REVIEW ARTICLE

Molecular farming: production of drugs and vaccines in higher plants

Atsuhiko Shinmyo and Ko Kato

On the basis of developments in plant biotechnology, drug and vaccine production by higher plants can be added to microbial and animal cell culture processes. When genes encoding drug or vaccine formation under a suitable promoter are introduced into plants, these useful compounds can be economically produced from CO₂ and inorganic chemicals using sunlight. The merits and demerits of the plant process are discussed in this paper.

The Journal of Antibiotics (2010) 63, 431–433; doi:10.1038/ja.2010.63; published online 30 June 2010

Keywords: chloroplast transformation; glycosylation; medicinal protein; molecular farming; plant; Prof. Demain; transgene expression

Although plants have been used for medicinal purposes for thousands of years, chemical and microbial medicines are being consumed more at present. Recombinant DNA technology in higher plants has opened up a new field of basic research and application in plant science. Basically, foreign genes can be introduced into any kind of plant and it is possible to produce therapeutic proteins, such as antibodies, blood products, cytokines, growth factors, hormones, vaccines and enzymes. The first recombinant plant-derived pharmaceutical protein was human serum albumin discovered in 1990 in transgenic tobacco and potato plants.¹

From an economic point of view, the production cost of biomolecules using plants is less than that of microbial and animal cell cultures, which require equipment, substrate and electric energy supply. Plants can synthesize any protein and metabolite from CO₂ and inorganic chemicals using solar energy. The production cost of immunoglobulin A by transgenic plants is estimated to be less than 1% of that by mammalian cell culture, and less than 5% of that by transgenic goats.²

PRODUCTION OF MEDICINAL PROTEINS

When protein encoding gene is introduced into plants under the control of a strong promoter of Cauliflower mosaic virus 35S RNA, foreign protein can be produced in plants. The production of recombinant proteins in plants has many advantages:

- (1) The plant system is more economical than industrial facilities using a fermentation system.
- (2) Technology for harvesting and processing on a large scale has already been established in agricultural and food industries.
- (3) Health risks arising from contamination with potential human pathogen and microbial end toxins are minimized when a suitable plant host is selected.

- (4) Purification can be eliminated when plant tissue such as tomato fruit containing recombinant protein is used as food (edible vaccines).

INCREASE OF ACCUMULATION OF FOREIGN PROTEIN IN PLANTS

Although the plant system for production of medicinal proteins has many advantages, there are several barriers that have to be overcome for industrial application. Accumulation of protein in transgenic plants is most important from an economic point of view. The amount of protein accumulated in plant tissue is not high enough; it is less than 0.1% of the total soluble protein in general.

Accumulation of protein is regulated in several steps in transcription, translation and posttranslation. The important element is promoter. As promoter activity is generally controlled by environmental stress, phytohormone and nutrients, and by specific tissue through *cis*-elements and transcription factors, a suitable promoter should be selected in each plant host.

We found that the 5'-untranslated region of the tobacco *alcohol dehydrogenase* (*ADH*) gene has translational enhancer activity. When the 5'-untranslated region of the *ADH* gene was placed upstream of the coding region of a foreign gene, accumulation of the protein increased 60–100 times in tobacco and *Arabidopsis* (dicotyledon), but not in rice (monocotyledon). Interestingly, the 5'-untranslated region of the *ADH* homolog in rice enhanced the translation of a foreign gene by 10 times in rice, but not in tobacco.^{3,4}

The terminator of bacterial gene *nos* (nopaline synthase) or *ocs* (octopine synthase) is generally used in vector construction for the transformation of plants. We have tested several terminators from plant genes, and found that the terminator of the heat-shock gene of

Arabidopsis thaliana shows an increase in transcription of a foreign gene by four times.⁵ Among 64 different combinations of nucleotides at positions -3 to -1 of ATG initiation codon, translation efficiency varied, and the optimum sequence was (A/G)(a/c)(a/g) AUG in *A. thaliana* and (A/G)(u/C)(g/C)AUG in *Oryza sativa*.⁶ Alteration of the codon of a mammalian gene to plant type is also important to increase translational activity. When a foreign gene is introduced into a plant by *Agrobacterium* infection or particle bombardment, the number of genes introduced deviates generally from single to multicopy. Multicopy genes are often involved in gene silencing, but single-copy genes show a high and stable expression in progeny.⁷ If we choose the optimum combination of transcription and translation units, we can expect to accumulate foreign protein in high concentration; hence, we succeeded in accumulating β -glucuronidase of more than 1% of total soluble protein in *Arabidopsis*.⁵

CHLOROPLAST TRANSFORMATION

Expression of foreign genes in chloroplast is expected to produce a high amount of proteins. An expanded plant leaf contains about 100 chloroplast organelles in a cell, and one chloroplast contains ~100 copies of circular chloroplast genome. Therefore, a foreign gene introduced into a chloroplast genome shows a strong gene dosage effect. Tobacco leaf accumulated green fluorescent protein of 30% of total soluble proteins when its gene was introduced into the chloroplast genome.⁸ Chloroplast transformation takes place by particle bombardment of gold particle coated by vectors against green organelle, and regeneration of transformed plants successfully takes place in several plants, such as tobacco, potato and lettuce.

Transformation of the chloroplast genome has another benefit in the cultivation of transgenic plants in the field. An environmental concern of transgenic plants is the crossing of transgenic pollen with weeds or related crops. As chloroplasts are not incorporated in the pollen (mother heritage), introduction of a foreign gene into chloroplast genome is significantly less harmful than introduction into nuclear genome.

Difference in glycosylation of protein in animals and plants

N-linked glycans in plants are different from those in animal cells. Plant N-glycans contain high mannose, β -1,2 xylose and α -1,3 fucose, which are not found in mammals. Plant glycan also lacks sialic acid, which represents ~10% of the sugar content of the mouse monoclonal antibody. Although there are several reports in which differences in glycan structure seem to have no effect on antigen binding, we cannot neglect the potential immunogenicity and allergenicity of plant protein used as human therapeutics. Isolation and introduction of mammalian-type transglycosylase gene into plant cells is expected to modify plant glycoproteins.^{9,10} Recently, synthesis of sialic acid in plant and sialylation of plant-expressed protein were reported.¹¹

Japanese national projects for production of valuable materials by plants

Transgenic plants are expected to produce not only protein but also other biological active compounds. The *Zea mays* phytoene synthase gene was introduced into rice grains. The resulting transgenic rice grain, which is called Golden rice, accumulated β -carotene at high levels and showed a yellow color.¹² Its consumption will be used to overcome vitamin A deficiency in developing countries.

The Japanese government's Ministry of Economy, Trade and Industry (METI) is conducting two national projects for the development of technology for production of bioactive compounds in

plants (see <http://www.ra-bio.or.jp/index.html>). The first project, 'Development of Fundamental Technologies for Controlling the Material Production Process of Plants' (2002–2009), was conducted by 10 industries and 17 universities and by national institutes including Chinese and Indonesian institutes under the support of the New Energy and Industrial Technology Development Organization (NEDO). The objective of the project is the development of basic technology for process control based on the analysis of the metabolic pathway for the production of useful compounds by higher plants using recombinant DNA technology. Target products include rubber, pulp, carotenoid, steroid, glycyrrhizin, hyaluronic acid and amino acids.

Outline of the research is as follows:

- Identification and analysis of genes involved in the production and control of useful metabolites by the analysis of transcriptome and metabolome in model plants, such as *Arabidopsis* and *Medicago*.
- Construction of an integrated database.
- Profiling of metabolites in the biosynthetic pathway of target compounds in practical plants, and profiling of gene expression required for production of useful compounds on the basis of the establishment of resources such as EST, cDNA and DNA sequence information.
- Identification of genes for production of useful compounds by comparative genomics using the integrated database in model and practical plants.
- Construction of regeneration and transformation systems in practical plants.
- Establishment of transgenic plants expressing structural and regulatory genes required for biosynthesis of industrial materials.
- Introduction of a gene or gene group involved in the production or control of target materials into the plant by DNA recombination and verification of the effect.
- Development of DNA microarray with high accuracy and quantitatively to be used for the above-mentioned analysis and verification.

The second METI project is 'Development of Fundamental Technologies for Production of High-value Materials using Transgenic Plants' (2006–2010) by 13 industries and 18 universities and institutes. The objective of the project is to develop basic technologies for the production of plant-derived high-value-added substances such as raw materials, reagents and enzymes for medical use in a currently available closed artificial environment. These technologies are expected to strengthen competitiveness in the manufacture of such useful materials and in the creation of a new bio-related industry. Target compounds and host plants can be used to produce vaccines for Alzheimer's disease (soybean) and porcine edema (lettuce), as well as avian influenza virus (potato), human thioredoxin (lettuce), miraculin (tomato) and sesamin (forsythia) vaccines.

- 1 Sijmons, P. C. *et al.* Production of correctly processed human serum albumin in transgenic plants. *Biotechnology (NY)* **8**, 217–221 (1990).
- 2 Daniell, H., Streatfield, S. J. & Wycoff, K. Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci.* **6**, 219–226 (2001).
- 3 Satoh, J., Kato, K. & Shinmyo, A. The 5'-untranslated region of the tobacco alcohol dehydrogenase gene functions as an effective translational enhancer in plant. *J. Biosci. Bioeng.* **98**, 1–8 (2004).
- 4 Sugio, T., Satoh, J., Matsuura, H., Shinmyo, A. & Kato, K. The 5'-Untranslated region of the *Oryza sativa* alcohol dehydrogenase gene functions as a translational enhancer in monocotyledonous plant cells. *J. Biosci. Bioeng.* **105**, 300–302 (2008).

- 5 Nagaya, S., Kawamura, K., Shinmyo, A. & Kato, K. The HSP terminator of *Arabidopsis thaliana* increases gene expression in plant cells. *Plant Cell Physiol.* **51**, 328–332 (2010).
- 6 Sugio, T. *et al.* Effect of the sequence context of the AUG initiation codon on the rate of translation in dicotyledonous and monocotyledonous plant cells. *J. Biosci. Bioeng.* **109**, 170–173 (2010).
- 7 Nagaya, S. *et al.* Shinmyo A: expression of randomly integrated single complete copy transgenes does not vary in *Arabidopsis thaliana*. *Plant Cell Physiol.* **46**, 438–444 (2005).
- 8 Yabuta, Y. *et al.* Molecular design of photosynthesis-elevated chloroplasts for mass accumulation of a foreign protein. *Plant Cell Physiol.* **49**, 375–385 (2008).
- 9 Misaki, R. ., Fujiyama, K. . & Seki, T. . Expression of human CMP-N-acetylneuraminic acid synthetase, and CMP-sialic acid transporter in tobacco suspension-cultured cell. *Biochem. Biophys. Res. Commun.* **339**, 1184–1189 (2006).
- 10 Fujiyama, K. *et al.* Production of mouse monoclonal antibody with galactose-extended sugar chain by suspension cultured tobacco BY2 cells expressing human b(1,4)-galactosyltransferase. *Biochem. Biophys. Res. Commun.* **358**, 85–91 (2007).
- 11 Castilho, A. *et al.* In planta protein sialylation through overexpression of the respective mammalian pathway. *J. Biol. Chem.* **285**, 15923–15930 (2010).
- 12 Al-Babili, S. & Beyer, P. Golden rice—five years on the road—five years to go? *Trends Plant Sci.* **10** (2005).