

# Establishment of a novel cell line of the red flour beetle for applications in insect gene functional analysis

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**A novel cell line Tc81 was established from embryos of the red flour beetle, *Tribolium castaneum*, and was used for the analysis of juvenile hormone (JH) signaling pathway. Because of the high efficiency in RNAi and gene transfection, this cell line is not only useful for the analysis of JH signaling, but also a promising tool for the functional analysis of various insect genes in general.**

Keywords: red flour beetle, *Tribolium castaneum*, cell line, RNAi, transgenesis, juvenile hormone

## Background

The red flour beetle *Tribolium castaneum*, a stored grain insect pest, has been widely used as a laboratory model next to the fruit fly. As an experimental organism, *T. castaneum* has many advantages such as easy rearing, a short life cycle, complete genome information, and highly efficient RNAi implemented by injecting dsRNA into individuals. However, the cell lines of *T. castaneum* available for gene function analysis have not yet been reported. In this study, we characterized a novel cell line Tc81 derived from embryos of *T. castaneum* and validate its utility by investigating the efficiencies of RNAi and gene transfection, and analyzing the JH signaling pathway.

## Results and Discussion

1. We have succeeded in establishing a novel cell line Tc81 from the embryos of *T. castaneum*. The Tc81 cells formed vesicles upon suspension in the culture medium (Fig. 1).
2. The Tc81 cells showed high efficiency in RNAi by simply soaking in low concentrations of dsRNA, and the effect persisted for more than one week (Fig. 2).
3. The Tc81 cells were highly sensitive to JH. The JH-inducible gene *Krüppel homolog 1* (*Kr-h1*), a JH-dependent repressor of insect metamorphosis, was rapidly induced by subnanomolar level of JH (Fig. 3).
4. Gene transfection and reporter assay of Tc81 cells showed a JH-dependent increase in the reporter activity in the cells transfected with the reporter plasmid carrying JH response element (JHRE) and a firefly luciferase gene (JHRE-reporter) (Fig. 4).
5. RNAi of JH receptor (*methoprene tolerant*, *Met*) and its partner (*steroid receptor co-activator*, *SRC*) reduced JH-dependent activities of JHRE-reporter in Tc81. This result revealed the presence of a common JH signaling pathway in *T. castaneum* and *B. mori*, in which JH-dependent interaction of *Met* and *SRC* to JHRE induces the expression of *Kr-h1*.
6. The Tc81 cell line did not show obvious change in morphology, efficiency of RNAi and gene transfection, and JH response after more than three years of culture (>150 passages) suggesting stability of the cells.

## Future prospects

1. The Tc81 cell line could be used as a general and powerful tool not only for the elucidation of JH signaling pathway but also for the functional analysis of various insect genes.
2. This cell line is useful for developing a novel insect growth regulators against Coleopteran insect pests.

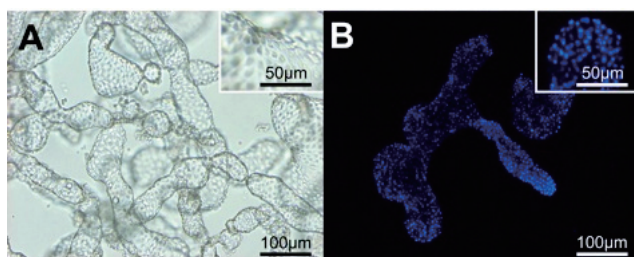


Fig. 1. Morphology of Tc81 cells. (A) Vesicle formation in Tc81 cells. (B) DAPI staining of the nuclei of Tc81 cells.

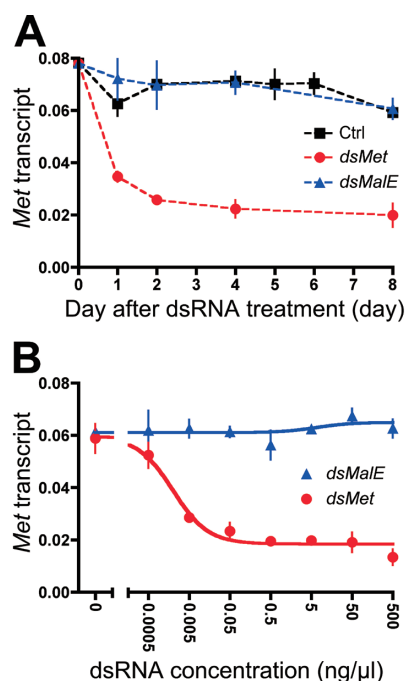


Fig. 2. RNAi efficiency in Tc81 cells. (A) In cells treated with dsRNA of *Met* gene (dsMet, final concentration 50 ng/μl), *Met* transcript level rapidly decreased to about one-fourth of initial level by day 2, and the effect was maintained for more than a week. (B) A near maximal RNAi effect was obtained at a concentration of 0.05 ng/μl dsRNA. *MalE*: negative control.

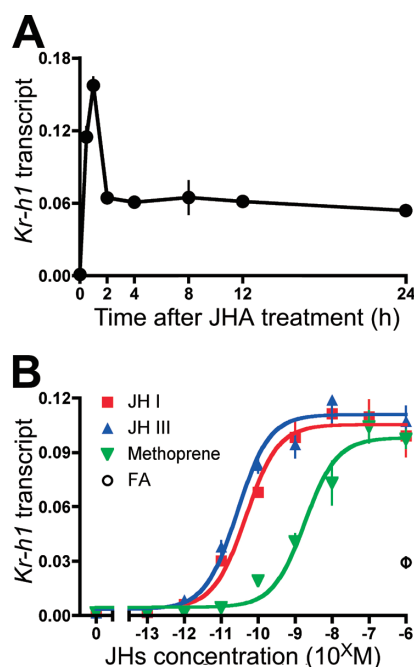


Fig. 3. JH response of Tc81 cells. (A) *Kr-h1* transcript rapidly reached a peak at 1 h after JH analog treatment (methoprene, final concentration 10 μM). (B) *Kr-h1* transcript was induced by low concentrations of JHs (10<sup>-11</sup>M).

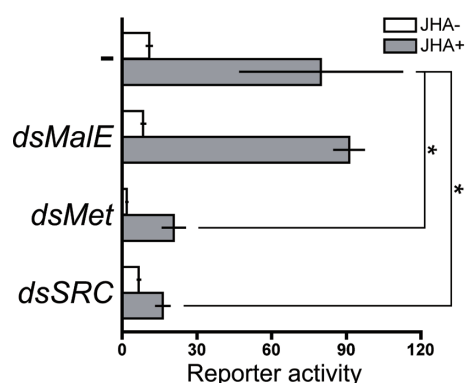


Fig. 4. Analysis of JH signaling pathway using Tc81 cells. The RNAi of *Met* and *SRC* genes significantly reduced the activities of JHRE-reporter. *dsMalE*: negative control.

## Reference

1. Kayukawa T, Tateishi K, Shinoda T (2013). Establishment of a versatile cell line for juvenile hormone signaling analysis in *Tribolium castaneum* *Scientific Reports* 3: 1570