Activation of Cyclin-dependent Kinase 2 (Cdk2) in Growth-stimulated Rat Astrocytes

GERANYLGEBERANYLATED Rho SMALL GTPase(s) ARE ESSENTIAL FOR THE INDUCTION OF CYCLIN E GENE EXPRESSION

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The role of the mevalonate cascade in the control of cell cycle progression in astrocytes has been investigated. Serum stimulation of rat astrocytes in primary culture induces the expression of cyclin E followed by the activation of cyclin-dependent kinase 2 (Cdk2) during G1/S transition. The expression of p27kip1, cyclin D1, and the activities of Cdk4 and Cdk-activating kinase (CAK), composed of Cdk7 and cyclin H, were not affected. Serum did, however, stimulate the expression of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase mRNA at mid-G1 phase. Moreover, an inhibitor of HMG-CoA reductase, pravastatin, reduced cyclin E expression and Cdk2 activation and caused G1 arrest in the astrocytes. In contrast, mevalonate and its metabolite, geranylgeranylated and translocated to membranes in the presence of GGPP during G1/S transition. The effect of GGPP on cyclin E expression was abolished by botulinum C3 exoenzyme, which specifically inactuates Rho. These data indicate that geranylgeranylated Rho small GTPase(s) are essential for the induction of cyclin E expression, Cdk2 activation, and G1/S transition in rat astrocytes.

The central nervous system is a complicated organ that consists of interacting neuronal and glial cells. Examination of the control of cell proliferation in neural tissue during development and maturation is particularly challenging because of the complex and multifactorial nature of the interactions between these cells. Although much is known about the molecular basis by cell cycle progression, little is known about cell cycle regulation in neural tissue during maturation. Since proliferation of glial cells is critical in brain development and in the repair of the damaged brain after injury (1, 2), we initially focused our studies on the control of cell cycle progression in glial cells only.

Cell cycle progression and the proliferation of eukaryotic cells are controlled by the sequential activation of cyclin-dependent kinases (Cdks)1 (3). Cdk activation is dependent on the formation of cyclin-Cdk complexes (4–6), the phosphorylation of Thr-160 mediated by Cdk-activating kinase (CAK), and the dephosphorylation of Tyr-15 by cdc25 in the case of Cdk2 (7–12). Cdk inhibitors such as Ink4 and Cip/Kip family proteins play roles as negative regulators of the cell cycle progression (13). Isoprenoid lipids produced by HMG-CoA reductase appear to control this activation process in neural tissue and glial cells. Thus, developing brain demonstrates extremely active synthesis of isoprenoid lipids, with this synthetic capacity confined to glial cells (14–17). There is a close temporal correspondence of DNA synthesis, isoprenoid synthesis, brain growth, and increases in glial cell number (1, 17). The inhibition of HMG-CoA reductase, the rate-limiting step for isoprenoid production, induces G1 arrest in glial cells (18–21), whereas the addition of mevalonate to the culture medium is sufficient to reverse the inhibition of cell cycle progression in glial cells (18–21); mevalonate is the common precursor of isoprenoid compounds. This mevalonate requirement is cell cycle phase-specific and located in mid to late G1 phase (20, 21).

Recently, we and other investigators (22, 23) demonstrated that among isoprenoid lipids, geranylgeranylpyrophosphatase (GGPP), not farnesylpyrophosphatase (FPP), plays an essential role in G1/S transition of proliferating cells. Moreover, a class of geranylgeranylated small GTP-binding proteins, termed Rho small GTPases, are proposed to be involved in the transition from G1 to S phase in mouse fibroblasts and rat thyroid FRTL-5 cells (22, 24). The present experiments were designed to obtain further insight into the relationship between the control of cell cycle regulators and the requirement of isoprenoids for cell cycle progression in glial cells and neural tissue. We used primary cultures of rodent astrocytes to define a system for investigation of the following questions. 1) Which cell cycle regulator(s) of the G1 phase are under control of the isoprenoids in glial cells? 2) Is protein isoprenylation involved in the control

1 The abbreviations used are: Cdk, cyclin-dependent kinase; GGPP, geranylgeranylpyrophosphate; FPP, farnesylpyrophosphate; CAK, Cdk-activating kinase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.
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of cell cycle regulator(s) that govern the G1/S transition of gial cells?

EXPERIMENTAL PROCEDURES

Materials—[3H]Geranylgeranylpiphosphorionate ([3H]GGPP) and [3H]farnesyl-pyrophosphorionate ([3H]FPP) were purchased from NEN Life Science Products. Geranylgeranylpiphosphorionate (GGPP) and farnesyl-pyrophosphorionate (FPP) were purchased from Sigma. Antibodies against cyclin D1, Cdk4, cyclin H, Cdk7, cyclin E, Cdk2, p27kip1, Rho A, Rho B were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-Ras (NCC-RAS-004) antibody was kindly provided from Dr. S. Hirohashi (National Cancer Center Research Institute, Tokyo, Japan) (29). Horseradish-linked anti-rabbit immunoglobulin goat antibody, enhanced chemiluminescence (ECL) detection reagent, random primer DNA labeling kit, [α-32P]dUTP, and [3H]thymidine were purchased from Amersham Corp. (Buckinghamshire, UK).

Preparation of Liposomes of Isoprenoids—To make liposomes containing each, an aliquot of a mixture of dipalmitoylphosphatidylcholine (5 μmol) and GGPP or FPP (200 μg) was added to a pear-shaped flask, and the solvent was removed by rotary evaporation and a vacuum pump. The dried lipid film was then dispersed in 0.5 ml of phosphate-buffered saline. Warming the flask to 50 °C facilitates smooth dispersion. The liposomes were sonicated and stored at 4 °C.

Cell Culture—Gial primary cultures were prepared as described previously (25). Brains were removed from 1-day-old SD rats obtained from timed pregnant females (Charles River Breeding Laboratories, Tokyo, Japan). The brain cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% horse serum, 2.5% fetal bovine serum, and 1% antibiotic/antimycotic in 100-mm diameter tissue culture dishes (Corning, Houston, TX) coated with 10 μg/ml of poly-L-lysine. On the 6th day, cultured cells were split and transferred to new 100-mm diameter culture dishes. In another 8 days, the population of astrocytes was more than 95% of the total cells as determined by staining with an antibody against glial fibrillary acid protein, a highly specific marker for gial cells of the astrocytic lineage (26, 27). Purified astrocytes were then cultured with serum-depleted medium Dulbecco’s modified Eagle’s medium (Corning) for 24 h, and then decreased (Fig. 1A). Serum stimulation, with cyclin E-I and cyclin E-s was increased by serum stimulation, with cyclin E-I being the dominant form. Cdk2 protein was detected as a single 34-kDa band in quiescent astrocytes. After serum stimulation, the expression of phosphorylated Cdk2 protein, which migrates as a single major 33-kDa band, was detected at 12 h in mid-G1, reached a maximum at 24 h, and then decreased (Fig. 1A).

The H1 histone kinase activity associated with Cdk2 was also detected at 12 h and reached a peak at 24 h (Fig. 1A). In contrast, the Cdk4 and CAK activities did not change after serum stimulation. p27 was detected in quiescent astrocytes, and serum stimulation did not influence the expression of p27 (Fig. 1A).

Northern blot analysis revealed that serum stimulation increased the expression of both cyclin E and Cdk2 transcripts at 12 h; their levels peaked at 24 h (Fig. 1B). The level of p27 mRNA did not, in contrast, change during the cell cycle progression from G1 to S phase (Fig. 1B).

The time course of thymidine incorporation and the induction of HMG-CoA reductase mRNA in serum-stimulated astrocytes are shown in Fig. 2. DNA synthesis began to increase 12 h after serum stimulation, reached a peak at 24 h, and gradually decreased thereafter (Fig. 2). The mRNA level of HMG-CoA reductase may play a role in activating the mevalonate cascade during G1/S transition in proliferating rat astrocytes.

The Effect of Pravastatin and Isoprenoids on Cell Cycle Progression and DNA Synthesis in Serum-stimulated Astrocytes—Pravastatin caused a dose-dependent suppression of DNA synthesis at 24 h in serum-stimulated astrocytes. More than 90% inhibition was observed at 1800 μM (Fig. 3A). The dose-dependent inhibition of DNA synthesis by pravastatin in rat astrocytes was very similar to that observed in both human peripheral lymphocytes and rat FRTL-5 cells (22, 23). The presence of pravastatin during the first 8 h of culture did not affect DNA synthesis in serum-stimulated astrocytes; however, the contin-
ued presence of pravastatin beyond 8 h inhibited DNA synthesis (data not shown). This inhibition was reversed by the addition of mevalonate also in a dose-dependent manner and was fully reversed at 0.03 mg/ml (Fig. 3B). Mevalonate fully reversed the pravastatin-induced inhibition only when it was added during 8–16 h in serum-stimulated astrocytes (data not shown). Mevalonate reversed the pravastatin-induced G1 arrest in the absence of upstream intermediates of cholesterol biosynthesis.

The inhibition by pravastatin on DNA synthesis was reversed by the addition of liposomes containing GGPP in a dose-dependent manner (data not shown). In contrast, liposomes containing FPP or liposomes containing vehicle did not affect the pravastatin-induced inhibition (Table I). The addition of GGPP (4 μM), but not FPP and vehicle, also reversed the pravastatin-induced G1 arrest (Table I). The inability of FPP to overcome pravastatin blockage did not reflect impaired entry into the cells from the liposomes, since incorporation of [3H]FPP into astrocytes was almost equal to that of [3H]GGPP (data not shown). Since GGPP is biosynthetically derived from the single condensation of FPP and isopentenyl-pyrophosphate, FPP could not be converted to GGPP in pravastatin-treated cells. These results, therefore, indicate that GGPP can reverse the pravastatin-induced G1 arrest in the absence of upstream intermediates of cholesterol biosynthesis.

Effect of Pravastatin and Isoprenoids on the Expression of Cell Cycle Regulators in Serum-stimulated Astrocytes—Pravastatin at the concentration of 1800 μM, which caused G1 arrest, suppressed the expressions of cyclin E in serum-stimulated astrocytes (Fig. 4A), whereas the expression of cyclin D1, Cdk4, cyclin H, Cdk7, and p27 was not affected. The expression of the phosphorylated form of Cdk2 and the kinase activity associated with Cdk2 was also inhibited by pravastatin as shown in Fig. 4A. Activities of Cdk4 and CAK were not affected by pravastatin.

Pravastatin-inhibited expression of cyclin E was restored by the addition of mevalonate and GGPP, not by FPP, whereas the expression of cyclin D1, Cdk4, cyclin H, Cdk7, and p27 was not affected. Mevalonate and GGPP reversed the inhibitory effect
of pravastatin on the expression of the phosphorylated form of Cdk2 and the kinase activity associated with Cdk2 (Fig. 3A).

In Northern blotting, pravastatin inhibited the transcription of both cyclin E and Cdk2 mRNA but did not influence the mRNA level of p27 in serum-stimulated astrocytes (Fig. 4B).

Table I

| Requirement of GGPP, not FPP, for thymidine incorporation into DNA and G1/S transition in rat cultured astrocytes |
|---|---|
| Thymidine incorporation into DNA | G1/S transition |
| dpm | % S phase |
| Control | 1592.1 ± 339.7 | 3.4 ± 0.5 |
| 10% serum | 13,371.2 ± 691.8 | 14.5 ± 2.8 |
| 10% serum + pravastatin (SP) | 1956.7 ± 346.3 | 3.5 ± 0.6 |
| SP + mevalonate | 12,901.8 ± 597.6 | 13.9 ± 1.2 |
| SP + GGPP | 10,931.4 ± 609.42 | 12.4 ± 1.8 |
| SP + FPP | 3745.5 ± 429.8 | 4.1 ± 0.9 |

Fig. 3. Effect of pravastatin on DNA synthesis in serum-stimulated rat astrocytes. A, dose-dependent effect of pravastatin on DNA synthesis by serum-stimulated astrocytes. Quiescent astrocytes were stimulated by serum for 24 h in the presence of the indicated concentrations of pravastatin. DNA synthesis of cultured astrocytes was determined as described under “Experimental Procedures.” Each histogram represents the mean ± S.D. of three independent experiments. B, dose-dependent effect of mevalonate to reverse the pravastatin-induced inhibition of DNA synthesis. Quiescent astrocytes were stimulated by serum for 24 h in the presence of 1800 μM pravastatin and with the indicated concentrations of mevalonate.

Fig. 4. Effect of pravastatin, mevalonate, and mevalonate metabolites on the expression of G1 cyclins and related molecules in serum-stimulated rat astrocytes. Quiescent astrocytes were stimulated by serum for 24 h in the presence of pravastatin (1800 μM) and with mevalonate (0.03 mg/ml), GGPP in liposomes (4 μM), FPP in liposomes (4 μM), or vehicle. A, cell lysates (10 μg) were analyzed by immunoblotting with antibodies against cyclin D1, Cdk4, cyclin H, Cdk7, cyclin E, Cdk2, and p27. Cdk2, Cdk4, and CAK activities were determined as described under “Experimental Procedures.” DNA synthesis of cultured astrocytes was also determined as described under “Experimental Procedures.” B, the mRNA levels were analyzed by Northern blot analysis using specific probes for Cdk2, cyclin E, and p27 as described under “Experimental Procedures.”
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Fig. 5. Geranylgeranylation of Rho small GTPase(s) during G1/S transition. Quiescent astrocytes were stimulated by serum for 24 h in the presence of pravastatin (1800 μM) and with mevalonate (0.03 mg/ml), GGPP in liposomes (4 μM), FPP in liposomes (4 μM), or vehicle. Crude membrane- and cytosol-containing fractions were prepared as described under “Experimental Procedures.” A, cell lysates (10 μg) were analyzed by immunoblotting with antibodies against Ras, Rho A, and Rho B. B, fluorography of immunoprecipitated [3H]geranylgeranlylated Rho A in membrane fraction. Molecular size standards (in kilodaltons) are indicated on the left.

translilation of Rho A and Rho B from the cytoplasm to membranes during the transition from G1 to S phase (Fig. 5A). Moreover, both mevalonate and GGPP reversed the pravastatin-induced blockage of their translocation (Fig. 5A). Ras small GTPase in membranes, which has been reported to be isoprenylated by FPP, was not affected by treatment with pravastatin. Although the effect was not as striking as for Rho A and Rho B, Ras small GTPase in cytosol was increased by pravastatin (Fig. 5A).

[3H]GGPP labeled a cluster of proteins between 23 and 26 kDa in membrane fraction of astrocytes in the presence of pravastatin (data not shown). High pressure liquid chromatography analysis of the lipids associated with these proteins showed that these proteins were geranylgeranylated but not farnesylated (data not shown). Immunoprecipitation analysis of Rho A in the membranes from growth-stimulated astrocytes with supplementation of [3H]GGPP demonstrated that one of the proteins labeled with [3H]GGPP was Rho A small GTPase (Fig. 5B).

Effect of Botulinum C3 Exoenzyme on the Expression of Cell Cycle Regulators and DNA Synthesis in Serum-stimulated Astrocytes—Botulinum C3 exoenzyme inactivates Rho small GTPase(s) (56). C3 exoenzyme abolished the ability of GGPP to reverse pravastatin-inhibited DNA synthesis in a dose-dependent manner. The maximal effect of C3 exoenzyme was observed at 50 μg/ml (Fig. 6). C3 exoenzyme also blocked the ability of GGPP to reverse the inhibition of cyclin E and Cdk2 expression in pravastatin-treated astrocytes (Fig. 7, A and B). The activation of Cdk2 by GGPP in pravastatin-treated cells was also diminished by C3 exoenzyme (Fig. 7A).

DISCUSSION

We have characterized the time-dependent expression of cell cycle regulators that govern G1/S transition in primary cultured rodent glial astrocytes. D-type cyclins have been shown to be induced earlier in G1 phase than cyclin E and to form complexes with Cdk4 and Cdk6 (35–38). In the present study, the expression of cyclin D1 and Cdk4 in rat astrocytes was not altered by the growth stimulation. The reason for this is not known at present. Two cyclin E isoforms (cyclin E-l and cyclin E-s) were detected in our system (Fig. 1A). They differ at their amino acid termini and are derived from alternatively spliced mRNAs (39). Only cyclin E-l was detected in quiescent astrocytes. In contrast to D-type cyclins, the expression of both cyclin E-l and cyclin E-s was increased by serum stimulation, with cyclin E-l being the dominant form. Northern blot analysis revealed that the expression of cyclin E mRNA was increased by serum stimulation (Fig. 1B). This was in agreement with the reports that the expression of cyclin E in mitotically proliferating cells is maximal at the G1 to S phase transition and that cyclin E-l and cyclin E-s were expressed in similar patterns during the cell cycle progression (40, 41). After serum stimulated the increased expression of cyclin E, Cdk2 was activated. Moreover, the expression of phosphorylated Cdk2 protein, which migrates as a single major 33-kDa band, was coincidentally increased by serum stimulation. The H1 histone kinase activity associated with Cdk2 was also increased. Among regulators of Cdk2 activity, the activity of CAK and the expression of p27 did not change after serum stimulation. We previously demonstrated that in growth-stimulated rat FRTL-5 cells, p27 was eliminated via accelerated degradation pathway as the cells enter the cell cycle (22, 42). In the present study, the possibility cannot be excluded that an undetectable decrement of p27 in immunoblotting might be enough to decrease its activity for G1/S transition in serum-stimulated astrocytes, since the number of astrocytes to enter the S phase was not large in our system. However, Soos et al. (44) and Poons et al. (43) reported that the amount of p27 was
cyclin E and the subsequent activation of Cdk2; other G1 cyc-
regulatory pathways. Pravastatin suppressed the expression of
cell cycle proteins under the control of mevalonate-dependent
ation. Further investigation was performed to identify a set of
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and p27. Cdk2 activities were determined as described under “Experi-
alyzed by immunoblotting with antibodies against cyclin E, Cdk2,
described under “Experimental Procedures.”
mblot analysis using specific probes for Cdk2, cyclin E, and p27 as
m constant during the cell cycle in MANCA hematopoietic B-cells
and in Swiss 3T3 mouse fibroblasts, respectively. Thus, p27
may serve as a buffer for cyclin-Cdk complexes in G1 phase, and
cyclin-Cdks are not active until their levels exceed that of p27
in rat astrocytes.
Pravastatin induced the inhibition of DNA synthesis and G1
arrest in serum-stimulated rat astrocytes; inhibition was, how-
ever, fully reversed by the addition of mevalonate. The kinetic
analysis of the inhibition of DNA synthesis by pravastatin and
its reversal by mevalonate demonstrated that mevalonate
and/or its metabolite(s) played important roles at G1/S transi-
union. Further investigation was performed to identify a set of
cell cycle proteins under the control of mevalonate-dependent
regulatory pathways. Pravastatin suppressed the expression of
cyclin E and the subsequent activation of Cdk2; other G1 cy-
lins and related molecules were not affected. The expression of
mRNAs of cyclin E and Cdk2 was also reduced by pravastatin. It
expression of cyclin E-Cdk2 complexes in other systems (45,
epithelial cells could abrogate the ability of transforming
growth factor-β to arrest cell cycle progression and lead to
reactivation of cyclin E-Cdk2 histone H1 kinase activity (45). In
the present study, the expression of cyclin D1 and Cdk4 was
not impaired by pravastatin. Activation of individual Cdks
was dictated in part by stage-specific phosphorylation and dephos-
phorylation events (47). In the present study, the expression of
Cdk7 and cyclin H, which are reported to comprise CAK (48), as
well as the activity of CAK, was not affected by pravastatin. It
seems, therefore, likely that pravastatin suppressed cyclin E-
Cdk2 activation through neither the inhibition of cyclin
D1-Cdk4 complex nor the inhibition of CAK in rat astrocytes.
The assembly of catalytically active cyclin E-Cdk2 complexes
was directly related to the abundance of the cyclin E protein in
exponentially proliferating cells (40, 49). From these data, and
the data described above, impaired Cdk2 activation in pravas-
tatin-treated astrocytes is most likely due to reduced induction
of cyclin E expression, and the expression of cyclin E in rat
astrocytes is under the control of mevalonate-dependent regu-
lar pathways.
The expression of p27 in the serum-stimulated astrocytes
was not affected by the treatment with pravastatin. These
findings were different from those of this HMG-CoA reductase
inhibitor observed in other mammalian cells, in which the
accumulation of p27 has been implicated in G1 arrest induced
by an HMG-CoA reductase inhibitor (50). Furthermore, when
we examined rat thyroid FRTL-5 cells, a normal rat thyroid cell
line, to observe the role of the mevalonate cascade in the cell
cycle progression, we did not observe that pravastatin also
caused the accumulation of p27 to induce G1 arrest (22). This
effect was reversed by the addition of mevalonate or GGPP, but
the expression of p27 mRNA was not influenced. In addition,
the expression of both protein and mRNA for cyclin E was not
inhibited by pravastatin, quite different from the findings in
cultured rat astrocytes. Recently Vogt et al. (51) reported that
p53-independent p21 accumulation might be essential for the
inhibition of proliferation without any effect of cyclin E expres-
sion in tumor cells treated with geranylgeranyltransferase I
inhibitors. Taken together, these results indicate that the role
of the mevalonate cascade, especially GGPP, in the regulation
of cyclins and its inhibitors may vary according to cell type and
experimental condition. In any case, the importance of GGPP
should be universal at G1/S transition for the proliferation in
mammalian cells.
A class of geranylgeranylated small GTP-binding proteins,
termed Rho small GTPase(s), were reported to be involved in
the transition from G1 to S phase (24, 52). The geranylgerany-
lation of Rho proteins is essential for their activation since it
has been reported that the activity of Rho proteins requires
their attachment to the inner leaflet of the plasma membrane.
Moreover, Rho proteins were shown to be translocated to the
membrane and activated after isoprenylation with GGPP,
which were derived from mevalonate (53, 54, 57). In the pres-
ent study, pravastatin clearly inhibited the translocation of
Rho small GTPase(s) from cytosol to membrane, whereas the
amounts of Ras small GTPase in membrane fraction was not
affected. Mevalonate and GGPP, not FPP, fully reversed the
pravastatin-induced inhibition of the translocation of Rho to
the membrane. The immunoprecipitation of Rho A in the mem-
brane fraction demonstrated that the protein was labeled by
the supplementation with [3H]GGPP in liposomes (Fig. 5B).
High pressure liquid chromatography analysis of the lipids
associated with the protein showed that the protein was ger-
anylgeranylated, but not farnesylated (data not shown). These
results indicate that Rho small GTPase(s) are geranylgerany-
lated and translocated to the membrane during G1/S transition.

![Diagram](image_url)

**Fig. 7. Effect of botulinum C3 exoenzyme on geranylgeranyl
pyrophosphate-dependent cyclin E expression and Cdk2 activa-
tion.** Quiescent astrocytes were stimulated by serum for 24 h in the
presence of pravastatin (1800 μM) and GGPP in liposomes (4 μM) and
with or without C3 exoenzyme (50 mg/ml). A, cell lysates (10 μg) were
analyzed by immunoblotting with antibodies against cyclin E, Cdk2,
and p27. Cdk2 activities were determined as described under “Experi-
mental Procedures.” B, the mRNA levels were analyzed by Northern
blot analysis using specific probes for Cdk2, cyclin E, and p27 as
described under “Experimental Procedures.”
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in rat astrocytes. Therefore, pravastatin may inhibit DNA synthesis, cyclin E expression, and Cdk2 activation by inactivation of Rho small GTPase(s), since they are not translocated to the membrane in the presence of pravastatin. GGPP, not FPP, reverses the pravastatin blockade.

In the present study, we examined the effect of botulinum C3 exoenzyme to clarify the roles of Rho small GTPase(s) in serum-stimulated astrocytes. Botulinum C3 exoenzyme was reported to inactivate Rho small GTPase(s) by ADP-ribosylating a specific asparagine residue in their putative effector domain and thereby interfering with their biological actions, presumably affecting their interaction with putative target protein(s) (55, 56). C3 exoenzyme abolished the effect of GGPP on pravastatin-induced inhibition of DNA synthesis. Furthermore, the effects of GGPP on cyclin E expression and Cdk2 activation in pravastatin-treated astrocytes were also completely blocked by the C3 exoenzyme. These results indicate that geranylgeranylated Rho small GTPase(s) are essential for cyclin E expression in serum-stimulated astrocytes.

In conclusion, we demonstrate that the Rho small GTPase(s), which are geranylgeranylated during G1/S transition, are one of the essential factors to regulate the expression of cyclin E and the activation of Cdk2 which are necessary to facilitate the G1/S transition during the proliferation of serum-stimulated rat cultured astrocytes. The present study might provide a new insight into the regulation of cyclin E and Cdk2 in proliferating neural cells, an involvement of the Rho-mediated pathway.

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