type in beetles. The two beetle AFPs have in common an extremely flat ice-binding surface comprising regular outward-projecting parallel arrays of threonine residues. The more active, wider RiAFP has four (rather than two) of these arrays between which the crystal structure shows the presence of ice-like waters. Molecular dynamics simulations independently reproduce the locations of these ordered crystallographic waters and predict additional waters that together provide an extensive view of the AFP interaction with ice. By matching several planes of hexagonal ice, these waters may help freeze the AFP to the ice surface,

Fionn –

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Dynamic Nucleotide-dependent Interactions of Cysteine- and Histidine-rich Domain (CHORD)-containing Hsp90 Cochaperones Chp-1 and Melusin with Cochaperones PP5 and Sgt1*

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Background: Hsp90 cochaperones are regulating interactors of Hsp90, but interactions between themselves are not well known.

Results: CHORD-containing Hsp90 cochaperones interact with cochaperones PP5 and Sgt1 in the presence of ATP.

Conclusion: Conformational changes induced by ATP binding play an important role in the regulation of interactions between cochaperones.

Significance: Interactions between cochaperones might have Hsp90-independent roles that are regulated by cellular ATP concentration.

Mammals have two cysteine- and histidine-rich domain (CHORD)-containing Hsp90 cochaperones, Chp-1 and melusin, which are homologs of plant Rar1. It has been shown previously that Rar1 CHORD directly interacts with ADP bound to the nucleotide pocket of Hsp90. Here, we report that ADP and ATP can bind to Hsp90 cochaperones Chp-1 and PP5, inducing their conformational changes. Furthermore, we demonstrate that Chp-1 and melusin can interact with cochaperones PP5 and Sgt1 and with each other in an ATP-dependent manner. Based on the known structure of the Rar1-Hsp90 complex, His-186 has been identified as an important residue of Chp-1 for ADP/ATP binding. His-186 is necessary for the nucleotide-dependent interaction of Chp-1 not only with Hsp90 but also with Sgt1. In addition, Ca^{2+}, which is known to bind to melusin, enhances the interactions of melusin with Hsp90 and Sgt1. Furthermore, melusin acquires the ADP preference for Hsp90 binding in the presence of Ca^{2+}. Our newly discovered nucleotide-dependent interactions between cochaperones might provide additional complexity to the dynamics of the Hsp90 chaperone system, also suggesting potential Hsp90-independent roles for these cochaperones.

Hsp90 (heat shock protein of 90 kDa) is a conserved, ubiquitous, and abundant molecular chaperone involved in a broad spectrum of key cellular functions. The Hsp90 homodimer works as a "molecular clamp" undergoing open and closed cyclic conformational changes driven by binding and hydrolysis of ATP (1). The activity of the Hsp90 chaperone machinery is regulated by dynamic association of various Hsp90 cochaperones during this chaperone cycle. Hsp90 cochaperones are known to modulate the ATPase activity of Hsp90, to stabilize certain conformations of Hsp90 dimer, to recruit particular classes of client proteins to Hsp90, and to exert post-transcriptional modifications on some client proteins or the Hsp90 chaperone complex (2, 3). Some cochaperones have their own chaperone activities, suggesting that cochaperones may also have Hsp90-independent cellular functions (4–8).

The Hsp90 cochaperones Sgt1 and Rar1 are involved in plant immunity by regulating the immune sensors called resistance proteins (R proteins)[3] (9–11). Sgt1 consists of the tetratricopeptide repeat (TPR), CS (CHORD-containing proteins and Sgt1), and SGS (Sgt1-specific) domains. Sgt1 acts as a client adaptor linking R proteins to Hsp90 by binding to R proteins through its SGS domain while interacting with Hsp90 through the CS domain (12, 13). It also bridges Skp1 and Hsp90 in yeast (14), revealing its function as a multidomain adaptor. Rar1, which contains two cysteine- and histidine-rich domains (CHORDs), has been suggested to facilitate the recruitment of R proteins to Hsp90 through interacting simultaneously with the Sgt1 CS domain and the Hsp90 N-terminal domain, supporting the adaptor function of Sgt1 (15).

In mammals, Sgt1 also plays an essential role in innate immune responses mediated by Nod-like receptor proteins such as Nod1 and Nalp3, the mammalian homologs of R proteins (16, 17). However, the roles for Chp-1 and melusin, the two mammalian CHORD-containing homologs of Rar1, in innate immunity have not yet been clarified. Chp-1 and melusin contain the additional C-terminal CS domain as well as the two conserved CHORDs (18). Both Chp-1 and melusin interact with Hsp90, suggesting their potential roles as Hsp90 cochap-

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[3] The abbreviations used are: R protein, resistance protein; TPR, tetratricopeptide repeat; CHORD, cysteine- and histidine-rich domain.

erones (5, 19). In addition, Chp-1 and melusin have been shown to interact with cochaperones PP5 (protein phosphatase 5) and Sgt1, respectively (5, 20). Melusin is a muscle-specific interactor of β-integrin (21) involved in the signaling pathway that senses and responds to mechanical stress in heart. Upon mechanical stress, melusin induces cardiac hypertrophy, while protecting heart from dilation and failure (22). On the other hand, Chp-1, also known as morgana, has been shown to inhibit Rho kinase II, preventing centrosome amplification (23). Like Chp-1, Sgt1 in *Drosophila* participates in centrosome maturation through stabilizing Polo, the upstream kinase of Rho kinase (24, 25). In addition, the Hsp90-Sgt1 chaperone complex is engaged in kinetochore formation in both yeast and mammals (26, 27). Therefore, both Chp-1 and Sgt1 seem to be involved in mitosis, although their genetic or physical connections *in vivo* remain elusive. In addition, it is not known whether melusin and Chp-1 carry out these functions as Hsp90 cochaperones or as Hsp90-independent regulators. Both Chp-1 and melusin have their own intrinsic chaperone activities (5, 8), but it has not been demonstrated whether they have Hsp90-independent functions related to this stand-alone chaperone activity.

In our previous study (20), we identified the interaction between Chp-1 and PP5 *in vivo*. PP5 is a Ser/Thr phosphatase involved in multiple cellular functions such as the MAPK signaling pathway, cell cycle progression, DNA damage repair, and regulation of transcription factors (28). The enzymatic activity of PP5 is autoinhibited by its TPR domain, which can be relieved by binding of the TPR domain to the Hsp90 C-terminal MEEVD sequence or fatty acids such as arachidonic acid (29, 30). Ppt1, the yeast homolog of PP5, dephosphorylates Hsp90 (31), whereas the known substrates of PP5 include the glucocorticoid receptor (32), Raf-1 (33), and tau (34, 35). In addition, both Ppt1 and PP5 dephosphorylate another Hsp90 cochaperone, Cdc37 (36).

In this study, we identified Chp-1 and PP5 as nucleotide-binding proteins. In addition to the previously known interaction between Chp-1 and PP5 (20), we newly identified the interaction between Chp-1 and Sgt1. Interestingly, these interactions were dramatically enhanced in the presence of ATP. Moreover, these nucleotide-dependent interactions were also conserved in melusin, revealing the similarities of the two CHORD-containing proteins. Furthermore, we show a regulatory role for Ca^{2+} in the melusin/Hsp90 and melusin/Sgt1 interactions. These results suggest that Hsp90 cochaperones retain the property of nucleotide-dependent interaction not only with Hsp90 but also with themselves, implying that each component of the Hsp90 chaperone machinery may participate in the dynamic assembly of multiple complexes depending on the cellular environment.

EXPERIMENTAL PROCEDURES

Construction of Expression Vectors—Plasmids containing cDNAs from mouse Hsp90a, mouse Chp-1, and human PP5 were described previously (20). Human Sgt1a was amplified from an I.M.A.G.E. expressed sequence tag clone (2985858) by PCR. To generate N-terminally His-tagged proteins, Hsp90a, PP5, and Sgt1 ORFs were cloned into pET28b vector. Mouse melusin, Chp-1, and Chp-1(H186A) ORFs were cloned into the pET15b vector. For the production of GST-tagged proteins, Hsp90, Sgt1, and melusin ORFs were cloned into the pGEX-4T-1 vector (GE Healthcare), and PP5 and Chp-1 ORFs were cloned into pGEX-3X.

Protein Expression and Purification—N-terminally GST-tagged proteins and N-terminally His-tagged proteins were expressed in *Escherichia coli* strain Rosetta gami2(DE3)pLysS. The bacteria were grown in LB medium at 37 °C and induced with 1 mM isopropyl 1-thio-β-D-galactopyranoside. Subsequently, the proteins were affinity-purified using glutathione-agarose resin (Novagen) for GST-tagged proteins and nickel-nitrilotriacetic acid affinity chromatography (GE Healthcare) for His-tagged proteins. His-tagged proteins were further purified using a Superdex 200 prep grade gel filtration column (GE Healthcare). Purified proteins were dialyzed against 20 mM Tris-HCl (pH 8.0) and 150 mM NaCl and stored at −70 °C.

ADP- and ATP-Agarose Binding Assay—The purified proteins were incubated with N^6-ADP (C_8-ADP)- or N^6-ATP-agarose resin (Sigma) at 4 °C in buffer A (20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 50 μM $ZnCl_2$, 1 mM DTT, and 5 mM $MgCl_2$) supplemented with 0.1% Nonidet P-40 and protease inhibitor mixture (Calbiochem). The incubated resin was washed with buffer B (20 mM Tris-HCl (pH 8.0) and 150 mM NaCl), and the bound proteins were eluted with 10 mM ADP or by boiling in SDS-PAGE sample buffer. The pulldown fractions were analyzed by Western blotting with anti-GST and anti-His antibodies (Santa Cruz Biotechnology).

Circular Dichroism Spectroscopy—The secondary structures of the proteins were analyzed using a JASCO J815 spectropolarimeter in the far-UV spectral region at room temperature. The spectra were recorded in buffer A with or without 1 mM ADP or ATP.

GST Pulldown Assay—The purified GST-tagged proteins were prebound to the resin by incubating the proteins with glutathione-agarose resin for 2 h at 4 °C in buffer A with 0.1% Nonidet P-40 and protease inhibitor mixture. The prebound resin was washed twice with buffer B. His-tagged proteins were then added to the prebound resin and incubated for 2 h at 4 °C in the presence or absence of 5 mM nucleotide. After washing the resin three times with buffer B, samples were analyzed by Western blotting with anti-GST and anti-His antibodies. To observe the effect of Ca^{2+} on the protein interactions, various concentrations of $CaCl_2$ was added to buffer A, and 1 mM $CaCl_2$ was added to buffer B used to wash the resin.

RESULTS

Hsp90 Cochaperones Chp-1 and PP5, but Not Sgt1, Bind to ADP and ATP—The crystal structure of the complex of the plant Hsp90 N-terminal, Sgt1 CS, and Rar1 CHORD-II domains shows that Rar1 CHORD-II directly interacts with the ADP bound to the nucleotide pocket of the Hsp90 N-terminal domain (15). In a recent proteomic analysis, Chp-1 was also shown to exhibit ADP-dependent interaction with Hsp90 (37). These results raised the possibility of the direct interaction of Chp-1 with nucleotides, independent of Hsp90. To test this, we examined the binding of Chp-1 and two other cochaperones, PP5 and Sgt1 (Fig. 1*A*), to ADP or ATP linked to agarose through the N^6-position of adenine. As shown in Fig. 1*B*, Chp-1

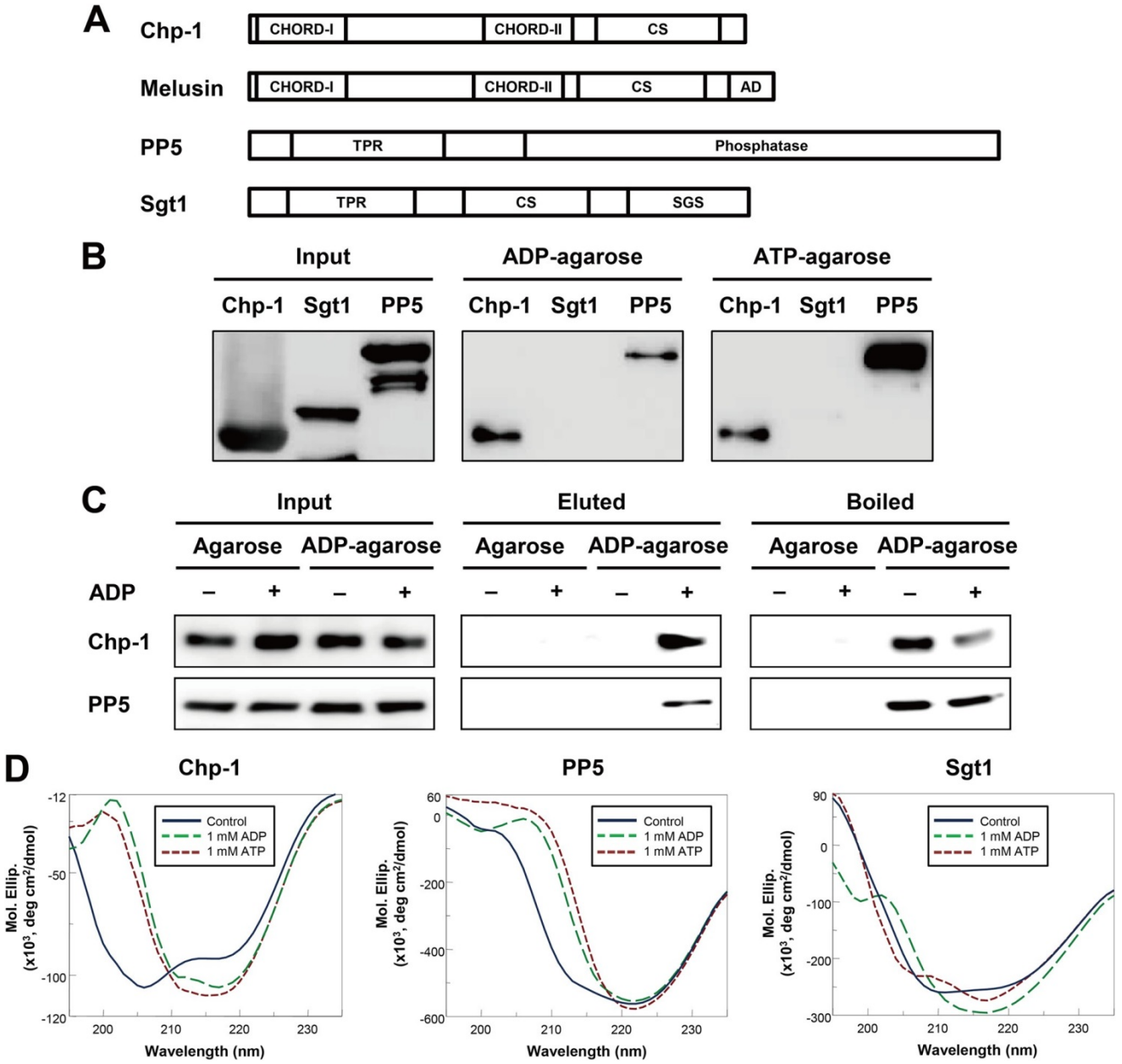

FIGURE 1. **Nucleotide binding property of Hsp90 cochaperones Chp-1 and PP5.** *A*, schematic diagram of the domain structures of Hsp90 cochaperones related to this work. *AD*, acidic domain. *B*, His-tagged mouse Chp-1, human PP5, and human Sgt1 proteins were incubated with N^6-ADP-agarose and N^6-ATP-agarose, and the bound proteins were eluted by boiling and detected by Western blotting. *C*, ADP elution of Chp-1 and PP5. Chp-1 or PP5 was incubated with control agarose or ADP-agarose and then eluted with 10 mM ADP to confirm specific binding. The remaining proteins bound to the agarose beads were detected after boiling. *D*, far-UV CD spectra of Chp-1, PP5, and Sgt1 in the absence or presence of 1 mM ADP or ATP. *Mol. Ellip.*, molar ellipticity; *deg*, degrees.

and PP5, but not Sgt1, bound to ADP- and ATP-agarose. Chp-1 and PP5 did not bind to the control agarose beads, and the proteins bound to ADP-agarose were eluted with ADP, confirming their specific binding to ADP (Fig. 1*C*). Furthermore, the changes in the CD spectra of Chp-1 and PP5 reflected the conformational changes in the proteins in the presence of ADP or ATP (Fig. 1*D*). In contrast, the CD spectra of Sgt1 showed little difference upon the addition of nucleotides (Fig. 1*D*), which is consistent with the previous experiment showing no interaction of Sgt1 with ADP- or ATP-agarose. These results suggest that not only Hsp90 but also Hsp90 cochaperones Chp-1 and PP5 possess the properties of nucleotide binding and subsequent conformational change. This might be a common regulatory mechanism shared by Hsp90 and its cochaperones Chp-1 and PP5, although these cochaperones have no ATPase activity.

ADP and ATP Enhance Interactions between Cochaperones—A previous study demonstrated the interaction between Chp-1 and the PP5 TPR domain *in vivo* (20), but its biological significance remains elusive. Other CHORD-containing proteins, plant Rar1 and mammalian melusin, have been shown to interact with Sgt1 (5, 11). Because ADP and ATP bind to Chp-1 and PP5, inducing their conformational changes, we asked whether ADP and ATP could affect the interactions between the cochaperones. Chp-1, PP5, and Sgt1 were purified as GST- and His-tagged proteins, and the interactions between the proteins

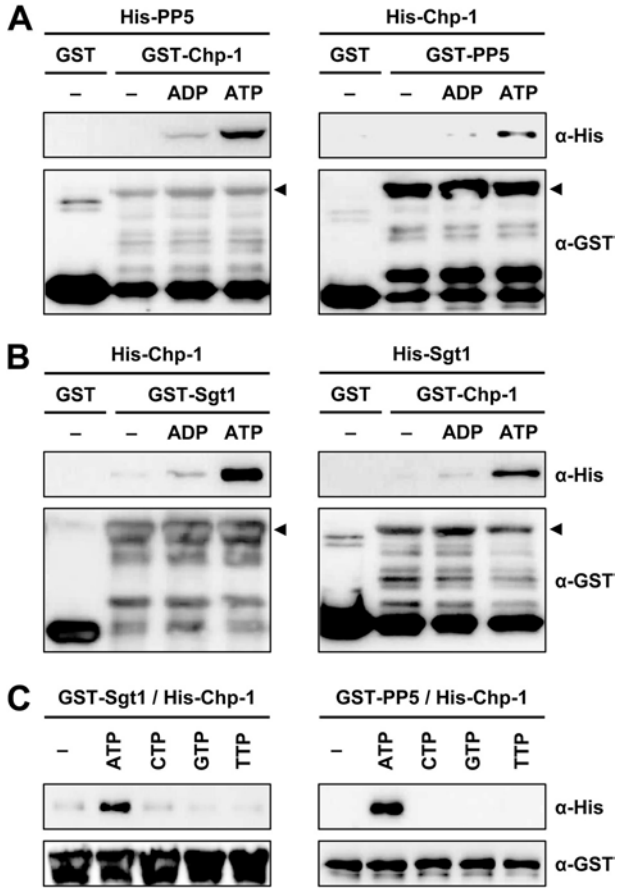

FIGURE 2. **Nucleotide-dependent interactions between Hsp90 cochaperones.** PP5/Chp-1 (*A*) and Chp-1/Sgt1 (*B*) interactions were examined by reciprocal GST pulldown experiments. Each GST-tagged cochaperone protein was incubated with another His-tagged cochaperone with or without 5 mM ADP or ATP. The full-length protein bands are indicated as *arrowheads*. Smaller bands are degradation products. *C*, the Chp-1/Sgt1 (*left*) and PP5/Chp-1 (*right*) interactions were analyzed by GST pulldown assays in the absence or presence of 5 mM ATP, CTP, GTP, or TTP. The pulldown fractions were analyzed by Western blotting.

were observed *in vitro* in the presence or absence of the nucleotides. Interestingly, the interaction between Chp-1 and PP5 increased in the presence of ADP and ATP in the reciprocal GST pulldown experiments (Fig. 2*A*). ATP enhanced the interaction more effectively compared with ADP. Furthermore, we newly identified the interaction between Chp-1 and Sgt1, which was also enhanced by ATP (Fig. 2*B*). Although ADP and ATP induced comparable levels of conformational changes in Chp-1 and PP5 in the CD spectra (Fig. 1, *B* and *C*), the interactions between the cochaperones showed preferential enhancement in the presence of ATP compared with ADP. Therefore, the)'-phosphate moiety of ATP might contribute to the enhancement of interactions between cochaperones. To further verify the nucleotide specificity in the interaction enhancement between the cochaperones, we tested the effects of all four NTPs in GST pulldown assays. Among the four NTPs, only ATP exclusively enhanced the interaction of Chp-1 with Sgt1 or PP5, suggesting the specific binding of the adenine base to Chp-1 (Fig. 2*C*).

Melusin Shows Nucleotide-dependent Interaction with PP5 and Sgt1—Melusin, a CHORD-containing Chp-1 homolog, has two N-terminal CHORDs, a central CS domain, and a C-terminal acidic domain (see Fig. 5*A*). The similarity of its structure to Chp-1 gave rise to the possibility that melusin might also have the property of nucleotide-dependent interactions with other cochaperones. Like GST-Chp-1, GST-melusin strongly interacted with PP5 and Sgt1 in the presence of ATP (Fig. 3). ADP also exerted a minor effect on enhancement of the interactions. Therefore, the ATP-dependent interactions with PP5 and Sgt1 are the conserved properties shared by Chp-1 and melusin.

Chp-1 and Melusin Can Form Homo- and Heterocomplexes in a Nucleotide-dependent Manner—Next, we tested the possibility of interaction between the CHORD-containing proteins. GST pulldown experiments showed interaction of His-Chp-1 with GST-melusin as well as GST-Chp-1 in the presence of ADP or ATP (Fig. 4*A*). As in the other interactions between cochaperones, ATP exerted a higher enhancement of the interaction compared with ADP. Moreover, His-melusin also exhibited a nucleotide-dependent interaction with GST-melusin (Fig. 4*B*). These results suggest that Chp-1 and melusin can form homo- or heterocomplexes in a nucleotide-dependent manner.

His-186 of Chp-1 Is Involved in the Specific Binding of ADP or ATP to Chp-1—The crystal structure of the complex of the Rar1 CHORD-II, Sgt1 CS, and Hsp90 N-terminal domains shows that the imidazole ring of His-188 of Rar1 CHORD-II interacts directly with the 13-phosphate of ADP that is bound to the Hsp90 N-terminal domain (15). Because this residue is conserved in Chp-1 as His-186 (Fig. 5*A*), we tested whether the H186A mutation could affect the nucleotide binding to Chp-1. Chp-1(H186A) had diminished affinity for ADP-agarose compared with wild-type Chp-1 (Fig. 5*B*). This result indicates that, like His-188 of Rar1, His-186 of Chp-1 contributes to the nucleotide binding property of Chp-1. Accordingly, the Chp-1(H186A) mutant showed a weaker nucleotide-dependent interaction with Hsp90 compared with wild-type Chp-1 (Fig. 5*C*). Unlike the interactions of Chp-1 with PP5 and Sgt1, which were stimulated mainly by ATP, the Chp-1/Hsp90 interaction was enhanced more by ADP than by ATP. Such ADP-dependent interactions of Hsp90 with Rar1 and Chp-1 have been demonstrated previously (15, 37).

The reduction in the ADP-agarose binding affinity of Chp-1(H186A) also suggests that His-186 is engaged in the binding to free ADP or ATP even in the absence of Hsp90. Therefore, we investigated whether the mutation of His-186 could also affect the nucleotide-dependent binding of Chp-1 to Sgt1. As shown in Fig. 5*D*, the nucleotide-dependent interaction with Sgt1 was greatly reduced in Chp-1(H186A) compared with wild-type Chp-1. These results suggest that conformational changes induced by ATP binding to His-186 might enhance the interaction of Chp-1 with Sgt1.

Ca^{2+} Further Enhances Nucleotide-dependent Melusin/Hsp90 and Melusin/Sgt1 Interactions—It is known that Ca^{2+} can bind to the C-terminal acidic domain of melusin, negatively regulating its interaction with the cytoplasmic domain of 13-integrin *in vitro* (21). Therefore, we examined the effect of Ca^{2+} on the nucleotide-dependent interactions of melusin with Hsp90, Sgt1, and PP5. Unlike the interaction between Chp-1 and Hsp90, ADP and ATP exerted similar levels of binding enhancement between melusin and Hsp90. The addition of

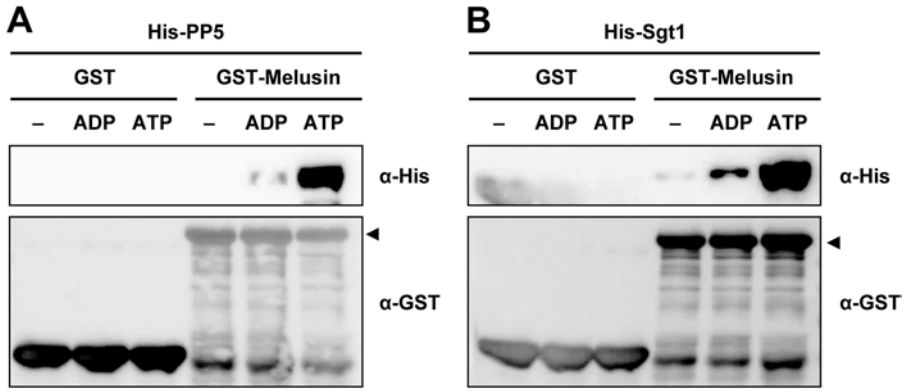

FIGURE 3. **Nucleotide-dependent interactions between melusin and other Hsp90 cochaperones.** GST-melusin was incubated with the His-tagged Hsp90 cochaperones PP5 (*A*) and Sgt1 (*B*) with or without 5 mM ADP or ATP. The pulldown fractions were analyzed by Western blotting.

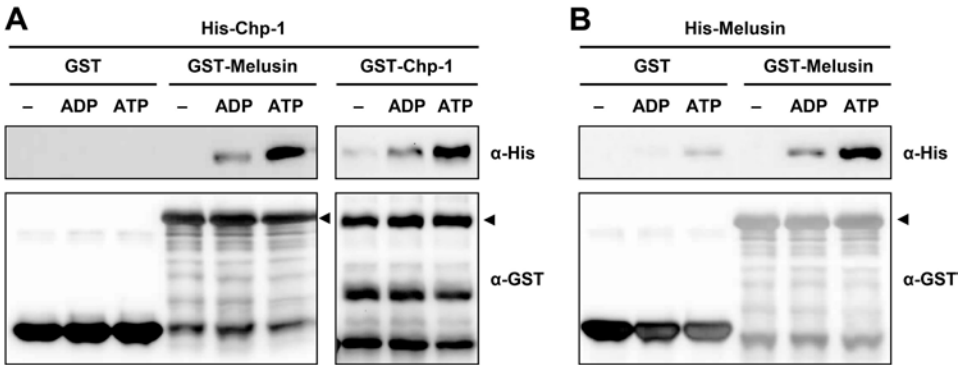

FIGURE 4. **Nucleotide-dependent homo- and heterocomplex formation by Chp-1 and melusin.** *A*, His-Chp-1 was incubated with GST, GST-melusin, or GST-Chp-1 with or without 5 mM ADP or ATP. *B*, His-melusin was incubated with GST or GST-melusin with or without 5 mM ADP or ATP. The pulldown fractions were analyzed by Western blotting.

Ca^{2+} further enhanced the nucleotide-dependent interaction between melusin and Hsp90, especially in the presence of ADP, resulting in a nucleotide preference similar to that of the Chp-1/Hsp90 interaction (Fig. 6*A*). This enhancement of the interaction was dependent on the concentration of Ca^{2+} to some extent (Fig. 6*D*). In contrast, Chp-1, which does not contain the C-terminal Ca^{2+}-binding acidic domain, showed no enhancement of the interaction upon the addition of increasing amounts of Ca^{2+} in the presence of ADP (Fig. 6*D*).

In addition, the ATP-dependent interaction between melusin and Sgt1 was further enhanced in the presence of Ca^{2+} in a concentration-dependent manner (Fig. 6, *B* and *D*). However, the addition of Ca^{2+} did not affect the interaction between melusin and PP5 (Fig. 6*C*). These results imply that melusin undergoes additional conformational change upon Ca^{2+} binding, which makes melusin more capable of interacting with Hsp90 and Sgt1.

DISCUSSION

Hsp90 cochaperones work as modulators of the Hsp90 chaperone machinery through dynamic association with the Hsp90 homodimer during its ATPase cycle. Various Hsp90 cochaperones are involved in multiple cellular functions, some of which are suggested to be Hsp90-independent (7).

In this study, we have demonstrated novel nucleotide binding properties of Hsp90 cochaperones Chp-1 and PP5. Interestingly, interactions of Chp-1 with other cochaperones such as PP5 and Sgt1 were greatly enhanced by ATP and, to a lesser extent, by ADP. Such nucleotide-dependent interactions with PP5 and Sgt1 were also conserved for melusin, another CHORD-containing protein in mammals. Furthermore, Chp-1 and melusin could form homo- and heterocomplexes by interacting in the same nucleotide-dependent manner. Therefore, conformational changes induced by ATP binding might play important roles in regulating the interactions among these Hsp90 cochaperones.

The structural determination of the complex between plant Rar1 CHORD-II and the Hsp90 N-terminal domain has revealed that Rar1 directly binds to ADP bound in the nucleotide pocket of Hsp90 (15). CHORD-I is also predicted to bind to Hsp90 through the same pattern of interaction, which allows binding of a single Rar1 molecule to both N-terminal binding sites of an Hsp90 dimer (15). His-188 in CHORD-II of Rar1, engaged in binding to the β-phosphate of ADP, is also conserved in Chp-1 and melusin (Fig. 5*A*). We demonstrated that His-186 of Chp-1, equivalent to His-188 of Rar1, plays an important role in the nucleotide-dependent binding of Chp-1 to Hsp90. For enhancement of the Chp-1/Hsp90 interaction, ADP played a more prominent role than ATP, consistent with a previous report (37). Such a nucleotide preference could possibly be related to the differences in the ADP- and ATP-bound conformations of Hsp90. A steric overlap between CHORD and the closed conformation of ATP-bound Hsp90 is observed when the complex of Rar1 CHORD-II and the Hsp90 N-terminal domain is superimposed on the structure of ATP-bound

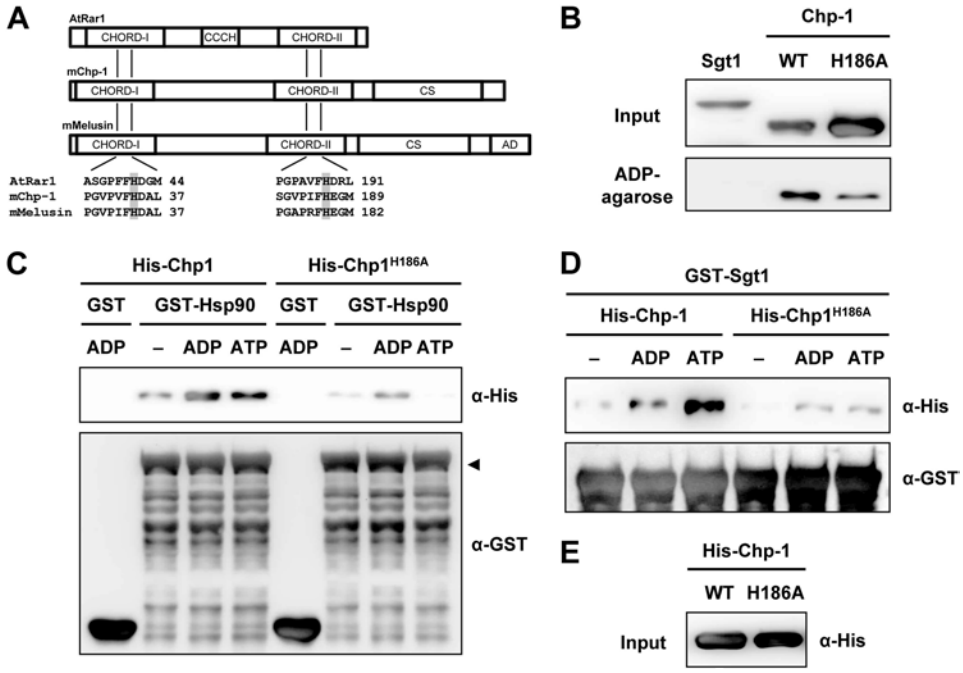

FIGURE 5. **Contribution of nucleotide binding property of Chp-1 to Chp-1/Hsp90 and Chp-1/Sgt1 interactions.** *A*, schematic diagram of the structures of the CHORD-containing proteins *Arabidopsis thaliana* (*At*) Rar1, mouse (*m*) Chp-1, and mouse melusin. The conserved His residues involved in nucleotide binding in *A. thaliana* Rar1 are *shaded*. *AD*, acidic domain. *B*, His-Sgt1, His-Chp-1, and His-Chp-1(H186A) were incubated with N^6-ADP-agarose, and the bound proteins were eluted and detected by Western blotting. His-Chp-1 and His-Chp-1(H186A) were incubated with GST-Hsp90 (*C*) or GST-Sgt1 (*D*) with or without 5 mM ADP or ATP. The pulldown fractions were analyzed by Western blotting. *E*, the amounts of His-Chp-1 and His-Chp-1(H186A) used for the GST pulldown experiments shown in *C* and *D* were analyzed by Western blotting.

full-length Hsp90 (15). Different preferences for different Hsp90 conformations are also known for other Hsp90 cochaperones, leading to sequential exchange of the cochaperones in the Hsp90 chaperone complex during progression of the Hsp90 cycle.

Remarkably, Chp-1(H186A) showed a reduced nucleotide-dependent interaction not only with Hsp90 but also with Sgt1. In the plant Hsp90-Rar1-Sgt1 ternary complex, the opposite faces of Rar1 CHORD-II bind simultaneously to Hsp90 and the Sgt1 CS domain (15). However, the residues involved in the CHORD-II/CS domain interaction are not conserved in Chp-1, melusin, and mammalian Sgt1. Therefore, it is not clear yet how Chp-1 or melusin interacts with Sgt1. However, if Chp-1 does not interact with both Hsp90 and Sgt1 through the same interacting surface where ADP or ATP acts as a bridge, the conformational changes in Chp-1 induced by nucleotide binding to His-186 might be responsible for the nucleotide-dependent interaction between Chp-1 and Sgt1. The fact that Chp-1(H186A) showed reduced binding affinity for ADP-agarose also supports the notion that His-186 is one of the residues involved in direct nucleotide binding even in the absence of Hsp90. Considering the results together, although the same nucleotide-binding sites in Chp-1 are involved in the regulation of Chp-1/Hsp90 and Chp-1/Sgt1 interactions, the mode of regulations might be quite different for these two interactions.

We have shown that Chp-1 and melusin share similar nucleotide dependence and preference for interactions with other cochaperones. On the other hand, unlike Chp-1, melusin did not show a clear preference of ADP in binding to Hsp90. Interestingly, we found that Ca^{2+} not only enhanced the melusin/Hsp90 interaction but also allowed melusin to acquire the ADP preference for Hsp90 binding. Ca^{2+} also enhanced the nucleotide-dependent melusin/Sgt1 interaction. This is opposite the interaction of melusin with β-integrin, which is diminished by Ca^{2+} (21). These results imply that nucleotides and Ca^{2+} act cooperatively in the regulation of melusin to be released from β-integrin and to bind Hsp90 or other cochaperones.

Several interactions between Hsp90 cochaperones have been shown to be crucial for the regulation of cellular functions. In yeast, the dimerization of Sgt1 has been demonstrated to be important for Sgt1/Skp1 binding and kinetochore assembly (38). In addition, the yeast Pih1/Tah1 cochaperone heterodimer plays a role in the recruitment of client proteins such as core ribonucleoproteins to Hsp90 (39). The interaction of plant Rar1 and Sgt1 in the Hsp90 chaperone complex is well known to be involved in plant resistance (9–11). The newly identified interactions between cochaperones might be part of the regulatory mechanisms to assemble the Hsp90 chaperone machinery, as in the case of the plant Rar1/Sgt1 interaction. The Chp-1/Sgt1 interaction can be expected to be a mammalian equivalent of the Rar1/Sgt1 interaction, although the role for Chp-1 in innate immunity needs to be clarified (16). The three CHORD-containing proteins Rar1, Chp-1, and melusin seem to interact with Sgt1, but, except for the Rar1/Sgt1 interaction, the physiological functions of these interactions are still elusive. In the case of PP5, some of its functions are mediated by its interaction with Hsp90 (32, 33, 36, 40), but it has not yet been clarified whether PP5 dephosphorylates all of its substrates as an Hsp90 cochaperone or as an Hsp90-independent phosphatase. Although the Hsp90-independent roles of the cochaperone/cochaperone interactions are largely unknown, we cannot rule out the possibility that these cochaperones could have their

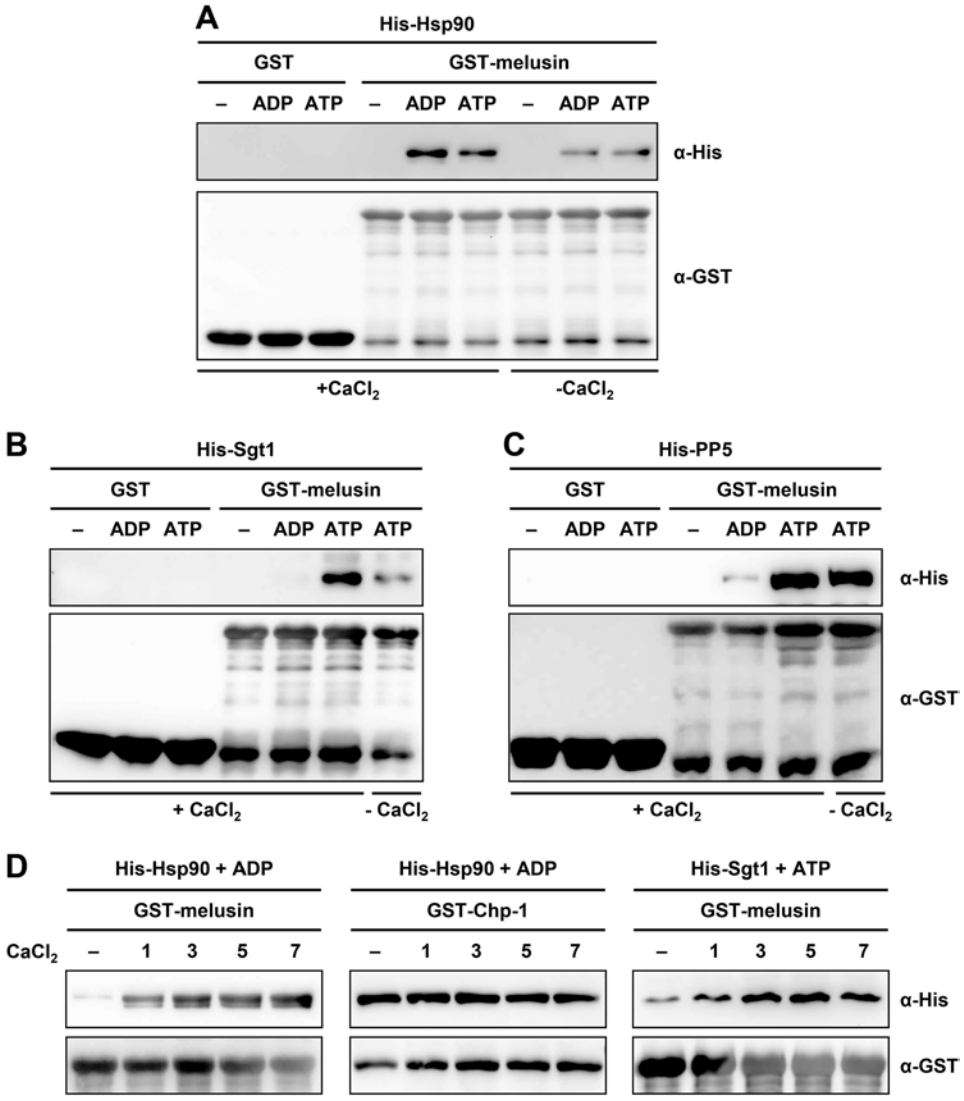

FIGURE 6. **Effect of Ca^{2+} on interaction of CHORD proteins with Hsp90 or Hsp90 cochaperones.** *A*, GST pulldown assay was performed with GST-melusin and His-Hsp90 with or without 1 mM Ca^{2+} in the presence of 5 mM ADP or ATP. GST-melusin was incubated with His-Sgt1 (*B*) or His-PP5 (*C*) with or without 3 mM Ca^{2+} in the presence of 5 mM ADP or ATP. The GST pulldown fractions were analyzed by Western blotting. *D*, GST-tagged melusin (*left*) or Chp-1 (*middle*) was incubated with His-tagged Hsp90 in the presence of 5 mM ADP, and GST-melusin was incubated with His-Sgt1 in the presence of 5 mM ATP (*right*) with increasing concentrations of Ca^{2+}. The GST pulldown fractions were analyzed by Western blotting.

own Hsp90-independent roles, which can be regulated by nucleotide-dependent interactions with other cochaperones demonstrated in our work. In fact, both Chp-1 and melusin possess their own chaperone activities (5, 8), implying their potential Hsp90-independent functions *in vivo*.

In this study, we have demonstrated interactions among cochaperones that are dependent mainly on ATP. Therefore, cellular ATP concentration, which can fluctuate depending on cellular energy status and the cell cycle (41), physiological conditions affecting mitochondrial function (42), and stresses such as metabolic or oxidative stress (43), could possibly influence the cochaperone/cochaperone interactions and the biological processes regulated by the cochaperones. In addition, cellular Ca^{2+} levels might play important roles in the regulation of melusin. Although the biological functions regulated by these interactions remain to be elucidated, this study will serve as a first step to investigate novel regulatory mechanisms for dynamic assembly of the Hsp90 chaperone machinery and to explore potential Hsp90-independent functions of these cochaperones.

REFERENCES

1. Prodromou, C., Panaretou, B., Chohan, S., Siligardi, G., O'Brien, R., Ladbury, J. E., Roe, S. M., Piper, P. W., and Pearl, L. H. (2000) The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. *EMBO J.* 19, 4383–4392
2. Wandinger, S. K., Richter, K., and Buchner, J. (2008) The Hsp90 chaperone machinery. *J. Biol. Chem.* 283, 18473–18477
3. Hahn, J. S. (2009) The Hsp90 chaperone machinery: from structure to drug development. *BMB Rep.* 42, 623–630
4. Bose, S., Weikl, T., Bügl, H., and Buchner, J. (1996) Chaperone function of Hsp90-associated proteins. *Science* 274, 1715–1717
5. Sbroggiò, M., Ferretti, R., Percivalle, E., Gutkowska, M., Zylicz, A., Michowski, W., Kuznicki, J., Accornero, F., Pacchioni, B., Lanfranchi, G., Hamm, J., Turco, E., Silengo, L., Tarone, G., and Brancaccio, M. (2008) The mammalian CHORD-containing protein melusin is a stress response protein interacting with Hsp90 and Sgt1. *FEBS Lett.* 582, 1788–1794
6. Chua, C.-S., Low, H., Goo, K.-S., and Sim, T. S. (2010) Characterization of

Plasmodium falciparum co-chaperone p23: its intrinsic chaperone activ- ity and interaction with Hsp90. *Cell Mol. Life Sci.* 67, 1675–1686
7. Picard, D. (2008) A stress protein interface of innate immunity. *EMBO Rep.* 9, 1193–1195
8. Michowski, W., Ferretti, R., Wisniewska, M. B., Ambrozkiewicz, M., Be- resewicz, M., Fusella, F., Skibinska-Kijek, A., Zablocka, B., Brancaccio, M., Tarone, G., and Kuznicki, J. (2010) Morgana/CHP-1 is a novel chaperone able to protect cells from stress. *Biochim. Biophys. Acta* 1803, 1043–1049
9. Takahashi, A., Casais, C., Ichimura, K., and Shirasu, K. (2003) HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11777–11782
10. Wang, Y., Gao, M., Li, Q., Wang, L., Wang, J., Jeon, J.-S., Qu, N., Zhang, Y., and He, Z. (2008) OsRAR1 and OsSGT1 physically interact and function in rice basal disease resistance. *Mol. Plant Microbe Interact.* 21, 294–303
11. Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K., and Schulze-Lefert, P. (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science* 295, 2073–2076
12. Bieri, S., Mauch, S., Shen, Q.-H., Peart, J., Devoto, A., Casais, C., Ceron, F., Schulze, S., Steinbi13, H.-H., Shirasu, K., and Schulze-Lefert, P. (2004) RAR1 positively controls steady state levels of barley MLA resistance pro- teins and enables sufficient MLA6 accumulation for effective resistance. *Plant Cell* 16, 3480–3495
13. Kadota, Y., Amigues, B., Ducassou, L., Madaoui, H., Ochsenbein, F., Guerois, R., and Shirasu, K. (2008) Structural and functional analysis of SGT1-HSP90 core complex required for innate immunity in plants. *EMBO Rep.* 9, 1209–1215
14. Catlett, M. G., and Kaplan, K. B. (2006) Sgt1p is a unique co-chaperone that acts as a client adaptor to link Hsp90 to Skp1p. *J. Biol. Chem.* 281, 33739–33748
15. Zhang, M., Kadota, Y., Prodromou, C., Shirasu, K., and Pearl, L. H. (2010) Structural basis for assembly of Hsp90-Sgt1-CHORD protein complexes: implications for chaperoning of NLR innate immunity receptors. *Mol. Cell* 39, 269–281
16. da Silva Correia, J., Miranda, Y., Leonard, N., and Ulevitch, R. (2007) SGT1 is essential for Nod1 activation. *Proc. Natl. Acad. Sci. U.S.A.* 104, 6764–6769
17. Mayor, A., Martinon, F., De Smedt, T., Pétrilli, V., and Tschopp, J. (2007) A crucial function of SGT1 and HSP90 in inflammasome activity links mammalian and plant innate immune responses. *Nat. Immunol.* 8, 497–503
18. Brancaccio, M., Menini, N., Bongioanni, D., Ferretti, R., De Acetis, M., Silengo, L., and Tarone, G. (2003) Chp-1 and melusin, two CHORD-con- taining proteins in vertebrates. *FEBS Lett.* 551, 47–52
19. Wu, J., Luo, S., Jiang, H., and Li, H. (2005) Mammalian CHORD-contain- ing protein 1 is a novel heat shock protein 90-interacting protein. *FEBS Lett.* 579, 421–426
20. Hahn, J. S. (2005) Regulation of Nod1 by Hsp90 chaperone complex. *FEBS Lett.* 579, 4513–4519
21. Brancaccio, M., Guazzone, S., Menini, N., Sibona, E., Hirsch, E., De An- drea, M., Rocchi, M., Altruda, F., Tarone, G., and Silengo, L. (1999) Me- lusin is a new muscle-specific interactor for 131 integrin cytoplasmic do- main. *J. Biol. Chem.* 274, 29282–29288
22. Brancaccio, M., Fratta, L., Notte, A., Hirsch, E., Poulet, R., Guazzone, S., De Acetis, M., Vecchione, C., Marino, G., Altruda, F., Silengo, L., Tarone, G., and Lembo, G. (2003) Melusin, a muscle-specific integrin 131-interact- ing protein, is required to prevent cardiac failure in response to chronic pressure overload. *Nat. Med.* 9, 68–75
23. Ferretti, R., Palumbo, V., Di Savino, A., Velasco, S., Sbroggiò, M., Sporto- letti, P., Micale, L., Turco, E., Silengo, L., Palumbo, G., Hirsch, E., Teruya- Feldstein, J., Bonaccorsi, S., Pandolfi, P. P., Gatti, M., Tarone, G., and Brancaccio, M. (2010) *morgana/chp-1*, a ROCK inhibitor involved in cen- trosome duplication and tumorigenesis. *Dev. Cell* 18, 486–495
24. Lowery, D. M., Clauser, K. R., Hjerrild, M., Lim, D., Alexander, J., Kishi, K., Ong, S. E., Gammeltoft, S., Carr, S. A., and Yaffe, M. B. (2007) Proteomic

screen defines the Polo-box domain interactome and identifies Rock2 as a Plk1 substrate. *EMBO J.* 26, 2262–2273
25. Martins, T., Maia, A. F., Steffensen, S., and Sunkel, C. E. (2009) Sgt1, a co-chaperone of Hsp90, stabilizes Polo and is required for centrosome organization. *EMBO J.* 28, 234–247
26. Rodrigo-Brenni, M. C., Thomas, S., Bouck, D. C., and Kaplan, K. B. (2004) Sgt1p and Skp1p modulate the assembly and turnover of CBF3 complexes required for proper kinetochore function. *Mol. Biol. Cell* 15, 3366–3378
27. Davies, A. E., and Kaplan, K. B. (2010) Hsp90-Sgt1 and Skp1 target human Mis12 complexes to ensure efficient formation of kinetochore-microtu- bule binding sites. *J. Cell Biol.* 189, 261–274
28. Hinds, T. D., Jr., and Sánchez, E. R. (2008) Protein phosphatase 5. *Int. J. Biochem. Cell Biol.* 40, 2358–2362
29. Chen, M. X., and Cohen, P. T. W. (1997) Activation of protein phospha- tase 5 by limited proteolysis or the binding of polyunsaturated fatty acids to the TPR domain. *FEBS Lett.* 400, 136–140
30. Ramsey, A. J., and Chinkers, M. (2002) Identification of potential physio- logical activators of protein phosphatase 5. *Biochemistry* 41, 5625–5632
31. Wandinger, S. K., Suhre, M. H., Wegele, H., and Buchner, J. (2006) The phosphatase Ppt1 is a dedicated regulator of the molecular chaperone Hsp90. *EMBO J.* 25, 367–376
32. Wang, Z., Chen, W., Kono, E., Dang, T., and Garabedian, M. J. (2007) Modulation of glucocorticoid receptor phosphorylation and transcrip- tional activity by a C-terminal-associated protein phosphatase. *Mol. En- docrinol.* 21, 625–634
33. von Kriegsheim, A., Pitt, A., Grindlay, G. J., Kolch, W., and Dhillon, A. S. (2006) Regulation of the Raf-MEK-ERK pathway by protein phosphatase 5. *Nat. Cell Biol.* 8, 1011–1016
34. Gong, C.-X., Liu, F., Wu, G., Rossie, S., Wegiel, J., Li, L., Grundke-Iqbal, I., and Iqbal, K. (2004) Dephosphorylation of microtubule-associated pro- tein tau by protein phosphatase 5. *J. Neurochem.* 88, 298–310
35. Liu, F., Iqbal, K., Grundke-Iqbal, I., Rossie, S., and Gong, C.-X. (2005) Dephosphorylation of tau by protein phosphatase 5. *J. Biol. Chem.* 280, 1790–1796
36. Vaughan, C. K., Mollapour, M., Smith, J. R., Truman, A., Hu, B., Good, V. M., Panaretou, B., Neckers, L., Clarke, P. A., Workman, P., Piper, P. W., Prodromou, C., and Pearl, L. H. (2008) Hsp90-dependent activation of protein kinases is regulated by chaperone-targeted dephosphorylation of Cdc37. *Mol. Cell* 31, 886–895
37. Gano, J. J., and Simon, J. A. (2010) A proteomic investigation of ligand-de- pendent HSP90 complexes reveals CHORDC1 as a novel ADP-dependent HSP90-interacting protein. *Mol. Cell. Proteomics* 9, 255–270
38. Bansal, P. K., Nourse, A., Abdulle, R., and Kitagawa, K. (2009) Sgt1 dimerization is required for yeast kinetochore assembly. *J. Biol. Chem.* 284, 3586–3592
39. Eckert, K., Saliou, J. M., Monlezun, L., Vigouroux, A., Atmane, N., Caillat, C., Quevillon-Chéruel, S., Madiona, K., Nicaise, M., Lazereg, S., Van Dorsselaer, A., Sanglier-Cianférani, S., Meyer, P., and Moréra, S. (2010) The Pih1-Tah1 cochaperone complex inhibits Hsp90 molecular chaper- one ATPase activity. *J. Biol. Chem.* 285, 31304–31312
40. Conde, R., Xavier, J., McLoughlin, C., Chinkers, M., and Ovsenek, N. (2005) Protein phosphatase 5 is a negative modulator of heat shock factor 1. *J. Biol. Chem.* 280, 28989–28996
41. Marcussen, M., and Larsen, P. J. (1996) Cell cycle-dependent regulation of cellular ATP concentration, and depolymerization of the interphase mi- crotubular network induced by elevated cellular ATP concentration in whole fibroblasts. *Cell Motil. Cytoskeleton* 35, 94–99
42. Leist, M., Single, B., Castoldi, A. F., Kühnle, S., and Nicotera, P. (1997) Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. *J. Exp. Med.* 185, 1481–1486
43. Tretter, L., Chinopoulos, C., and Adam-Vizi, V. (1997) Enhanced depo- larization-evoked calcium signal and reduced [ATP]/[ADP] ratio are un- related events induced by oxidative stress in synaptosomes. *J. Neurochem.* 69, 2529–2537