2007

Production of a feline interferon in transgenic silkworms

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Systems for producing recombinant proteins have been developed in many organisms. Transgenic organisms are thought to be useful for producing large amounts of proteins and in some cases it is also possible to produce proteins containing the mammalian types of sugar chains. The protein production systems in transgenic silkworm have been developed because the silkworm possesses a very efficient organ adapted for the production of proteins. The systems developed in the silkworm use a middle part of the silk gland and sericin 1 and fibroin L chain gene promoters have been used for gene expression. However, the most abundant protein in the silk gland is the fibroin H chain. The system using the fibroin H chain gene has not been used for the production of globular proteins because of the difficulty in purification of products. Therefore, we applied the *piggyBac* vector utilizing the fibroin H gene for production of recombinant proteins. The vector possesses the DsRed gene under the control of the eye-specific expression promoter 3xP3 as a marker and the long upstream region of the fibroin H chain gene. The gene for the production of the recombinant protein is inserted into the middle part of the fibroin H gene. In this experiment, we produced feline interferon as a model pharmaceutical protein in the transgenic silkworm and investigated the possibility of recovering the active form of protein from the cocoon and silk gland.

The structure of piggyBac transposon vector for the production of feline interferon is shown in Fig. 1. We constructed two types of vectors. One possessed the interferon gene without a protein cleavage site and the gene was fused with N and C terminal sequences of fibroin H chain gene. We used short sequences in both terminal regions but the product still had short N and C terminal sequences of amino acids of fibroin H chain. The other vector possessed the feline interferon gene with the two protease cleavage sites in the N and C terminals. The product was expected to be produced as a fusion protein with rather long N and C terminal sequences of fibroin H chain. Using these vectors, we successfully constructed the transgenic silkworms. The protein was extracted from the cocoon and analyzed by SDS-PAGE and Western blotting. As shown in Fig. 2A and B, a rather large amount of interferon was produced in both strains. The molecular weight of the products precisely agreed with the products estimated from the gene structure. Then we extracted the proteins from the silk gland and measured the activity of interferon. As expected the activity of the silk solution in both strains was rather low. However, the intact size of the interferon was detected when we treated the solution with PreSciccion protease. The activity of the cleaved product possessed very high interferon activity that was not much different from standard interferon.

From the result, we concluded that the production of pharmaceutical protein is possible using the system of fibroin H chain gene. However, the protein must be tolerant to the detergent and treatment with a specific protease. We note that this is the first experiment using fibroin H chain gene for the production of recombinant protein. The active protein was recovered from cocoons of transgenic silkworm.

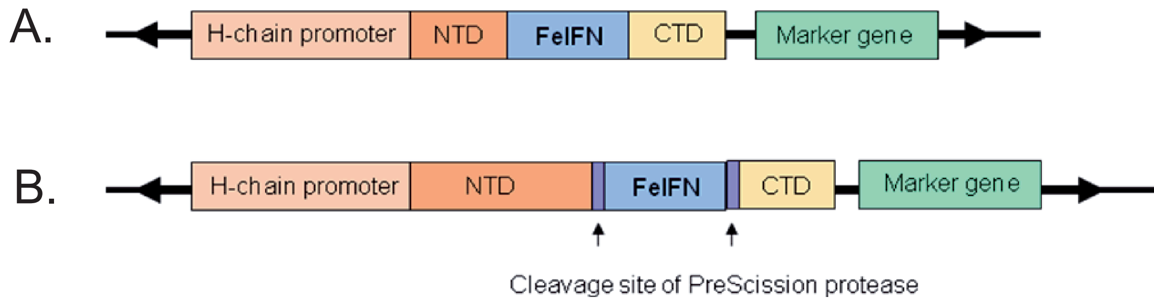

Fig. 1 Structure of *piggyBac* transposon vector for the production of feline interferon (FeIFN)
A. Vector of feline interferon without protease cleavage site. B. Vector for the production of feline interferon with protein cleavage site. N-terminal and C-terminal domains were shown by NTD and CTD, respectively.

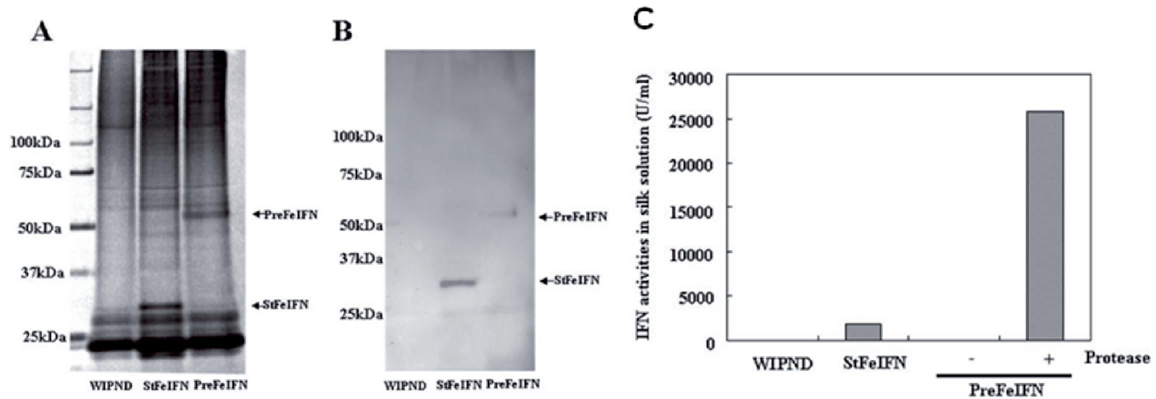

Fig. 2 Analysis of feline interferon extracted from the transgenic cocoons by SDS-PAGE (A), Western-blotting (B) and interferon activity(C)
Treatment of protease was shown by +. StFeIFN and PreFeIFN indicate the products of the transgenic strains possessed the interferon gene without or with protease cleavage sites, respectively.