Heterologous expression systems for the production of functional antibody fragments

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Introduction Antibody fragments (scFv), either selected from phage display libraries or derived from antibody-secreting hybridoma cell lines, can be cloned and functionally expressed in heterologous systems (see Ann. Rep., 1995, 125-127). These reagents are now being used in numerous biotechnological and biomedical applications, such as diagnosis and therapy. ScFv can be a source of standardised reagents for use in plant pathology and other areas of the plant sciences. Furthermore, by expressing anti-viral scFv in their cells, plants can be made disease-resistant. ScFv that recognise molecules naturally found in plants, can be expressed in plants to immunomodulate plant metabolism.

We have produced novel diagnostic reagents for plant viruses and plant enzymes by selecting antibody fragments from phage display libraries, or by cloning genes that encode scFv from existing hybridoma cell lines. The scFv reagents have been expressed in *Escherichia coli* cells and used successfully for virus detection by various immunological methods, such as ELISA, Western blotting and tissue printing (see Ann. rep., 1997/98, 111-113). The reagents were extensively tested (EC AIR3 project CT94-1046) and found to perform as well as monoclonal or polyclonal antibodies.

Heterologous expression of scFv Antibody fragments that are to be used in disease diagnosis or therapy have to be produced in large quantities and the preparations must be of consistently good quality. This report describes scFv expression in four different systems.

E. coli

Traditionally the most widely used organism for the heterologous expression of antibody fragments, the advantages of *E. coli* include easy manipulation, rapid growth of the bacteria, and simple media requirements. An scFv (scSCR20), derived by molecular cloning from a hybridoma cell line that secretes an anti- African cassava mosaic virus monoclonal antibody, was expressed in *E. coli*. The yield in simple shake flasks was 1 mg scFv per litre of culture.

However, there are also potential disadvantages with the bacterial system. For example, loss of the plasmid during culture, or toxicity of some scFv sequences, can lead to low or no accumulation of scFv, or to the production of insoluble protein aggregates. Also, the post-translational modifications of the recombinant protein that could occur in eukaryotic cells are not possible in prokaryotic systems such as *E. coli*. Therefore, in parallel with the expression in *E. coli*, alternative methods of scFv production such as use of insect cells and plants were compared.

Insect cells

Two different systems were compared: the Baculovirus expression system (Invitrogen), which adapts an insect virus for the transient expression of recombinant protein in infected cells, and the *Drosophila* expression system (DES, Invitrogen), in which insect cell lines are stably transformed.

BACULOVIRUS SYSTEM: The *Baculovirus* vectors for the infection of *Spodoptera frugiperda* (or other insect) cells contained the genetic information for a melittin signal peptide derived from the honey bee. This sequence facilitates the secretion of the expressed protein to the culture medium. However, when scSCR20 was expressed from the *Baculovirus* vector in any of three different insect cell lines, the scFv was retained within the cells (Fig. 1) and could not be detected in the culture medium. This could be a result of the baculovirus infection affecting protein

secretory mechanisms in the infected insect cell. However, other proteins can be secreted using the baculovirus system. Probably, specific features such as the amino acid sequence of the scFv can modify the behaviour of the expressed fusion protein.

DROSOPHILA SYSTEM: In contrast to the Baculovirus system, the *Drosophila* system relies on stable cell lines that express the foreign protein. Since the *Drosophila* cells will spontaneously incorporate hundreds of copies of the transfected genetic information, the expression levels should be very high, and no extensive testing of cell lines for expression is required. Stable cell lines can be established after co-transfection with a plasmid that confers resistance to the antibiotic hygromycin B. The expression of the foreign protein is under the control of the metallothionein promoter, and the system can be induced by the addition of non-toxic concentrations of metal ions such as copper. For scSCR 20, a yield of 20 mg per litre of cell culture was obtained. The bulk of the scFv was found secreted to the culture medium (Fig. 1) as expected, which allowed easy purification of the recombinant scFv.

Plants

Plants, too, can be used as alternatives to microbial fermentation for the cost-effective production of large amounts of therapeutic proteins, including antibodies. We have investigated the effects of the expression of scFv in plants on virus replication and on plant metabolism, using scFv specific for virus-encoded replicase and a scFv that binds to a plant enzyme, potato granule-bound starch synthase (GBSS).

Figure 1 Immunoblot of scSCR20 produced in insect cells. D Drosophila system B Baculovirus system.

However, not every antibody sequence is equally well suited for high-level expression in plants. There are, as yet, no general rules for the design of effective constructs that result in accumulation of functional scFv. In order to assess quickly the performance of a number of different constructs, a transient expression system based on the plant virus, Potato virus X, was employed (see Ann. Rep., 1995, 125-127).

The system allows a variety of different constructs to be evaluated (Fig. 2) in a short time. The influence of signal sequences and fusion proteins on the localisation, level of accumulation and functionality of the product can be analysed quickly.

Using the PVX system, an scFv against GBSS that had been selected from an antibody phage display library, was expressed in *N. benthamiana* as a fusion with an IgG human kappa constant region. A murine IgG leader sequence was used to target the scFv to the apoplast. ScFv's recovered from plant extracts were found to be functional in both ELISA and Western blot for the detection of GBSS (Fig. 3). Also, a number of constructs for the expression of scSCR20 were successfully tested using the PVX system.

ScFv constructs found suitable in the PVX system were used for *Agrobacterium* mediated transformation of potato. Expression levels reached 0.5% of total soluble protein.

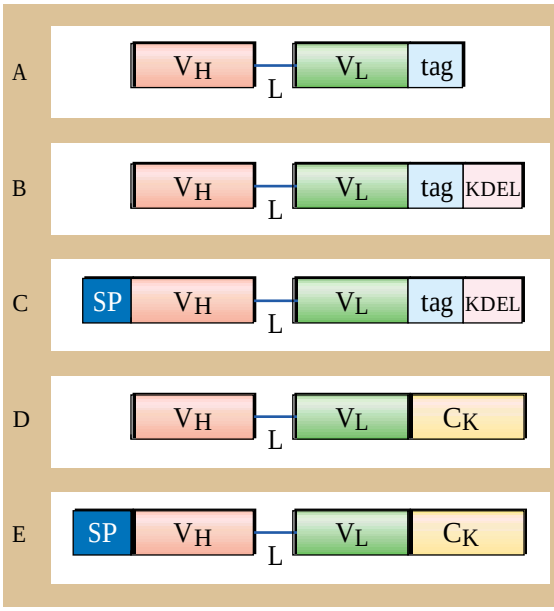

Figure 2 Structure of different scFv constructs tested in the PVX expression system.
$V_H \overline{L} V_L$ = scFv
SP = murine IgG leader
tag = peptide epitope tag for detection
KDEL = signal peptide for retention in endoplasmic reticulum
C_K = IgG human kappa constant region

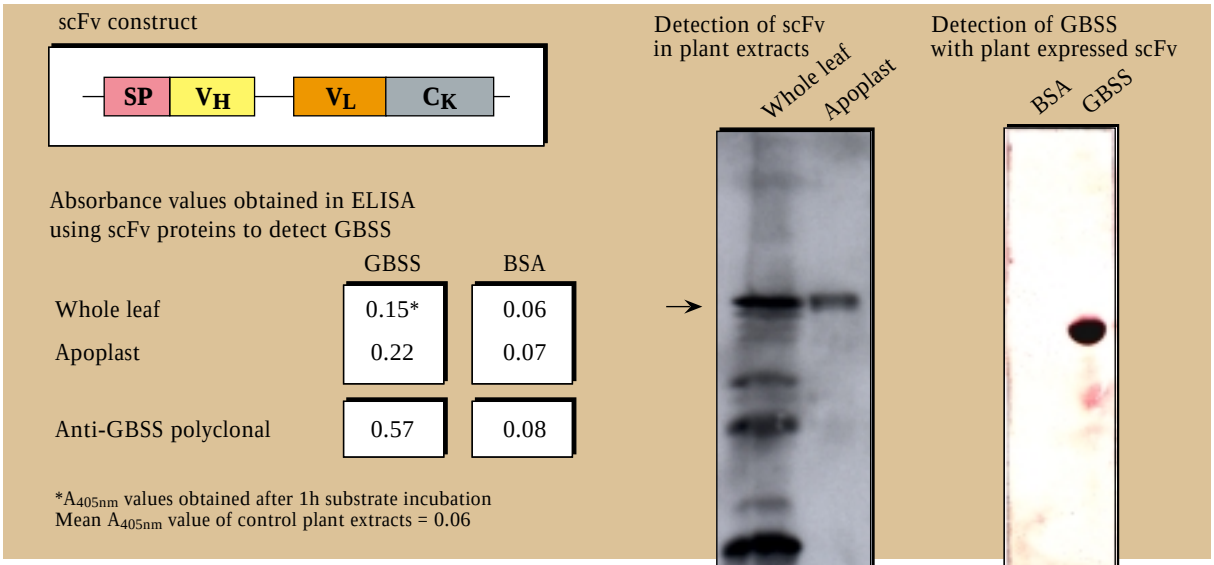

Figure 3 PVX vector expression of GBSS-specific single chain Fv.

Conclusions The choice of expression system depends on a number of factors, such as the projected use of the scFv (e.g. diagnosis, therapy or antibody mediated resistance). Also, the quantities of scFv required and the methods of purification and validation that are available for each system. Once a system has been chosen, it has to be optimised for each scFv sequence. There is no one expression protocol that is ideally suited for every scFv.

Insect cells offer a cost effective, high efficiency alternative to *E. coli* expression.

Unlike *E. coli*, insect cells are capable of performing post-translational modifications similar to mammalian cells. The insect cells can be grown in stirrer vessels at room temperature, which can help to stabilise the scFv, so there is no need for specially equipped incubators.

For scSCR20, the yield using the *Drosophila* system was 10x the yield from the *E. coli* expression. With the PVX system, testing of constructs for plant expression of scFv can be done before committing time and resources to the production and analysis of stable transformed lines using *Agrobacterium*, a process that usually takes many months.

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