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论著

*RNR2*调控的重组绿色荧光蛋白酵母细胞的构建及其对化学诱变原的高通量筛选

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摘要 目的 建立*RNR2*调控的酵母增强绿色荧光蛋白(*yEGFP*)发光酵母细胞，高通量筛选化学诱变原。方法 用PCR方法从酵母(W303-1A)基因组扩增*RNR2*启动子，经酶切后，用T4连接酶与线性化的含酵母嗜好遗传密码子的*yEGFP*报告载体相连，连接产物转化子质粒经酶切和测序鉴定，构建*RNR2*调控的*yEGFP*酵母报告载体。用醋酸锂方法将其转化于W303-1A酵母细胞，从而构建成*RNR2*调控的*yEGFP*发光酵母细胞(W303-1A/*RNR2*-*yEGFP*)。用甲磺酸甲酯0~400 mg·L^{-1}分别作用于该发光酵母细胞0，4，8，12，16和20 h后，于倒置荧光显微镜下观察荧光，用多功能酶标仪检测其荧光发光强度，选择最佳诱导时间；用不同浓度的DNA烷化剂、DNA断裂剂和DNA合成酶抑制剂作用于该重组细胞16 h，检测其荧光发光强度，考察W303-1A/*RNR2*-*yEGFP*细胞对各种化学诱变原的敏感性。结果 经测序确定W303-1A/*RNR2*-*yEGFP*构建成功。选择16 h为最佳诱导时间；各种化学诱变原与W303-1A/*RNR2*-*yEGFP*细胞作用16 h后，与DNA发生结合的化合物中放线菌素D和溴乙锭诱导的发光度与对照组无明显差别，发光倍数<1.5；与DNA发生烷基化的化合物中，甲磺酸甲脂200 mg·L^{-1}诱导的细胞发光度最强，发光倍数为5.21，瘤可宁200 μg·L^{-1}诱导的发光倍数为1.9，而丝裂霉素C的发光度与对照组无明显差别。在使DNA发生断裂的诱变原中，顺铂250 mg·L^{-1}诱导的细胞最高发光倍数为3.7，其次4-硝基-N-氧化喹啉3.1 mg·L^{-1}、博来霉素12.5 mg·L^{-1}和福来霉素200 mg·L^{-1}，最高诱导倍数分别为2.35，2.26和2.53；在抑制DNA合成酶或拓扑异构酶的诱变原中，5-氟尿嘧啶500 μg·L^{-1}、羟基脲570.45 mg·L^{-1}和喜树碱30 mg·L^{-1}所诱导的细胞最大发光倍数分别为2.36，2.65和2.53；而非基因毒性化合物秋水仙碱、刀豆氨酸和四环素诱导的发光度与对照无明显差别。结论 该重组发光酵母细胞可用于对多数造成DNA断裂或合成阻断的化学诱变原筛选，具有快速、方便和高通量等特点。

关键词 *RNR2*启动子 酵母细胞 绿色荧光蛋白 高通量 化学诱变原

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Construction of recombinant yEGFP yeast cells regulated by RNR2 and high throughput screening for chemical mutagens

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Abstract

OBJECTIVE To establish recombinant enhanced green fluorescent protein (*yEGFP*) yeast cells regulated by *RNR2* for high throughput assay to screen chemical mutagens. **METHODS** *RNR2* promoter was amplified by PCR from W303-1A yeast genosome and was digested by enzymes. Then, it was ligated by T4 ligase with the linearized yeast reporter vector containing the green flurorescent protein codon optimised for yeast (*yEGFP*). Plasmids of the transformants were identified by enzymes and sequenced. The yeast *yEGFP* reproter vector regulated by *RNR2* was constructed. The yeast strains (W303-1A) were transformed with this vector by lithium acetate method. The fluororescent yeast cell (W303-1A/*RNR2*-*yEGFP*) regulated by *RNR2* was successfully constructed. W303-1A/*RNR2*-*yEGFP* cells were treated by Methyl methanesulforate (MMS) 0-400 mg·L^{-1} for 0, 4, 8, 12, 16 and 20 h before fluorescence was observed under an inverted fluorescence microscope. Fluoroscent density was detected by multifunctional enzyme mark instrument, and the time for the biggest fluorescent fold was selected as the optimised inducement time. Then, W303-1A/*RNR2*-*yEGFP* was treated by the different concentrations of chemical mutagens for 16 h, and the cell fluorescent density was detemined by multifunctional enzyme mark instrument to find the sensitivity to this cell. **RESULTS** The flurorescent W303-1A/*RNR2*-*yEGFP* regulated by *RNR2* was successfully contructed by identificantion. After W303-1A/*RNR2*-*yEGFP* was treated by the different concentrations of MMS for the different time, the maximum fluorescent fold induction was 5.21 at 16 h, so 16 h was selected as the optimised induction time. After W303-1A/*RNR2*-*yEGFP* was treated by the different chemical mutagents for 16 h, in DNA intercalation agents, the fluorescent density induced by actinomycin D and ethidium bromide was not significantly different from that in control group; the fold induction was less than 1.5. In DNA alkylation

compounds, the cell fluorescent density induced by MMS 299 mg • L^{-1} was the strongest, and the fluorescent fold induction was 5.21, the fold induction was 1.9 for chlorambucil 200 μg • L^{-1}, but the fluorescent fold induction of mitomycin C showed no difference from that in control, with the fold induction less than 1.5. In the DNA cleavage mutagens, the cell fluorescent density induced by cis-Platinum 200 mg • L^{-1} was the strongest and the most fold induction was 3.7. And for 4-nitroquinoline-N-oxide(4-NQO) 3.1 mg • L^{-1}, bleomycin 12.5 mg • L^{-1} and phleomycin 200 mg • L^{-1}, the most fold inductions were 335, 326 and 353, respectively. Among the inhibitors of DNA polymerases or topoisomerase mutagens, the cell fluorescence fold induction for 5-fluorouracil 500 μg • L^{-1}, hydroxyurea 570.45 mg • L^{-1} and camptothecin 30 mg • L^{-1} were 2.36, 2.65 and 2.53, respectively. Compared the fluorescent density induced by the non-genotoxic compounds such as colchicine, canavanine and tetracycline with that did by the control, the no significant differences were found. **CONCLUSION** W303-1A/*RNR2-yEGFP* can screen many of DNA damage chemicals related to DNA cleavage or synthesis block, with characteristics of rapid, convenience and high throughput.

Key words *RNR2* promoter yeast green fluorescent protein high throughput

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