Interaction between the Ga Subunit of Heterotrimeric G12 Protein and Hsp90 Is Required for Ga12 Signaling*

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The Ga subunit of G12 protein, one of the heterotrimeric G proteins, regulates diverse and complex cellular responses by transducing signals from the cell surface, presumably involving more than one downstream effector. Yeast two-hybrid screening of a human testis cDNA library identified a large fragment of Hsp90 as a protein that interacted with Gα12. The interaction between Gα12 and Hsp90 was further substantiated by a co-immunoprecipitation technique. We have determined that Hsp90 is not required for the interaction of Gα12 with its binding partners, p115 RhoGEF and the Gβ subunit. Importantly, Hsp90 is required for Gα12-induced serum response element activation, cytoskeletal changes, and mitogenic response. Closely related to Gα12, the Gα13 subunit did not interact with Hsp90 and did not require functional Hsp90 for serum response element activation. Thus, our results identify a novel signaling module of Gα12 and Hsp90.

Gα12 protein, one of the heterotrimeric G proteins, regulates diverse and complex cellular responses by transducing signals from the cell surface, presumably involving more than one signaling pathway. Gα12 regulates Na+/H+ exchange activity (1), regulates the ERK (2, 3) and JNK pathways (3, 4), and promotes assembly of actin stress fibers (5). It also induces mitogenesis and neoplastic transformation (2, 6) and apoptosis (7, 8). Recently, the guanine nucleotide exchange factor for RhoA, p115 RhoGEF, was shown to interact with and act as a GTPase-activating protein for Gα12 (9). Another Gα12-interacting protein is cadherin, a protein that is involved in cell-cell adhesion (10).

Many cellular events depend on the regulated protein-protein interaction. Discovering novel protein interaction facilitates the understanding of the molecular mechanisms underlying biological processes. The yeast two-hybrid system has been widely used for the identification of protein interaction (11). Here, using the yeast two-hybrid approach, we have determined that Gα12 interacts with Hsp90 (heat shock protein of 90 kDa), a molecular chaperone that interacts with multiple signal-transducing molecules and is often essential to a variety of signal transduction pathways (12).

Hsp90 is a highly conserved protein that is ubiquitously expressed and is required for viability of eukaryotes (for review, see Ref. 13). Hsp90 can act as a molecular chaperone to promote refolding of denatured protein, to hold denatured proteins in a folding-compotent state for other chaperones, and to prevent protein unfolding and aggregation (12). Hsp90 modulates protein structure, thus increasing the half-life of proteins and facilitating their interactions. A remarkably large subset of known Hsp90 substrates are signaling molecules (12). Geldanamycin is a specific inhibitor of Hsp90 that interacts with its ATP-binding site, which is important in regulating Hsp90 functions (14). Geldanamycin-bound Hsp90 cannot form heterocomplexes with proteins that require Hsp90. Therefore, geldanamycin has been successfully used both to identify proteins that might interact with Hsp90 and to determine the physiological significance of such interaction (12). Here, the interaction between Gα12 and Hsp90 found using the yeast two-hybrid system was further substantiated by a co-immunoprecipitation technique. We have determined that Hsp90 is not required for the interaction of Gα12 with its binding partners, p115 RhoGEF and the Gβ subunit. Importantly, Hsp90 is required for Gα12-induced SRE activation, cytoskeletal changes, and mitogenic response.

EXPERIMENTAL PROCEDURES

Materials—Anti-Myc, anti-Gα12, anti-Gp, anti-common, and anti-Raf-1 antibodies were purchased from Santa Cruz Biotechnology. Anti-HA antibody was purchased from BabCo. Anti-Hsp90 antibody was purchased from BD Transduction Laboratories. Glutathione-Sepharose 4B was purchased from Amersham Pharmacia Biotech. Protein A-agarose was purchased from Life Technologies, Inc. The SRE-L-luciferase reporter plasmid was kindly provided by Paul Sternweis. HA-tagged Gα12 and Gα5 were kindly provided by Silvio Gutkind. Sources of HA-tagged Gαq and Raf-1 are described elsewhere (3, 15). Myc-tagged Gq2 and Gα1 subunits were kindly provided by Janet Robishaw.

Yeast Two-hybrid Screening—The yeast two-hybrid MATCHMAKER LexA system (CLONTECH) was employed to detect specific protein-protein interaction (16). The Gα12 two-hybrid bait was constructed by subcloning the cDNA for human mutationally activated Gα12 Q225L into the pLexA polylinker region. A human testis library in the vector pB42AD was screened; all colonies were assayed for lacZ and LEU2 reporter gene activities; double-positive clones were picked; and plasmids were isolated and sequenced.

Cell Culture and Transfections—Transient transfections of NIH3T3 and COS-7 cells were performed using LipofectAMINE 2000 reagent (Life Technologies, Inc.) according to the manufacturer’s instructions. Stable transfection of NIH3T3 cells with constitutively activated human Gα12 was performed as described earlier (2).

Immunoprecipitation and Western Blotting—An immunoprecipitation assay was performed as described (17). Briefly, HA-tagged wild-type Gα12 was transiently expressed in COS-7 cells for 48 h. Cells were lysed in 50 mM Hepes (pH 7.5), 1 mM dithiothreitol, 50 mM NaCl, 5 mM MgCl2, and 1% Lubrol. Where indicated, AlF4 (10 mM NaF and 30 μM AlCl3) was included. Lysates were normalized for protein concentration, and proteins were immunoprecipitated with anti-HA antibody and protein A-agarose for 16 h at 4°C. Immunoprecipitates were washed, separated by 8–10% SDS-PAGE, and immunoblotted with appropriate antibodies.

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‡ The abbreviations used are: ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; SRE, serum response element; HA, hemagglutinin.
**RESULTS**

**Ga12 Interacts with Hsp90**—To further delineate components of signaling pathways that are important for apparently diverse cellular functions, we used yeast two-hybrid screening of a human testis cDNA library (3.5 × 10^6 clones) to search for proteins interacting with constitutively activated Ga12, Q229L. Using full-length Ga12 as "bait," the screening yielded six positive clones, one of which was a fragment (amino acids 378–732) of the molecule chaperone Hsp90. The interaction between Ga12 and the C-terminal domain of Hsp90 was specific since neither the Ga13 nor Hsp90 clones alone could activate transcription of LEU2 and lacZ in yeast (data not shown).

To determine whether Ga12 can interact with endogenous Hsp90, HA-tagged Ga12 was immunoprecipitated from COS-7 cells, and immunoprecipitates were probed for the presence of Hsp90 (Fig. 1). In cells transfected with HA-tagged Ga12 antibody against HA specifically immunoprecipitated endogenous Hsp90, whereas in vector-transfected cells, anti-HA antibody did not precipitate Hsp90 (Fig. 1). We next examined whether other Gα subunits could also interact with Hsp90. For this purpose, we immunoprecipitated the HA-tagged constructs of Ga13 or Ga9, transiently expressed in COS-7 cells. Our results showed that Hsp90 displayed very little binding to Ga13 and did not bind to Ga9 (Fig. 1). Importantly, the expression of both Ga13 and Ga9 was much higher than that of Ga12 as determined by Western blotting of total cell lysates with anti-HA antibody (Fig. 1). These data suggest that Ga12 exists in a specific complex with endogenous Hsp90 under physiological, non-stress conditions.

**Hsp90 Is Constitutively Bound to Ga12 and Is Not Required for the Interaction of Ga12 with Its Binding Partners, p115RhoGEF and the Gβγ Subunit**—The α subunits of heterotrimeric G proteins are molecular switches and clocks, and these functions are impaired by conformational changes that result from binding and hydrolysis of GTP (18, 19). Ga subunits are inactive in a GDP-bound state because of reduced affinity for downstream effectors and enhanced affinity for Gβγ subunits. Binding of Gβγ subunits to GDP-bound Ga occupies sites for interaction with downstream effectors. In contrast, GTP-bound Ga subunits have increased affinity for the effectors and decreased affinity for Gβγ subunits. To understand the physiological significance of Ga12–Hsp90 interaction, it was essential to learn if Ga12 binding to Hsp90 is dependent on the activation state of Ga12. To determine whether Hsp90 interaction depends on the activation state of Ga12, we performed co-immunoprecipitation assays in the absence and presence of AlF4, an activator of the Ga subunit that promotes a conformation similar to that of the transition state for GTP hydrolysis (20). The data showed that Hsp90 interacted with Ga12 in both the absence and presence of AlF4 (Fig. 2A), suggesting that Ga12 is constitutively bound to Hsp90 in both conformationally active and inactive states.

Recently, the guanine nucleotide exchange factor of Rho, p115RhoGEF, was shown to interact with a conformationally active form of Ga12 (21). Therefore, we next examined whether Hsp90 binding to Ga12 can be influenced by p115RhoGEF, another Ga12-binding partner. In these experiments, cells were transfected with HA-tagged Ga12 and Myc-tagged p115RhoGEF. The data showed that Ga12 interacted with p115RhoGEF only in the presence of AlF4 (Fig. 2A); this is consistent with previous observations (21). Importantly, the amount of Hsp90 bound to Ga12 was not changed by the p115RhoGEF binding (Fig. 2A), suggesting that Hsp90 and p115RhoGEF may have non-overlapping binding sites on Ga12.

To determine whether inhibition of Hsp90 binding can affect the Ga12 interaction with p115RhoGEF, we performed co-immunoprecipitation assays in the presence of geldanamycin, an inhibitor that disrupts Hsp90 interactions with target proteins (12). Cells transfected with HA-tagged Ga12 and Myc-tagged p115RhoGEF were pretreated with 1 μg/ml geldanamycin for 1 h, lysed, and immunoprecipitated with anti-HA antibody. Our data showed that pretreatment of the cells with geldanamycin disrupted the interaction of Ga12 with Hsp90; however, the interaction of p115RhoGEF with Ga12 was not affected (Fig. 2B). These data suggest that Hsp90 binding is not required for the interaction of Ga12 with p115RhoGEF, which further supports the notion that Hsp90 and p115RhoGEF may have non-overlapping binding sites on Ga12.

As GDP-bound Ga subunits have higher affinity for Gβγ subunits, we examined whether Hsp90 binding to Ga12 could be influenced by Gβγ binding. In these experiments, cells were transfected with HA-tagged Ga12 and immunoprecipitated with anti-HA antibody in the presence of either AlF4 or geldanamycin (Fig. 2, C and D). Immunoprecipitation of GDP-bound Ga12 showed the complex formation between Ga12 and the endogenous Gβ subunit (Fig. 2C). As expected, immunoprecipitation of Ga12 in the presence of AlF4 resulted in the dissociation of the Gβ subunit from the complex (Fig. 2C). Consistently, Hsp90 was bound to Ga12 in both the presence and absence of AlF4 (Fig. 2C). When Ga12 was precipitated from the cells pretreated with 1 μg/ml geldanamycin, the complex between endogenous Hsp90 and Ga12 was disrupted,
from the cytoplasmic fraction of rat liver (22) and because the endogenous G\(\alpha_{12}\) subunit could form a complex with exogenous HA-tagged G\(\alpha_{12}\), it was possible that Hsp90 detected in the immunocomplex formed after the interaction with anti-HA antibody could be a result of the Hsp90 interaction with the G\(\beta\) subunit. To test this possibility, Myc-tagged G\(\gamma\) and G\(\delta\) subunits were cotransfected into COS-7 cells. Immunoprecipitation with anti-Myc antibody showed that the G\(\beta\) subunit formed a complex with the G\(\gamma\) subunit (Fig. 2E). However, Hsp90 was not detected in this complex, suggesting that it is likely that Hsp90 interacts with the G\(\alpha_{12}\) subunit, but not with G\(\beta\gamma\) subunits (Fig. 2E). Together, these data (i) suggest that Hsp90 binding is not required for the interaction of G\(\alpha_{12}\) with the endogenous G\(\beta\) subunit and (ii) further support the notion that Hsp90 and other G\(\alpha_{12}\)-binding partners may have non-overlapping binding sites on G\(\alpha_{12}\).

Hsp90 Is Required for G\(\alpha_{12}\)-induced SRE Activation, Cytoskeletal Changes, and Mitogenic Response—As our data clearly showed that geldanamycin disrupted the interaction between G\(\alpha_{12}\) and Hsp90, we used geldanamycin to determine the functional significance of G\(\alpha_{12}\)-Hsp90 interaction. Mutational activation of G\(\alpha_{12}\) stimulates proliferation of NIH3T3 fibroblasts (2, 6). To analyze the role of Hsp90 in G\(\alpha_{12}\)-dependent cell growth rate, NIH3T3 cells stably transfected with constitutively activated G\(\alpha_{12}\) Q229L were plated in medium containing 0.5% calf serum, and cells were counted at 24 and 48 h after plating. The overexpression of G\(\alpha_{12}\) Q229L was 2-fold more compared with endogenously expressed G\(\alpha_{12}\) as determined by Western blot analysis (data not shown). In the presence of 0.5% calf serum, G\(\alpha_{12}\) Q229L-expressing cells continued to proliferate for an additional 48 h, whereas vector only-expressing cells did not proliferate at all (Fig. 3A), suggesting that the observed cell growth is exclusively dependent on the G\(\alpha_{12}\) function. Treatment of the cells with geldanamycin did not affect cell growth parameters of the vector-transfected cells; however, it significantly inhibited G\(\alpha_{12}\) Q229L-induced cell growth (Fig. 3A).

Another important cellular function of G\(\alpha_{12}\) is its ability to induce actin stress fiber formation. Therefore, we next examined whether geldanamycin can affect the G\(\alpha_{12}\) Q229L-mediated actin cytoskeleton reorganization. NIH3T3 cells expressing activated G\(\alpha_{12}\) or the pCDNA3 vector were seeded onto coverslips and starved for 24 h. Polymerized actin was visualized by staining of the fixed cells with rhodamine-conjugated phallolidin. Under these conditions, vector-transfected cells displayed staining of polymerized cortical actin; however, actin stress fibers were not detected (Fig. 3B). G\(\alpha_{12}\) Q229L-expressing cells clearly displayed pronounced actin stress fibers formation, consistent with previously reported observations (5). Pretreatment of the cells with 1 \(\mu\)g/ml geldanamycin for 6 h did not affect the actin cytoskeleton in the vector-expressing cells, but abolished actin stress fibers in G\(\alpha_{12}\)-expressing cells (Fig. 3B).

The G\(\alpha_{12}\) subunit regulates gene expression by transcriptional activation of distinct transcriptional control elements such as the SRE (23). We examined the role of Hsp90 in the regulation of SRE-dependent expression by G\(\alpha_{12}\). In SRE-mediated transcription of a luciferase reporter gene, an altered c-fos SRE, SRE.L, was placed in front of the luciferase gene (24). SRE.L binds only to the transcriptional serum response factor, and not to the tertiary complex factor. Expression of constitutively activated mutants of G\(\alpha_{12}\), G\(\alpha_{13}\), and Rho induced pronounced SRE activation (Fig. 3C), consistent with previously reported data (23, 24). Treatment of the cells with geldanamycin significantly inhibited G\(\alpha_{12}\)-induced SRE activation; however, it did not affect the SRE activation induced by G\(\alpha_{13}\) or by Rho proteins (Fig. 3C), suggesting that Hsp90 is specifically involved in the G\(\alpha_{12}\) function.
Geldanamycin does not decrease the G\(_{12}\)/H9251 presence of stress fiber formation by geldanamycin. NIH3T3 cells stably transfected with the pcDNA3 vector or G\(_{12}\), Q229L (50,000 cells/well, 24-well plates) were grown in 0.5% calf serum in the absence or presence of 1 \(\mu\)g/ml geldanamycin. At the indicated time points, cells were harvested and counted using a hemocytometer. The data shown are from three experiments performed in triplicates. ■, untreated vector-transfected cells; ●, untreated G\(_{12}\), Q229L-transfected cells; □, geldanamycin-treated vector-transfected cells; ▲, geldanamycin-treated G\(_{12}\), Q229L-transfected cells. Error bars are S.D. B, inhibition of G\(_{12}\)-dependent actin stress fiber formation by geldanamycin. NIH3T3 cells stably transfected with the pcDNA3 vector or G\(_{12}\), Q229L were seeded onto gelatin-coated coverslips and serum-starved for 24 h, and 1 \(\mu\)g/ml geldanamycin was added for an additional 6 h. Cells were fixed and stained with rhodamine-conjugated phalloidin. Ten random microscopic fields (~600 cells) were analyzed. Three independent experiments gave similar results. C, inhibition of G\(_{12}\)-mediated SRE gene transcription by geldanamycin. NIH3T3 cells were cotransfected with 50 ng of SRE-L-luciferase reporter gene, 50 ng of lacZ, and 100 ng of the indicated G protein. Cells were serum-starved overnight, and 1 \(\mu\)g/ml geldanamycin was added for an additional 8 h. Luciferase activity was measured in the cell extracts and normalized to \(\beta\)-galactosidase activity and is expressed as fold stimulation from the basal level. Data represent means ± S.E from one of at least three experiments performed in triplicates. AU, arbitrary units. D, geldanamycin does not decrease the G\(_{12}\) expression level. NIH3T3 cells (60-mm dishes) were transfected with 1 \(\mu\)g of HA-tagged G\(_{12}\) or Raf-1. Forty-eight hours after transfection, cells were treated with 1 \(\mu\)g/ml geldanamycin for the indicated periods of time. Thereafter, cells were collected for Western blotting with anti-HA or anti-Raf-1 antibody.

Geldanamycin disruption of the Hsp90 interaction with its client proteins, including Raf-1, results in protein destabilization and degradation (25). Therefore, we addressed the possibility that treatment with geldanamycin could result in G\(_{12}\) degradation. As shown in Fig. 3D, treatment of NIH3T3 cells with 1 \(\mu\)g geldanamycin for 1–12 h did not result in a measurable decrease in G\(_{12}\). In contrast, after a 12-h treatment, geldanamycin significantly reduced the amount of Raf-1, consistent with previously published data (25).

Hsp90 Is Required for Thrombin-mediated Actin Stress Fiber Formation in Human Endothelial Cells—It was recently demonstrated that thrombin receptors induce actin stress fiber formation via G\(_{12}\) protein (26, 27). Using G\(_{a}\) minigenes expressing C-terminal peptides that can serve as specific inhibitors of G protein signal transduction events, we have recently shown that, in endothelial cells, thrombin mediates actin stress fiber formation via G\(_{12}\) and G\(_{13}\) proteins (28). Therefore, we examined the role of Hsp90 in thrombin-induced actin stress fiber formation in human microvascular endothelial cells. These cells were treated with geldanamycin for 1 or 4 h and then challenged with thrombin for 10 min. Thereafter, the cells were fixed and stained for F-actin with rhodamine-conjugated phalloidin. Thrombin rapidly induced stress fiber formation and cell rounding, the phenotype that is consistent with previous reports (27). Geldanamycin did not affect the actin cytoskeleton in non-stimulated cells; however, it blocked the thrombin-induced stress fiber formation (Fig. 4).

**DISCUSSION**

This study describes for the first time that the G\(_{a}\) subunit of heterotrimeric G\(_{12}\) protein specifically interacts with Hsp90. This interaction does not depend on the conformational state of the G\(_{a}\) subunit and is required for the G\(_{12}\) functions.

Based on amino acid sequence similarities, G\(_{12}\) and G\(_{13}\) proteins belong to a distinct class of G\(_{a}\) subunits, separate
from the Goα, Gαq, and Gα13 classes, because they are <45% homologous to other Gα subunits (29). Although Gα12 and Gα13 share only 67% sequence identity, they were originally combined in one group of Gα subunits (29). Indeed, Gα12 and Gα13 regulate similar intracellular and cellular responses, including neoplastic transformation, apoptosis, Na+/H+ exchange, and cytoskeletal reorganization. Involvement of the downstream small GTPases Ras, Rho, Rac, and Cdc42 has been often reported. Regulatory molecules such as p115RhoGEF and Ras GTPase-activating protein-1 have been shown to directly interact with Gα12 and Gα13 proteins (9, 21, 30). Most recent studies show that Gα12 proteins can also interact with and regulate the function of the ERM (ezrin/radixin/moesin) family of proteins (17). ERM proteins function as cross-linkers between the actin cytoskeleton and the plasma membrane and regulate organization of the cortical actin (31). Another novel and important protein partner of Gα12 proteins is cadherin, a protein that mediates cell-cell interactions (10). One united feature of all these Gα12 partners is their ability to form multimolecular complexes with other signaling proteins. For example, ERM proteins interact with the Rho guanine nucleotide dissociation inhibitor and the p85 subunit of phosphatidylinositol 3-kinase (32, 33).

In this study, we provided evidence that Gα12 specifically interacted with another protein that is capable of forming complex protein modules, Hsp90. Using yeast two-hybrid screening with constitutively activated Gα12 as bait, we observed that Hsp90 was one of the Gα12-interacting proteins. This observation was supported by co-immunoprecipitation studies. Hsp90 is different from other chaperones in that many of its known substrates are signal transduction proteins such as Src kinase and Raf-1 kinase (for review, see Ref. 34). Because Hsp90 is essential for maintaining the activity of numerous signaling proteins, it plays a key role in cellular signal transduction networks. It is believed that because signaling molecules with multiple regulatory states often undergo a conformational switch, the structural flexibility needed for these steps may render them less stable and thus more likely to be recognized by Hsp90 (34). This makes Gα subunits perfect candidates for interaction with Hsp90. Interestingly, only Gα12 could form a complex with endogenous Hsp90, and Hsp90 was required for Gα12 (but not Gα13 or Rho)-induced activation of gene transcription.

The possible involvement of Hsp90 in G protein-mediated signaling has been suggested in several studies. 1) Hsp90 was shown to copurify with Gβγ from the cytoplasmic fraction of rat liver (22). However, we have not been able to detect co-immunoprecipitation of Hsp90 with Gβγ subunits, but consistently detected interaction between Hsp90 and Gα12 using a co-immunoprecipitation technique. Although these data do not disprove the ability of Gβγ subunits to interact with Hsp90, they suggest that Gα12 forms a constitutive and stable complex with Hsp90, whereas the apparent interaction of Gβγ with Hsp90 could be less stable or of lower affinity. 2) It was recently reported that geldanamycin can induce degradation of the heterotrimeric Gα12 subunit via the proteasome pathway (35). Our data showed that geldanamycin disrupted the interaction of Gα12 and Hsp90, resulting in the inhibition of the Gα12 functional responses: actin cytoskeleton reorganization, cell proliferation, and SRE activation. However, we did not detect a decrease in the amount of Gα12 protein after geldanamycin treatment, suggesting that protein degradation is not likely to be a reason for the geldanamycin-induced inhibition of the Gα12 responses. Importantly, although geldanamycin induced Gαo degradation, coprecipitation of these two proteins was not detected (35), suggesting that the apparent interaction of Gαo with Hsp90 could be less stable or of lower affinity than that of Gα12. 3) When this study was in progress, it was reported that thrombin receptor signaling to the cytoskeleton requires Hsp90 (36). It was shown that Hsp90 forms a complex with the thrombin receptor protease-activated receptor-1 and that geldanamycin inhibits thrombin-induced cytoskeletal changes, but not calcium release (36).

The future challenge will be understanding the molecular mechanism that underlies the interaction of Gα12 with Hsp90. The mechanism of Hsp90 functioning as a molecular chaperone is complex and not completely understood. The in vivo function of Hsp90 is believed to be dependent on ATP binding and ATP hydrolysis (37). Thus, mutants defective in ATP binding and ATP hydrolysis are unable to replace wild-type Hsp90 in yeast in vivo (37). Importantly, ATPase activity requires the complete Hsp90 protein because C-terminal deletions result in the loss of ATPase activity (37). Thus, cellular effects of geldanamycin could be interpreted as the consequences of the interruption of the Hsp90 ATPase cycle because geldanamycin binds to the ATP-binding site with high affinity and inhibits ATP binding to Hsp90 (14). Therefore, it would be significant to analyze the interaction between Hsp90 mutants defective in ATP binding/hydrolysis and Gα12.

Another important task would be to determine the Gα12-interacting domain of Hsp90. Our yeast two-hybrid screening of a human testis cDNA library showed that fragment 378–732 of the molecule chaperone Hsp90 interacted with Gα12. Using a pull-down assay, we then determined that purified Gα12 bound to purified glutathione S-transferase-Hsp90 fusion protein (C-378); there was no detectable binding of Gα13 to control glutathione S-transferase (data not shown). The C-terminal location of the binding may seem counterintuitive because we later determined that the interaction between Gα12 and endogenous Hsp90 could be abolished by geldanamycin, which in turn bound to the N-terminally located ATP-binding site of Hsp90. We are testing the possibility that both the N- and C-terminal domains of Hsp90 may interact with Gα12 based on the following observations. It was reported that Hsp90 contains two substrate-binding sites that are located in the N- and C-terminal domains (38, 39). Similar results were obtained by Scheib et al. (40), who reported that both N- and C-terminal fragments of Hsp90 interact with substrate proteins in vitro, although with different specificity and ATP dependence. Importantly, both the N- and C-terminal domains of Hsp90 display the dominant-negative phenotype in several yeast strains (40). Interestingly, yeast two-hybrid screening determined that the cytoplasmic domain of the thrombin receptor protease-activated receptor-1 interacts with the C-terminal part (amino acids 369–721) of Hsp90 (36).

An additional intriguing goal would be to locate the Hsp90-interacting domain of Gα12. Our data clearly showed that the interaction of Hsp90 and Gα12 did not depend on the conformational state of Gα12. Moreover, Hsp90 binding to Gα12 was not affected by and was not required for the interaction of Gα12 with its binding partners, p115RhoGEF and the Gβ subunit. Therefore, it is likely that Hsp90, p115RhoGEF, and the Gβ subunit may have non-overlapping binding sites on Gα12. Recent progress in understanding the molecular mechanisms of G protein action has been made by the solution of crystal structures of several Gα subunits in active GTP-bound, inactive GDP-bound, and transitional AlF4−-complexed conformations (41–43). Three regions of Gα subunits called switches I–III change their conformation upon GTP-GDP exchange (42, 43). One or more of these switches is likely to participate in effecter interaction and/or Gβ interaction. Thus, the available crystal
structures and sequence comparison of Ga subunits may help to predict the possible Hsp90-interacting domain of Ga12.

In conclusion, we have characterized a novel signaling module of Ga12 and Hsp90. Because both of these proteins are ubiquitously expressed and are involved in the regulation of multiple intracellular and cellular events, understanding the fundamental molecular mechanism underlying the interaction of Ga12 with Hsp90 is important, as it will provide basic knowledge required for the successful determination of Ga12 function in a specific tissue in vivo.

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REFERENCES

1. Voyno-Yasenetskaya, T. A., Conklin, B. R., Gilbert, R. L., Hooley, R., Bourne, H. R., and Barber, D. (1994) Oncogene 9, 2559–2565
2. Voyno-Yasenetskaya, T. A., Pace, A. M., and Bourne, H. R. (1994) J. Biol. Chem. 269, 4721–4724
3. Voyno-Yasenetskaya, T. A., Faure, M., Ahn, N., and Bourne, H. R. (1996) J. Biol. Chem. 271, 21063–21067
4. Voyno-Yasenetskaya, T. A., Faure, M., Ahn, N., and Bourne, H. R. (1996) J. Biol. Chem. 271, 21063–21067
5. Buhl, A. M., Johnson, N. L., Dhanasekaran, N., and Johnson, G. L. (1995) Science 270, 24631–24634
6. Xu, N., Bradlet, L., Ambdukar, I., and Gutkind, J. S. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6741–6745
7. Altheofer, H., Eversole-Cire, P., and Simon, M. I. (1997) J. Biol. Chem. 272, 24380–24386
8. Berestetskaya, Y. V., Faure, M. P., Ichijo, H., and Voyno-Yasenetskaya, T. A. (1998) J. Biol. Chem. 273, 27816–27823
9. Kozaa, T., Jiang, X., Hart, M. J., Sterreweiss, P. M., Singer, W. D., Gilman, A. G., Bollag, G., and Sterreweiss, P. C. (1998) Science 280, 2109–2111
10. Meigs, T. E., Fields, T. A., McKee D., and Casey, P. J. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 519–524
11. Tohy, G. G., and Goledis, E. A. (2001) Methods 24, 201–217
12. Pratt, W. B. (2000) Proc. Soc. Exp. Biol. Med. 217, 420–434
13. Buchner, J. (1999) Trends Biochem. Sci. 24, 136–141
14. Podremou, C., Ren, S. M., O’Brien, R., Ladbury, J. E., Piper, P. W., and Pearl, L. H. (1997) Cell 89, 65–75
15. Lewis, M. J., and Bourne, H. R. (1992) J. Cell Biol. 119, 1297–1307
16. Finley, R. L., and Brent, R. (1995) in DNA Cloning, Expression Systems: A Practical Approach (Hames, B. D., and Glover, D. M., eds) Vol. 2, pp. 169–203, Oxford University Press, Oxford
17. Vaikunthai, R., Adarichev, V., Forthmayr, H., Kozasa, T., Gudkov, A., and Vassylo-Yasenetskaya, T. A. (2000) J. Biol. Chem. 275, 26206–26212
18. Bourne, H. R., and Nicoll, R. A. (1993) Cell/Neuron 10, 65–75
19. Berman, D. M., and Gilman, A. G. (1998) J. Biol. Chem. 273, 1269–1272
20. Berman, D. M., Kozasa, T., and Gilman, A. G. (1996) J. Biol. Chem. 271, 27209–27212
21. Hart, M. J., Jiang, X., Kozasa, T., Roseow, W., Singer, W. D., Gilman, A. G., Strenweis, P. C., and Bollag, G. (1998) Science 280, 2112–2114
22. Inanobe, A., Takahashi, K., and Katada, T. (1994) J. Biochem. (Tokyo) 115, 486–492
23. Fromm, C., Coso, O. A., Montaner, S., Xu, N., and Gutkind, J. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10098–10103
24. Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
25. Schulte, T. W., Blagkosklonny, K. M., Romanova, L., Mushinski, J. F., Monia, B. P., Johnston, J. F., Nguyen, P., Trepel, J., and Neckers, L. M. (1996) Mol. Cell. Biol. 16, 5839–5845
26. Gohla, A., Offermanns, S., Wilkie, T. M., and Schultz, G. (1999) J. Biol. Chem. 274, 17901–17907
27. Majumdar, M., Seasholtz, T. M., Buckmaster, C., Tokoz, D., and Brown, J. H. (1999) J. Biol. Chem. 274, 26815–26821
28. Gilchrist, A., Vanhuwe, J. F., Li, A., Thomas, T. O., Vassylo-Yasenetskaya, T. A., and Hamn, H. E. (2001) J. Biol. Chem. 276, 25672–25679
29. Strathmann, M. P., and Simon, M. I. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5582–5586
30. Jiang, Y., Ma, W., Wan, Y., Kozasa, T., Hattori, S., and Huang, X. Y. (1998) Nature 395, 808–813
31. Tsukita, S., and Yamemura, S. (1999) J. Biol. Chem. 274, 34507–34510
32. Takahashi, H., Sasaki, T., Mamamoto, A., Takakishi, K., Kameyama, T., Tsukita, S., Tsukita, S., and Takai, Y. (1997) J. Biol. Chem. 272, 23731–23735
33. Gourraje, A., Poulet, P., Loeuverd, D., and Arpin, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 7300–7305
34. Young, J. C., Moarefi, I., and Hartl, F. U. (2001) J. Cell Biol. 154, 267–273
35. Busconci, L., Guan, J., and Denker, B. M. (2000) J. Biol. Chem. 275, 1565–1569
36. Pai, K. S., Mahajan, V. B., Lau, A., and Cunningham, D. D. (2001) J. Biol. Chem. 276, 32642–32647
37. Obermann, W. M. J., Sondermann, H., Russo, A. A., Pavletich, N. P., and Hartl, F. U. (1998) J. Biol. Chem. 273, 12919–12924
38. Young, J. C., Schneider, C., and Hartl, F. U. (1997) FEBS Lett. 418, 139–143
39. Scheibel, T., Weikl, T., and Buchner, J. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1485–1499
40. Scheibel, T., Weikl, T., Rimnerman, R., Smith, D., Lindquist, S., and Buchner, J. (1999) Mol. Microbiol. 34, 701–713
41. Noel, J. P., Hamm, H. E., and Sigler, P. B. (1993) Nature 366, 654–663
42. Lambricht, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) Nature 369, 621–628
43. Sondek, J., Lambricht, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) Nature 372, 276–279
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