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Biopharmaceuticals from Plants: A Multitude of Options for Posttranslational Modifications

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Abstract

In 1982 the first recombinant therapeutic, human insulin, was introduced into the market and started a new branch of pharmaceutical development, manufacture, and therapy options. To date, more than 130 recombinant protein therapeutics have been approved by the US Food and Drug Administration (FDA) and many more are being developed world wide. With the increasing number of protein therapeutics the number of potential production organisms is also expanding, and posttranslational modification of proteins has become a topic of special focus. One major difference between small-molecule drugs and protein therapeutics is that the latter are reliant on a host organism for their production and this can have a large influence on the final structure and can ultimately affect the pharmacokinetics, immunogenicity, and the function of the protein depending on the production process. Plants can be efficiently used as production systems for recombinant proteins thereby offering a variety of options for transgene targeting and modification. This review is intended to give an overview about the potential of plants to serve as a production system for therapeutic and prophylactic biopharmaceuticals with respect to posttranslational modifications.

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Abbreviations: CaMV, cauliflower mosaic virus; ER, endoplasmic reticulum; GCD, glucocerebrosidase; GT, glycosyl transferase; HPV, human papillomavirus; hST, human somatotropin; mAb, monoclonal antibody; PTM, posttranslational modification; TSP, total soluble protein; VLP, virus-like particle.

Introduction

Several life-threatening diseases can be successfully medicated by the application of therapeutic proteins, e.g. diabetes with insulin, or blood clotting disorders by the application of coagulation factors. Such protein therapeutics can be obtained from their natural sources only in minute amounts and in most cases the use of human tissue for the extraction is not an option. Therefore, alternative sources have had to be found and for a long time, only animal tissue was available for this. Until the early 1980s, bovine and porcine pancreas tissue was the sole valid source for the extraction of insulin but due to the exiguous difference in the amino acid sequence between animal and human insulin side effects like allergic reactions occurred in recipients which reduced the suitability of these animal-derived drugs. Humanization of porcine insulin was feasible by the combination of an enzymatic and a chemical reaction (Morihara *et al.*, 1979) but the supply with porcine pancreatic tissue was still the bottleneck for industrial production. With the introduction of recombinant DNA technology it became possible for the first time to produce any given protein in virtually unlimited amounts in a recombinant host organism by simply expressing the target cDNA. Human insulin (Goeddel *et al.*, 1979) was the first recombinant pharmaceutical introduced into the market in 1982 and paved the way for many more biopharmaceuticals. To date, there have been more than 160 protein therapeutics approved worldwide for use in humans and many more candidates are currently being tested (Lawrence, 2007). The estimated market size for recombinant therapeutics was $44 billion in 2004 (Lawrence, 2005) and even larger values are projected for the coming years.

Moreover, many diseases can be treated or prevented by novel therapeutics and vaccines for the first time. For example, most traditional prophylactic vaccines for viral diseases rely on tissue culture propagated, attenuated, or killed forms of virus particles. In the case of human papillomaviruses, no such effective tissue culture system exists (Schiller and Okun, 1996). The only option is to heterologously express virus genes and produce subunit vaccines. The recently approved vaccines against papillomavirus infection and cervical cancer, Gardasil® (Sanofi Pasteur MSD) and Cervarix® (GlaxoSmithKline), are produced recombinantly in yeast or insect cells, respectively, by the expression of the major capsid protein L1 (Inglis *et al.*, 2006). The ability of the L1 protein to self-assemble into non-infectious, virus-like particles when expressed in a recombinant system enables the production of a safe and abundant prophylactic vaccine.

Although theoretically any given organism could serve as a production platform for recombinant proteins, today only a few host organisms are routinely in use for the production of protein therapeutics. The gram-negative bacterium *Escherichia coli* is the most prominent since it is easy to transform, its growth characteristics are well studied and it can be cultivated in large quantities at relative low costs (excellent reviews can be found (Baneyx, 1999; Swartz, 2001)). However, the major drawback of its use is that *E. coli* does not perform the often required posttranslational modification (PTM) of proteins, which could be necessary for the function, stability, and antigenicity of the therapeutics. Proteolytic cleavage of preproteins, correct disulfide linkage, and glycosylation does not take place in *E. coli* and often proteins accumulate in the form of inclusion bodies, which makes tedious *in-vitro* reconstitution necessary. Moreover, careful purification of the target proteins is mandatory since even trace amounts of bacterial lipopolysaccharides could act as immunogens and must be avoided. However,

between 2003 and 2006, nine of the 31 approved therapeutic proteins were produced in *E. coli* (Walsh, 2000).

The unicellular yeast *Saccharomyces cerevisiae*, also known as "brewer" or "baker´s yeast", is used since ancient times for the making of food and beverages. For the production of recombinant therapeutics it has been used since the early 1980s (Hitzeman *et al.*, 1981) and has qualified as a valuable production host due to relative simple culture conditions, rapid growth, and low costs. Several more yeast genera are used today, e.g. *Pichia pastoris* (Daly and Hearn, 2005). As eukaryotic organisms, yeast cells are capable to produce more complex proteins since they perform PTMs, like protein processing and glycosylation. However, glycan structure greatly differs from mammalian glycosylation and is supposed to be antigenic in humans (Liu, 1992).

Hence, when it comes to authenticity of the recombinant proteins of human origin, mammalian cell cultures are the system of choice and the major production vehicle for glycosylated, injectable protein therapeutics. As a major downside, culture conditions are more complex and the costs of fermentation are much higher than for any other recombinant system.

During the last two decades a novel production host for protein therapeutics came into focus: transgenic plants or tissue cultures thereof. Although there were numerous concerns about this technology, tremendous improvements have been made regarding its transformation into a production platform which ultimately led to the first approval for a plant made therapeutic, a vaccine against Newcastle Disease in poultry (*nomen nescio*, 2006).

Plants have a number of potential advantages over conventional production systems. They are inexpensive to produce, generating vast amounts of biomass at very low costs, and usually do not harbor human pathogens. It is also possible to store recombinant proteins in specialized organs like seeds and tubers, a feature which could simplifying storage and transport of raw material. Moreover, also plant cell cultures are an option for recombinant protein production. Although fermenters and nutrients are necessary for cell propagation, as for the other systems, plant cell cultures combine the advantages of the unique host system plant with the needs of a production process like batch-to-batch product consistency and containment (Hellwig *et al.*, 2004).

Plant hosts and transformation options

Other than for bacteria or yeast, there is no single plant species which could be considered as the "working horse" for recombinant protein expression and which could be utilized to define standards. Several plant species are used for recombinant protein production, among them the most prominent tobacco, tomato, potato, maize, rice, lettuce, and spinach. Frequently, those plants are utilized which are easy to transform, which have short generation times, and which yield reasonable biomass with standard cultivation techniques. Naturally, especially economic crops plants are well studied regarding cultivation and post-harvest processing and therefore, are frequently used as production system for recombinant proteins. *Arabidopsis thaliana*, the plant genomics model organism for which whole genome data are available, would be also an ideal system but is less favorable due to its very low biomass.

Transformation of plants can be achieved by several different techniques, and its choice is dependent on the plant species and its regenerability in tissue culture, and

also the targeted organelle for transgene insertion (*Figure 1*). The most frequently used transformation method utilizes the soil bacterium *Agrobacterium tumefaciens*. Its ability to infect a plant by delivering a circular DNA called Ti-plasmid into the plant cell is capitalized by the *Agrobacteria*-mediated transformation method. For its biotechnological application, a disarmed Ti-plasmid harbours the gene of interest in an expression cassette under control of a strong promoter (Tzfira and Citovsky, 2006). After delivery of the plasmid into the plant cell by the bacterium, the DNA-stretch is integrated into the host nuclear genome in a rather erratic manner, resulting in various integration events at different locations and in various numbers. Hence, any transformation generates numerous individuals which greatly differ regarding their viability and regarding the level of recombinant protein produced. Therefore, a large number of transformants have to be tested to identify those with suitable properties for further cultivation.

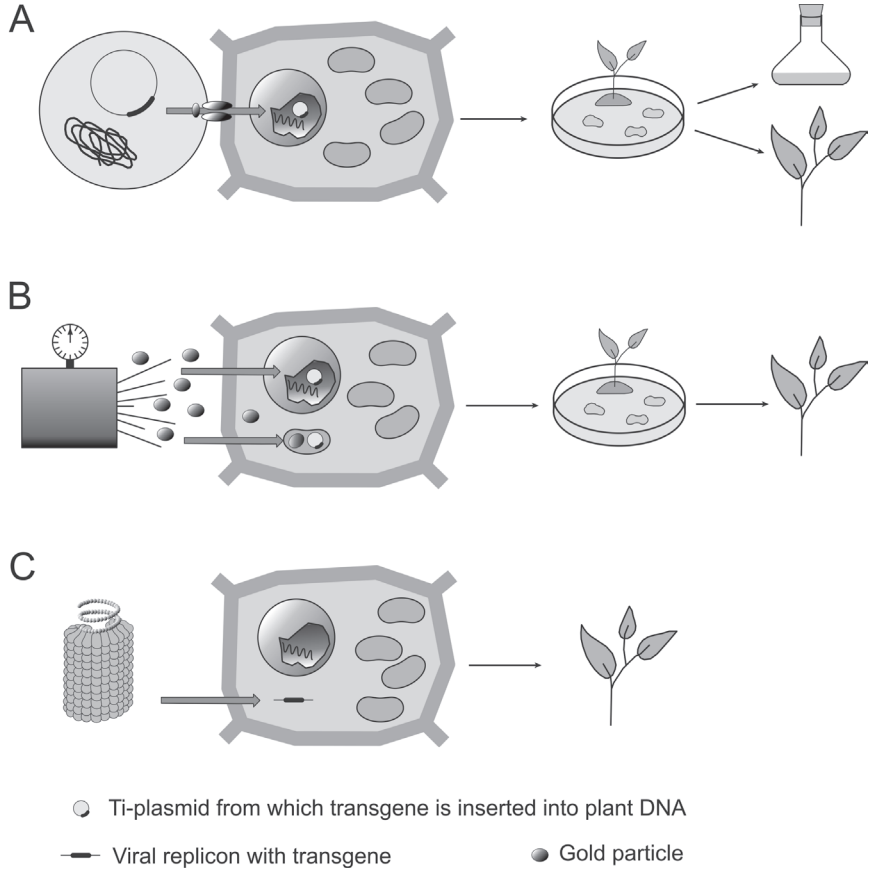

Figure 1. Scheme of three major methods to transform plant cells for the production of therapeutic proteins. **A**. *Agrobacterium*-mediated. The bacterium injects a modified Ti-plasmid into the plant cell, where the expression cassette is integrated into the nuclear genome. Plants are regenerated *in vitro* from single cells via callus tissue under antibiotic selection. Alternatively, calli can be transformed to cell suspension cultures. **B**. Biolistic gene delivery. Plasmid-coated gold particles are brought into either the nucleus or the plastids, where integration of the expression cassette takes place. Regeneration of transformants is achieved under antibiotic selection and several rounds of regeneration are necessary for plasmid transformants to eliminate wild type plasmid genomes. Cell suspension cultures from chloroplast transformants are not beneficial since cultures are usually not photoautotrophic and therefore do not contain many plastids. **C**. Transient expression. A non-transgenic plant is infected with a modified plant virus. Transgene expression occurs within days.

Since the amount of recombinant protein accumulating after nuclear transformation is often inadequate, other transformation techniques have been developed which benefit from unique features of the plant cell. Chloroplasts as semi-autonomous compartments of all photosynthetic active higher plants can also serve as the integration locus for transgenes (Daniell *et al.*, 1990; Svab *et al.*, 1990). Several features of chloroplasts make them superior to nuclear transformation: i) transgene insertion into the chloroplast genome takes place by homologous recombination, therefore circumventing positional effects as observed for T-DNA-insertions in the nucleus. ii) Leaf mesophyll cells may contain up to hundred chloroplasts and each of them contains up to a hundred copies of their genome. The large number of transgene copies per cell usually results in high levels of recombinant proteins. iii) Chloroplast transformants are considered superior regarding their safety since the organelles are maternally inherited in most crop species and therefore, transgene spread through pollen is omitted (Ruf *et al.*, 2007; Svab and Maliga, 2007). The downside of this technique is that the generation of transplastomic plants is tedious and longsome and that to date only a few species can be transformed routinously. So far, only tobacco has been used to produce recombinant therapeutics in its chloroplasts (Daniell, 2006).

Another technique resulting in usually extraordinary amounts of recombinant protein is the use of plant viruses. In this case, a plant virus like tobacco mosaic virus (TMV) is modified in a way that the gene for the highly expressed coat protein is replaced by the gene of interest. Inoculation of a non-transgenic host plant and viral replication initiates transgene expression within days. Several varieties of this technique have been developed (an overview can be found in (Pogue *et al.*, 2002)) but the downside is the need for manual inoculation of plants with virus particles or *Agrobacteria* which contain the viral replicon.

The examples given above show that several options exist by which plants could be transformed and used for the production of recombinant proteins. If an industrial application is aspired it might be necessary to take into account that subsequent commercialization of any product could be hampered by intellectual property rights applying to the techniques used for transformation (Dunwell, 2005). For the product itself the amount of protein obtained and the structural property of the protein is extremely important, both highly depending on the localization of the protein.

Transgene localization and its influence on posttranslational modification

Plants offer a multitude of localization choices where the recombinant protein could be accumulated, both on cellular and tissue level. Tissue- or organ-specific expression is highly dependent on the promoter used. The used constitutive CaMV35S promoter facilitates more or less even expression in all plant tissues, but yields are rarely exceeding levels of 0.1-1.0% of total soluble protein (TSP). Also regulated promoters have been used which enable localized expression and accumulation of the transgene, e.g. in ripe tomato fruits (E8-promoter, (Deikman *et al.*, 1992; Sandhu *et al.*, 2000)), or in maize endosperm (Ramessar *et al.*, 2008). It has been shown that the localization could have a great influence on the stability of a given protein, which is due to specific conditions predominant in this tissue. Seeds for example are viable for a long period in their mature dry state and protect the recombinant proteins during storage and transport. Moreover, this localization enables extremely high protein accumulation

(Stoger *et al.*, 2005). Up to 36% of TSP could be achieved when a recombinant single-chain antibody was expressed under the control of a seed-specific promoter from *Phaseolus vulgaris* (De Jaeger *et al.*, 2002). Seed seems to be ideal not only for protein stability but also for protein glycosylation due to the large endomembrane system of the endosperm cells.

THE SECRETORY PATHWAY AND PROTEOLYTIC CLEAVAGE

The subcellular localization of the recombinant protein greatly influences the posttranslational modification and therefore, is an important point to consider for the production of recombinant proteins. Most therapeutic proteins of mammalian origin are secreted and need to travel through the endoplasmic reticulum (ER). In plants, as in any other eukaryotic organism, cotranslational transport of the peptide into the ER is facilitated by a short N-terminal signal peptide which is subsequently cleaved. Mammalian signal peptides are also functional in plants and could guide proteins to the plant ER, as it was shown for erythropoietin (Matsumoto *et al.*, 1995) and human acetylcholine esterase (Mor *et al.*, 2001). For recombinant proteins, usually a genuine plant SP is translationally fused to the protein of interest to allow efficient ER-localization. While the SP is efficiently removed, further proteolytic processing of mammalian preproteins does not necessarily take place in plants.

The ER enables most proteins to adopt the correct structure including disulfide bond formation. In plants, this has been shown for a large number of human proteins, like insulin (Nykiforuk *et al.*, 2006), or collagen (Ruggiero *et al.*, 2000). Obviously, the correct folding of animal proteins in plants is not a major concern. It has to be noted that in the case of collagen a gene encoding a prolyl-4-hydroxylase (P4H) had to be coexpressed to provide the correct prolyl hydroxylation of the human protein enabling formation of the correct structure (Merle *et al.*, 2002). Although plants do harbor an endogenous P4H, the enzyme does not ensure the correct modification of the repetitive sequence X-Pro-Gly in human collagen. Therefore, a chimeric P4H comprised of two α-subunits from *Caenorhabditis elegans* and two human β-subunits was co-introduced into the plants, which efficiently modified prolyl residues *in planta* and additionally had a reduced temperature optimum compared to the sole human enzyme. This example shows that even if plant cells do not have the enzymatic capacity to modify large amounts of recombinant proteins, engineering of a particular pathway can provide a tailor-made expression host.

PROTEIN GLYCOSYLATION

The most widespread PTM in biopharmaceutical proteins is glycosylation. Not only can glycosylation vary depending on the organism where the protein is expressed but also it can vary between the tissues from the same organism. Hence, glycosylation introduces a great portion of heterogeneity into any protein population. Oligosaccharides are attached cotranslationally either through serine/threonine residues (O-linked) or through asparagine in the glycosylation consensus sequence Asn-X-Ser/Thr (N-linked). Mainly the latter is of interest for therapeutical proteins. The initial glycan, the “core” oligosaccharide transferred to asparagine, is similar in mammals and plants (Faye *et al.*, 2005). The subsequent trimming and the addition

of various sugars during the proteins transit through the ER and the Golgi results in very different structures (*Figure 2*). This is true between organisms as well as between proteins of the same organism. Hence, the same protein will have significant differences when expressed in either mammalian cells, yeast, insect-cells, or plants. Additionally also, the plant species has an influence on the glycosylation patterns. Human lactoferrin exhibits variable glycan structures when expressed in either tobacco or maize (Samyn-Petit *et al.*, 2003) and *Aspergillus niger* phytase showed different glycan spectrum if produced in either leaves or seeds of *Oryza sativa* (rice) (Drakakaki *et al.*, 2006).

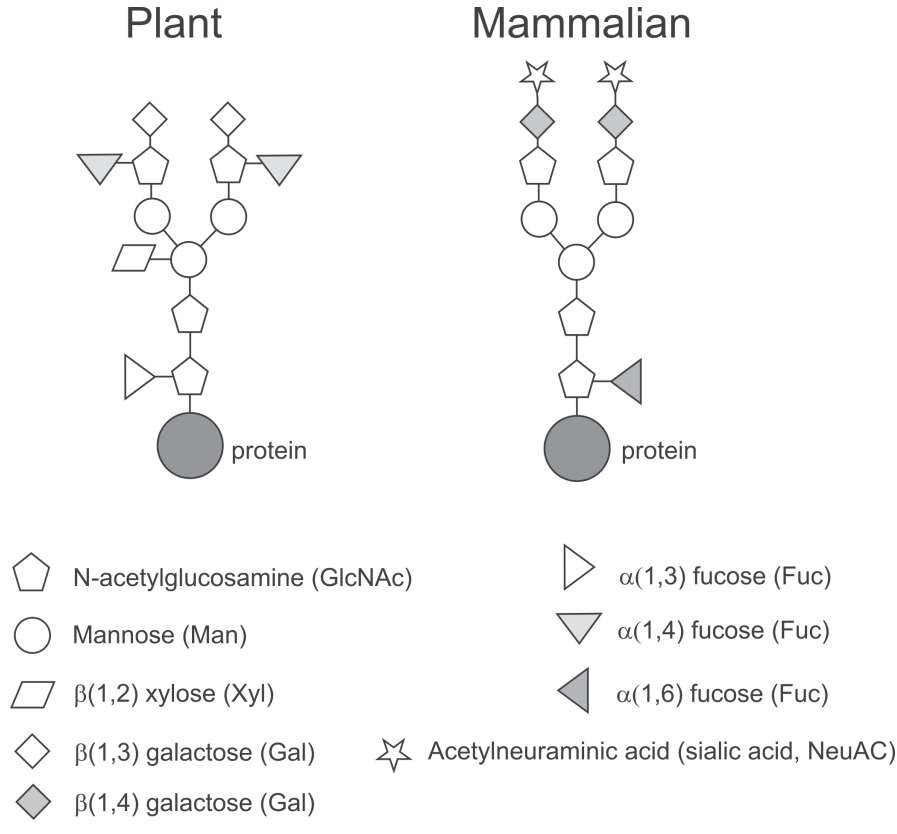

Figure 2. Basic scheme of the glycosylation pattern of mammalian and plant glycoproteins. It has to be noted that for both cases various modifications and variations occur, depending on the grade of processing and the localization within the cells.

Glycosylation has an impact on the stability, immunogenicity, and activity of several therapeutic proteins. One of the major concerns about recombinant proteins from hosts other than mammalian cells is the immunogenicity they could induce in the recipient. This has been described for yeast glycan structures (Podzorski *et al.*, 1990), and for plants (Bardor *et al.*, 2003). It can be assumed that any biotechnologically produced therapeutic may exhibit some form of immunogenicity and an antibody response can result in reduced efficacy or potentially in the inactivation of endogenous proteins by neutralizing antibodies (Schellekens, 2003, 2005).

Avoiding protein glycosylation could be one option when the glycan has no influence on the activity of the therapeutic. There are examples of naturally glycosylated human proteins which retain their activity when produced in *E.coli* as non glycosylated proteins, e.g. IFN-α, IL-2, or TNF-α. When expressed in plants, non-glycosylation could be achieved when the protein is directed to another compartment than the ER, namely the plastids, where disulfide bond linkage takes place but no glycosylation. This will be discussed later. However, some therapeutics have shown remarkable pharmacokinetic differences if either glycosylated or not, although the sugar moiety has no influence on receptor binding or enzymatic activity. Erythropoietin can be produced in *E. coli* as a non-glycosylated protein, which shows full activity *in vitro*. Though, *in vivo* this particular erythropoietin has no activity due to a reduced half life and rapid clearance from body fluids (Sasaki *et al.*, 1987; Wasley *et al.*, 1991). A similar influence of glycosylation on a particular protein could be observed with antibodies. The C_H2-domain of an IgG is glycosylated at Asp297 (Nezlin and Ghetie, 2004) and the glycan residue is integral to the F_c structure and essential for adopting the conformation necessary for efficient binding of effector ligands. Omitting glycoprotein formation is therefore not suitable for therapeutic monoclonal antibodies.

Considering the above mentioned examples, it would be highly beneficial to develop plant hosts which are capable of producing a mammalian-like carbohydrate structure on proteins. Glycoengineering describes the process of inactivating endogenous glycosyl transferases (GT) and/or the expression of heterologous GTs. There are already reports describing the generation of plants in which the enzymes β(1,2)-xylosyl transferase and α(1,3)-fucosyl transferase are knocked-down by RNA interference (Strasser *et al.*, 2004). Hence, the plants produce monoclonal antibody (mAb) with N-glycans devoid of the plant-specific residues. Cox et al. (Cox *et al.*, 2006) have modified the aquatic plant *Lemna minor* to produce a mAb against human CD30 which is indistinguishable to a mAb produced in mammalian CHO-cells. Moreover, the glycan structure is highly consistent, which is not necessarily the case for mammalian cells (Patel *et al.*, 1992) and therefore, the plant-made mAb seems to be superior to those conventionally made.

Other typical mammalian glycan-structures which do not occur in plants are the penultimate β(1,4) galactose and the terminal sialic acid residues. Attempts were first made to introduce the human β(1,4)-galactosyl transferase into plants (Palacpac *et al.*, 1999; Misaki *et al.*, 2003). *In vitro* β(1,4)-galactosylation of a recombinant human antibody in tobacco was to 30% efficient (Bakker *et al.*, 2001), but the due to the overall difference and the heterogeneity of the glycans to be found in tobacco (Cabanes-Macheteau *et al.*, 1999) the mAb could not be considered humanized and represent a complex mixture of different glycoforms. The addition of a terminal sialic acid displays an even more complex task, since multiple enzymes are required which are not present in plants (Seveno *et al.*, 2004; Zeleny *et al.*, 2006). However, there are recent reports proving that with transgene stacking this particular pathway can be built in plants (Castilho *et al.*, 2008). Taken together, by choosing the appropriate plant species and via glycoengineering, the production of therapeutic proteins with authentic mammalian glycans in plants should be feasible in the future.

There are examples of therapeutic proteins for which it is an advantage if they do not harbor the full mammalian glycan structure. Glucocerobrosidase (GCD) is used in enzyme replacement therapy with morbus Gaucher, a lysosomal storage disorder. For efficient uptake by macrophages GCD has to bind to mannose receptors on the

cell surface, facilitating efficient uptake of the enzyme. For the recombinant GCD produced in CHO-cells, *in vitro* deglycosylation via α-neuraminidase, β-galactosidase, and β-N-acetylglucosaminidase needs to be performed to expose terminal mannose residues and to obtain a functional enzyme (Friedman *et al.*, 1999). When the GDC-gene is expressed in carrot cell suspension cultures and the protein is targeted to the storage vacuole, a product with terminal mannose residues is obtained *in vivo*. Thus, expression in this plant cell system precludes the need for post-production enzymatic modification *in vitro* (Shaaltiel *et al.*, 2007). Moreover, comparison of the plant-derived therapeutic with the commercial GCD Cerezyme® proved that both enzymes are equivalent.

CHLOROPLAST TARGETING AND PTM

Chloroplasts are a cellular compartment unique to plants. If a nuclear encoded transgene ought to be targeted to the chloroplast, it needs to be translationally fused to an N-terminal chloroplast transit peptide. Chloroplast localization can have a tremendous impact on protein stability and accumulation, as it was shown for the L1 protein of human papillomavirus HPV. An efficient vaccine against cervical cancer can be generated by the expression of the capsid proteins L1 of different HPV subtypes in heterologous systems and subsequent self-assembly into virus-like particles (VLP) (Stanley *et al.*, 2006). With plants, several attempts have been undertaken to produce papilloma VLPs (Biemelt *et al.*, 2003; Varsani *et al.*, 2003; Warzecha *et al.*, 2003; Varsani *et al.*, 2006) but although VLP-self-assembly and immunogenicity of the particles could be demonstrated, the yield was far too low for any reasonable application. When the L1 protein was targeted to the chloroplast by the transit peptide of *rbc*S, accumulation of up to 17% of the total soluble protein (TSP) could be achieved (Maclean *et al.*, 2007). Moreover, assembly into higher order structures like capsomers (pentamers of L1) and VLPs could be observed and purified particles exhibited immunogenic properties in mice. This example clearly shows that subcellular targeting of a therapeutic protein can increase its yield dramatically.

Biopharmaceuticals can also be efficiently produced by direct targeting of the transgene to the chloroplast genome. When human somatotropin (hST) was expressed from a chloroplast-located transgene, TSP-levels of 7% where obtained, which is 300fold more than accomplished from the same gene located in the nucleus (Staub *et al.*, 2000). Moreover, the chloroplast-derived hST was biologically active and exhibited the correct disulfide linkage as the native human enzyme, showing that chloroplast are capable of folding complex eukaryotic enzymes. Hence, chloroplast seems to be the ideal compartment to obtain correct disulfide linkage without any glycan-modification, as it would be the case in the ER. Also bacterial proteins like alkaline phosphatase are correctly assembled including disulfide linkage (Bally *et al.*, 2008) and this was true for its localization either in the chloroplast stroma and the thylakoid lumen. In fact, several more bacterial proteins with potential use as vaccines or adjuvants have been shown to be correctly folded in the chloroplast, like *E. coli* heat-labile enterotoxin (LT-B) (Kang *et al.*, 2004), the fragment C of the tetanus toxin (Tregoning *et al.*, 2003; Tregoning *et al.*, 2005), or the cholera toxin B subunit (Daniell *et al.*, 2001). These results again demonstrate the potential of plants to serve as a production system for a large number of recombinant therapeutics of different origin.

In general, chloroplasts seem to be the ideal compartment for the expression of bacterial transgenes due to their heritage from bacterial ancestors (Goksoyr, 1967), a suitability based on their more prokaryotic mode of gene expression (Bock, 2001). They seem also to have inherited part of a mechanism which cannot usually be found in eukaryotic organisms, namely the formation of bacterial lipoproteins. Bacterial lipoproteins contain a typical N-terminal signal peptide recognized by the enzyme diacylglyceryl transferase (Lgt), which attaches a diacylglycerol to the thiol of a conserved cysteine, an invariant part of the lipobox motif (Taylor *et al.*, 2006). Cleavage of the signal peptide by signal peptidase II results in the unique Pam2Cys-structure at the amino terminus of the protein. Contingently, a third palmitic acid could be attached to the free amino group in Gram- bacteria, resulting in a Pam3Cys-structure. Other than the signaling function of palmitoylated proteins from eukaryotes, bacterial lipoproteins are surface-exposed and are mostly involved in adhesion, invasion, cell wall synthesis and sensing (Sutcliffe and Harrington, 2004). Due to their localization bacterial lipoproteins are important targets for the immune system and for several pathogens, lipoproteins have been identified as potential vaccine candidates, e.g. from *Streptococcus sp*. (Rodriguez-Ortega *et al.*, 2006) or *Neisseria meningitides* (Masignani *et al.*, 2003). The most prominent example is the outer surface protein A (OspA) of the Lyme disease agent *Borrelia burgdorferi*, which has been already used as a recombinant vaccine in humans - Lymerix (Sigal *et al.*, 1998; Steere *et al.*, 1998). It has been shown that OspA does not accumulate to very high levels when expressed in *E. coli*, possibly due to interference with the export of host proteins (Dunn *et al.*, 1990). Hence, production of large amounts of lipoprotein is limited by the processing capacity of the host bacterium. As an alternative system, plants have been utilized for the production of OspA. Navarre and coworkers (Navarre *et al.*, 2006) expressed the *ospA*-gene in tobacco cell suspension cultures but did not achieve high level accumulation of the protein (0.006% of TSP). Moreover, lipidation of OspA has not been investigated in this study. In the case of OspA it has been shown that the unique lipid moiety is essential for immunogenicity and that a non-lipidated form did not induce protective immunity in mice (Erdile *et al.*, 1993; Weis *et al.*, 1994). It has recently been demonstrated that recombinant OspA can be produced at 1% TSP in tobacco via the chloroplast and that the protein is lipidated and immunogenic in mice (Glenz *et al.*, 2006). Further enhancement of protein level up to 10% was feasible but drastically interfered with plant viability (Hennig *et al.*, 2007): it might be necessary to modify the signal peptide to circumvent this effect. Although the PTM of this lipoprotein is only partial, it shows that plants have an enormous capacity of introducing PTM into any given recombinant protein and combine eukaryotic and prokaryotic features.

Conclusion

During the last decade it has been demonstrated that transgenic plants can be developed to produce extraordinary high amounts of recombinant proteins, facilitating the rational production of therapeutics. Moreover, the first vaccine for animal use has gained approval, clearly demonstrating that this technology can provide pharmaceuticals of sufficient quality. Groundbreaking work has been performed to produce engineered and optimized host plants which yield authentic proteins regarding their PTM, expanding

the number of potential therapeutic proteins to be produced in plants. Although the use of plants will not be a one-for-all production platform, it will definitely be a valuable add-on to the established use of host organisms like bacteria, yeast, and mammalian cells.

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