昆虫学报
ACTA ENTOMOLOGICA SINICA
ISSN 0454-6296
CN 11-1832/Q
主办：中国科学院动物研究所
中 国 昆 虫 学 会

昆虫学报 » 2012, Vol. 55 » Issue (2): 139-146 DOI:
研究论文

斜纹夜蛾铁硫亚基蛋白的表达及功能鉴定

左洪亮, 陈永, 高璐, 刘海远, 钟国华

Expression and function identification of the Rieske iron-sulfur protein from the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae)

ZUO Hong-Liang, CHEN Yong, GAO Lu, LIU Hai-Yuan, ZHONG Guo-Hua

全文: PDF (5771 KB) HTML (1 KB) 输出: BibTeX | EndNote (RIS) 背景资料

摘要 铁硫亚基蛋白(rieske iron-sulfur protein, RISP) 是线粒体复合物Ⅲ的关键蛋白亚基之一， 在呼吸链电子传递过程中起到重要作用。本研究通过RT-PCR克隆得到斜纹夜蛾*Spodoptera litura* RISP基因*SlitRISP*的ORF， 构建了pET32a-*SlitRISP*原核表达载体， SDS-PAGE和Western blot检测结果显示， *SlitRISP*原核表达蛋白以包涵体的形式存在于菌体沉淀中， 且RISP抗体可成功用于该蛋白的免疫印迹检测。为了进一步鉴定SlitRISP在斜纹夜蛾离体细胞系SL-1中的功能， 通过向细胞内转染siRNA， 利用RNAi技术沉默SL-1中的*SlitRISP*。qRT-PCR结果表明， 分别经50 nmol/L和100 nmol/L siRNA处理48 h后， SL-1中*SlitRISP*的表达均几乎完全被抑制； Western blot结果显示， SL-1中*SlitRISP*含量显著低于CK。当SL-1 *SlitRISP*被成功沉默后， 通过检测SL-1线粒体膜电位、 细胞ATP含量和细胞增殖抑制率鉴定RISP在线粒体电子传递过程中的重要作用。流式细胞仪测定结果表明，经50 nmol/L和100 nmol/L siRNA处理24 h后， SL-1线粒体膜电位相对于CK分别降低23.52%和11.32%， 而处理48 h后，SL-1线粒体膜电位则分别升高5.58%和27.66%； siRNA处理24 h和48 h后， SL-1 ATP含量相对于CK分别降低82.71%和84.50%， 最终导致SL-1细胞增殖抑制率分别为53.64%和67.94%。这些结果表明SlitRISP在SL-1中参与线粒体膜电位的形成和细胞ATP的合成。介于RISP在线粒体电子传递链中的重要作用， 其可能成为新型杀虫作用靶标， 这可为研制新型呼吸抑制剂提供参考。

关键词： 斜纹夜蛾 铁硫亚基蛋白 原核表达 蛋白功能 SL-1细胞

Abstract： The Rieske iron-sulfur protein (RISP) is a key protein subunit of mitochondrial complex Ⅲ, which plays an important role in the respiratory electron transport chain. The opening reading-frame (ORF) of *SlitRISP* was cloned by RT-PCR from *Spodoptera litura* for the construction of prokaryotic expression vector pET32a-*SlitRISP*. SDS-PAGE and Western blot analysis showed that the prokaryotic protein SlitRISP was mainly present as inclusion body in the bacteria precipitate, and the antibody of RISP could be applied to the immunoblot analysis of SlitRISP successfully. In order to identify the function of SlitRISP in the cultured cell line SL-1 of *S. litura*, RNAi was used to silence *SlitRISP* by transfecting siRNA into SL-1 cells. The qRT-PCR result showed that at 48 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA, respectively, the expression levels of SlitRISP mRNA were all inhibited effectively compared with the control. The Western blotting result showed that the content of SlitRISP in SL-1 cells was obviously lower than the control. The mitochondial membrane potential (MMP), ATP content and inhibition rate of cell proliferation were detected based on the obvious silence of *SlitRISP* in SL-1 cells to identify the important roles of RISP in the electron-transport chain of mitochondria. The changes of MMP in SL-1 cells were monitored by flow cytometry (FCM), which decreased by 23.52% and 11.32% at 24 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA, respectively, compared with control. However, at 48 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA, the MMP increased by 5.58% and 27.66%, respectively. The ATP content in SL-1 cells at 48 h after treatment with 50 nmol/L and 100 nmol/L siRNA was decreased by 82.71% and 84.50%, respectively, measured by luminometer. Owning to the suppression of ATP synthesis by siRNA in SL-1 cells, the inhibition rates of cell multiplication reached to 53.64% and 67.94%, respectively, at 48 h after the SL-1 cells were treated with 50 nmol/L and 100 nmol/L siRNA. These results demonstrate that the RISP plays an important role in the MMP formation and ATP synthesis in SL-1 cells. As RISP plays important roles in the electron-transport chain of mitochondria, it could become a new target for pest control, and this may provide reference for developing new respiration inhibitors.

Key words： *Spodoptera litura* Rieske iron-sulfur protein prokaryotic expression protein function; *Spodoptera litura*

服务
把本文推荐给朋友
加入我的书架
加入引用管理器
E-mail Alert
RSS
作者相关文章
左洪亮
陈永
高璐
刘海远
钟国华

cultured cell line (SL-1)

收稿日期: 2011-11-30; 接受日期: 2012-02-05

通讯作者: 钟国华　E-mail: guohuazhong@scau.edu.cn

作者简介: 左洪亮， 男， 1987年生， 硕士研究生， 研究方向为天然源农药与农药毒理， E-mail: zhlzuo@163.com

引用本文:

左洪亮,陈永,高璐等. 斜纹夜蛾铁硫亚基蛋白的表达及功能鉴定[J]. 昆虫学报, 2012, 55(2): 139-146.

ZUO Hong-Liang,CHEN Yong,GAO Lu et al. Expression and function identification of the Rieske iron-sulfur protein from the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae)[J]. ACTA ENTOMOLOGICA SINICA, 2012, 55(2): 139-146.

链接本文:

http://www.insect.org.cn/CN/　或　http://www.insect.org.cn/CN/Y2012/V55/I2/139

没有本文参考文献

[1] 白润娥, 王雄雅, 李静静, 刘晓华, 熊大斌, 李冬兵 . 烟粉虱MED隐种铁蛋白基因克隆、 不同发育阶段和吡虫啉胁迫下的表达及原核表达[J]. 昆虫学报, 2013, 56(7): 738-746.

[2] 纪萍, 刘靖涛, 谷少华, 朱晓强, 张永军, 郭予元. 绿盲蝽气味结合蛋白AlucOBP7的表达及气味结合特性[J]. 昆虫学报, 2013, 56(6): 575-583.

[3] 陈玲, 李红亮, 周宇翔, 赵磊, 张林雅, 倪翠侠, 商晗武. 桔小实蝇气味结合蛋白BdorOBP2的cDNA克隆、组织表达及配基结合特性[J]. 昆虫学报, 2013, 56(6): 612-621.

[4] 姬继超, 安世恒, 李为争, 罗梅浩, 原国辉, 郭线茹. 棉铃虫P450基因*HarmCYP9A33*的克隆、 序列分析及原核表达[J]. 昆虫学报, 2013, 56(5): 465-474.

[5] 刘艳荷, 王利华, 方继朝. 自然界斜纹夜蛾核型多角体病毒的基因型多态性[J]. 昆虫学报, 2013, 56(4): 372-378.

[6] 刘海远, 舒本水, 姜春来, 李良德, 钟国华. 斜纹夜蛾水通道蛋白1（AQP1）基因的克隆、 分子特性和表达分析[J]. 昆虫学报, 2013, 56(4): 339-349.

[7] 胡颖颖, 徐书法, 李薇, Abebe Jenberie WUBIE, 国占宝, 周婷. 中华蜜蜂感觉神经元膜蛋白基因克隆、组织表达分析及原核表达[J]. 昆虫学报, 2013, 56(1): 9-17.

[8] 许小霞, 王爽, 张文庆, 孙强, 王超, 金丰良. 斜纹夜蛾抗菌肽基因*Moricin*和*Cecropin*时空表达模式及微生物敏感性的分析[J]. 昆虫学报, 2012, 55(9): 1008-1013.

[9] 陈全梅, 程道军, 马振刚, 胡晓明, 查幸福, 赵萍. 家蚕和野桑蚕脂肪酸脱氢酶*desat4*全长cDNA和启动子的克隆及其原核表达[J]. 昆虫学报, 2012, 55(8): 885-894.

[10] 张国辉, 刘彦飞, 仵均祥. 梨小食心虫化学感受蛋白cDNA的克隆、 序列分析及原核表达[J]. 昆虫学报, 2012, 55(6): 668-675.

[11] 高璐, 左洪亮, 姜春来, 刘海远, 钟国华. 斜纹夜蛾蜕皮响应基因*E75D*的克隆、 原核表达分析及microRNA作用位点预测[J]. 昆虫学报, 2012, 55(5): 510-519.

[12] 罗梅, 董章勇, 宾淑英, 廖泓之, 林进添. 扶桑绵粉蚧组织蛋白酶B基因的克隆、 原核表达和不同发育阶段表达分析[J]. 昆虫学报, 2012, 55(3): 276-283.

[13] 王涛, 邱秀翠, 焦艳艳, 刘辉, 刘永杰. 斜纹夜蛾蜕皮激素受体与超气门蛋白的原核表达及活性检测[J]. 昆虫学报, 2012, 55(11): 1239-1245.

[14] 刘艳荷, 郭慧芳, 方继朝. C型斜纹夜蛾核型多角体病毒X_{17}病毒株部分基因的序列分析[J]. 昆虫学报, 2011, 54(9): 1010-1017.

[15] 孟翔, 胡俊杰, 金丰良, 任顺祥. 绿僵菌素A和B对斜纹夜蛾SL-1细胞的增殖抑制和致凋亡作用[J]. 昆虫学报, 2011, 54(9): 1003-1009.