

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

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Molecular characterization, prokaryotic expression analysis and miRNA binding site prediction of an ecdysone inducible gene *E75D* from *Spodoptera litura* (Lepidoptera: Noctuidae)

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摘要 E75是昆虫蜕皮级联反应中的早期转录因子之一。本研究运用RT-PCR和RACE技术,首次获得了斜纹夜蛾*Spodoptera litura* *E75D*基因,命名为*Sli-E75D* (GenBank 登录号: JQ266225),其开放阅读框全长1 836 bp,编码611个氨基酸残基,3'非翻译区全长为358 bp,同时获得其5'非翻译区共341 bp。经核苷酸序列比对分析,E75在鳞翅目昆虫间保守性较高,尤其3'非翻译区具有高保守性,但由于启动子不同,E75异构体mRNA 5'端序列存在差异;经氨基酸序列比对,*Sli-E75D*与棉贪夜蛾*Spodoptera littoralis*、烟草天蛾*Manduca sexta*、家蚕*Bombyx mori* *E75D*的一致性分别为98.4%、79.3%和76.5%。基于E75 3'非翻译区的高保守性,利用PITA和RNAhybird程序预测了最有可能调控鳞翅目昆虫*E75*基因的3种miRNAs: miR-14, miR-33和miR-87。构建pET28a-*Sli-E75D*表达载体,分别转化BL21(DE3)和Transetta(DE3)菌株,检测大肠杆菌*Escherichia coli*稀有密码子对*E75D*原核表达的影响,SDS-PAGE结果显示,转化Transetta(DE3)菌株的pET28a-*Sli-E75D*可高效表达大小约74.79 kD (含预测的67.19 kD *Sli-E75D*, 7.6 kD T7·Tag 和His·Tag)的重组蛋白,与其理论分子质量基本吻合,而转化BL21(DE3)菌株的pET28a-*Sli-E75D*质粒只见微量重组蛋白表达。由于Transetta(DE3)菌株可补充大肠杆菌6种稀有密码子的tRNA,较BL21(DE3)更适合于*E75D*的外源表达。qPCR检测了斜纹夜蛾从未龄幼虫到成虫发育过程中各时间点*Sli-E75*的相对表达水平:*Sli-E75*在6龄幼虫期的表达量较低,从预蛹开始,表达量急剧升高,并在蛹中期达到最高峰,之后迅速下降,但成虫期表达水平又出现回升。这些结果有助于深入研究E75在昆虫蜕皮级联反应中的作用。

关键词: 斜纹夜蛾 *E75D* 克隆 原核表达 microRNA

Abstract: *E75D* is one of the important early transcription factors in the molting process of insects. The cDNA of *E75D* was cloned from *Spodoptera litura* by RT-PCR and RACE technology for the first time in this experiment and named *Sli-E75D* (GenBank accession no. JQ266225). *Sli-E75D* consists of a 1 836 bp open reading frame encoding 611 amino acids, with a 341 bp 5' untranslated regions (UTR) and a 358 bp 3' UTR. *E75*, especially its 3' UTR, is highly conserved among the Lepidoptera insects; however, it shows significant difference in 5' UTR among four isoforms because of the difference of their promoter. The deduced amino acid sequence of *E75D* in *S. litura* share 98.4%, 79.3% and 76.5% identity with the homologues in *Spodoptera littoralis*, *Manduca sexta* and *Bombyx mori*, respectively. The miRNAs miR-14, miR-33 and miR-87, which are the most possible regulator of *E75*, were predicted by PITA and RNAhybird programs based on the highly conserved 3' UTR of *E75*. The recombinant vector pET28a-*Sli-E75D* was constructed and transformed into *Escherichia coli* BL21(DE3) and Transetta(DE3), respectively, to study the effects of rare codons on *E75D* expression. SDS-PAGE analysis of prokaryotic protein showed that the Transetta(DE3) which transformed pET28a-*Sli-E75D* recombinant vector expressed much more prokaryotic recombinant *E75D* protein (consisting of 67.19 kD *Sli-E75D*, and 7.6 kD T7·Tag and His·Tag) than BL21(DE3). The result of SDS-PAGE analysis demonstrated that Transetta(DE3) is better for the prokaryotic expression of *E75D* because it supplies tRNAs corresponding to six rare codons in *E. coli*. The expression levels of *Sli-E75* in developmental stages from the last instar larva to adult were detected by qRT-PCR. The results of qRT-PCR revealed that *Sli-E75* was expressed at a low level in 6th instar larva, and its expression increased rapidly from prepupa and reached the peak in the middle pupal stage. Then, the expression level of *Sli-E75* decreased quickly in the last stage of pupa and rebounded again in the adult. These results can contribute to the in-depth study of *E75* in molting process in insects.

Key words: *Spodoptera litura* *E75D* cloning prokaryotic expression microRNA

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