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Large-scale Expression System of the Foreign Protein in *C. elegans*

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Purpose

The free-living nematode *Caenorhabditis elegans*, whose entire genome sequence has been determined, is a tractable experimental model system for the study of both vertebrate and invertebrate biology. Although several host systems are available, the problems (e.g., protein folding, post-translational modification) have not been resolved in expression of the eukaryotic protein. *C. elegans* is a multicellular eukaryote, and also proliferated in large-scale culture as well as bacteria.

We have been studying about mitochondrial Complex II of the parasitic nematode *Ascaris suum*. *A. suum* adults inhabit the host small intestine where there is limited oxygen tension (0～10 mmHg), and employ anaerobic metabolism. Particularly, mitochondrial Complex II, which shows fumarate reductase activity, plays a important role in adaptation to the anaerobic environment. In this study, to evaluate the expression system of the foreign protein in *C. elegans*, we tried to express recombinant *A. suum* Complex II in *C. elegans*.

Method

1) The expression plasmids were constructed as follows: 2 kb promoter region of the gene for the Ip subunit of *C. elegans* Complex II was inserted into a Fire vector (pPD 49.26) MCS1. And, each cDNA region of the four subunits of *A. suum* Complex II was inserted to this construct. 2) A mixture of these constructs was injected into *C. elegans* according to the standard microinjection method. Transmitting lines were established and maintained by selection for the GFP expression. The integrated line was generated by UV irradiation (Fig. 1). Further expression analyses were conducted with western blotting and enzymatic activity analysis.

Result

The promoter of the *C. elegans* Ip subunit induced GFP expression in the whole body of the worm. Plasmid mixture was transformed to Bristol N2 strain by microinjection and GFP expression strains were obtained. UV irradiation was conducted in order to obtain the integrated line. By standard separation procedure, two integrated lines, which contains all four subunits, were obtained. Western blot analysis showed that each subunit was translated and localized in mitochondria. And, the fumarate reductase activity of the transgenic line was about three-fold upregulated compared to that of wild-type. Furthermore, chromatographic analysis showed that each elution peak of four subunits was identical.

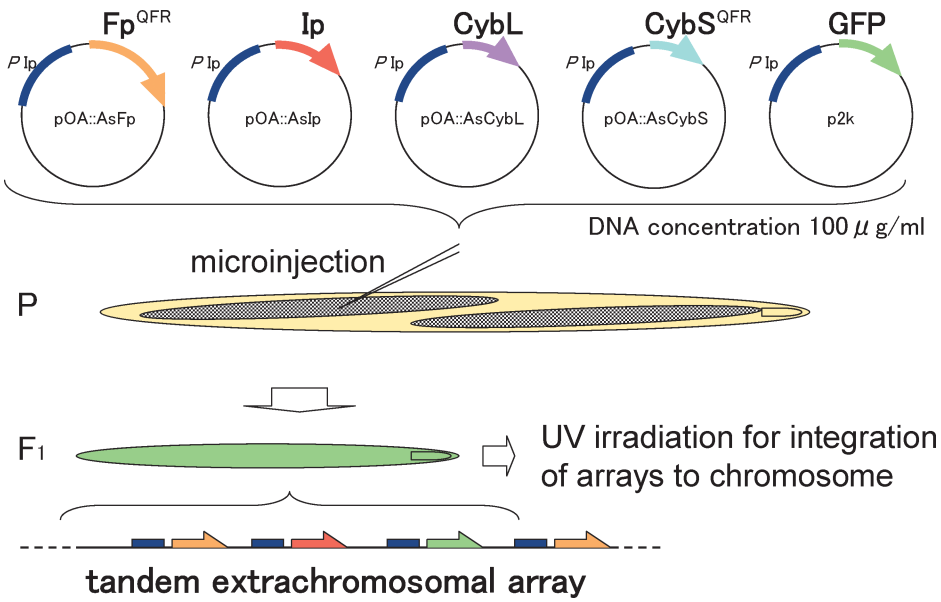

Fig. 1. Scheme of obtaining transgenic line.

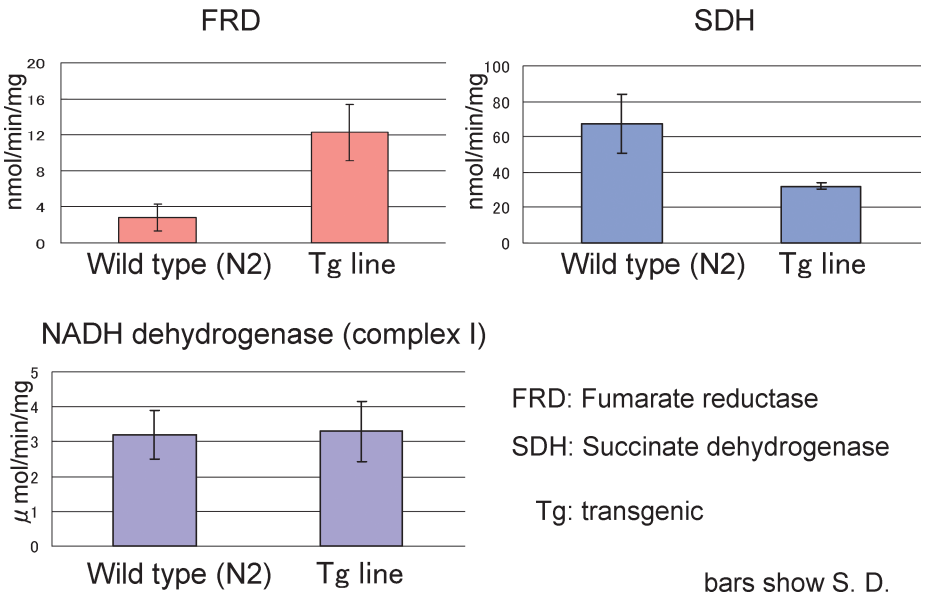

Fig. 2. Comparison of enzymatic activities.

Conclusion

A. suum FRD complex II was functionally expressed in *C. elegans*. Although the expression level was not high enough for biochemical analysis, this is the first report of expression of mitochondrial membrane protein in *C. elegans*. These results indicate that *C. elegans* could be used as an expression host for the multi subunit protein of eukaryote.