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Cellular stress increases RGS2 mRNA and decreases RGS4 mRNA levels in SH-SY5Y cells

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Abstract

Modulation of the expression of regulator of G-protein signaling (RGS) proteins is a major mechanism used to modulate their actions. Besides control by second messengers, the expression of RGS proteins, particularly RGS2, can be regulated by cell stress. Because RGS2 and RGS4 expression can be regulated by the cell cycle, we examined if cell cycle signals are involved in their regulation following stress. Treatment of SH-SY5Y cells with camptothecin increased RGS2 mRNA and decreased RGS4 mRNA levels. This effect on RGS2 mRNA was blocked by the cyclin-dependent kinase-2 (cdk2) inhibitors roscovitine and purvalanol. Cell cycle arrest was further implicated in regulating RGS mRNA levels because geldanamycin, which causes cell cycle arrest by inhibiting the actions of heat shock protein 90, caused changes in the mRNA levels of RGS2 and RGS4 similar to, and additive with, the effects of camptothecin. Overall, these results indicate that cell cycle arrest regulates the expression of RGS2 and RGS4, and that the expression of these two RGS family members is oppositely regulated by stress that causes cell cycle arrest.

Keywords: RGS2; RGS4; Camptothecin; Roscovitine; cdk2; Geldanamycin; Cell cycle arrest

Regulator of G-protein signaling (RGS) proteins attenuate the signaling activities of many G-protein-coupled receptors through their action as GTPase activating enzymes to deactivate G-proteins [10]. RGS proteins themselves are regulated in a number of ways, one of the best characterized being by control of their transcription. Thus, many RGS proteins, such as RGS2 and RGS4, are expressed at a low level but this can be increased by activation of G-protein-coupled receptors, presumably to provide a feedback mechanism to attenuate receptor-mediated signaling [5,14].

In addition to their classical association with plasma membrane receptor-coupled signal transduction systems, emerging evidence suggests that RGS proteins have additional actions and are regulated by additional cellular stimuli. In this respect, RGS2 is especially interesting because it has often been found to be located in the nucleus rather than at the plasma membrane associated with G-protein-coupled receptors [3]. Additionally, the expression of RGS2 has been shown to be regulated by several cell stressors [15,21,22], by the cell cycle [22], and by the differentiation state of cells [15]. Since some cell stressors can cause cell cycle arrest, and RGS2 expression changes during the cell cycle in unstressed cells [22], these characteristics raised the possibility that cyclin-dependent kinases associated with the cell cycle may have a regulatory influence on the expression of RGS2. We examined this by using the topoisomerase 1 inhibitor camptothecin to stress cells, which activates the tumor suppressor p53 and increases RGS2 mRNA levels in human neuroblastoma SH-SY5Y cells [22], and roscovitine, an inhibitor of cyclin-dependent kinases (cdk). Roscovitine is a purine analog which competitively binds at the ATP binding site [11,16] and at concentrations up to approximately 10 μM roscovitine is a specific inhibitor of cdk2, while at higher concentrations it can inhibit other kinases [1]. Furthermore, we compared changes in RGS2 mRNA to those of RGS4 mRNA because we recently reported that the expression level of RGS4 is regulated by cell stress in an opposite direction from that of RGS2 [22].

Human neuroblastoma SH-SY5Y cells were grown in RPMI medium (Cellgro, Herndon, VA) supplemented with 10% horse serum (Invitrogen, Carlsbad, CA), 5% fetal clone II (Hyclone, Logan, UT), 2 mM L-glutamine, 100 units/ml penicillin and

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100 μg/ml streptomycin, and were maintained in humidified, 37 °C chambers with 5% CO_2 prior to incubation in serum-free media overnight before treatments. Experimental agents used included camptothecin, roscovitine, purvalanol, LiCl, kenpaullone (Sigma, St. Louis, MO), and indirubin-3′-monoxime (Alexis Biochemicals, San Diego, CA).

RGS2 cDNA was generously provided by Dr. D.R. Forsdyke (Queen's University, Kingston, Ont., Canada) and RGS4 cDNA was obtained from the Guthrie cDNA Resource Center (Guthrie, Sayre, PA). The methods for measuring mRNA levels using Northern blots were as described previously [14]. Total RNA was extracted using Trizol reagent (Invitrogen) according to the manufacturer's instructions. RNA was separated by electrophoresis in 1.2% agarose gels containing formaldehyde and transferred to nitrocellulose membranes. cDNA was random prime-labeled with [^{32}P]dCTP (Amersham Pharmacia Biotech). Blots were hybridized with labeled probes at 42 °C for 18 h and then washed in two changes of 2× saline-sodium citrate and 0.1% SDS at 20 °C for 20 min and once in 1× saline-sodium citrate and 0.1% SDS at 55 °C for 10 min. Results were obtained using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA), and bands were quantitated using ImageQuant.

DNA damage was induced by treatment with the topoisomerase 1 inhibitor camptothecin, which we previously reported causes a concentration-dependent increase in the levels of p53 and p21, indicative of DNA damage, with a maximal effect produced by 1 μM camptothecin in SH-SY5Y cells [17]. Treatment with camptothecin causes a large, rapid increase in RGS2 mRNA levels and reduction in RGS4 mRNA levels [22]. Since regulation of cdk2 is a key component of the DNA damage response [2], we tested if the cdk2 inhibitor roscovitine affected these responses to DNA damage. Fig. 1A shows the concentration-dependent inhibitory effect of roscovitine in SH-SY5Y cells treated with camptothecin to induce the expression of RGS2. As previously reported [22], treatment with camptothecin (1 μM; 2 h) caused a large (3-fold) increase in the level of RGS2 mRNA. Pretreatment with roscovitine (1 h) caused a concentration-dependent inhibition of camptothecin-induced increases in RGS2 mRNA, with an IC50 near 1 μM. This potency indicates that the effect of roscovitine is highly likely due to inhibition of cdk2 [1]. A high concentration (10 μM) of roscovitine completely eliminated RGS2 mRNA expression, but this strong effect may be due to the inhibition of additional proteins besides cdk2. Furthermore, the inhibitory effect of 1 μM roscovitine on camptothecin-induced RGS2 mRNA levels was relatively fast and long-lasting, as it was evident at the shortest time tested after camptothecin treatment (1 h) and throughout a 4 h time course of camptothecin treatment (Fig. 1B).

We noted previously that the expression of RGS4 is regulated oppositely to that of RGS2 following cell stress [22]. In agreement with this observation, camptothecin treatment decreased RGS4 mRNA levels, as opposed to the increase in RGS2 mRNA levels (Fig. 1A and B). However, treatment with roscovitine initially had little effect on the camptothecin-induced reduction in RGS4 mRNA levels (Fig. 1A), although reductions were observed with longer treatment times of 3 and 4 h (Fig. 1B). Neither camptothecin nor roscovitine altered actin mRNA lev-

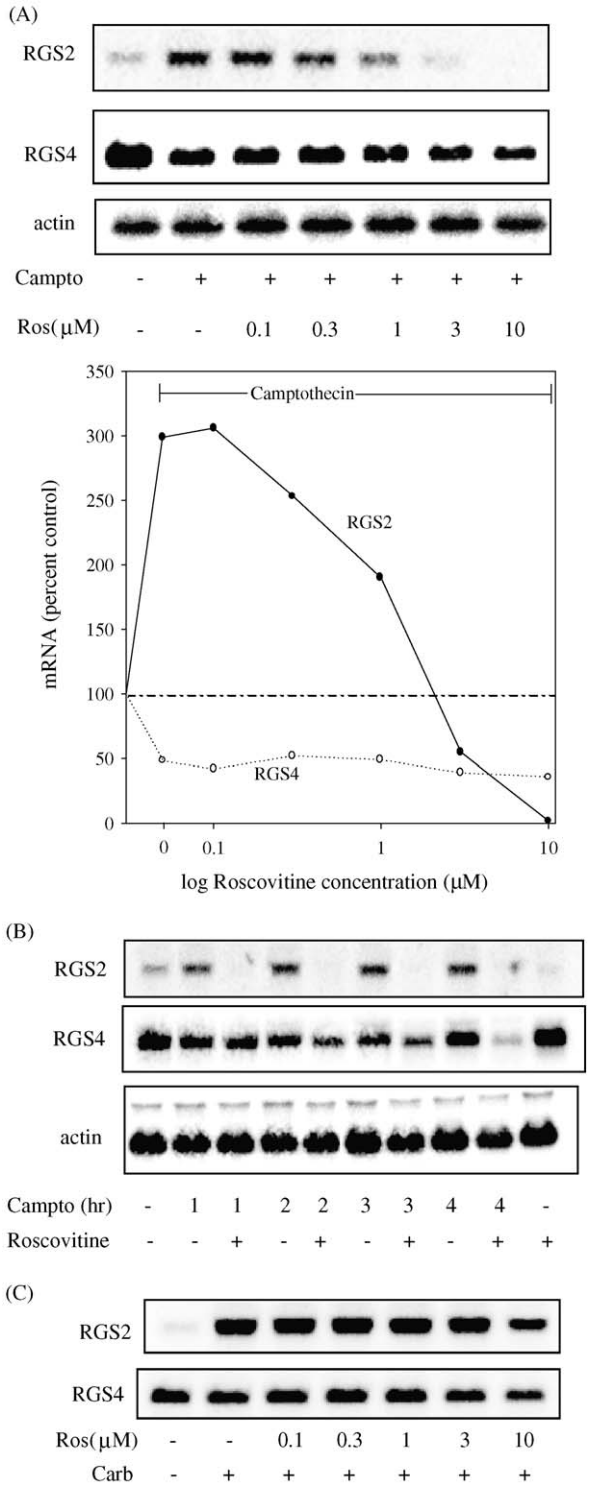

Fig. 1. Inhibition of camptothecin-stimulated RGS2 expression by roscovitine. (A) SH-SY5Y cells were pretreated for 1 h with 0–10 μM roscovitine, followed by treatment with 1 μM camptothec (Campto) in for 2 h and measurements of RGS2 (ROS), RGS4, and actin mRNA levels by Northern blots. Quantitative values are presented as the percent of mRNA levels in cells not treated with camptothecin or roscovitine. (B) Cells were pretreated with 1 μM roscovitine for 1 h followed by treatment with 1 μM camptothecin for 1–4 h and measurements of RGS2, RGS4, and actin mRNA levels by Northern blots. (C) Cells were pretreated for 1 h with 0–10 μM roscovitine followed by treatment with 1 mM carbachol (Carb) for 1 h and measurements of RGS2 and RGS4 mRNA levels by Northern blots.