Dual Mechanisms of ABCA1 Regulation by Geranylgeranyl Pyrophosphate*

ATP-binding cassette transporter A1 (ABCA1) mediates an active efflux of cholesterol and phospholipids and is mutated in patients with Tangier disease. Expression of ABCA1 may be increased by certain oxysterols such as 22(R)-hydroxycholesterol via activation of the nuclear hormone receptor liver X receptor (LXR). In searching for potential modulators of ABCA1 expression, we have studied the effects of various mevalonate metabolites on the expression of ABCA1 in two human cell lines, THP-1 and Caco-2 cells. Most of the tested metabolites, including mevalonate, geranyl pyrophosphate, farnesyl pyrophosphate, and ubiquinone, failed to significantly change the expression levels of ABCA1. However, treatment with geranylgeranyl pyrophosphate resulted in a dose- and time-dependent reduction of ABCA1 expression. Geranylgeranyl pyrophosphate appears to reduce ABCA1 expression via two different mechanisms. One of these mechanisms is by acting directly as an antagonist of LXR since it reduces the interaction between LXRα or -β with nuclear coactivator SRC-1. Another mechanism appears to involve activation of the Rho GTP-binding proteins since treatment of Caco-2 cells with inhibitors of geranylgeranyl transferase or the Rho proteins significantly increased the expression and promoter activity of ABCA1. Further studies showed that mutations in the DR4 element of the ABCA1 promoter completely eliminate the inducible activities of these inhibitors. These data indicate that activation of the Rho proteins may change the activation status of LXR.

Plasma concentration of high density lipoprotein cholesterol is inversely related to the incidence of coronary heart disease (1, 2). Our understanding of the mechanisms that regulate HDL1 cholesterol has received a major advance with the elucidation of the cause of Tangier disease (TD). Patients with TD are characterized by near or complete absence of circulating HDL and by the accumulation of cholesteryl esters in many peripheral tissues (3, 4). Recently three groups independently reported identification of the ATP-binding cassette transporter A1 (ABCA1) as the defective gene responsible for TD (5–7). ABCA1 is a member of the ATP-binding cassette superfamily. These proteins couple the energy provided by ATP hydrolysis to the transport of a wide variety of molecules across membranes (8–11). ABCA1 is thought to mediate the active efflux of cholesterol and phospholipids to apolipoprotein (apo) acceptors, most importantly apoA-I, the major apo of HDL (12, 13). Due to mutations, however, the function of ABCA1 in patients with TD is impaired. Therefore, cellular cholesterol efflux in TD patients is defective, which leads to accumulation of excess cellular cholesterol and defective formation of HDL (14–16).

ABCA1 is widely expressed and is particularly abundant in monocytes and macrophages (17). Studies in macrophages have shown that the expression of ABCA1 is sterol-dependent (17). Expression of ABCA1 is up-regulated by modified low density lipoprotein and down-regulated by HDL (17, 18). The mevalonate pathway supplies substrate for the sequence of reactions leading to the formation of polyisoprenoid lipids such as ubiquinone, cholesterol, and oxysterols (for review, see Ref. 19). Recently it was found that expression of ABCA1 may be up-regulated by certain oxysterols including 22(R)-hydroxycholesterol (22(R)-Hch) and 20(S)-Hch. Further studies demonstrated that such induction of ABCA1 may be mediated by the activation of the nuclear hormone receptor LXR (20, 21). Understanding the regulation of ABCA1 expression is a critical step toward a better comprehension of its role in lipid metabolism. We have thus decided to search for additional endogenous modulators that may regulate the expression of ABCA1. We found that geranylgeranyl pyrophosphate (GGPP), one of the major products of the mevalonate pathway, potently suppressed ABCA1 expression. Furthermore, we demonstrated that GGPP may regulate ABCA1 expression via two different mechanisms.

MATERIALS AND METHODS

Cell Culture—THP-1 cells, a human monocytic leukemia cell line, and Caco-2 cells, a human intestinal epithelial cell line, were obtained from the American Type Culture Collection (Manassas, VA). Cells were maintained in RPMI 1640 (THP-1 cells) or OPTI-MEM medium (Caco-2 cells) with 10% heat-inactivated fetal calf serum, 50 units/ml penicillin G, 50 μg/ml streptomycin sulfate in an atmosphere containing 5% CO2 and 95% air. Before assay, cultured cells were harvested, washed once with phosphate-buffered saline, and then resuspended in the assay medium (RPMI 1640 or OPTI-MEM medium with 0.5% heat inactivated fetal calf serum, 50 units/ml penicillin G, and 50 μg/ml streptomycin sulfate). All reagents used in the experiments were diluted in the same assay medium.

RNA Isolation—Total RNA was extracted from the cultured cells using TRIZOL reagent according to the protocol provided by the manufacturer (Life Technologies, Inc.). RNA was then treated with DNase I.
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(Ambion, Austin, TX) for 30 min at 37 °C. DNase I was inactivated and removed from the reaction with the addition of DNase inactivation reagent (Ambion). RNA concentrations were determined spectrophotometrically at A260 and A230 using a DU 640 Spectrophotometer (Beckman).

**Primers and Fluorogenic Probes—Oligonucleotide primers and TaqMan probes for human ABCA1 were designed using Primer Express software (PerkinElmer Life Sciences) and were synthesized by PerkinElmer Life Sciences. Primer and probe sequences (5'–3') are as follows: ABCA1 forward primer, GTGCCAGTCGTAGTTGTTGTG; ABCA1 reverse primer, AAGGGAGATGTCGCGATT; ABCA1 probe, 6FAM-ACACTTGAGAGAACTCTTTACACCGAGTACTCCG-TAMRA; ABCG1 (ABC8) forward primer, TCGAGCTTCTGCGCATATTTGA; ABCG1 reverse primer, CCAGGCAGCTTCTGTAGTCA; ABCG1 probe, 6FAM-TACCCACACCACTCAGATTTGTCATGGA-TAMRA. Primers and probes for human IL-8 and glyceraldehyde-3-phosphate dehydrogenase were purchased from PerkinElmer Life Sciences.

**Real-time Quantitative PCR—**Real-time quantitative TaqMan PCR analysis (22) was used to determine the relative levels of ABCA1, ABCG1, and IL-8 mRNA. Reverse transcription-PCR and TaqMan PCR reactions were performed according to the instructions of the manufacturer (PE Biosystems, TaqMan Gold reverse transcription-PCR protocol and TaqMan Universal PCR Master Mix). Sequence-specific amplification was detected with an increased fluorescent signal of FAM (reporter dye) during the amplification cycle. Amplification of the gene for human glyceraldehyde-3-phosphate dehydrogenase was performed in the same reaction on all samples tested as an internal control for variations in RNA amounts. Levels of the different mRNAs were subsequently normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA levels and were presented as fold difference of treated cells against untreated cells.

**Costimulator Association Assays—**A homogeneous time-resolved fluorescent assay-based nuclear receptor coactivator assay was used to examine the interaction of LXR with GGPP. A complete description of this assay has been published elsewhere (23). Briefly, 198 μl of reaction mixture (100 mM HEPES, 125 mM KF, 0.125% (w/v) CHAPS, 0.05% dry milk, 5 mM GST-LXRα ligand binding domain (LBD) or 5 mM GST-LXRβLBD, 2 mM anti-GST-(Eu/IK, 10 mM biotin-SRC-(568-568), 20 mM SAXL665) were added to each well of a 96-well plate followed by the addition of 2 μl of methanol or GGPP or GPP (in methanol) in appropriate wells in the presence or absence of 500 nM 22(R)-Hch (about 1.5 × EC₅₀). Plates were mixed by hand and covered with TapSeal. The reaction was incubated overnight at 4 °C followed by measurement of a fluorescence reading on a Discovery instrument (Packard Instrument Co.). Data were expressed as the ratio, multiplied by a factor of 10⁴, of fluorescence reading on a Discovery instrument (Packard Instrument Co.).

**DNA Transfection and Reporter Gene Assays—**Transfections were performed in 96-well plates with FuGENE 6 reagent according to the instructions of the manufacturer (Roche Diagnostics Corp.). Caco-2 cells were maintained in complete OPTI-MEM medium containing 10% heat inactivated fetal calf serum, 50 units/ml penicillin G, and 50 μg/ml streptomycin sulfate. Immediately prior to transfection, the complete medium was replaced by serum and antibiotic-free medium to a concentration of 5 × 10⁵ cells/ml, and 200 μl were added per well of 96-well plate. The constructs ABCA1-Luc or DR4Mut1-Luc were cotransfected in a 4:1 ratio with a control reporter plasmid, pRL-TK (Renilla luciferase, Promega, Inc.), in the concentrations recommended by the manufacturer to maintain a FuGENE to DNA ratio of 3:1. Five hours after transfection, cell treatments with compound were initiated, and treatments were stopped at 48 h from the beginning of transfection. Cells were harvested using Passive Lysis Buffer and assayed for relative luciferase activities with the dual-luciferase reporter assay system purchased from Promega and read with a luminometer (Dynex Technologies, Chantilly, VA). All transfections were performed in triplicate.

**RESULTS**

GGPP Reduces Expression of ABCA1—Consistent with previous reports (12), THP-1 and Caco-2 cells expressed readily detectable levels of ABCA1 mRNA. When cells were exposed to the LXR ligand 22(R)-Hch, a marked increase of ABCA1 mRNA was observed (data not shown). Overnight (16 h) treatment of cells with most products from the mevalonate pathway including mevalonate, farnesyl pyrophosphate, GPP, and ubiquinone failed to significantly change the expression levels of ABCA1 mRNA. Addition of GGPP, however, significantly reduced the expression of ABCA1 (Fig. 1). The inhibitory effect of GGPP on ABCA1 expression was dose-dependent (Fig. 2). In THP-1 cells, an apparent reduction was detected at concentrations as low as 0.3 μM GGPP. At 10 μM, GGPP reduced ABCA1 expression by greater than 80%. The concentration needed for a half-maximal inhibition was ~2 μM (Fig. 2). In the same experiment, we found that GGPP also inhibited the expression of ABCG1 (ABC8) with a similar potency (Fig. 2A). ABCG1, another member of the ABC transporter family, was recently shown to be induced by cholesterol loading and activation of LXR (24, 25). Our results suggest that ABCA1 and ABCG1 may be subjected to similar mechanisms of regulation by GGPP. An additional study showed that GGPP also had a similar but less potent inhibitory effect for ABCA1 expression in Caco-2 cells (Fig. 2B). The GGPP-mediated reduction of ABCA1 or ABCG1 expression apparently is not due to cell toxicity since parallel studies showed that GGPP fails to significantly change the ability of cells harvested using Passive Lysis Buffer and assayed for relative luciferase activities with the dual-luciferase reporter assay system purchased from Promega and read with a luminometer (Dynex Technologies, Chantilly, VA). All transfections were performed in triplicate.

**FIG. 1.** Effect of mevalonate metabolites on expression of ABCA1 in THP-1 cells. THP-1 cells (5 × 10⁵ cells/well) were plated in six-well plates and mixed with the indicated metabolites (mevalonate, 100 μM; farnesyl pyrophosphate (FPP), 15 μM; GPP, 15 μM; GGPP, 10 μM; ubiquinone, 50 μM). After overnight incubation (~16 h) at 37 °C, RNA samples were prepared from the cultured cells, and mRNA for ABCA1 was measured as described under “Materials and Methods.” Results are described as fold of control (untreated cells), and data are shown as the means ± S.D. of duplicate determinations.
An additional study showed that the inhibitory effect of GGPP on ABCA1 expression was dependent upon the duration of treatment (Fig. 3A). At 3.5 h, 10 μM GGPP reduced expression of ABCA1 in THP-1 cells by about 40%. Expression of ABCA1 was further reduced as the incubation proceeded, and it reached maximal reduction levels at about 16 h. Results from a similar time course study demonstrated an apparently more rapid kinetic effect of 22(R)-Hch on ABCA1 expression (Fig. 3B). Maximal increase of ABCA1 expression was observed at 3.5 h postexposure to 22(R)-Hch.

**GGPP Suppresses Interaction of LXRα and LXRβ with Nuclear Coactivators**—Induction of ABCA1 by certain oxysterols including 22(R)-Hch may be mediated by the activation of LXR (20, 21). Recently, Forman et al. (28) reported that GGPP may reduce transactivation of LXRα. We thus questioned whether GGPP would reduce the expression of ABCA1 by acting as an antagonist of LXR. Because ligand-dependent interactions between nuclear receptors and members of a family of nuclear receptor coactivators are associated with transcriptional activation (29, 30), we thus asked whether GGPP would prevent the interaction of LXRs or -β with nuclear coactivators. Interaction of LXRs or -β with the nuclear coactivator steroid receptor coactivator-1 (SRC-1) was quantified using the fluorescence resonance energy transfer approach as described under “Materials and Methods.” As shown in Fig. 4, addition of GGPP effectively reduced the basal and LXR ligand 22(R)-Hch-induced interaction of LXRs and LXRβ with SRC-1. The GGPP concentration needed for a half-maximal inhibition of LXRs and LXRβ is about 0.5 and 0.7 μM under basal condition and 0.7 and 0.9 μM in the presence of 0.5 μM of 22(R)-Hch, respectively. In the same study, we found that another mevalonate metabolite, GPP, failed to significantly affect the interaction of LXRs and LXRβ with SRC-1 (Fig. 4). In a similar study and using conditions essentially identical to those for LXR as described above, we found that GGPP at the same concentrations failed to significantly affect the interaction of peroxisome proliferator-activated receptors with nuclear coactivator SRC-1 or cAMP-response element-binding protein (CREB)-binding protein (data not shown, see Ref. 23 for assay details). These observations thus establish a direct and specific effect of GGPP on LXRs and LXRβ.

**Partial Reduction of GGPP on LXR Agonist-mediated Induction of ABCA1**—To ask whether the inhibitory effect of GGPP on LXR accounts for its inhibitory effect on ABCA1 as described above, cells were treated with increasing concentrations of GGPP in the presence of a fixed amount of LXR ligand 22(R)-Hch (Fig. 5). As concentrations of GGPP increased, expression of ABCA1 induced by the treatment with 22(R)-Hch decreased. However, a further increase of GGPP concentration failed to mediate additional reduction of ABCA1 expression. These observations suggest that GGPP may not compete for the same binding site as that of 22(R)-Hch. It also suggests potential involvement of an additional mechanism for regulating the expression of ABCA1.

**Inhibition of Geranylgeranylation Increases Expression of ABCA1**—Since GGPP may be used for posttranslational modification of signaling molecules (31), we questioned whether GGPP would reduce expression of ABCA1 through a mechanism involving prenylation of the signaling molecules. We...
tested a potential prenylation effect of GGPP on ABCA1 in Caco-2 cells by using specific inhibitors of geranylgeranyl transferase, L-839,867 and L-836,978 (32). Geranylgeranyl transferase catalyzes the transfer of the geranylgeranyl group from GGPP to various signaling molecules such as the Rho proteins (31). As shown in Fig. 6A, we found that treatment of Caco-2 cells with L-839,867 led to a dose-dependent increase of ABCA1 expression. Prenylation of signaling molecules may also be catalyzed by farnesyl transferase, an enzyme that transfers a farnesyl group from farnesylpyrophosphate (31). Consistent with a negligible effect of farnesyl pyrophosphate on ABCA1 (see Fig. 1), we found that addition of α-hydroxyfarnesyl phosphonic acid, a specific inhibitor of farnesyl transferase, failed to significantly change the expression of ABCA1 (Fig. 6B).

Among the isoprenylated proteins, the Rho and the Rab proteins are known to be the most extensively geranylgeranylated (33). To determine whether the effects of GGPP or L-839,867 on ABCA1 expression may be related with their influence on the prenylation of Rho proteins, ABCA1 expression was measured in the presence of C3 exoenzyme, a specific inhibitor of Rho proteins (34). As shown in Fig. 6B, addition of

**Fig. 4. Effect of GGPP on interaction of LXRα or LXRβ with nuclear coactivator SRC-1.** 5 nM purified GST-LXRαLBD (A and B) or LXRβLBD (C and D) were incubated with 2 nM anti-GST-(EeuK, 10 nM biotin-SRC-(568–680), 20 nM SA/XL665, and various concentrations of GPP (C) or GGPP (A) in the presence (B and D) or absence (A and C) of 500 nM 22(R)-hydroxycholesterol. After overnight incubation at 4 °C, interaction of LXRα or LXRβ with SRC-1 was analyzed as described under “Materials and Methods.” Data are expressed as the ratio, multiplied by a factor of 104, of the emission intensity at 665 nm to that at 620 nm. Each value represents the mean ± S.D. of three determinations. Similar results were obtained in additional experiments.
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Inhibition of Geranylgeranylation Increases Promoter Activity of ABCA1—To further delineate a mechanism that underlies prenylation-mediated regulation of ABCA1, we prepared human ABCA1 promoter-luciferase constructs (−928 to +101 bp) with or without mutation in the LXR response element DR4 and transected them into Caco-2 cells. Consistent with earlier reports (20, 21), addition of 22(R)-hydroxycholesterol significantly increased promoter activity of ABCA1 (Fig. 7A). Mutation of the DR4 element from the promoter of ABCA1 completely eliminated the inducible activity of 22(R)-hydroxycholesterol, thus confirming an LXR-dependent regulation of ABCA1 transcription by 22(R)-hydroxycholesterol (Fig. 7A). We next studied the role of geranylgeranylation on the promoter activity of ABCA1 by using the aforementioned specific inhibitors of geranylgeranyl transferase or Rho proteins. As shown in Fig. 7, B and C, addition of L-839,867 or C3 exoenzyme resulted in a significant increase of the ABCA1 promoter activity. Further studies showed that mutation of the DR4 element from the promoter of ABCA1 completely eliminated the inducible activity of these inhibitors (Fig. 7, B and C). These data indicate that a prenylation-mediated ABCA1 regulation also involves activation of LXR.

DISCUSSION

In view of the potential importance of ABCA1 in the regulation of HDL cholesterol, we studied the molecular mechanisms that regulate expression of the ABCA1 gene. Consistent with earlier reports (20, 21), we show that ABCA1 expression is effectively up-regulated by 22(R)-hydroxycholesterol, a product of the mevalonate biosynthetic pathway. Furthermore, we report an interesting finding that expression of ABCA1 is potently down-regulated by a distinct product of the mevalonate pathway, GGPP. Our data suggest the presence of endogenous machinery that may counterbalance the expression level of ABCA1. Since both the positive regulator, such as 22(R)-hydroxycholesterol, and the negative regulator, GGPP, are important products of the cholesterol biosynthesis pathway, these data support an important role of ABCA1 in cholesterol homeostasis.

Expression of ABCA1 may be regulated by the activation of LXR. This is attributed to the presence of a unique DR4 element in the ABCA1 promoter. Upon activation of LXR, such as with the ligand 22(R)-Hch, LXR forms a heterodimer with another nuclear receptor, RXR, and binds to the DR4 element, mediating transactivation of the ABCA1 gene (20, 21). Results from our study indicate that at least part of the inhibitory effect of GGPP on ABCA1 expression may occur via direct inhibition of LXR. We showed that addition of GGPP inhibited the interaction of LXRα and LXRβ with nuclear coactivator SRC-1 (Fig. 4). Interestingly we found that GGPP was only partially capable of inhibiting the inducible effect of LXR ligand 22(R)-Hch on ABCA1 mRNA expression (Fig. 5), suggesting that the binding site for GGPP and 22(R)-Hch on LXR may be different. In support of our view, our preliminary experiments suggest that GGPP fails to displace the binding of a synthetic LXR ligand from LXRα or -β.2 Recently Forman et al. (28) reported that GGPP may reduce the transactivation of LXRα. It was found that upon GGPP binding to LXRα the binding of LXRα-RXR complex to DNA was inhibited. These observations are thus fully consistent with findings reported here. While detailed mechanism(s) by which GGPP inhibits LXR remains unclear, together our data suggest that one of the mechanisms used by GGPP to suppress the expression of ABCA1 is by acting directly as an antagonist of LXR. Apparently such antagonizing activity is equally potent to LXRα and LXRβ (Fig. 4).

C3 exoenzyme (4 μg/ml) significantly increased ABCA1 expression. These data thus suggest that prenylation of Rho proteins suppresses ABCA1 expression and that inhibition of Rho with geranylgeranyl transferase inhibitors or C3 exoenzyme enhances ABCA1 expression. The ability of GGPP to suppress ABCA1 expression may thus derive, in part, from enhanced prenylation and function of Rho.

Fig. 5. Effect of GGPP on LXR agonist-mediated expression of ABCA1. THP-1 cells (5 × 10⁵ cells/well) were plated in six-well plates and mixed with 22(R)-hydroxycholesterol (5 μM) and increasing concentrations of GGPP. After overnight incubation (~16 h) at 37 °C, RNA samples were prepared from the cultured cells, and mRNA for ABCA1 was measured as described under “Materials and Methods.”

Fig. 6. Effect of prenylation inhibitors on expression of ABCA1 in Caco-2 cells. Caco-2 cells (5 × 10⁵ cells/well) were plated in six-well plates and mixed with increasing concentrations of L-839,867 (A) and buffer, L-839,867 (1 μM), α-hydroxyfarnesyl phosphonic acid (HFP A) (6 μM), and C3 exoenzyme (4 μg/ml) (B). After overnight incubation (~16 h) at 37 °C, RNA samples were prepared from the cultured cells, and mRNA for ABCA1 was measured as described under “Materials and Methods.”

K. MacNaul and T. Q. Cai, unpublished observation.
Experiments were performed.

Three to four independent experiments were performed. Luciferase activities, and data are shown as the means ± S.D. of triplicates of a representative experiment. The resulting plasmid was cotransfected with a control reporter plasmid (Renilla luciferase) in Caco-2 cells. Cells were treated with 22(R)- or 22(S)-hydroxycholesterol (10 μM) (A), increasing concentrations of C3 exoenzyme (C). After 48 h, cells were lysed, and activities for relative luciferase were measured. Results are expressed as a ratio between the firefly and Renilla luciferase activities, and data are shown as the means ± S.D. of triplicates of a representative experiment. Three to four independent experiments were performed.

Interestingly, results from our additional studies indicate that this may not be the only mechanism used by GGPP to regulate ABCA1 expression. While inhibition of geranylgeranyl transferase may potentially increase the intracellular concentration of its substrate, GGPP, thus triggering an LXR-dependent down-regulation of ABCA1 as described above, we nevertheless found that expression and promoter activity of ABCA1 was increased by the treatment with L-839,867, a potent and specific inhibitor of geranylgeranyl transferase (Figs. 6 and 7). These data suggest that a prenylated protein serves as a negative regulator of ABCA1 expression.

The geranylgeranyl groups may be transferred to specific sequences at the carboxyl terminus of many proteins. Two geranylgeranyl transferases, types I and II, have been identified. Type I is a cytosolic enzyme and types I and II, have been identified. While type I requires a carboxyl-terminal sequence of -Cys-Ala-Ala-X, the type II enzyme requires a double Cys motif, such as -Cys-Cys-, -Cys-X-Cys-, or -Cys-Cys-X-X- (for review, see Ref. 31). Thus, it is unlikely that either LXR or RXR can be modified in this fashion since both LXR and RXR receptors lack a consensus sequence for protein prenyl transferase (LXRα and LXRβ: Trp-Asp-His-Glu-COOH; RXR: Pro-His-Gln-Met-Thr-COOH). Therefore, we have turned our attention to the small GTP proteins. Among the isoprenylated proteins, the Rho and the Rab proteins are major substrates for post-translational modification by geranylgeranyl transferase (33). Upon geranylgeranylation, these signaling molecules are activated, and they go on to play an important role in a number of functional processes such as the regulation of membrane traffic, exocytic and endocytic transport processes (35), actin stress fiber formation, focal adhesion assembly, and reorganization of the actin cytoskeleton (36, 37). Consistent with an inducible effect of the geranylgeranyl transferase inhibitor, we showed that the treatment of cells with C3 exoenzyme, a specific inhibitor of the Rho proteins, also increased the expression and promoter activity of ABCA1 (Figs. 6 and 7). Furthermore, we showed that mutation of the functional DR4 element completely diminishes activity of the ABCA1 promoter induced by inhibitors of geranylgeranyl transferase or Rho proteins (Figs. 7, B and C). These data indicate that regulation of ABCA1 expression by Rho proteins is mediated through LXR.

It is unclear how activation of the Rho proteins may change the activation status of LXR. The Rho proteins have been shown to regulate the c-Jun NH2-terminal kinase and the p38 mitogen-activated protein kinase cascades (38–40). Because protein kinases are involved in the phosphorylation of transcription factors such as RXR (41), it is conceivable that there may be cross-talk between Rho protein-regulated kinase signaling cascades and LXR activation. As mentioned previously, the Rho proteins play an important role in a number of functional processes such as vesicular transport. Thus, it may also be possible that the Rho proteins could potentially regulate the activation status of LXR by changing trafficking and distribution of an endogenous pool of cholesterol or ligand of LXR.

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![Fig. 7. Effect of prenylation inhibitors on promoter activity of ABCA1 in Caco-2 cells.](image-url)
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