CCTγ, a Novel Soluble Guanylyl Cyclase-interacting Protein*

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Nitric oxide (NO), a simple diatomic gas, plays anything but a simple role in signal transduction. NO is produced by the enzyme nitric-oxide synthase. The main target of NO, mediating most of its downstream effects, is a cytosolic heterodimer of α and β subunits known as soluble guanylyl cyclase (sGC). Upon NO binding, the activated sGC catalyzes the formation of cGMP from GTP (1–3). Cyclic GMP is then involved in the activation of a variety of effectors such as cyclic nucleotide-gated channels, protein kinases, and phosphodiesterases (4).

There has been evidence suggesting that there are in vivo mechanisms other than nitric oxide that regulate sGC activity. Bellamy et al. (5) show that deactivation of sGC on the removal of NO occurred 25-fold faster in intact cerebellar cells than the fastest estimate for purified sGC. These data suggest that there is probably another protein or group of proteins that regulate sGC activity, because it occurs in the order of seconds. In addition to the indirect evidence of protein regulation of sGC provided by Bellamy et al. (5), PSD95 (post-synaptic density protein 95) (6) and heat shock protein 90 (HSP90) (7) have both been shown to interact with isoforms of soluble guanylyl cyclase. Both PSD95 and HSP90 was shown to enhance the response of sGC to NO. In both of these cases, the interaction with sGC is modulated by interaction with a nitric-oxide synthase isoform. We hypothesized that a protein existed that modified sGC activity, independent of any interaction with nitric-oxide synthase isoforms.

To find this regulator of sGC activity, we performed a yeast two-hybrid screening with the β subunit of sGC as bait against a human brain cDNA library. The screening revealed CCT (chaperonin containing t-complex polypeptide) subunit η to be an interacting protein. Immunoprecipitation and immunohistochemical studies suggest that this interaction occurs in vivo. This interaction inhibited NO-stimulated sGC activity both in vitro and in vivo, whereas basal activity or activation by allosteric regulator was not affected by CCTγ. CCTγ had no effect on the constitutively active αγ sGCmutant that lacks a heme group. These studies suggest that CCTγ, in cooperation with some other factor, can mediate a novel type of NO-dependent inhibition of sGC.

MATERIALS AND METHODS

All of the materials were supplied by Sigma unless otherwise specified.

Yeast Two-hybrid Screen—The full-length cDNA of the β subunit of sGC was cloned in-frame with the GAL4 DNA binding domain of the pGBK7 vector between the EcoRI and Sall sites (pGβBj). This vector was transfected into AH109 yeast cells (Clontech). A human brain cDNA library cloned in-frame with the GAL4 activation domain of the pACT2 vector was transfected into yeast containing the pGβBj vector. The resulting transfected yeast were then plated on quadruple dropout agar (-Trp/-Leu/-His/-Ade) with 5 mM 3-aminotriazole. The yeast were then allowed to grow for 3 weeks at 30 °C at which time the positive clones from this screening were then transfected into AH109 containing pGBKT7 as controls to see whether any of these positive clones could activate the reporter gene in AH109 by itself or in

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1 The abbreviations used are: NO, nitric oxide; CCT, chaperonin containing t-complex polypeptide; SNP, sodium nitroprusside; sGC, soluble guanylyl cyclase; BAY41-2272, 5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1H-pyrazolo[3,4-b]pyridin-3-yl]-pyrimidin-4-ylamine; PBS, phosphate-buffered saline; IBMX isobutylmethylxanthine; GST, glutathione S-transferase; DEA, diethylamine dianzeniumdiolate.

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conjunction with the binding domain alone. This step further reduced the number of false positives.

Deletions of β-subunit—To identify the sGC-binding region(s) at CCTγ, progressive C-terminal deletions of gC β subunit were generated. The truncated β subunits were cloned into pGPTV to generate pGβB366–619, pGβB366–619, and pGβB366–619 constructs carrying the indicated deletions.

α-Galactosidase Assay—The plasmids with full-length and truncated β subunit, pGβB366–619, pGβB366–619, pGβB366–619, and pGβB366–619 were transfected into AH109 containing pACT-CCTγ and grown in selective liquid culture (Trp/-Leu) overnight. The yeast were then centrifuged at 10,000 × g for 2 min, and 8 µl of the conditioned growth medium were mixed with 24 µl of assay buffer (33 mM 5-nitrophenyl-α-D-galactoside and 333 mM sodium acetate). This mixture was then incubated in a water bath of 30 °C for 30 min, and the reaction was stopped by the addition of 960 µl of stop solution (100 mM sodium carbonate). The cell lysate protein concentration was determined via Bradford assay. The mouse brains used for co-immunoprecipitation were prepared in the cell culture buffer and then followed by centrifugation; the lysate was centrifuged at 2,900 × g for 20 min at 4 °C. The supernatant was then taken and centrifuged for 45 min at 29,000 × g and 4 °C.

Co-immunoprecipitation—20 µl of a 50% protein G-Sepharose beads (Amersham Biosciences) slurry was added to control and experimental tubes. 20 µl of acetic serum with rat mononclonal CCT antibody (Stressgen) or rat preimmune serum were added to each of the four plates. The tubes were then spun down for 15 min at room temperature. The mixture was spun down for 5 s, and the supernatant was discarded. 940 µl of PBS was then added to each tube along with 780 µg of mouse brain lysate prepared as described above and incubated with constant mixing at 4 °C overnight. The mixture was then spun down at 10,000 g and washed with 500 µl of PBS five times. Immunoprecipitated proteins were then eluted with 50 µl of Laemmli buffer. 20 µl of the eluted samples and 5 µl of lysate were loaded and resolved by SDS-PAGE and subjected to immunoblot analysis as described above.

Human sGC Expression and Purification—Human recombinant sGC expression and lysate preparation were done using the SF9 baculovirus system and were previously described (27). The cDNA was then shaker-harvested and the pellets were dissolved in detergent buffer.

**GTG Conversion Assay**—Purified CCTγ (3.25 µg) and either sGC-expressing SF9 lysate (5–8 µg) or aSFγ-hexon 100–619 mutant sGC-expressing SF9 lysate (5–8 µg) or purified sGC (1.2 µg) were incubated together in 20 µl of sample buffer (2 mg/ml albumin, 50 mM triethanolamine, and 100 mM EGTA) for 10 min at room temperature. Following the incubation, 40 µl of reaction buffer (125 mM triethanolamine, 250 mM Eta, 2.5 mM MgCl2, 2.5 mM MnCl2, 0.3 µg/ml proteinase K, 1.25 µg/ml creatine kinase, 12.5 mM creatine phosphate, and 7.5 mM magnesium chloride) were added. The mixture of sample buffer and incubation buffer, 40 µl of substrate buffer containing various concentrations of DEA-NO (as indicated) or 100 µM SNP or 2 µM BAY and 200 µM GTP/0.01 µCi of [α-32P]GTP were added. This mixture was then incubated in a circulating water bath at 37 °C for 45 min. The reaction was stopped by the addition of 1 ml of 100 mM zinc acetate and 1 ml of 120 mM sodium carbonate. The tubes were then centrifuged at 1,000 × g for 5 min, and the supernatant was loaded on to 1.5-ml aluminum columns. Synthesized cGMP was eluted with 10 µl of 100 mM Tris, pH 7.5, and was quantified on a β scintillation counter.

**Cell Culture and Stable Transfection**—FLAG-tagged CCTγ was amplified using the 5′ primer, 5′-TAAGCTAGCCACATGATGGAT-TAACAAGGATGACGACGATAAAGCCACCACACTGATTTCTATTGAAA-3′, and 3′ primer, 5′-TGCGCCCGCCGTACGTTGGGGCGCACG-3′, and cloned into the mammalian expression vector pMVGH2 (Invigen). BE2 cells (ATCC number CRL-2268) were plated at a density of 5 × 10^5 cells/ml in cell culture medium (50% Dulbecco’s modified Eagle’s medium, 50% Ham’s F-12, 10% fetal bovine serum, 2.5 mM l-glutamine, 1.5 g/liter sodium bicarbonate, 0.5 mM sodium pyruvate, and 0.05 mM non-essential amino acids). Four 100-mm plates of 80% confluent BE2 cells were transfected using LipofectAMINE-plus (Invitrogen) according to the manufacturer’s instructions. Two were transfected with PMG-CCTγ plasmid, and two were transfected with PMG2H vector alone. To select the stable transfected clones, media with 400 µg/ml hygromycin B were added to each of the plates every 4 days for 3 weeks. Stable colonies were then picked, transfected to fresh plates, and grown to confluency with 300 µg/ml hygromycin B.

**RNA Isolation and Reverse Transcription—**RNA was isolated from confluent plates of BE2 cells stably transfected with a PMG-CCTγ construct and PMG2H2 vector using RNeasy kit (Qiagen) according to manufacturer’s instructions. The expression of FLAG-tagged CCTγ was tested by a reverse transcription reaction using the following primers: 5′-CA-
Western blot with lysates from different C57BL/6 mouse organs. 15 μg of homogenates prepared from various organs from C57BL6 mice were displayed on SDS-PAGE. The presence of CCT1 in homogenates was tested with polyclonal antibody against CCT1.

Co-immunoprecipitation of sGC with CCT1. CCT1 was immunoprecipitated from mouse brain using rat preimmune serum (lane 2), or rat monoclonal CCT antibodies (lane 3). Western blotting (WB) with rabbit polyclonal anti-CTT1 antibodies (A) or rabbit polyclonal anti-sGC antibodies (B) was used to detect the co-precipitation of CCT1 and sGC. 5 μl of load and 20 μl of immunoprecipitated (IP) elution were loaded in each case. C, specificity of anti-sGC antibodies was confirmed by the blocking of sGC signal with an excess of antigenic peptide (lanes 1 and 2).

RESULTS

Yeast Two-hybrid Screening—To find the putative protein(s) that interacts with human sGC, we performed a yeast two-hybrid screen using human sGC as bait from a cDNA library (Clontech). Twelve million cDNAs were screened against full-length human β1 protein, and 334 of these clones grew on selective media. 12 of these clones were positive for the Gal4 binding domain without bait and also into yeast with no plasmid. Both of these transfections did not grow on selective media, indicating that the interaction is specific in yeast (data not shown).

Because CCT1 is a novel interacting partner of α1β1 sGC identified via a yeast-two-hybrid screen, we next wanted to determine the expression pattern of CCT1 versus the ubiquitously expressed α1β1 sGC. We found that CCT1 was abundant in many tissues. CCT1 was not expressed at all in the small intestine, but it was expressed in the testis, heart, brain, kidneys, and lungs (Fig. 1).

sGC Co-immunoprecipitates with CCT1—After the expression pattern of CCT1 was determined, we tested the interaction between sGC and CCT1 by co-immunoprecipitation. Immunoprecipitation of CCT1 protein from the C57BL6 mouse brain revealed the co-precipitation of sGC. On the contrary, when immunoprecipitation was performed with the preimmune serum, neither CCT1 nor sGC was precipitated, confirming the specificity of sGC/CCT1 precipitation (Fig. 2). Based on the standard curves obtained from the Western blotting of purified CCT1 and sGC, we calculated that ~6% of the total CCT1 was precipitated together with ~2% of the β subunit of sGC (data not shown).
CCT\(\eta\)-mediated Inhibition of Soluble Guanylyl Cyclase

Fig. 3. Co-localization and confocal microscopy of sGC and CCT\(\eta\) in rat hippocampus. Sections of rat hippocampus were stained with anti-CCT\(\eta\) (A, D, and G) or anti-sGC (B, E, and H) antibodies and viewed at several increasing magnifications. First row, viewed at \(\times 10\) magnification; second row, viewed at \(\times 120\) magnification, third row, viewed at \(\times 300\) magnification. Co-localization of sGC and CCT\(\eta\) is presented in panels C, F, and I. Control staining without primary antibodies for CCT\(\eta\) and sGC are shown at lowest magnification in panels J and K, respectively. A quantitative representation of the amount of co-localization between the two proteins is shown in L with the units of both axes pixel-numbered. The bar beneath the graph indicates the color scheme for an increasing number of co-localized pixels with red being the highest. The white bar in A, B, and C is 270 \(\mu\)m, the bar in D, E, and F is 20 \(\mu\)m, and the bar in G, H, and I is 8 \(\mu\)m in scale. The scale bar of controls (J and K) is 270 \(\mu\)m.

Co-localization of sGC and CCT\(\eta\)—After confirming the in vivo interaction between sGC and CCT\(\eta\) by immunoprecipitation, we next decided to visualize the co-localization of CCT\(\eta\) and sGC in rat brain by immunohistochemistry and confocal microscopy (Fig. 3). As seen in the image from all of the magnifications (Fig. 3, C, F, and I) CCT\(\eta\) and sGC proteins do co-localize in the rat hippocampus along the dentate gyrus. From the highest magnification, it appears that sGC has cytosolic and perinuclear localization, whereas CCT\(\eta\) localizes mainly in the cytosolic compartment (Fig. 3F). Such subcellular localization suggests that only the cytosolic fraction of sGC enzyme could efficiently interact with CCT\(\eta\) protein.

Region of \(\beta_1\) Interacting with CCT\(\eta\)—We next determined the region of the \(\beta_1\) subunit of sGC that interacts with CCT\(\eta\). As seen in Fig. 4, the first 100 amino acids of \(\beta_1\) subunit are not sufficient to interact with CCT\(\eta\) protein. However, the truncated \(\beta_1\) subunit carrying 266 residues interacts with CCT\(\eta\) with the same efficiency as the truncated \(\beta_1\) subunit with 366 residues. The highest strength of interaction was observed when full-length \(\beta_1\) subunit was tested. Thus, these data indicate that there are at least two regions in the \(\beta_1\) subunit, one between the residues 101 and 266 and another between residues 367 and 619, that are interacting with CCT\(\eta\) protein.

CCT\(\eta\) Inhibits NO-stimulated sGC Activity—To determine whether or not CCT\(\eta\) has an effect on sGC activity, we expressed a full-length CCT\(\eta\) tagged with hexahistidine at the N terminus in BL21 bacterial strain. Hexahistidine-tagged \(\beta\)-galactosidase was also expressed in the same strain and used as negative control. The results shown in Fig. 5 indicate that \(\beta\)-galactosidase and buffer have a similar effect on DEA-NO-stimulated cGMP formation activity of sGC, whereas CCT\(\eta\) inhibits this cGMP formation by 30–50% in the 10–100 \(\mu\)m range of DEA-NO (Fig. 5). Furthermore, CCT\(\eta\) seems to inhibit sGC-expressing SF9 lysate by decreasing the \(V_{\text{max}}\) of NO-stimulated-sGC and not by affecting the EC\(_{50}\) of sGC for NO. Note that there is no difference in cGMP formation activities among buffer, \(\beta\)-galactosidase, and CCT\(\eta\) under basal conditions. When BAY41-2272 was used to stimulate the sGC-expressing SF9 lysate, no difference in cGMP formation was seen between controls and CCT\(\eta\) (data not shown). The fact that CCT\(\eta\) inhibited NO-stimulated sGC, a heme-dependent agonist, but did not inhibit BAY-stimulated sGC, an NO-independent allosteric activator, or any of the basal activities of sGC implies that the mechanism of CCT\(\eta\) inhibition may work through a modification of the NO-stimulated heme group or the consequent conformational changes that occur after NO binds to the heme group.

This mechanism of CCT\(\eta\) inhibition of NO-stimulated sGC was further supported by the effect seen on \(\alpha\beta_Cys^{105}\) sGC. This mutant lacks the heme moiety because of the substitution of His-105 residue coordinating heme iron and is insensitive to NO stimulation (11). However, it was shown that \(\alpha\beta_Cys^{105}\) sGC has a constitutively increased activity, even in the absence of any stimulators, suggesting that the mutant sGC is already in an activated conformation (11). These properties of the mutant enzyme are also summarized in the Fig. 6A. Preincubation of the mutant enzyme with CCT\(\eta\) has no effect on cGMP-forming activity of basal or NO-treated \(\alpha\beta_Cys^{105}\) sGC similar to the control buffer or \(\beta\)-galactosidase protein (Fig. 6B). However, preincubation of wild type enzyme with CCT\(\eta\) inhibit the SNP-stimulated activity versus buffer and \(\beta\)-galactosidase controls. This lends further evidence to the hypothesis that CCT\(\eta\) inhibits sGC activity through modification of an NO-activated heme group or a modification of the conformational changes induced by nitrosyl heme.

N-terminal Portion of CCT\(\eta\) Is Crucial for sGC Inhibition—Further characterization of the inhibitory effect of CCT\(\eta\) on sGC was done via deletional analysis. The original 1,350-bp coding region of CCT\(\eta\), retrieved from yeast two-hybrid screen, was subcloned into the pGEX5X-3 vector to express the GST fusion product, GST-CCT\(\eta\)gal, in bacteria. This GST-fused fragment of CCT\(\eta\) lacked 94 N-terminal residues. We compared the effects of this truncated CCT\(\eta\)gal with the full-length CCT\(\eta\) on the activity of SNP-stimulated sGC (Fig. 7). Bacterial lysate-expressing GST-CCT\(\eta\)gal product decreased sGC activity by only 20% in comparison with the lysate-expressing control GST protein. However, the full-length CCT\(\eta\) inhibited the activity of sGC-expressing SF9 lysate by 68% (Fig. 7).

CCT\(\eta\) Alone Requires Other Factors to Inhibit NO-stimulated sGC—We next compared the effects of purified CCT\(\eta\) on pure sGC. Interestingly, we found that purified CCT\(\eta\) had no inhibitory effect on pure sGC unless SF9 lysate was provided (Fig. 8). Thus, it appears that CCT\(\eta\) plays a role of the adaptor protein, which recruits additional factors necessary for sGC inhibition.

In Vivo Inhibition of sGC—After showing the inhibitory ef-
Fig. 4. Defining the region of interaction between the β₁ subunit of sGC and CCT. Progressive C-terminal deletions of the sGC β₁ subunit were cloned in-frame to the GAL4 binding domain of the bait vector pGBK7 of the yeast two-hybrid system. The full-length CCT was cloned in-frame to the GAL4 activation domain of the target pACT vector of the yeast two-hybrid system. Both bait and target vectors were transfected into the AH109 strain of yeast, and the strength of the interaction was measured in vivo by the α-galactosidase assay. Interaction of p53 and the small T-antigen was used as positive control for in vivo interaction, whereas interaction of sGC β₁ subunit and actin was used as negative control. Data are presented as the mean ± S.D. of two independent experiments performed in triplicates.

Fig. 5. CCT inhibits sGC Vₘₐₙ but does not affect the affinity to NO. Inset, purity of CCT preparation was tested by Coomassie Blue staining (lane 2), and the identity of the purified protein was confirmed by Western blotting (lane 1) using anti-CCT antibodies. Protein molecular size markers are shown in lane 3. SF9 lysate-expressing sGC enzyme was treated with increasing concentration of NO-donor DEA-NO in the presence of equal amounts of purified CCT (triangles), β-galactosidase (squares), or control buffer (diamonds). sGC activity was determined as described under “Materials and Methods.” Data are presented as the mean ± S.D. of three independent experiments performed in triplicates.

The effect of CCT on sGC in crude lysates, we wanted to see whether this inhibition occurred in intact cell as well. Full-length CCT with an N-terminal FLAG was cloned into a mammalian expression vector, pMCH2. This pMCH-CCT construct, along with the vector alone, was then transfected into BE2 cells, a neuroblastoma line that endogenously expresses sGC (11). The stable lines cells were selected on hygromycin for 3 weeks. Several Hyg colonies were expanded, and the mRNA was purified. The expression of FLAG-tagged CCT in these stable lines was confirmed by reverse transcriptase-PCR specific only for flagged-CCT (Fig. 9A). Western blot analysis indicated that the expression level of sGC enzyme was not affected in these lines (Fig. 9B). We also performed Western blots to check the expression level of sGC in the stable line expressing CCT versus the control line with vector alone and found the expression to be approximately equal (Fig. 9B).

After establishing successful expression of CCT in BE2, in vivo cGMP accumulation was measured via radioimmunoassay to determine the effect of CCT on sGC in vivo. Both the control cell line and the CCT-expressing cell line (BE2-CCT) were treated with IBMX or 100 μM SNP and IBMX for 15 min at 37 °C with 5% CO₂. The cells were then lysed, and cGMP level was estimated via cGMP radioimmunoassay. The results, shown in Fig. 9C, illustrate that CCT does inhibit sGC in vivo upon activation but has no affect on the basal activity of sGC. This concurs with the in vitro data presented in Fig. 5, which
also indicated that CCT inhibits SNP-stimulated sGC but not the basal activity of sGC. Furthermore, the extent of observed inhibition is very similar in both cases.

DISCUSSION

To date, there have been no reports of a systematic search for proteins interacting with sGC, let alone affecting its activity. The yeast two-hybrid screening described in this report identified the η subunit of CCT as a novel protein directly interacting with sGC. The association of CCT with sGC was demonstrated not only through interaction in yeast but also through immunoprecipitation (Fig. 2) and colocalization in the hippocampal area of the brain (Fig. 3).

CCT is a eukaryotic cytosolic protein involved in the folding of other proteins. It is composed of two rings, one on top of the other, forming a cylinder in which the environment is amenable to protein folding. Each of the rings is composed of eight different 60-kDa subunits: α, β, δ, ε, γ, η, ε, and θ (9). Actin and tubulin have been identified as two major substrates of CCT (13).

The CCT complex has the same function as the GroE$_6$/GroEL complex in that both are formed by two oligomeric rings to create a closed compartment in which protein folding can occur (14). In light of this fact, the reason for having eight different subunits to do the job of protein folding where two seem to suffice indicates that each of the subunits may have individual functions within the CCT complex or that these subunits may provide some specificity to the function of CCT complex. There is precedence for subunits of the CCT complex to have specific functions. CCTα has been detected in microtubule structures, and when antibodies to CCTα are added in vitro, microtubule polymerization is inhibited (15). CCTα has been shown to be important for yeast morphogenesis (16). Interestingly, the subunits of CCT do not always display the same cellular localization, e.g. CCTα and CCTε were detected only in the particulate fraction associated with microtubules (17, 18). This finding suggests that the subunits of the CCT complex do not necessarily always form an oligomer or that this oligomer is not always composed of all eight subunits.

Numerous GST pull-down assays were attempted with GST-tagged CCT and purified sGC. However, sGC had a high affinity not only to GST alone but also to glutathione column used for these purposes and was consistently detected in control samples (data not shown). The only conditions that decreased the sGC background to acceptable levels (500 mM NaCl and 0.5% Nonidet P-40) were prohibitive of any protein interactions.

In addition to evidence of association between CCT and sGC in brain homogenates, we have also shown in vivo evidence for their association through co-localization in rat brains (Fig. 3). Interestingly, although sGC and CCT displayed a superimposable localization in rat hippocampus, both proteins showed only partial overlapping on a subcellular level. The immunohistochemical staining presented here (Fig. 3G) corrob-
orates well with previously described cytosolic localization of CCT\(\eta\) (17). Whereas CCT\(\eta\) was fully cytosolic, sGC showed cytosolic and perinuclear localization. Such distribution suggests that most probably the cytosolic sGC is regulated by CCT\(\eta\). Although traditionally regarded as cytosolic protein, a number of studies also report that sGC can be associated with particulate fractions. Schmidt and co-workers (12) suggest a calcium-dependent sequestration of sGC to the membrane in platelets and endothelial cells. Koesling and colleagues (6) show sGC activity in the membrane fraction of the brain homogenates, which they attributed to the association between the \(\alpha_2\beta_3\) isoform of sGC and PSD95, a protein localized at the plasma membrane.

From the deletional analysis, it appears that the association between sGC and CCT\(\eta\) requires the N-terminal 94 residues of CCT\(\eta\) (Fig. 7) as well as the sGC regions between the residues

**Fig. 8.** CCT\(\eta\) requires crude lysate to inhibit sGC. Equal amounts of purified CCT\(\eta\) were added to purified wild type sGC or Sf9 lysates containing the same amount of wild type sGC. The activity was measured in the presence of 100 \(\mu\)M SNP as described under “Materials and Methods.” 100% activity for purified sGC is defined as 18 \pm 1.5 \(\mu\)mol cGMP/\(\mu\)g·min, and 100% activity for sGC-containing Sf9 lysate is defined as 22 \pm 1.8 nmol cGMP/\(\mu\)g·min. Data from three independent measurements performed in duplicates are presented.

**Fig. 9.** Effect of CCT\(\eta\) overexpression on sGC in vitro. A, a 300-bp amplicon specific for the FLAG-tagged CCT\(\eta\) is detected in a reverse transcriptase-PCR reaction from the CCT\(\eta\)-FLAG-transfected stable line but not from the line transfected with control vector. B, expression of sGC is equal in BE2 line transfected with pMG-CCT\(\eta\) plasmid or control pMG vector. C, SNP-dependent increase in cGMP levels in BE2 cells is decreased in cells transfected with pMG-CCT\(\eta\) plasmid but not the control pMG vector.
indicate a role for HSP90 in sGC regulation (7). Because sGC is involved in such a variety of physiological signals ranging from immunomodulation to vasodilatation to the inhibition of primary hemostasis, the regulation of sGC activity should likewise be complex. Interaction of sGC with CCT and the resulted inhibition described in this paper provide a novel aspect of this regulation.

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