Mosses Work Miracles

Most recombinant biopharmaceuticals are complex human glycoproteins and are usually produced by mammalian production lines, such as CHO cells, derived from Chinese Hamster Ovaries (CHO).

Plants do not seem to be such likely candidates for the production of human glycoproteins, and yet the use of plants as alternative expression hosts is on the rise. Here are some of the reasons:

- They are easier to cultivate,
- They are less expensive,
- There is no risk of infectious agents being introduced,
- Purification processes (downstream processing) and safety tests are more straightforward and thus less expensive.

Several plant-produced pharmaceuticals are currently undergoing clinical studies. The first product, Taliglucerase alfa, an enzyme to treat Gaucher’s disease, was introduced to the market by Pfizer/Protalix in 2012.

Major challenges for plants

In order to be widely used as alternative production sources for glycoproteins, plants must meet the following three criteria:

- They must be able to produce large quantities of the recombinant product.
- Although the core structure of glycoproteins in plants is basically the same as in humans, there are differences that may affect their stability and efficacy, as well as the patient’s immune response.
- For biopharmaceuticals to be licensed, a production protocol must be in place that complies with good manufacturing practice (GMP) guidelines, and this includes self-contained production systems.

Moss: millions of years older than spermatophytes (seed plants)

Our team in Freiburg works with the moss Physcomitrella patens as a production host for glycoproteins. I have been focusing my research on this plant since the 1980s, when most researchers looked at Arabidopsis thaliana as a plant model. Although mosses have never been truly fashionable as models, a lot of knowledge and insight into mosses has accumulated over centuries. What attracted me to mosses was their small size, their ability to grow on pure mineral media in Petri dishes, and, last but not least, the fact that they have extended haploid life cycles.

On the evolutionary tree, mosses are found halfway between single-celled algae and complex seed plants. The latter developed about 1 billion years later. Mosses more or less retained their appearance during the last 400 million years and are among the oldest living land plants. They were around when dinosaurs came into being and also saw their demise. Although they never caught the limelight, they were and are masters of survival.

Enabling technologies

Over the years, we were able to develop our moss plant model into our flagship model, unperturbed by various short-lived trends in science and technology. Our mosses grow not only in Petri dishes and Erlenmeyer flasks, but also in industrial size bioreactors in pure mineral media. They do not require any organic additions such as antibiotics, carbon sources, or growth regulators, or a highly controlled environment.

P. patens was transformed by conferring antibiotic resistance to the wild-type moss, so that it can be easily identified. The moss is particularly suitable for gene targeting (GT). As its haploid growth phase occurs frequently and extensively, GT was often used to disrupt genes of interest and infer gene functions from these “knockout mosses” in a reverse genetics approach. This application became the first example for precision in genome engineering in plants, dating back to 1998.

The high rate of GT is a clear advantage for glycoengineering of moss when compared to similar approaches in seed plants.

Surprisingly, P. patens suits a wide variety of components of the transcription, translation, and secretion machineries, which were originally developed and optimized for recombinant production in CHO cells—a real evolutionary surprise!

The Physcomitrella genome comprises 500 mega base pairs. It became the third plant genome to be fully sequenced after A. thaliana and Populus, the poplar. Its full genome information is freely available via www.cosmoss.org.

A living and production environment for mosses

Like plant cell cultures and hairy root cultures, mosses can be grown in photobioreactors, which makes containment easy in a controllable environment. Good manufacturing practice is easier to establish under such conditions. The first batch was cultured in a 2 L foil reactor. This was then followed by culturing in 5, 10, and 20 L stirred glass tanks, which are the workhorses in the laboratory. Currently, for commercial production under GMP-certified conditions 100 and 500 L disposable wave reactors are used.

Recombinant protein products from moss

Several proteins have been produced in moss. Bacterial beta-glucuronidase (GUS), bacterial alpha-amyrase (AMY), and the human placental secreted alkaline phosphatase (SEAP) were used as quantifiable reporter proteins. As a product as well as a stabilizing agent for secreted biopharmaceuticals, human serum albumin (HSA) has been coexpressed in the production process. Further moss-produced human proteins include tumor-directed monoclonal antibodies with enhanced antibody-dependent cytotoxicity (ADCC), vascular endothelial growth factor (VEGF), complement factor H (FHL), keratinocyte growth factor (FGF7/KGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), asialo-erythropoietin (asialo-EPO), alpha-galactosidase (aGal), and beta-glucocerebrosidase (GBA). Moreover,
an Env-derived multiple-epitope HIV protein was produced in moss as a candidate vaccine.

Secondary metabolites

Mosses contain far more genes involved in secondary metabolism than seed plants. Some of these metabolites possess well-known human health benefits. Therefore, one aspect of the field is the metabolic engineering of moss to enhance the production of secondary metabolites with commercial value. A breakthrough was the expression of taxadiene synthase from Taxus brevifolia, an enzyme responsible for the synthesis of a precursor of paclitaxel, a widely used anticancer drug.

Another major market for engineered mosses is the fragrance industry. Moss-expressed patchoulol synthase and alpha/beta-santalene synthase are two sesquiterpenoids used in the production of perfume and other fragrant products.

Human complement factor and vaccines produced in moss

Human complement factor H (FH) is the key regulator of the alternative pathway of complement activation and a protectant against oxidative stress. Because it is a large protein (155 kDa) and contains 40 disulfide bonds, it is a difficult-to-express protein. Therefore, attempts are ongoing to produce truncated but bioactive versions in insect cells. In vitro studies showed that full-length FH with full biological activity can be produced in moss. In a subsequent step, the protein will be further evaluated in FH-deficient knockout mice. FH could be essential in the treatment of kidney diseases such as atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathies, and for age-related macular degeneration (AMD).

Moss-produced FH may be a cost-effective and more compliant alternative to the monoclonal antibody Eculizumab, which is limited to the treatment of aHUS and has severe side effects. Eculizumab is the most expensive biopharmaceutical worldwide, with treatment costs of about $450,000 per patient per year.

Several human growth factors (like EGF and HGF) that are used in mammalian cell culture have been produced in the moss system. FGF7/KGF (keratinocyte growth factor) is the first commercially available moss-produced human protein, intended for in vitro use (www.greenovation.com).

Based on these experiences, moss has been suggested as a potential production host for vaccines. Because no adverse effects of moss consumption are known, vaccine-producing moss may be directly administered as an oral vaccine. Thus, expensive protein purification could be avoided.

Biobetters from moss

Plants as alternative production hosts are becoming more popular because they allow cheaper production while also providing greater safety. Human proteins can be produced in a way similar to that of CHO cells. For the production of such biosimilars, extensive glycoengineering approaches have to be taken.

The inherent differences between plants and mammals may favor plant cell factories. At least in some cases, they produce superior biopharmaceuticals, so-called biobetters. Here are some examples: a glyco-optimized monoclonal antibody that was developed to recognize tumor-associated glycosylation patterns was produced in moss. It was 40 times more effective at inducing lysis in three different tumor cell lines than the same antibody produced in CHO cells.

Gaucher's disease and Fabry's disease are serious orphan diseases that respond to enzyme replacement treatment. Both human enzymes, α-galactosidase (aGal) for Fabry and β-glucocerebrosidase for Gaucher, are being produced in moss. A detailed analysis of glycan structures from different batches showed higher homogeneity and significantly enhanced batch-to-batch stability compared to commercially available drugs that are produced in mammalian cell lines. Thus, moss is able to produce superior biopharmaceuticals. Moss-produced Gal has successfully passed toxicity testing. It is the first moss-produced pharmaceutical in clinical trials.

Asialo-EPO, a useful and safe EPO

Erythropoietin (EPO) is a hormone (cytokine) involved in the maturation of red blood cells in bone marrow and has a wide range of other effects—including better kidney function, angiogenesis, and neurogenesis. It has been shown that it enhances the immune response and prevents apoptosis—not to mention its illegal use in various sports. Functional EPO has been produced in moss.

The resulting asialo-EPO was of outstandingly high uniformity. This EPO glycoform does not promote the maturation of red blood cells, and thus cannot be abused for doping, but exerts neuroprotective and antiapoptotic functions. Therefore, moss-produced asialo-EPO may be a safe biobetter for a variety of indications (see cartoon at the end of this chapter).

Conclusions

Over the past two decades, the moss Physcomitrella patens has been developed from scratch into a flagship model species in basic research and in biotechnology. Some of the key features of the moss system are a fully sequenced genome, outstanding possibilities for targeted genome-engineering via homologous recombination, certified GMP production in bioreactors, successful upscaling to 500L wave reactors, outstanding homogeneity of protein glycosylation and batch-to-batch stability, and safe cryopreservation for master cell banking.

Ralf Reski (b. 1958) studied biology, chemistry and pedagogics.
He was Heisenberg Fellow of the German Research Foundation DFG and was appointed as Distinguished Professor for Plant Biotechnology at the University of Freiburg, Germany in 1999.

In 2011 he was appointed as a Senior Fellow at the Freiburg Institute for Advanced Studies (FRIAS) and as a lifetime member of the Heidelberg Academy of Sciences and Humanities. Since 2013, he has been a member of the University of Strasbourg Institute for Advanced Study (USIAS).

**Worth Thinking About**

But it is illusion to think

that there is anything fragile about the life of the Earth;

surely this is the toughest membrane imaginable in the universe, opaque to probability, impermeable to death.

We are the delicate part, transient and vulnerable as cilia.

Nor is it a new thing for man to invent an existence that he imagines to be above the rest of life;

this has been the consistent intellectual exertion down the millennia.

As illusion, it has never worked out to his satisfaction in the past,

any more than it does today.

Man is imbedded in nature.

**Lewis Thomas (1913–1993)**

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Cited and Recommended Literature


Useful Weblinks

- AlgaeBase—a comprehensive database of over 35,000 algae, including seaweeds, with over 5000 images and some 40,000 references. [http://www.algaebase.org/](http://www.algaebase.org/)
- The European Union’s website on GMOs, the work of independent science journalists [http://www.gmo-compass.org/eng/home](http://www.gmo-compass.org/eng/home)
- Everything you wanted to know about GM organisms, provided by *New Scientist*. [http://www.newscientist.com/channel/life/gm-food](http://www.newscientist.com/channel/life/gm-food)
- Greenpeace about GMOs: [http://www.greenpeace.org/international/footer/search?q=GMOs](http://www.greenpeace.org/international/footer/search?q=GMOs)
- A fantastic collection of historic and modern biology books: Kurt Stüber’s Online Library. Now over 100,000 scanned pages available: [http://www.biolib.de/](http://www.biolib.de/)

8 Self-Test Questions

1. Why don’t famine regions “just” look after themselves and set up algae farms?
2. Why did the bright idea of producing protein from oil fail? Which bioproduct is a health food and a recent result of this single cell protein research?
3. What is the name of the bacterium that causes root gall, and how can it be used for gene transfer in plants?
4. Does the gene transfer in question three also apply to corn or rice? What is the way out?
5. Is the cloning of plants completely new? Were genetic engineers the first to discover it?
6. What is the principle that was used to create antimush tomatoes? Which enzymes are blocked by this?
7. Name three “well intentioned” transgenic plant products created by scientists.
8. What is the connection between frost damage in plants and infestations with bacteria? What are antifrost bacteria?

Moss cells can be easily transformed to produce many important drugs (see Box 7.14 by Ralf Reski!!)
factor deficiency. In severe forms, minimal injuries can lead to unstoppable life-threatening external bleeding or hemorrhages into the tissues or the joints. With milder forms, hemorrhage needs to be taken into account predominantly during surgical procedures. Treatment consists of the intravenous administration of the missing clotting factor, and the dose depends on the risk of bleeding, i.e., it needs to be relatively high for children and before operations.

Less than 500 g (1.10 lb) of factor VIII should cover the needs of the world, but this small amount had so far cost 170 million dollars without biotechnology!

The factor VIII protein consists of 2,332 amino acid components. It is therefore one of the largest proteins whose structure has been elucidated so far. Each person has 1 mg of factor VIII in his approximately 10 pints (6 L) of blood.

A hemophiliac must be injected with 1 mg of the factor twice a week in order to live a normal life. If one donation of blood is 1 pint (½ L), we theoretically need 24 blood donors a week for a single hemophiliac! However, the extraction and processing of donated blood is not just expensive, it is also extremely risky: the main danger used to be infection with hepatitis B viruses which cause jaundice, but today there is the risk of infection with the AIDS virus or hepatitis C (see Chapter: Viruses, Antibodies, and Vaccines).

It is estimated that 60% of hemophiliacs have been tragically infected by donated blood, some of those in France criminally so. In the United States, nearly 90% of Americans with severe hemophilia became infected with AIDS in the 1980s when the nation’s blood supply was contaminated. More than 50% of those infected with HIV have died.

On the other hand, the genetically engineered factor VIII is virus-free and safe. As factor VIII is a glycoprotein, it is created in mammalian cell lines and used for the treatment and prophylaxis of bleeding in hemophiliac A patients.

Factor VIII has also been produced recently by transgenic pigs (see Chapter: Embryos, Clones, and Transgenic Animals). The complete human cDNA was combined with the promoter for whey acid protein from the mammary glands of pigs, and factor VIII was expressed in the milk of the pigs.

9.5 EPO for Kidney Patients and in Sports

Other genetically engineered products have followed factor VIII, such as the hormone erythropoietin (EPO), which is formed in the kidneys and stimulates the body’s own erythrocyte production (erythropoiesis) in the bone marrow. The glycoprotein EPO is a growth factor for red blood cells and induces the formation of hemoglobin in the bone marrow (Box 9.1).

EPO is important for the lives of dialysis patients, who suffer anemia as a result of dialysis.

In the past, athletes were sent to train at high altitudes, since the “thin air” promotes the formation of red blood cells for a perfect source of oxygen in subsequent competitions. That can now be achieved more cheaply. EPO is produced, like factor VIII, in mammalian cells. The EPO sugar chains are important, since they protect EPO from rapid degradation in the liver and EPO is inactive without sugar.

In the Tour de France in 1998, whole teams dropped out of the race when they discovered testing was to be carried out by the police and independent doping experts. Ralf Reski in Freiburg now produces EPO in bioreactors, using transgenic moss (see Box 7.14).

Hundreds of thousands of patients have used the life-saving growth factor, GM-CSF (granulocyte macrophage colony stimulating factor), which stimulates the formation of eosinophils, neutrophils, and macrophages.

Both genetic engineering products have a world market value of 2 billion dollars each. They are top products!