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论著

白纹伊蚊蜕皮激素拮抗剂筛选酵母模型的建立

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摘要
目的 在毕赤酵母体内构建白纹伊蚊蜕皮激素转录活化系统，建立高通量杀虫剂筛选模型，用于筛选蜕皮激素代谢途径拮抗药物。 方法 人工合成果蝇蜕皮激素响应元件（EcRE）5次重复的序列，与果蝇热激蛋白基因启动子（pHSP27）序列连接，以绿色荧光蛋白（GFP）报告基因，将EcRE-pHSP27-GFP片段亚克隆入pPIC3.5k，整合入毕赤酵母染色体构建阴性酵母A。人工合成白纹伊蚊蜕皮激素受体(EcR)及超螺旋蛋白（USP）编码序列，两个基因以双表达盒形式亚克隆入组成型表达质粒pGAPZ，整合入酵母染色体的另一位点，使EcR与USP在酵母中组成型表达，构建模型酵母B。制备蜕皮激素拮抗剂虫酰肼悬液（浓度为0.83 mg/ml），分别施加于模型酵母B与阴性酵母A，荧光显微镜下目测荧光强度，与未施加药物的对照组比较。同时提取各组RNA，半定量RT-PCR检验GFP基因的转录效率。 结果 模型酵母B发出绿色荧光， 而阴性酵母A与空白酵母GS115未见荧光，表明在模型酵母体内表达的EcR与USP形成复合二聚体，作用于EcRE启动GFP报告基因表达荧光蛋白。施用虫酰肼，模型酵母B荧光强度明显减弱，表明GFP的表达量减少。施用虫酰肼的模型酵母B，GFP与内参灰度比值(为0.614)低于对照组(1.134)，表明虫酰肼可降低模型酵母B体内GFP基因的转录水平。 结论 在酵母体内建立了白纹伊蚊蜕皮激素转录活化系统，该酵母模型可用于筛选作用于蜕皮激素代谢途径的药物。

关键词　白纹伊蚊　蜕皮激素受体　超螺旋蛋白　蜕皮激素受体响应元件　虫酰肼　蜕皮激素拮抗物

分类号

Construction of a Yeast Model for Screening *Aedes albopictus* Ecdysone Agonist Pesticides

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Abstract
Objective To reconstitute a transactivation system in yeast （yeast model） for screening the pesticides acting on ecdysone metabolism route and eventually influencing the process of ecdysis. Methods The fragment of 5 times repeated EcRE from *Drosophila melanogaster* was synthesized and the HSP27 promoter from *D. melanogaster* genome was amplified with PCR. The two sequences were connected and followed by a reporting gene——green fluorescence protein(GFP) gene. The EcRE-HSP27 promoter-GFP fragment was inserted into the expression plasmid pPIC3.5 and integrated into the yeast chromosome to construct yeast A. EcR and USP coding sequences of *Aedes albopictus* were synthesized, and these two fragments were inserted into *Pichia pastoris* expression plasmid pGAPZ as two respective reading frames. The two reading frames were integrated into *Pichia pastoris* chromosome in another recombinant site（pGAPZ and pPIC3.5k share different recombinant sites while being integrated into *Pichia pastoris* yeast chromosome). EcR and USP were constituted and expressed in the yeast. This recombinant yeast was called yeast B. The model yeast was thus constructed. A known ecdysone agonist-tebufenozide was used to test the yeast model. The effect of tebufenozide on the model yeast was observed under fluorescent microscope. Semi-quantitative RT-PCR was used to test the transcrip-tion level of GFP in the tebufenozide affected yeast and the control. Results In the model yeast， the intracellular expressed EcR and USP constituted EcR/USP heterodimer interacting with EcRE， the expression of GFP was activated， and green fluorescence was observed in model yeast under fluorescent microscope. Tebufenozide affected model yeast showed less fluorescence in comparison to the control model yeast， indicating that the transcription of GFP was suppressed by tubufenozide. Yeast housekeeping gene Actin-1 was used as inner control， semi-quantitative RT-PCR was operated and the result was scanned. The ratio of the brightness of GFP to Actin-1 was calculated automatically， and that of

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tubufenozide added yeast and the control yeast was 0.614 and 1.134 respectively. This result showed a low transcription level of GFP in tebufenozide affected model yeast, comparing to that of the control. Conclusion The ecdysone-related transacting system in yeast has been constructed, and the model yeast can be used to screen the ecdysone agonists which can act on the ecdysone metabolic route.
Key words *Aedes albopictus* Ecdysone receptor （EcR） Ultraspiracle protein （USP） Ecdysone recepter element（EcRE） Tebufenozide Ecdysone agonist

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