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Reverse genetics of *Bombyx mori* calreticulin in insect cells using small interfering RNAs

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As the sequencing of the Bombyx mori genomes will be completed in a short time there is an increasing demand for quick and efficient mechanisms to analyze gene function in insect cell culture. So far various methods to study gene function are shown such as gene deletion, transgenes, antisense techniques and microinjection. However there are no effective methods are developed for the Bombyx mori's gene study, and a lot of cloned cDNAs provide insufficient information on protein function. The method of RNA interference is recently developed to study of sequence-specific gene silencing, which provided practical tools to silence gene expression in cultured cells. And it is opening new possibilities for functional studying of unidentified genes and proteins by reverse genetics. In fact, although the mechanism of small interfering RNA (siRNA) method is not yet clear, it is very simple to use in vitro experiment; may silence only one mRNA that is homologous in the region complementary to the siRNA. So far the information of siRNA method in mammalian cells is considering, but not in insect cells. Thus, we have established the siRNA method for the insect cells using artificially synthesized 22 bp RNA fragments encoding Bombyx mori calreticulin (b-calreticulin). These approaches involve introducing silent mutations in which no expression of b-calreticulin. The result will be available for the study of insect sciences including insect biochemistry & molecular biology, insect physiology and pest management. In addition, this technology in future will give powerful opportunity to understand genomewide analysis of insect gene function in cultured cells.