ELSEVIER

Plant-based material, protein and biodegradable plastic

Jürgen Scheller[1] and Udo Conrad[2]

Fibrous proteins from spiders, proteins with synthetic multiple repeats and mammalian structural proteins such as collagen have been produced in transgenic plants. Recent advances in the production of biodegradable plastic in plants also show the potential of molecular farming for research into and production of materials. Selection of a growing variety of such products, optimization of expression, and the development of effective purification strategies will further promote this growing field of biotechnology.

Addresses
[1] Institut für Biochemie, Christian-Albrechts Universität zu Kiel, Olshausenstrasse 40, D-24098 Kiel, Germany
[2] Institut für Pflanzengenetik und Kulturpflanzenforschung Gatersleben (IPK), Corrensstrasse 3, D-06466 Gatersleben, Germany

Corresponding author: Udo Conrad (conradu@IPK-gatersleben.de)

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Introduction

Plant materials such as cellulose, starch or even latex from rubber trees have traditionally been used for several purposes by mankind. In the past century, the chemical industry opened the way to modify plant-based materials, and products such as cellulose acetate or cellulose nitrate were invented. The development of genetic engineering technology and efficient transformation methods for several crops has opened the way for the genetic modification of traditional plant products; for example, the development of modified starch. Furthermore, interesting proteins originating from animals and novel polymers can now be synthesized in transgenic plants [1]. Silk proteins, from either spiders or insects, are especially interesting as basic materials for the production of silk fibers that have mechanical properties superior to those of chemical fibers. Synthetic proteins that have multiple repeats, elastin derivatives for example, could be used as basic material for medical use in artificial organ technology or for coating transplanted tissue. Similar applications are imagined for collagen produced by plants. Biodegradable plastics isolated from transgenic plants, which might have packaging and coating applications for example, might be environmentally friendly alternatives to synthetic petrochemical polymers and a further field of an imagined plant-based material economy. Different agricultural expression systems that can be used for large-scale production of recombinant proteins have been developed in recent years. These systems include promoters, intracellular targeting and organ-specific expression (for review see [2]). In this review, we focus on recent work on the production of new biopolymers in transgenic plants.

New protein-based materials from plants

Naturally occurring proteins often exhibit interesting properties as fibers or adhesives. For example, larvae from insects produce fibrous glues in the form of sericin (produced by *Bombyx mori* for example [3]) or Balbiani Ring Gene proteins (produced by *Chironomus tendans* [4]). Other fibrous proteins, such as silks from spiders and insects or elastin and collagen from mammals, have remarkable importance for the survival of the animals that produce them. They are therefore in the focus of evolutionary research [5]. All of these proteins are also useful for the creation of new biomaterials because of their exceptional material properties including toughness, strength and elasticity [1]. The proteins consist of short blocks of amino-acid repeats [5]. Some animals, such as spiders, combine different proteins into one material. This opens the way for biotechnology to join several protein-based materials by gene fusions, by posttranslational combination and by mixing on a technical level.

Silk proteins from spiders and insects and their production in transgenic plants

During evolution, spiders have become highly diverse in production and use of silks (for review see [6,7]). This diversity is forced by the central roles played by silk throughout the whole life of spiders. Silk is used by spiders not only to capture prey but also to construct shelter and in reproduction. Araneoid spiders can spin seven types of silk from their spinnerets. One of the strongest fibers, dragline silk, is used by spiders for the frames of their webs and as safety lines [8–11]. Dragline silk is stronger than and one-tenth the weight of high tensile steel; it has a tensile strength similar to that of the high-performance p-aramid fiber KEVLAR [12]. The capture spiral silk (i.e. flagelliform silk) has a lower tensile strength than dragline silk but can be stretched to more than twice its length before breaking [13,14]. Aciniform silk is used by araneoid spiders to wrap and immobilize prey, to construct web decorations and to encase eggs [15]. X-ray diffraction and NMR data from spider silk, as well as from insect fibroin silk, show that the fibers contain crystalline and non-crystalline components [5]. The crystalline arrays are thought to be responsible for

the stiffness of the fiber. The amorphous regions (which make up 55–60% of dragline spider silk) are more or less kinetically free and can change their shape under the influence of external load, but they show entropic elasticity [16].

The structures of the genes encoding several spider silk proteins and insect fibroins from different species have been determined, and the implications of this information on our evolutionary understanding has been discussed (for reviews see [5,9,11,15,17,18]). All these proteins are typified by a modular nature. The repetitive portions of 11 characterized silk proteins from spiders can be generalized as typical sets of consensus repeats containing six types of amino acid motifs as poly-A, poly-(GA), GGX, GPGXX, GPX and spacers. The repetitive amino-acid motifs of the different silk proteins are thought to be directly responsible for the mechanical properties of spider silk fibers [19]. Poly-A regions and poly-(GA) sequences were found in β-sheet regions of major and minor ampullate silks [20]. β-sheet regions are able to link and form the crystalline areas in the fibers. Crystalline regions might contribute to the extremely high tensile strength of silk fibers [11]. Furthermore, the GPGXX motif is also thought to contribute to the mechanical properties of silks. Every GPGXX motif could form a type II β-turn. GPGXX motifs that occur in tandem could result in a series of β-turns [21]. Such consecutive turns could form a structure showing homology at a structural level to the β-spiral of elastin [22]. To date, only major ampullate (MaSpI and MaSpII proteins) and flagelliform silks (Flag) have been found to contain GPGXX motifs. These silks are the stretchiest of spider silks [11,21,23]. The function of the non-repetitive carboxy-terminal regions of MaSpI, MaSpII and Flag is still unclear. The sequencing of Flag has revealed that it also contains a non-repetitive amino-terminal region of unknown function. It is therefore likely that MaSpI and MaSpII also contain non-repetitive amino-terminal regions.

In general, all fibrous proteins of spiders and insects are large (several hundred kDa) and have highly repetitive amino-acid sequences. When expressed in microorganisms such as *Escherichia coli* [P1] [24–27] or in lower eukaryotes such as *Pichia pastoris* [28], transgenic spider-silk-like proteins accumulated only to low levels. Because spider-silk proteins largely consist of the hydrophobic amino acids glycine and alanin, an extended pool of these two building blocks must be provided if spider-silk proteins are to be produced by fast-growing microorganisms such as bacteria or yeast. Alternatively, fragments of the spider-silk proteins MaSpI, MaSpII and Adf3 of 60–140 kDa were produced in cultured mammalian cells [29]. These experiments might lead to the generation of transgenic animals that secrete spider-silk proteins into their milk. However, the mass production of a structural protein for technical purposes from animal cells or transgenic animals seems to be too expensive and time consuming in terms of fermentation or animal breeding. Nevertheless, the genetically programmed synthesis of protein polymers allows precise control of molecular mass and amino-acid monomer composition, and therefore could lead to the design and synthesis of polymers whose differing physical and functional properties are exactly controlled. Such controlled synthesis is difficult using chemical technologies [30]. To date, the development of protein-based biomaterials for commercial application has often been limited by difficulties in producing sufficient material to allow the study of structure-dependent functions [1].

We believe that transgenic plants are powerful protein factories that can overcome the problems mentioned above. Therefore, we constructed stable transgenic tobacco and potato lines to express various synthetic spider-silk genes ranging from 420 to 3600 basepairs in size. The genes were assembled so as to achieve very high homology to the native MaSpI gene from *Nephila clavipes* (more than 90%). Accumulation of up to 2% spider-silk protein of total soluble protein (TSP) in the endoplasmic reticulum (ER) of tobacco leaves was achieved, and this production was relatively independent of the size of the spider-silk protein. The recombinant spidroins exhibited extreme heat stability. This property, together with resistance against acidification and fractionated ammonium sulfate precipitation, was used to purify the plant-produced spidroins by a simple and efficient procedure [31]. Barr *et al.* [32•] showed the expression of synthetic spider silk proteins in *Arabidopsis thaliana* driven by the ubiquitous CaMV35S promoter and by the seed-specific β-conglycinin α′ subunit promoter. In *Arabidopsis* leaves, a 64 kDa artificial silk protein mimicking MaSpI was accumulated to 1.65% TSP and a 127 kDa protein to 0.06% TSP [32•]. In seeds, the 64 kDa protein was accumulated to 1.4% TSP and the 127 kDa protein to 1% TSP. The 64 kDa protein could also be detected in transformed somatic embryos of soybean.

A protocol similar to that described by Scheller *et al.* [31] has been used for successful purification of spidroins from plant tissues. Menassa *et al.* [33] reported the production of native MaSpI and MaSpII in transgenic tobacco plants in the greenhouse and in a field trial. However, the expression levels achieved in these trials were relatively low (69 g per hectare resulting from 0.1% TSP) in comparison to the levels achieved with artificial spider silk proteins [31,32•]. It is not yet clear if the small sequence differences between artificial spider silk proteins and 'native' MaSpI and MaSpII are responsible for this difference in expression levels. In further experiments, we constructed a fusion protein of 94.4 kDa made of the synthetic spider silk protein SO1 (51.2 kDa) and the synthetic elastic biopolymer 100xELP (100 repeats of pentapeptide Val-Pro-Gly-Xaa-Gly [where Xaa is Gly,

Val or Ala]) [34]. The 'best-producer' plants accumulated up to 4% of TSP as spider-silk–elastin fusion proteins [35•].

Natural elastin fibers provide elasticity to many tissues that require the ability to be deformed repetitively and reversibly [36]. Synthetic elastin-like polypeptides consist of oligomeric repeats of the pentapeptide Val-Pro-Gly-Xaa-Gly (where Xaa is any amino acid except for proline) [37]. Even when expressed as a fusion protein, elastin-like polypeptides become reversibly insoluble if the temperature is raised above their transition temperature [34]. When the temperature decreases, the elastin-like polypeptides and their fusion proteins become soluble again ('inverse transition cycling') [37]. The proposed secondary structure of elastin is the β-turn spiral. A first step towards the production of new biomaterials that have useful industrial and medical properties has been taken by combining a spider silk protein that exhibits a high tensile strength with an elastic biopolymer, such as an elastin-like polypeptide.

A new purification strategy has been developed using the unique properties of ELP fusions. First, the spider silk proteins from plants were enriched by boiling. We used heat treatment at 95 °C for 60 min and clearance by centrifugation of tobacco leaf extracts to enrich the spider-silk–ELP fusion protein in the supernatant. For the selective precipitation of the SO1-100XELP, NaCl was added to a final concentration of 2 M and the temperature was raised to 60 °C. Under these conditions, the recombinant spider-silk–elastin fusion proteins aggregated and could be precipitated by centrifugation. Cellular proteins remained in the supernatant. The precipitated recombinant proteins were resolved at a lower temperature and without salt to a final concentration of 1 mg/ml of nearly homogenous product. Dialysis against water and drying led to the formation of silk-proteins in the form of storable membranes. Extraction of 1 kg tobacco leaf resulted in 80 mg pure recombinant spider-silk–elastin protein. Transgenic plants for spider silk and spider-silk–elastin fusion proteins were phenotypically indistinguishable from wildtype plants. In addition, the expression of recombinant proteins in second generation plants was comparable to that in first-generation plants. In summary, expression and purification systems for plant-based silk proteins have been developed. Expression in storage organs such as tubers and seeds has been generally shown.

The selection of suitable spidroin and fibroin sequences for defined applications and commercialization is still at an early stage. To date, there are no reports of the successful spinning of plant-derived silk proteins. The first example of possible medical use has, however, been reported [35•]. Adherent mammalian cells need extra cellular matrix (ECM) proteins for attachment, proliferation and differentiation *in vivo* as well as *in vitro*. For successful medical application, recombinant ECM-like proteins should enhance cell growth in cell culture, inhibit differentiation and exhibit a high biocompatibility. The production of such proteins in plants will avoid contamination with mammalian viruses. In a first attempt to test spider-silk–ELP fusion proteins as substitutes for original ECM proteins, the growth of human chondrocytes (HCH-371) and CHO cell lines on this plant-derived fusion protein were compared with the growth of these cells on fetal calf serum and collagen. The growth of the cells on spider-silk–ELP was comparable to that on to collagen. The plant-derived spider-silk–elastin fusion protein seems to be an effective, biocompatible matrix that promotes the growth of mammalian cells of various characters. Studies of the surface-coating of implants and of *in vivo* immunological acceptance are in preparation.

Production of mammalian-derived structural proteins in plants

Elastin is a strong elastic fiber that is present in ligaments and in arterial walls. Synthetic proteins that are constructed from multiple repeats also show elastic properties. Such polymers have also been shown to prevent post-surgical adhesions and scars in rats [38]. A bioelastic protein-based polymer with the amino-acid sequence 121xGVGVP was successfully expressed in transgenic tobacco plant either after nuclear or after chloroplastic transformation [39,40], but the purification of this protein has not yet been reported. 100xELP could also be produced and purified from transgenic tobacco leaves and from transgenic tobacco tubers in a similar process to that described for spidroin–ELP fusions (J Scheller, M Rakhimova, U Conrad, unpublished). Possible applications for this protein in cell cultivation are currently under study.

Collagens form a family of extra-cellular matrix proteins that are detectable in all connective tissues of mammals. They represent 30% of total body weight of proteins. Collagen molecules consist of three polypeptides called α chains, which assemble to form triple helical domains. The collagen α chains contain the repeating triple sequence Gly-X-Y, where X and Y are frequently proline and hydroxyproline residues, respectively. This sequence is necessary for the correct formation of the triple helix [41]. Fibrillar collagen I, the collagen that is most widely distributed in tissues, consists of either two α1(I) chains and one α2(I) chain or of the homotrimer $(\alpha1[I])_3$. Even the homotrimer can form a stable triple helix. Ruggiero *et al.* [42] showed that tobacco plants transformed with a cDNA that encodes the human preproα1 chain of collagen are able to produce fully processed triple helical molecules. The recombinant collagen was synthesized in plantlets as a precursor, which was disulfide-linked via its C-propeptides. The precursor was further processed by removing the C-propeptides but retaining the complete N-propeptide. The final recombinant product was fully processed to collagen. As a consequence of the lack of

prolyl-hydroxylation in plants, the thermal stability of the recombinant product was decreased in comparison to that of the homotrimer purified from bovine tissues [42]. To solve this problem, Merle *et al.* [43] produced tobacco plants that are co-transformed with a human type-I collagen and a chimeric proline-4-hydroxylase. By this technique, the thermal stability of recombinant collagen I from plants was significantly improved. Hydroxylation was necessary for the receptor-binding properties of collagen. Prolyl hydroxylation was required for efficient binding to integrin α1β1 and glycoprotein VI [44].

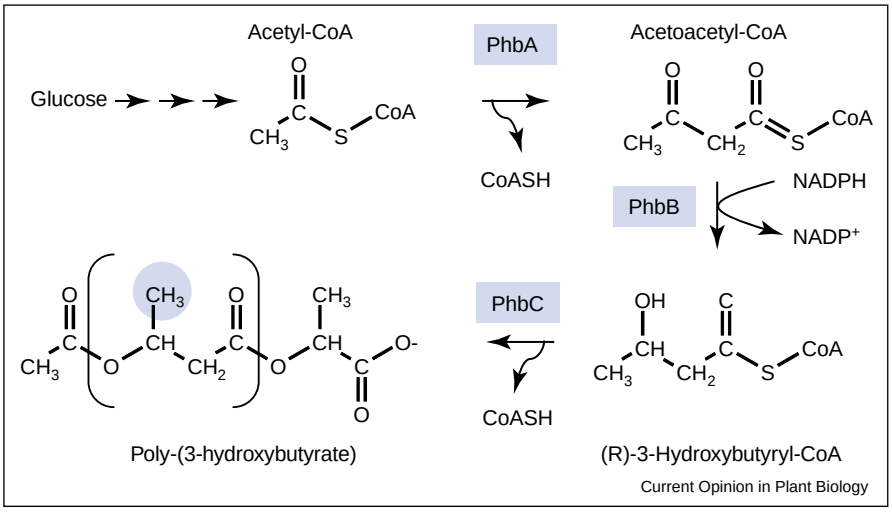
Biodegradable plastics (PHB): natural producers and properties

Biodegradable plastics have been proposed as environmentally friendly alternatives to synthetic petrochemical polymers for use, for example, in packaging and coatings. Poly(3-hydroxyalkanoates) (PHAs) are a group of biodegradable, structurally simple macromolecules that are synthesized by several microorganisms. These molecules have material properties that are similar to those of some common plastics, such as polypropylene [45]. Physiologically, PHAs are believed to play a role as a sink for carbon and energy [46]. Since the discovery of PHAs by Lemoigne [47] at the Institute Pasteur in 1926, a growing number of mainly linear, head-to-tail polyesters that are composed of 3-hydroxy fatty acid monomers have been described. In head-to-tail polyesters, the monomers are assembled in such a way that they are all oriented in the same direction. The molecular mass of PHAs varies with PHA producer but is generally between 50 and 1000 kDa. Historically, poly(3-hydroxybutyrate) (PHB) has been studied most extensively and has triggered commercial interest in this class of polymers. The bacterium *Ralstonia eutropha* can synthesize PHB from glucose and store it as discrete granules to levels as high as 85% of the cell dry weight. The homopolymer PHB from bacteria has characteristics that are similar to those of petrochemical plastics such as polypropylene, but the material is somewhat brittle, making it less stress resistant for industrial application [48]. Therefore Imperial Chemical Industries (ICI) has developed the PHB copolymer poly(3-hydroxybutyrate-co-hydroxyvalerate ([PHBV], also known as Biopol), which is less stiff and less brittle than bacterial PHB [49]. This copolymer has been produced by *Ralstonia eutropha* on glucose and propionate, leading to the incorporation of 3HV (3-hydroxyvalerate) into the polymer [50]. Because of the high-production cost of PHB from natural producers in comparison to the cost of synthetic plastics, alternative hosts for the production of PHBs have been evaluated. Enzymes that are responsible for the biosynthesis of PHB have therefore been transferred to *E. coli* [51,52], *Saccharomyces cerevisiae* [53] and the cells of insects [54], organisms that normally do not synthesize PHB. Furthermore, molecular breeding of transgenic plants that express functionally active biopolyester pathways has been carried out and might be an economically viable alternative for the production of biopolyester [1].

The biosynthetic pathway of PHB

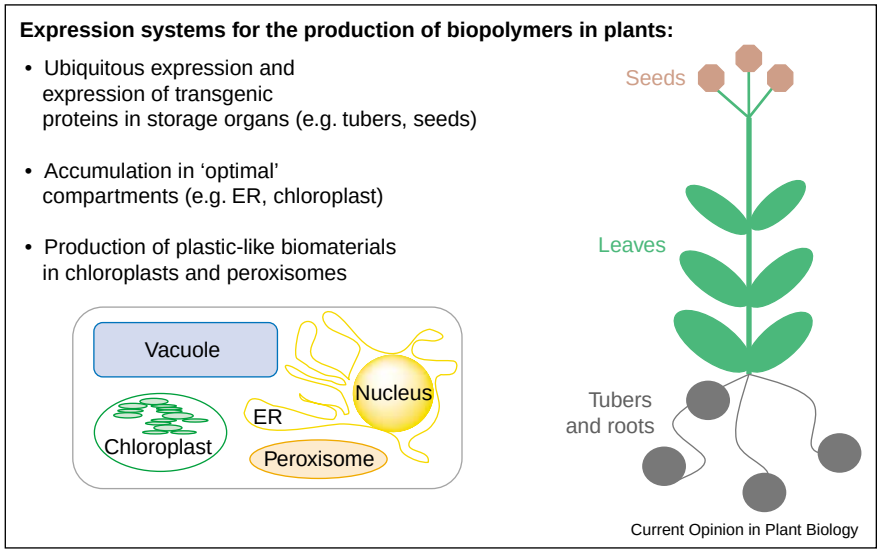
In general, the biosynthetic pathway for PHB comprises the three enzymes β-ketoacyl-CoA thiolase (PhbA), acetoacetyl-CoA reductase (PhbB) and PHB-polymerase/synthase (PhbC), which are often clustered in bacterial genomes ([55]; Figure 1). PhbA catalyzes the condensation of two acetyl coenzyme A (acetyl CoA) molecules into acetoacetyl-CoA. PhbB catalyses the reduction of acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA. Finally, the (R)-3-hydroxybutyryl-CoA monomers are polymerized into PHB by PhbC (Figure 2; [56]). PHAs can differ

Figure 1



Biosynthetic pathway for PHB. PHB is synthesized in a three-step pathway that is catalyzed by the enzymes β-ketoacyl-CoA thiolase (PhbA), acetoacetyl-CoA reductase (PhbB) and PHB-polymerase/synthase (PhbC) [56].

Figure 2



Optimal expression systems for proteins in transgenic plants [2] include ubiquitous expression, expression in storage organs and targeting to specific compartments [80]. This knowledge has been partially applied to the expression of polymeric proteins in plants [31,40]. Biodegradable plastic production has been shown in the chloroplasts and peroxisomes of transgenic plants [66–70].

in the length of their side chains, the illustrated PHB in Figure 2 carries a methyl-group but even larger side-chain groups have been found. Monomers ranging from 3–5 carbons are called short-chain-length PHAs (SCL-PHAs), monomers ranging from 6–16 carbons are called medium-chain-length PHAs (MCL-PHAs). In general, longer monomers tend to form more elastic and sticky polymers whereas shorter monomers form stiffer polymers [49]. The wide range of material properties found among different PHAs make them interesting biomaterials. Beside the bacterial protein machinery for PHB production, the same bacteria provide proteins for the degradation of these macromolecules to CO_2 and H_2O under optimal conditions.

Biodegradable plastics and PHB from transgenic plants

Until now, the commercial production of PHBs has been restricted by high production costs, making bacterial fermentation of PHB at least five times more expensive than chemical synthesis of polyethylene. Plants might be a suitable alternative for the low-cost production of PHBs. It was calculated that polymer concentrations in plants will need to reach at least 15% of dry weight for economically useful production.

The general feasibility of the production of PHB in plants was shown in 1992 in pilot experiments in which PHB synthesis was directed into the cytoplasm of *Arabidopsis thaliana*. The accumulation of 0.1% of leaf dry weight as PHB was low in comparison to PHB accumulation in bacteria. Nevertheless, the plant-produced PHB formed granules whose size and appearance were comparable to those of the granules formed by bacteria. Unfortunately, these *Arabidopsis* plants displayed a stunted-growth phenotype, leading to the assumption that the redirection of cytoplasmic acetyl-CoA to PHB production results in insufficient production of isoprenoids and flavoids, which are needed for normal growth [57].

Major improvements in PHB accumulation were achieved by targeting the bacterial PHB-biosynthesis pathway to other compartments of plant cells in transgenic plants. In 1994, Nawrath *et al.* [58] produced constitutive expression of the enzymes of the PHB biosynthetic pathway in chloroplasts of *A. thaliana* by nuclear transformation and fusion of transit peptides to the three PHB enzymes. They reported PHB accumulation of up to 14% of leaf dry weight.

In contrast to the cytoplasm, plastids are compartments in which there is a high flux of acetyl-CoA, which is mainly utilized as precursor for the fatty-acid biosynthesis of membrane lipids. Plants in which the bacterial PHB-biosynthesis pathway was targeted to plastids showed nearly wildtype growth and fertility but, in high PHB producers, chlorosis of the leaves was observed. One crucial issue that was not addressed by Nawrath *et al.* [58] is whether stable, high-level polymer production in plants could be maintained over many generations. Later, transgenic *A. thaliana* plants were directly screened for high accumulation of PHB in the plastids of leaves using gas chromatography and mass spectrometry (GC-MS). Accumulation of up to 40% of leaf dry weight as PHB

was reported. Unfortunately, a strong negative correlation between PHB accumulation and plant growth was detected once again, and even moderate concentrations of PHB (i.e. 3% of leaf dry weight) were accompanied by reduced plant growth, which was probably caused by exhausted acetyl-CoA pools in the plastids. In addition, T_2 progeny plants were analyzed and had moderately reduced PHB levels, indicating that PHB production might be lost or reduced to physiologically compatible amounts over the generations [59].

Although most work on PHB biosynthesis has been done in the model plant *A. thaliana*, there are also some reports of high accumulation of PHB in commercial crop plants. By targeting PHB synthesis to the plastids of maize and rape (*Brassica napus*) PHB accumulation of up to 5.7% and 7.7%, respectively, was achieved [1,60]. Growth retardation was not observed. An integrated processing system to extract PHB, oil and meal from rapeseeds was proposed, but any effect of PHB production on oil yield was not analyzed. Accumulation of PHB up to a concentration of 5% of dry weight has also been produced in the plastids of the hairy roots of sugar beet (*Beta vulgaris* L.) by nuclear transformation [61].

Even though attempts have been made to produce PHB by introducing the PHB-biosynthetic pathway into the plastid genome of transgenic tobacco, this strategy has not yet led to PHB production in desirable amounts [62] and strong growth defects were observed [63].

The syntheses of PHB in transgenic cotton and flax were examples of introducing a new polymer into existing fibers [64,65]. The transgenic cotton fibers exhibited normal strength, length and micronaire (i.e. fineness) but had enhanced insulating properties. The changes to thermal properties were small, however, perhaps because only small amounts of PHB accumulated in the cytoplasm of fiber cells (0.34% fiber weight) [64]. The transgenic flax showed a clear correlation of plastidial PHB content and growth retardation, the best-producer plants accumulated PHB to 0.5% of dry weight. In addition, the amounts of glucose, starch and linolenate, the most abundant fatty acid in flax seeds, decreased with increasing PHB content [65].

There is an obvious need to further improve transgenic PHB production by producing higher accumulation without side-effects such as chlorosis, male sterility and growth retardation, but genetic engineering has already yielded the first examples of the production of PHA-copolymers in transgenic plants (SLC- and MCL-PHA). PHB is a highly crystalline polymer whose usefulness is restricted by its physical properties, being relatively stiff and brittle. SLC- and MCL-PHA are less stiff and brittle, even having rubber-like properties, and therefore exhibit a greater potential for commercial applications.

In 1999, Slater and colleagues [66] from Monsanto succeeded in the production of a PHBV-copolymer (SLC-PHA) in the plastids of *A. thaliana* and *B. napus* (at up to 3% of plant tissue dry weight or seed weight, respectively). This was achieved by transformation of the plants with four different genes: *ilvA*, *bktB*, *phbB* and *phbC*, the last three being standard enzymes of the PHB biosynthetic pathway (a ketoliase, a reductase and a synthase). *IlvA* from *E. coli* encodes the protein threonine deaminase and converts threonine to 2-ketobutyrate. The endogenous pyruvate dehydrogenase complex catalyzes the synthesis of propionyl-CoA from 2-ketobutyrate. Acetyl-CoA and propionyl-CoA are then further processed and copolymerized into PHBV [66]. Whether the accumulation of PHB remained stable in the progeny was not analyzed.

In contrast to peroxisomal fatty acids in mammals, those in plants can be completely oxidized by the acyl-CoA oxidase (SC-ACOX) to acetyl-CoA (a process known as β-oxidation) [67]. This system has been evaluated for the production of SCL-PHAs in transgenic plants. Transgenic *Arabidopsis* plants that accumulate up to 0.23% of leaf dry weight as the SLC-PHB poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) have been engineered [68]. Therefore, SLC-PHA accumulation in transgenic plants indicates that SLC intermediates of the β-oxidation of SCL-fatty acids exist in peroxisomes. Furthermore, peroxisomal production of MCL-PHAs by β-oxidation has been observed, leading to a broad range of (R)-3-hydroxyacyl-CoA intermediates. These intermediates were further processed by the transgenic PHA-biosynthetic pathway, but the overall yield was still low (0.6% of dry weight) compared to that of PHB synthesis in plastids [69–71].

Recently, the synthesis of SCL-MCP-copolymers by *E. coli*, with glucose as carbon source, was reported [72,73]. The enzyme 3-ketoacyl-acyl-carrier protein synthase (fabH) normally catalyses the condensation of malonyl-CoA and acetyl-CoA, generally accepting substrates of 2–4 carbons in length. A genetically engineered fabH enzyme was obtained that accepted substrates of 2–10 carbons in length. When co-expressed with the *phaC* enzyme, PHA-copolymers accumulated that contained SCL (C_4) and MCL ($C_{6–10}$) monomers. Nomura and colleagues now aim to study how this system might be transferred to plants. Because the accumulation of PHB and simple PHBV copolymers in plastids is more effective than SLC and MCL-PHA accumulation in peroxisomes, plastids might serve as suitable sites for improved production of SLC-MCL-PHA co-polymers.

Plastic-like biomaterial from polymerization of amino acids

Biomaterials that are composed of polyamino acids, such as poly-γ-glutamate, polyaspartate or poly-γ-lysine, are components of dispersants, thickeners or hydrogels

[74,75]. Although poly-γ-glutamate and polyaspartate can be produced by bacteria, polyaspartate has to be produced by chemical synthesis or by hydrolysis of cyanophycin under mild conditions [P2]. The synthesis of cyanophycin, which accumulates mainly in a water-insoluble form in cyanobacteria, is dependent on the action of the cyanophycin synthetase (*cphA*) [76–79]. Recently, the expression of a water-soluble form of cyanophycin was shown in *E. coli* transformed with cyanophycin synthetase [79]. In addition, tobacco and potato transgenics for cyanophycin synthetase can produce cyanophycin in high amounts (I Broer, K Neumann, EK Pistorius, personal communication).

Conclusions and perspectives

The production of biomaterials from transgenic plants has been developed in recent years. Spider-silk proteins, elastins and collagen have been expressed in transgenic plants. In the case of spider-silk proteins, only the major ampullate spidroins I and II or their artificial derivatives have been produced in plants. A systematic study of the expression of a greater variety of spider-silk or insect-silk proteins in plants is still lacking. This is one of the challenges in the further development of the biotechnical potential of these interesting biomolecules. The expression of spider-silk or insect-silk proteins in some individual storage organs, such as seeds and tubers, has already been studied, but a general study of the expression of a collection of different molecules that includes targeting to different compartments (such as chloroplasts, ER and storage vacuoles) would also help to define optimal expression systems. Extraction and purification methods have been developed for spider-silk proteins and ELP, but extraordinary properties, such as heat resistance and temperature-dependent solubility, could be explored further. The silk produced from transgenic plants must have properties that are clearly better then those of technical fibres, otherwise the production and purification costs of proteins from transgenic plants will be too high for them to compete successfully with those from other sources.

Collagens have also been produced and processed in transgenic plants, in which the introduction of a second gene for successful processing was necessary. In case of biodegradable plastic-like compounds, high expression of PHB has been achieved but, in most cases, a negative correlation between PHB accumulation and plant growth has been reported. There is a need for further improvements in PHB production in transgenic plants aimed at higher accumulation without side-effects such as chlorosis, male sterility and growth retardation. Success in producing biopolymers in plants on a commercial scale depends not only on high levels of production of well-processed polymers but also on the development of efficient extraction and purification strategies that keep costs, especially energy costs, low.

The world market price of plastics derived from petroleum is below US $1 per kilogram, whereas the bacterial production of biodegradable plastics costs 5–10 times this figure. It has been estimated that the polymer concentrations that are required for commercialisation are at least 15% of dry weight. At present, it is not clear whether stable high-level polymer production can be obtained without severe side-effects, such as growth retardation.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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