

论文

小鼠截短型CDC25B蛋白PKA体外磷酸化分析

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摘要:

目的 通过体外磷酸化研究和放射自显影技术证实小鼠CDC25B蛋白是蛋白激酶A(PKA)的直接作用底物。方法 构建小鼠截短型pGEX-4T-2-CDC25B₂₀₁原核表达载体,异丙基-β-D-硫代半乳糖苷(IPTG)诱导表达,glutathione Sepharose 4B亲和层析纯化CDC25B蛋白,与PKA进行体外磷酸化反应,分别采用十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(SDS-PAGE)、蛋白免疫印迹(western blot)和放射自显影鉴定CDC25B是否为PKA直接磷酸化底物。结果 在E.coli BL21细胞内,采用0.1 mmol/L的IPTG在27℃条件下诱导表达3 h即可达到较高表达水平;SDS-PAGE显示在约50 kD处有明显GST-CDC25B₂₀₁融合蛋白特异条带;放射自显影显示GST-CDC25B₂₀₁融合蛋白的磷酸化条带大约位于55 kD处;Western blot分析,在重组质粒表达的总蛋白、载体表达的GST蛋白、纯化蛋白及磷酸化蛋白的相应位置均出现GST抗体的杂交条带。结论 小鼠CDC25B蛋白是PKA的直接作用底物。

关键词: 小鼠 CDC25B PKA 体外磷酸化

Phosphorylation of recombinant mouse cell division cycle 25 homolog(CDC25B) protein *in vitro*

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Abstract:

Objective To verify mouse CDC25B acting as a direct protein kinase A(PKA) substrate by phosphorylation *in vitro* and autoradiography. Methods Prokaryotic expression of the pGEX-4T-2-CDC25B₂₀₁ fusion protein was expressed in the presence of isopropyl-β-D thiogalactopyranoside(IPTG) and purified by the glutathione sepharose 4B protein with chromatography and phosphorylated with PKA *in vitro*, and then identified by sodium dodecyl sulfate polyarylamide gel electrophoresis (SDS-PAGE), western blotting, and autoradiography. Results The glutathione S-transferase (GST) CDC25B₂₀₁ was expressed highly for 3 hours at 27℃ in *Escherichia coli* BL21 (DE3) transformed with pGEX-4T-2CDC25B 201 in the presence of 0.1mmol/L IPTG. The results of SDS-PAGE showed a clear band of GST-CDC25B 201 fusion protein in 50 kD of the expected size, whereas the band of phosphorylated GST-CDC25B₂₀₁ protein was seen at about 55kD by autoradiography. Total protein induced by IPTG, vector-expressed protein, and purified protein were identified by western blotting and GST bands were emerged in corresponding position. Conclusion Mouse CDC25B is the direct downstream substrate of PKA.

Keywords: mouse, CDC25B, PKA, phosphorylation *in vitro*

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