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Selection of Differentially Expressed Clones during *Bombyx mori* Embryogenesis

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Objectives

To understand the molecular mechanism of diapause determination and termination during embyogenesis of the silkworm, *Bombyx mori*, mRNA from maintained or activated eggs of diapause was compared using differential expression.

Materials and Methods

1. Materials : *Bombyx mori* diapausing eggs and diapause activated eggs
   cDNA library
2. Methods : cDNA library screening, Dot blot analysis, RNA isolation,
   and Northern blot analysis

Results and Discussion

We selected 22 different cDNA clones which express differentially from mRNAs of maintained or activated eggs of diapause. Among these clones, we have isolated several clones showing high similarity with caspase of *D. melanogaster*, cytochrome oxidase subunit I (*Bombyx mori*) and etc. The selected clones were used to generate randomly primed probes and to screen northern blots to confirm the results of the differential screening. The probes hybridized with blotted total RNA of diapause-maintained eggs (diapausing egg) and diapause-activated eggs (developing eggs) on nylon membrane. As a results, we found that cytochrome oxidase subunit I hybridized the position of 2kb. Cytochrome oxidase subunit I was expressed at low level in diapausing eggs, but was begin to increase the expression level at 7-day in developing eggs. We suggest that cytochrome oxidase subunit I is involved in embryogenesis of *Bombyx mori* eggs during termination of diapause.

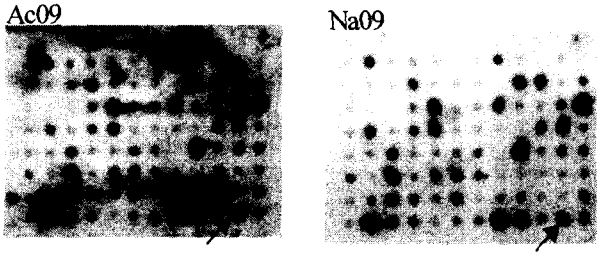

Fig. 1. Differential screening by cDNA library from developing eggs (Ac09) and diapausing eggs (Na09). Arrow indicate the selected clone, cytochrome oxidase subunit I.

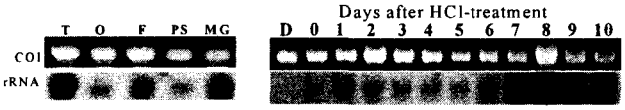

Fig. 2. Expression of cytochrome oxidase subunit I in several tissues and each egg stages. HCl-treatment was used for development at 20h after oviposition. Total RNA (20μg) isolated from several tissues(left) and developing eggs (right) was separated on a 1.2% formaldehyde-agarose gel and transferred onto nylon membrane. rRNA was used as the control gene. COI: cytochrome oxidase subunit I, T: testis, O: ovary, F: fat body, PS; posterior silk gland, MG: mid gut, D: diapausing eggs.

Reference

Masaki Yara, Eiichi Kosegawa,/yasushi Sugimoto, Katsymi Kaga, 1994, Comparison of translation products of mRNA from unfertilized eggs of *Bombyx mori*. J. Seric. Sci. Jpn, 63(4), 336-340.