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酵母组蛋白乙酰基转移酶GCN5和去乙酰化酶RPD3在大肠杆菌中的表达 Cloning and Prokaryotic Expression of Yeast GCN5 and RPD3

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摘要　以酵母基因组DNA为模板，通过PCR方法分别扩增出在5′端带有6xHis标签的gcn5和rpd3基因，将其克隆到pBV220质粒中，分别构建成表达质粒pBVgcn5和pBVrpd3,转化大肠杆菌（Escherichia coli）并进行诱导表达。SDS-PAGE显示，含重组质粒的菌株经热诱导后分别过量表达出约50kDa的蛋白。利用6xHis亲和层析纯化了这两种重组酶。对组蛋白乙酰基转移酶GCN5进行体外活性检测，证实其具有组蛋白乙酰基转移酶活性。本工作为通过体外实验研究乙酰化和去乙酰化修饰与基因转录调控的关系奠定了基础。

Abstract:The yeast histone acetyltransferase (HAT) gene gcn5 and histone deacetylase (HDAC) gene rpd3 were cloned from yeast genomic DNA by PCR amplification.The two genes,both with additional 6xHis tag,were subcloned into pBV220 vector to construct expression plasmids pBVgcn5 and pBVrpd3,respectively.Both pBVgcn5 and pBVrpd3 were over-expressed in Escherichia coli upon temperature induction,as revealed by SDS-PAGE.The recombinant GCN5 and RPD3 were purified by using a 6xHis affinity column.The purified GCN5 was tested to possess the HAT activity by using a 14C-labeling assay.This work has laid down the basis for further in vitro studies into roles of histone acetylation/deacetylation in modulating chromatin conformation and transcription activity.

关键词　组蛋白乙酰基转移酶　组蛋白去乙酰化酶　GCN5　RPD3　基因克隆 Key words　histone acetyltransferase　histone deacetylase　GCN5　RPD3　gene cloning

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