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生长抑制因子(GIF)与G蛋白Rab3a直接相互作用

Growth Inhibitory Factor (GIF) Directly Interacts with G-protein Rab3a

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作者 单位

康巧华 北京大学生命科学学院,蛋白质工程与植物基因工程国家重点实验室,北京 100871

陈巧林 扬州大学畜牧兽医学院,扬州 225009

任宏伟 北京大学生命科学学院,蛋白质工程与植物基因工程国家重点实验室,北京 100871

茹炳根 北京大学生命科学学院,蛋白质工程与植物基因工程国家重点实验室,北京 100871

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中文摘要:

生长抑制因子(growth inhibitory factor, GIF),又称金属硫蛋白-3,为68个氨基酸组成的脑特异性金属硫蛋白,具有广泛的生理功能;GIF可能与阿尔茨海默氏症(Al zhei mer's)病理相关,在Al zhei mer's脑提取物存在下,还对神经细胞具有特异的生长抑制活性.然而,对其发挥生长抑制作用的分子机制并不清楚.运用酵母双杂交系统从人脑cDNA文库中筛选与GIF相互作用因子,从4.1×10⁶个人脑cDNA文库转化子中,首次筛选到Ras家族G蛋白Rab3a C端,包含87个氨基酸的片段能与GIF相互作用;用PCR自人胎盘总cDNA中获得包含完整Rab3a编码序列的cDNA;通过酵母双杂交实验表明,全长Rab3a蛋白亦能与GIF相互作用.免疫共沉淀和蛋白质印迹实验进一步验证了GIF与Rab3a在哺乳动物细胞中可以相互作用;而且,Rab3a是以GTP结合形式(GTP-Rab3a)与GIF发生相互作用.

英文摘要:

Growth inhibitory factor (GIF), renamed as metallothionein-3, is a 68-amino acids brain specific member of the metallothionein family. GIF plays comprehensively physiologic roles and is deemed to be implicated in Alzheimer's disease. When supplemented with Alzheimer's brain extracts, GIF demonstrates its *in vitro* growth inhibitory activity on cultured neuron; but the underlying molecular mechanism remains unknown. To probe this mystery, the yeast two-hybrid system was employed to screen a human brain cDNA library for GIF-interacting proteins. From 4.1× 10⁶ transformants of library screening, a C-terminal fragment of small G-protein Rab3a was obtained, harboring 87 amino acids that interacted with GIF in yeast. The full-length Rab3a was amplified by polymerase chain reaction (PCR) using human total placenta cDNA as template and con comitantly cloned. In yeast two-hybrid test, full-length Rab3a also interacted with GIF. Through coimmunoprecipitation and Western blotting experiments, interaction between GIF and Rab3a was confirmed in mammalian cell. Furthermore, the result indicated that Rab3a interacted with GIF in a GTP-binding form.

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