Thrombin-induced Rapid Geranylgeranylation of RhoA as an Essential Process for RhoA Activation in Endothelial Cells

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RhoA plays a critical signaling role in thrombin-induced endothelial dysfunction. The possible thrombin regulation of geranylgeranylation, a lipid modification, of unprocessed RhoA and the significance of the geranylgeranylation in RhoA activation in endothelial cells (ECs) are not well understood. The amounts of the unprocessed and geranylgeranylated forms of RhoA in non-stimulated cultured human aortic ECs were 31 ± 8 and 69 ± 8% total cellular RhoA, respectively (n = 6, p < 0.0001), as determined by the Triton X-114 partition method. Thrombin-induced rapid conversion of most of the unprocessed RhoA into the geranylgeranylated form within 1 min through stimulating geranylgeranyltransferase I (GGTase I) activity. Thrombin-induced rapid geranylgeranylation was inhibited by acute short term (3 min) pretreatment with atorvastatin as well as by an inhibitor of GGTase I (GGTI-286). Thrombin also rapidly stimulated GTP loading of RhoA, which was blocked by acute pretreatment with either atorvastatin or GGTI-286. These observations indicate the dependence of thrombin stimulation of RhoA on the rapid geranylgeranylation of unprocessed RhoA. Importantly, the addition of geranylgeranylpiphosphosphate to ECs pretreated with atorvastatin quickly reversed the atorvastatin inhibition of thrombin stimulation of RhoA. These results suggest that geranylgeranylation of unprocessed RhoA may limit thrombin-induced full activation of RhoA in ECs. Cytoskeleton analysis demonstrated that atorvastatin and GGTI-286 inhibited thrombin-induced stress fiber formation. We provide the evidence that, in thrombin-stimulated ECs, the unprocessed form of RhoA is rapidly geranylgeranylated to become the mature form, which then is converted into GTP-bound active RhoA.

Thrombin regulates various responses in the vasculature including endothelial cell (EC) activation, vascular constriction, platelet aggregation, and activation of the coagulation cascade, all of which contribute to the pathogenesis of coronary atherosclerosis and acute coronary syndrome (1–3). Moreover, thrombin has been shown to induce the rapid activation of small GTPase RhoA, which is involved in endothelial dysfunction including hyperpermeability and impaired nitric oxide synthesis (4–9).

RhoA plays a critical role in thrombin signaling for activation of ECs. Geranylgeranylation is a post-translational lipid modification of proteins that is required for subsequent activation of RhoA (10–15). Geranylgeranylpiphosphosphate (GGPP) is an isoprenoid that is a substrate for geranylgeranylation of the unprocessed GDP-bound form of RhoA (GDP-RhoA) catalyzed by geranylgeranyltransferase I (GGTase I) (16, 17). Activation, i.e. GTP loading of geranylgeranylated mature GDP-RhoA, occurs through the activity of a guanine-nucleotide exchange factor (GEF), which can bind to and be activated by the α-subunit of G12/G13 (18–20). The beneficial effects of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors (statins) on endothelial dysfunction of cardiovascular disease are mediated through depletion of GGPP for geranylgeranylation of small GTP-binding proteins such as RhoA and Rac (21–23).

Thrombin rapidly activates RhoA in ECs and other cells within 1 min via p115RhoGEF (20). Previously, we demonstrated the unusually acute effect of a statin, namely the rapid inhibition of lysophosphatidylcholine-induced RhoA activation in ECs by a statin (22). The effect of a statin was mediated through its action on GGPP metabolism, because it was reversed by the addition of GGPP. However, little is known regarding how rapidly geranylgeranylation of RhoA is regulated and how important geranylgeranylation is in the regulation of RhoA activity.

In this study, we investigated the rapid effect of thrombin on geranylgeranylation of RhoA, the role of geranylgeranylation in thrombin-induced RhoA activation, and the effects of a statin and a GGTase I inhibitor on geranylgeranylation and RhoA activation in ECs.

EXPERIMENTAL PROCEDURES

Materials—The sources of most of the conventional reagents were described previously (22, 24). Thrombin was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Atorvastatin was kindly provided by Pfizer (New York, NY). GGPP was purchased from Sigma, and GGTase I inhibitor GGTI-286 was from Calbiochem.

Endothelial Cell Culture—Human aortic ECs were cultured accord-

GFP, guanine-nucleotide exchange factor; PBS, phosphate-buffered saline; TRITC, tetramethylrhodamine isothiocyanate; ANOVA, analysis of variance.
ing to the supplier’s instructions (Clonetics, Inc., Walkersville, MD, and Sanko Junyaku Co. Ltd., Tokyo, Japan) as described previously (22). They were used for experiments after 5–10 passages.

**Separation of Unprocessed and Geranylgeranylated RhoA Protein—** Unprocessed RhoA and geranylgeranylated RhoA were separated by the Triton X-114 partition method as described previously (25, 26). Cells were washed twice in ice-cold phosphate-buffered saline (PBS) and lysed in a buffer containing 50 mmol/liter Tris-HCl, pH 7.5, 150 mmol/liter NaCl, 5 mmol/liter MgCl₂, 200 μmol/liter GDP, 1 mmol/liter dithiothreitol, 1 mmol/liter Pefabloc, 20 μmol/liter each of leupeptin, aprotinin, and soybean trypsin inhibitor and then sonicated. Triton X-114 (11% w/v) was added to the lysate to a final concentration of 1% (v/v). The lysates were mixed for 10 min at 4 °C and centrifuged at 15,000 × g at 4 °C for 30 min to remove insoluble materials. The supernatant was warmed at 37 °C for 2 min until it became cloudy and then centrifuged at 400 × g for 4 min at room temperature to separate the upper (unprocessed) phase from the lower (processed) phase. Both phases were adjusted to 1% (v/v) Triton X-114 on ice, and protein concentrations were determined by the Bradford method. The level of Rho protein in each phase was determined by Western blotting followed by densitometric analysis as described previously (22, 27). A mouse monoclonal antibody to RhoA (Santa Cruz Biotechnology Inc., Santa Cruz, CA) diluted 1:500 was used for immunoblotting. The signals from immunoreactive bands were visualized with ECL (Amersham Biosciences).

In addition, ECs were labeled with 5 μmol/liter [³H]GGPP (22 mCi/mmol, Amersham Biosciences) for 3 min and stimulated with or without thrombin for 1 min followed by Triton X-114 partition. The radiolabeled band was detected by autoradiography after SDS-PAGE, and using the same sample, Western blotting was performed simultaneously to determine whether the radiolabeled band was identical to RhoA.

**GGTase I Activity Assay—** The activity of GGTase was determined by a pull-down assay as described previously (22, 27–29). ECs were washed with ice-cold PBS three times and lysed in a lysis buffer (50 mmol/liter Tris, pH 7.2, 500 mmol/liter NaCl, 10 mmol/liter MgCl₂, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 20 μg/ml antipain, 20 μg/ml leupeptin, 1 μmol/liter phenylmethylsulfonyl fluoride). After centrifugation at 15,000 × g at 4 °C for 10 min, the extracts were incubated at 4 °C for 45 min with glutathione-Sepharose 4B beads coupled with glutathione S-transferase-rhotekin fusion protein to determine RhoA activity. Bound RhoA proteins were quantitated by Western blotting as described above.

**Determination of Actin Stress Fibers—** The content of actin stress fibers was determined in confluent cells grown in a slide chamber (Nalge Nunc International, Naperville, IL) as described previously (31). ECs were washed with PBS, fixed in 4% formaldehyde/PBS for 10 min, and permeabilized for 5 min at room temperature in Triton X-100 (0.05% in PBS). The ECs then were stained for 3 min with 1 μg/ml tetramethylrhodamine isothiocyanate (TRITC)-phalloidin in PBS. After two washes in PBS, coverslips were mounted using Dako Faramount mounting medium. Stained cells were stored in the dark until they were analyzed by confocal microscopy (Olympus, Tokyo, Japan).

**Densitometric Analysis—** After scanning blots into a computer (EPSON GT5500ART, Tokyo, Japan), the optical densities of individual bands in Western blots were analyzed using National Institutes of Health IMAGE program (Bethesda, MD). The area of each band analyzed was kept constant for each blot analyzed. Background density was subtracted from the densitometric data obtained for each band.

**Statistical Analysis—** Statistical analyses were performed using ANOVA with Scheffe’s post hoc test if appropriate. A value of p < 0.05 was considered significant. Data are expressed as the means ± S.D.

**RESULTS**

**Proportions of Unprocessed and Geranylgeranylated Forms of RhoA—** Fig. 1 shows the proportions of the unprocessed and geranylgeranylated forms of RhoA in cultured human aortic ECs, as determined by the Triton X-114 partition method. The percentages of the unprocessed and the geranylgeranylated forms of RhoA in ECs were 31 ± 8 and 69 ± 8%, respectively (n = 6, p < 0.0001).

**Effects of Thrombin and Atorvastatin on Geranylgeranylation and GTP Loading of RhoA—** Treatment with 1 unit/ml thrombin for 1 min stimulated the conversion of most of the unprocessed form of RhoA into its geranylgeranylated form (Fig. 2A, lane 2). This rapid geranylgeranylation was markedly suppressed by acute treatment with 10 μmol/liter atorvastatin for 3 min before adding thrombin (Fig. 2A, lane 3). Adding GGPP alone to ECs did not alter the levels of geranylgeranylated RhoA (Fig. 2A, lane 4).

Autoradiography from the [³H]GGPP labeling experiment showed the radiolabeled band of geranylgeranylated RhoA, which was identified by anti-RhoA antibody in Western blotting analysis, in the detergent phase in response to thrombin within 1 min (Fig. 2B). This is direct evidence that thrombin rapidly alters the level of RhoA prenylation in ECs.

Pull-down analysis revealed that thrombin increased the levels of the GTP-bound form of RhoA (GTP RhoA) within 1 min. Acute pretreatment with atorvastatin also decreased thrombin-stimulated GTP loading of RhoA by 46 ± 17% (Fig. 2C, n = 4, p < 0.005), suggesting that depletion of the geranylgeranylated form of RhoA inhibited thrombin-induced RhoA activation.

Inhibition of thrombin-induced GTP loading of RhoA by atorvastatin (Fig. 2D, lane 2, n = 4, p < 0.005) was completely reversed by 5 μmol/liter GGPP (Fig. 2D, lane 3, n = 4, p < 0.005), indicating that this effect of atorvastatin was attributed to the depletion of GGPP. The GTP loading was not affected by the addition of GGPP alone (data not shown). The fact that the effect of atorvastatin on geranylgeranylation of RhoA was already evident after 3 min (Fig. 2A) suggests that the cellular GGPP pool was quite rapidly down-regulated by statin. Thus, taken together these results indicate that thrombin rapidly stimulated geranylgeranylation of the unprocessed form of RhoA.
RhoA as well as GDP/GTP exchange in RhoA and that the cellular level of the geranylgeranylated form of RhoA had a profound influence on thrombin-stimulated GTP loading of RhoA. In addition, atorvastatin antagonized thrombin stimulation of RhoA, at least by inhibiting its geranylgeranylation.

**Stimulation of GGTase I Activity in Response to Thrombin**—We measured the activity of GGTase I, an enzyme responsible for geranylgeranylation of unprocessed Rho, in thrombin-stimulated ECs. Thrombin significantly increased the activity of GGTase I within 1 min, which persisted for at least 5 min (Fig. 3A, n = 5, p < 0.01). This increase in response to thrombin within 1 min was prevented by pretreatment with 3 μmol/liter GGTI-286 for 3 min (Fig. 3B, lane 3, n = 8, p < 0.005). However, atorvastatin did not significantly affect thrombin stimulation of GGTase I activity after 3 min or overnight (Fig. 3B, lanes 5 and 7, n = 8, p < 0.05, each).

**Effects of Thrombin and GGPP on Geranylgeranylation and GTP Loading in ECs Pretreated with Statin Overnight**—To deplete cellular GGPP more completely, ECs were pretreated with 10 μmol/liter atorvastatin overnight. Fig. 4A shows that the percentages of the unprocessed and geranylgeranylated forms of RhoA in ECs pretreated with atorvastatin were 83 ± 12 and 17 ± 12%, respectively (n = 3, p < 0.005). Atorvastatin pretreatment overnight markedly increased the percentages of unprocessed RhoA compared with those of control cells (Figs. 1 and 4A, 83 ± 12 versus 31 ± 8%, p < 0.0001). Moreover, the thrombin-induced increase in the geranylgeranylated form of RhoA was markedly attenuated in ECs by pretreatment with atorvastatin compared with non-pretreated ECs. Namely, thrombin induced a small but significant increase in the geranylgeranylated form of RhoA from 17 ± 12% total RhoA to 36 ± 4% in statin overnight treatment (Fig. 4A, p < 0.005), although almost all of the unprocessed form of RhoA was converted into geranylgeranylated form in control ECs (Fig. 2A).

Importantly, the addition of 5 μmol/liter GGPP to atorvastatin-pretreated ECs markedly increased thrombin-stimulated GTP loading within 1 min compared with thrombin stimulation, as determined by pull-down analysis (Fig. 4B, lanes 3 and 4, n = 3, p < 0.005). Thus, these results provided further evidence that geranylgeranylation of unprocessed RhoA is required for thrombin-induced full activation of RhoA.

**Effect of GGTI-286 on Thrombin-induced RhoA Activation**—Fig. 5A shows that pretreatment of ECs with 3 μmol/liter GGTI-286 for 3 min suppressed thrombin-induced geranylgeranylation of unprocessed RhoA, which was similar to the effect of atorvastatin (Fig. 5A). We examined the effect of GGTI-286 on thrombin-induced GTP loading of RhoA. Pull-down analysis demonstrated that pretreatment with 3 μmol/liter GGTI-286 for 3 min partially inhibited thrombin-induced RhoA activation (Fig. 5B). These observations, together with thrombin stimula-

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**Fig. 2** A, effect of atorvastatin on thrombin-induced geranylgeranylation of unprocessed RhoA. ECs were treated with 10 μmol/liter atorvastatin (ATV) for 3 min before thrombin stimulation for 1 min or 5 μmol/liter GGPP. The levels of unprocessed GDP-RhoA and geranylgeranylated RhoA separated by the Triton X-114 partition method were determined by Western blotting. Bars are the means ± S.D. of quantitative densitometric analyses from four separate experiments. *, p < 0.005. Immunoblots are from an experiment representative of four similar experiments. UP, unprocessed. B, effect of thrombin on geranylgeranylation of unprocessed RhoA. ECs were labeled with 5 μmol/liter [3H]GGPP for 3 min and stimulated with or without thrombin followed by Triton X-114 partition. The radiolabeled band was detected by autoradiography after SDS-PAGE, and Western blotting of RhoA was performed simultaneously. A representative autoradiogram from three separate experiments is shown. C, effect of ATV on GDP/GTP exchange in rapid RhoA activation caused by thrombin. ECs were treated with 10 μmol/liter ATV for 3 min before thrombin stimulation for 1 min. Pull-down assays were performed to determine the levels of the GTP-bound active form of RhoA. GTP-RhoA indicates the GTP-bound active form of RhoA, and Total represents total cellular RhoA. Representative immunoblots from four separate experiments are shown at the top. Bars are the means ± S.D. of quantitative densitometric analyses from four separate experiments. *, p < 0.005. D, effect of GGPP on reduced GTP loading induced by ATV. ECs were treated with 10 μmol/liter ATV for 3 min before thrombin stimulation or with GGPP (5 μmol/liter) for 3 min before ATV plus thrombin. GTP loading was determined by pull-down analysis. Representative immunoblots from four separate experiments are shown at the top. Bars are the means ± S.D. of quantitative densitometric analyses from four separate experiments. *, p < 0.005.
tion of GGTase I activity, indicate that thrombin-induced rapid geranylgeranylation of RhoA is mediated via stimulation of GGTase I and lend further support to the notion that thrombin-induced RhoA activation is dependent on rapid geranylgeranylation of RhoA.

Effect of GGTI-286 and Statin on Thrombin-triggered Assembly of Actin Stress Fibers—As shown in Fig. 6, thrombin reorganized actin stress fibers in ECs within 1 min and this was suppressed by pretreatment with GGTI-286 for 3 min (Fig. 6, B and C). Atorvastatin also had a similar inhibi-
They tested the addition of GGPP to ECs pretreated with atorvastatin overnight. This significantly increased the assembly of actin stress fibers in response to thrombin compared with thrombin stimulation alone (Fig. 6, G and H).

**DISCUSSION**

This study provides evidence that, in response to thrombin, the unprocessed form of RhoA is rapidly geranylgeranylated to become the mature form, which is subsequently converted into GTP-bound RhoA in human aortic ECs.

It has been reported that, in MCF-7 breast cancer cells, 3T3-L1 fibroblasts, and vascular smooth muscle cells, ~35–45% total cellular RhoA is in the geranylgeranylated mature form (17, 30, 32, 33). In Jurkat cells, the majority of RhoA is in the geranylgeranylated form (34). However, little is known regarding the proportions of the unprocessed and geranylgeranylated forms of RhoA in ECs. Therefore, we first determined the amounts of the unprocessed and geranylgeranylated forms of RhoA in cultured human aortic ECs using the Triton X-114 partition method. The cellular geranylgeranylated form of RhoA includes both the inactive GDP-bound and active GTP-bound forms. Our results showed that there was twice as much of the geranylgeranylated form of RhoA (69% total cellular RhoA) than the unprocessed form (31% RhoA). Overnight treatment with atorvastatin induced an increase in the amount of the unprocessed form of RhoA from 31% to 31% (Fig. 4A). This finding is consistent with the report by Liu et al. (34) that treatment with lovastatin for 2.5 days changed the majority of geranylgeranylated RhoA to the ungeranylgeranylated form in Jurkat cells.

We and others (4, 7, 20) demonstrated that thrombin activated RhoA in ECs within 0.5–5 min and increased GEF activity within 1 min in HEK-293T cells (20, 35). Holinstat et al. (20) showed that thrombin phosphorylated p115RhoGEF in human umbilical vein ECs within 1 min. However, they did not determine whether thrombin stimulated the geranylgeranylation of unprocessed RhoA.

We examined the effect of thrombin on geranylgeranylation of the unprocessed form of RhoA as determined by the Triton X-114 partition with labeling ECs with [3H]GGPP and GGTase I assay. Thrombin rapidly stimulated geranylgeranylation of unprocessed RhoA with a concomitant rapid increase in cellular GGTase I activity, resulting in nearly total conversion of the unprocessed form into the geranylgeranylated form of RhoA within 1 min (Figs. 2, A and B, and 3). Thrombin also increased the level of the GTP-bound active form of RhoA within 1 min (Fig. 2C). Conversion of the unprocessed form into the mature geranylgeranylated form was necessary for thrombin-induced full activation of RhoA, because either depletion of GGPP by...
at orvastatin or inhibition of GGTase I with GGTT-286, both of which inhibited thrombin-induced generation of the geranylgeranylated form from the unprocessed form, substantially decreased thrombin-induced GTP loading of RhoA (Figs. 2, A and C, and 5). These results strongly suggest that the unprocessed form of RhoA (GDP-RhoA) is made available for the GDP/GTP exchange reaction through its geranylgeranylation in response to thrombin. This idea was supported by the observation that the replenishment of GGPP in atorvastatin-treated ECs in which cellular GGPP was depleted effectively reversed the response to thrombin with respect to RhoA activation (Fig. 2D). Of particular note, the addition of GGPP to ECs treated with atorvastatin overnight in which GGPP was depleted profoundly dramatically reversed the thrombin stimulation of GTP loading and actin stress fiber formation in a relatively short time (3 min) (Figs. 4B and 6). These observations clearly indicate that thrombin rapidly stimulates geranylgeranylation of the unprocessed immature form of RhoA, resulting in an increase in the amount of the geranylgeranylated form, which is efficiently converted into the GTP-bound active form by the stimulated activity of a GEF.

This study provides information regarding the cellular pools of the unprocessed form of RhoA and GGPP in ECs. Acute treatment with thrombin induced a rapid conversion of almost all of the unprocessed form of RhoA into the geranylgeranylated form in ECs. Depletion of cellular GGPP by a statin and inhibition of GGTase I by GGTT-286 with treatments for short times (3 min) almost completely reversed the conversion. This finding suggests that the cellular pools of unprocessed RhoA and GGPP may be relatively small in ECs.

Previously, we reported that, within 1 min, lysophosphatidylcholine rapidly increased GTP-bound RhoA in membrane fractions in cultured human aortic ECs. This was inhibited by a statin, and GGPP reversed the suppressive effect of a statin on RhoA activation induced by lysophosphatidylcholine (11). Taken together with previous reports (4, 7, 17, 20), our results suggest that, in ECs, receptor agonist-induced rapid activation of unprocessed GDP-RhoA occurs through stimulation of two sequential steps, geranylgeranylation of RhoA and GDP/GTP exchange in RhoA.

Consistent with the rapid conversion of unprocessed RhoA into the geranylgeranylated form in response to thrombin, we observed that thrombin significantly increased the activity of GGTase I in ECs for up to 5 min (Fig. 3A). Overnight treatment with atorvastatin markedly increased the amount of the unprocessed form of RhoA by dramatically depleting GGPP (Fig. 4A). However, atorvastatin did not alter the activity of GGTase I with or without thrombin stimulation compared with control (Fig. 3B), suggesting a negligible effect of endogenous amount of GGPP on GGTase I activity. In addition, Western blotting showed that thrombin did not change the amounts of GGTase I within 5 min compared with control (data not shown). These results suggest that thrombin increased the specific activity of GGTase I, resulting in the alteration of prenylation in ECs.

Our results also demonstrated distinct pathways of GGTT-286 and statin inhibition of geranylgeranylation (Fig. 3). Specifically, the statin depleted the isoprenoid essential for geranylgeranylation, whereas GGTT-286 inhibited the enzyme that catalyzes geranylgeranylation. Begum et al. (17) reported that thrombin promoted GGTase I activity and increased the proportion of geranylgeranylated RhoA by ~15% in smooth muscle cells. However, they did not show the profile of unprocessed and geranylgeranylated forms of RhoA in response to thrombin. In this study, thrombin increased the amount of the geranylgeranylated form of RhoA from 69 to almost 100% (Figs. 1 and 2, A and B). Pretreatment with GGTT-286 for 3 min almost totally prevented the rapid geranylgeranylation and partially inhibited thrombin-induced GTP loading of RhoA, similar to atorvastatin (Fig. 5, A and B). These observations strongly suggest that geranylgeranylation is a rate-limiting step in thrombin-induced activation of unprocessed GDP-RhoA in ECs. We also examined the time course of conversion of unprocessed RhoA into the geranylgeranylated form and GTP loading. After 60 min of thrombin stimulation, the mature geranylgeranylated RhoA remained at a high level, whereas the level of active GTP form of RhoA markedly fell compared with 1 min of stimulation (data not shown). Consistent with the result of the GGTase I assay, these findings suggest that thrombin-induced geranylgeranylation of RhoA is sustained and that the increase in prenylation level may act as a memory of thrombin action. The data also may suggest that there is no significant stimulation of de-prenylating activity, at least within 60 min in response to thrombin in ECs. However, the issue of de-prenylating activity needs to be resolved.

Cytoskeleton analysis clearly demonstrated that acute treatment with thrombin induced the assembly of actin stress fibers and that this was partially reversed by the inhibition of geranylgeranylation with GGTT-286 or atorvastatin for 3 min (Fig. 6), suggesting that rapid activation of unprocessed RhoA may play an important role in the cytoskeletal reorganization, which contributes to endothelial activation.

In conclusion, we provide evidence that unprocessed GDP-RhoA is rapidly activated in response to thrombin in ECs. We suggest that inhibition of this rapid activation may be a strategy for treating endothelial dysfunction in cardiovascular disease.

REFERENCES

1. Coughlin, S. R. (2000) Nature 407, 258–264
2. Sonel, A., Sasseen, B. M., Fineberg, N., Bang, N., and Wilensky, R. L. (2000) Circulation 102, 1107–1113
3. Ardissino, D., Merlini, P. A., Bauer, K. A., Bramucci, E., Ferrari, M., Coppola, R., Feiteveu, R., Lucrezio, S., Rosenberg, R. D., and Mannucci, P. M. (2001) Blood 98, 2726–2729
4. van Nieuw Amerongen, G. P., van Delft, S., Veerme, M. A., Collard, J. G., and van Hinsbergh, V. W. N. (2000) Circ. Res. 87, 335–340
5. Harisson, D. G. (1997) J. Clin. Invest. 100, 2153–2157
6. Laufs, U., and Liao, J. K. (1998) J. Biol. Chem. 273, 24206–24271
7. Eto, M., Barandier, C., Rathgeb, L., Konai, T., Joch, H., Yang, Z., and Luscher, T. F. (2001) Circ. Res. 89, 583–590
8. Qiao, J., Huang, F., and Lum, H. (2003) Am. J. Physiol. 284, L192–L1980
9. Mehta, D., Rahman, A., and Malik, A. B. (2001) J. Biol. Chem. 276, 22614–22620
10. van Aelst, L., and D’Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
11. Hall, A. (1998) Science 279, 509–514
12. Kaito, K., Kuroda, S., and Amano, M. (1999) Ann. Rev. Biochem. 68, 459–486
13. Gosser, Y. Q., Nomanbakh, T. K., Aghazadeh, B., Manor, D., Combs, C., Cerione, R. A., and Rosen, M. K. (1997) Nature 387, 814–819
14. Chant, J., and Stowers, L. (1995) Cell 81, 1–4
15. Golstein, J. L., and Brown, M. S. (1990) Nature 343, 425–430
16. Moures, S. L., Schaber, M. D., Messer, S. D., Rands, E., Ohara, M. B., Garsky, V. M., Marshall, M. S., Pompliano, D. L., and Gibbs, J. B. (1991) J. Biol. Chem. 266, 14603–14610
17. Begum, N., Sandu, O. A., and Duddy, N. (2002) Diabetes 51, 2256–2263
18. Hart, M. J., Jiang, X., Kozasa, T., Rosse, W., Singer, W. D., Gilman, A. G., Sternweis, P. C., and Baltimore, D. (1991) Science 258, 2112–2114
19. Majumdar, M., Seasholtz, T. M., Buckmaster, C., Toksoz, D., and Brown, J. H. (1999) J. Biol. Chem. 274, 26815–26821
20. Holinstat, M., Mehta, D., Konai, T., Minshall, R. D., and Malik, A. B. (2003) J. Biol. Chem. 278, 28793–28798
21. Park, H. J., Kong, D., Iruela-Arispe, L., Bergley, U., Tang, D., and Galper, J. B. (2002) Circ. Res. 91, 143–150
22. Yokoyama, K., Ishibashi, T., Okawara, H., Kimura, J., Matsuoka, I., Sakurada, T., Nagata, K., Sugimoto, K., Sakurada, S., and Maruyama, Y. (2002) Circulation 105, 962–967
23. Wolfrum, S., Jensen, K. S., and Liao, J. K. (2003) Arterioscler. Thromb. Vasc. Biol. 23, 729–736
24. Nagata, K., Ishibashi, T., Sakamoto, T., Nakazato, K., Seino, Y., Yokoyama, K., Okawara, H., Teramoto, T., and Maruyama, Y. (2003) J. Hypertens. 19, 775–783
25. Bordier, C. (1981) J. Biol. Chem. 256, 1604–1607
26. Gutierrez, L., Magree, A. I., Marshall, C. J., and Hancock, J. F. (1989) EMBO J. 8, 1093–1096
27. Ishibashi, T., Sakamoto, T., Okawara, H., Nagata, K., Sugimoto, K., Sakurada, S., Sugimoto, N., Watanabe, A., Yokoyama, K., Sakamoto, N., Kurabayashi, M., Ikuwasa, Y., and Maruyama, Y. (2003) Arterioscler.
Role of Rapid Geranylgeranylation in RhoA Activation

28. Okamoto, H., Takuwa, N., Yokomizo, T., Sugimoto, N., Sakurada, S., Shigematsu, H., and Takuwa, Y. (2000) Mol. Cell. Biol. 20, 9247–9261
29. Ryu, Y., Takuwa, N., Sugimoto, N., Sakurada, S., Usui, S., Okamoto, H., Matsui, O., and Takuwa, Y. (2002) Circ. Res. 90, 325–332
30. Chappell, J., Golovchenko, I., Wall, K., Stjernholm, R., Leitner, J. W., Goalstone, M., and Draznin, B. (2000) J. Biol. Chem. 275, 31792–31797
31. Nebes, C. D., and Hall, A. (1995) Cell 81, 53–62
32. Goalstone, M. L., Leitner, J. W., Berhanu, P., Sharma, P. M., Olefsky, J. M., and Draznin, B. (2001) J. Biol. Chem. 276, 12805–12812
33. Golovchenko, I., Goalstone, M. L., Watson, P., Brownlee, M., and Draznin, B. (2000) Circ. Res. 87, 746–752
34. Liu, L., Moesner, P., Kovach, N. L., Bailey, R., Hamilton, A. D., Sebti, S. M., and Harlan, J. M. (1999) J. Biol. Chem. 274, 33334–33340
35. Chikumi, H., Fukuhara, S., and Gutkind, J. S. (2002) J. Biol. Chem. 277, 12463–12473