G-substrate, a substrate for cGMP-dependent protein kinase, shuttles between cytosol and nuclear in cerebellar Purkinje cells.

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Introduction

NO-sGC-cGMP-PKG pathway plays an essential role in the induction of cerebellar long-term depression (LTD). However, the downstream component of PKG (cGMP-dependent protein kinase) was not elucidated. G-substrate, one of a few preferred of PKG resides in cerebellar Purkinje cell. We have cloned G-substrate cDNA and have been characterizing its physiological and biochemical roles in cerebellar Purkinje cells and cerebellar functions.

G-substrate distributes not only in cell soma but also in dendrites of cerebellar Purkinje cells. G-substrate is an excellent substrate of PKG in cerebellar Purkinje cells, was observed both in cytosol and nuclear of cerebellar slices. And CRM1 was observed mainly in nuclei of cerebellar Purkinje cells.

Conclusions

G-substrate, an excellent substrate of PKG in cerebellar Purkinje cells, was observed both in cytosol and nuclear of cerebellar slices. And CRM1 was observed mainly in nuclei of cerebellar Purkinje cells.

G-substrate in cell nuclear was reduced by treatment with 8-Br-cGMP, an membrane permeable analog of cGMP. However, TPA or forskolin did not cause the reduction of G-substrate in nuclear. This may suggest that a negative charge added specifically by PKG phosphorylation disrupted a cluster of positive charges in potential NLS in G-substrate.

Leptomycin B, an inhibitor of CRM1-dependent transport, resulted in drastic accumulation of G-substrate in Purkinje cell nuclear. The results suggest the functional leucine-rich NES in G-substrate and CRM1-dependent export of G-substrate in Purkinje cells.

G-substrate may shuttle with functional NLS and NES. The shuttling may be regulated by phosphorylation through PKG.

Potential nuclear localization signal (NLS) and nuclear export signal (NES) of G-substrate.

G-substrate is an acid-stable and heat-stable protein with 155-160 amino acid. Major class of NLSs contains a cluster of basic amino acid such as Arg and Lys (shown in Blue). G-substrate has three potential NLSs and two of them are near the phosphorylation sites by PKG, cGMP-dependent protein kinase. In addition, G-substrate has three potential NESs containing a cluster of hydrophobic amino acid such as Leu, Ile, Phe, Met and Val (shown in Red). The functional significance of each NLS and NES in the translocation of G-substrate is unknown.

The distribution of CRM1 in cerebellar Purkinje cells in culture.

The cells were stained with anti CRM1 antibody and anti calbindin antibody. The immunoreactivity was visualized using Alexa Fluor 488 labeled (green) and Alexa Fluor 546 labeled secondary antibodies (red).

Cellular distribution of G-substrate in mouse Purkinje cells.

The cerebellar slices were stained with anti G-substrate antibody and anti calbindin antibody. The immunoreactivity was visualized using Alexa Fluor 488-labeled (green) and Alexa Fluor 546-labeled secondary antibodies (red).

Shuttling of G-substrate between nuclear and cytosol via CRM1-dependent pathway.

The translocation of G-substrate was observed in cerebellar Purkinje cells in culture. 8Br-cGMP, a membrane permeable analog of cGMP, induced the translocation of G-substrate in cytosol. On the other hand, Leptomycin B, an inhibitor of the CRM1-mediated nuclear export, induced the accumulation of G-substrate in the nuclear. Nuclear is stained with propidium iodide.

The intracellular distribution of G-substrate was altered by a variety of stimuli.

The fluorescence intensity obtained in pictures in the previous panel was quantified.

A. Treatments are as follows; 0.5 mM 8Br-cGMP for 30 min; 0.5 mM TPA; 50 mM quisqualate; 50 mM forskolin.

**, p<0.01; compared with the control.

B. Time course of the accumulation of G-substrate in nuclear in the presence of leptomycin B (50 ng/ml). The values are average ± SD.

Introduce NO-sGC-cGMP-PKG pathway plays an essential role in the induction of cerebellar long-term depression (LTD). However, the downstream component of PKG (cGMP-dependent protein kinase) was not elucidated. G-substrate, one of a few preferred of PKG resides in cerebellar Purkinje cell. We have cloned G-substrate cDNA and have been characterizing its physiological and biochemical roles in cerebellar Purkinje cells and cerebellar functions.

G-substrate distributes not only in cell soma but also in dendrites of cerebellar Purkinje cells. G-substrate is an excellent substrate for PKG and the phosphorylated G-substrate work as protein phosphatase inhibitor for Ser/Thr protein phosphatase, PP-1 and PP2A. We recently observed that G-substrate localized in Purkinje cell nuclear as well as cytosol. In addition, G-substrate have potential nuclear localization signals (NLS) and nuclear export signals (NES). To elucidate further the role of G-substrate in cerebellar Purkinje cells. We characterized the potential shuttling of G-substrate between cytosol and nuclear in cerebellar Purkinje cells.