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细胞内物质转运调节分子ARFGAP3的克隆表达及生化活性

Cloning, Expression and Biochemical Activity of ARFGAP3, a Regulator of Intracellular Transport

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中文摘要：

ADP核糖基化因子-GTP酶活化蛋白(ARF GAP)是重要的细胞内物质转运调节分子.在22周孕龄人胎肝cDNA文库中发现一种新基因，其编码的氨基酸序列与大鼠ARF1 GAP有32%同源性.将这种新基因命名为“ARFGAP3”，对其进行功能研究，利用逆转录-聚合酶链式反应(RT-PCR)，从人胎盘总RNA中扩增ARFGAP3全长cDNA序列，并将其亚克隆到pGEM-T载体；采用RNA印迹法和斑点杂交法，检测其组织表达谱，发现在多种腺体和睾丸中有很高水平ARFGAP3基因转录，并且只有一种约2.7 kb的转录本.利用基因重组技术，构建表达质粒pBAD/Thio-ARFGAP3，在大肠杆菌中表达，采用亲和层析法纯化表达产物，利用肠激酶切除重组融合蛋白N端引导序列.检测重组ARFGAP3的生化活性，证实ARFGAP3对ARF1具有GAP活性，促进ARF1结合的GTP水解为GDP，磷脂酰肌醇二磷酸(PIP2)增强其GAP活性，而磷脂酰胆碱(PC)抑制其GAP活性.

英文摘要：

ARF GAP is a kind of important regulator of introcellular transport. Recently, a novel human gene has been found from a cDNA library of second trimester human fetal liver. The amino acid sequence encoded by the novel gene has 32% similarity to rat ARF1 GAP, was thus termed as ARFGAP3. Functional studies of the new gene were performed. The full-length cDNA of ARFGAP3 was amplified from the human total placenta RNA by RT-PCR technique, then subcloned into pGEM-T vector and sequenced. The RNA Master blot and multiple tissue Northern blot analysis were used to define the expression profile and the transcript size of ARFGAP3 in human tissues. It was shown that ARFGAP3 was strongly expressed in glands and testis and that ARFGAP3 mRNA existed as only one kind of transcript of 2.7 kb in various human tissues. Then, the expression and purification of the recombinant human ARFGAP3 (rhARFGAP3) were performed. It was demonstrated that rhARFGAP3 exhibited strong GTPase-activating protein (GAP) activity towards the recombinant ARF1 *in vitro* by an assay of a single round of GTP hydrolysis on recombinant ARF1, and that GAP activity of ARFGAP3 was stimulated by PIP2 and inhibited by PC.

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