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Production of Industrial Materials in Transgenic Plants

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Production of industrial materials in transgenic plants

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SUMMARY

Millions of tonnes of organic chemicals, or polymeric derivatives, are produced annually from agricultural plants. Genetic engineering methods can be used to modify the chemical composition of the storage compounds in many plant species. This will create opportunities to expand the uses of biomaterials as renewable and environmentally more benign alternatives to some uses of petrochemicals. Many of the most promising opportunities, in this respect, involve relatively minor modifications of the chemical composition of plant oils. The introduction of a single functional group into a fatty acid may create new industrial uses for the fatty acid and, therefore, significantly increase the value of the oil. Because different species of higher plants accumulate at least 210 different kinds of fatty acids, the genetic resources are available to support the production of a wide variety of modifications of agricultural oilseed species by genetic engineering. In addition, a large number of useful modifications may be produced by introducing genes for fatty-acid modifying enzymes, or related aspects of metabolism, from microorganisms.

1. INTRODUCTION

The wide range of organic chemicals upon which the developed world is dependent are largely derived from petroleum, natural gas and coal. For a number of reasons it may be useful to substitute biomaterials increasingly for fossil fuels as feedstocks for the chemical industry. First, biomaterials are renewable. Although the proven reserves of petroleum appear to be adequate to meet our needs for approximately fifty years at current rates of consumption, it seems worthwhile to attempt to conserve this resource by replacement with renewable alternatives. Second, in many cases the production of industrial chemicals from fossil fuels results in the production of large volumes of toxic waste. In some cases it is possible that the same or equivalent compounds can be produced from biomaterials with processes which generate less toxic by-products. Also, in some instances industrially useful compounds can be produced from biomaterials at lower cost than from petrochemicals. Third, the developed world has excess agricultural capacity which results in a variety of economic and environmental problems. The excess capacity depresses commodity prices which ultimately results in the necessity for large agricultural subsidies and environmental degradation of agricultural lands. In principle, these and related problems would be ameliorated if high value alternative crops were available. Because of excess food production in the developed world, the most likely mechanism for creating high-value alternative crops is to produce non-food commodities.

The production of large volumes of non-food commodities from higher plants is exemplified by the production of cellulose and crude fibre from plants such as cotton, ramie, sisal, hemp, and flax and the production of wood, fibre and cellulose from forest species. In addition, industrial-scale quantities of tall oils, rubber and tannins are extracted from woody species. Except for these examples, most plants which are adapted to modern agricultural practices are currently used to produce large quantities of starch, sucrose, lipid, or protein (table 1). Silk and wool are the only non-food proteins for which the magnitude of current demand would justify production on the scale which could be achieved by high-level expression in agricultural plants. Although it is possible that silk, or other polypeptides with similar properties may eventually be produced in transgenic plants, there are likely to be many technical hurdles associated with achieving high level expression of the genes, the correct tertiary structure of the protein and with procedures for extracting the proteins in a useful form. Therefore, in the short term, most applications of genetic engineering to the production of novel high-volume industrial commodities will probably be directed toward either modifying the structure or composition of starch or storage lipids, or toward diverting the biosynthetic capabilities of plant storage organs toward the production of new kinds of compounds. The uses of starch as a chemical feedstock are well-established (Otey & Doane 1984) and the general considerations associated with the production of new starches by genetic engineering have been reviewed recently (Kishore & Somerville 1993). Therefore, in the following sections I have outlined the rationale, recent advances and some of the potential opportunities in genetic engineering of fatty acids and related

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Table 1. *Estimated world consumption of biological raw materials by the chemical industry (Baumann* et al. *1988)*

raw material	consumption (k tonnes per year)
sugar	800
starch	1750
cellulose	5014
oils and fats	9500

compounds. Extensive information on the industrial uses of oleochemicals can be found in an excellent recent review (Baumann *et al.* 1988). Certain of the genetic or biochemical aspects of this subject have been described in other recent reviews (Murphy 1992; Willmitzer & Töepfer 1992). General information on basic aspects of plant lipid metabolism can be found in several reviews (Browse & Somerville 1991; Harwood 1988).

2. DEFINING GOALS

Many industrial uses of fatty acids are well established and have been designed to exploit inexpensive sources of large-volume commodities such as animal tallow (primarily saturated and monounsaturated C16 and C18 fatty acids), marine oils, and storage oil from the seeds of field crops, olive, palm and coconut. Although higher plants accumulate more than two hundred structurally different kinds of fatty acids (Van de Loo *et al.* 1993), the plants used for the production of oils accumulate only about ten to any significant extent (12:0, 14:0, 16:0, 18:0, $18:1^{\Delta 9}$, $18:2^{\Delta 9,12}$, $18:3^{\Delta 9,12,15}$, $20:1^{\Delta 11}$, $22:1^{\Delta 13}$, 12-hydroxy-$18:1^{\Delta 9}$). Except for ricinoleic acid (12-hydroxy oleic acid) these fatty acids differ only by chain length and degree of unsaturation. Thus, the relatively large numbers of industrial uses for these oils reflects considerable ingenuity on the part of industrial chemists who have designed robust methods for converting fatty acids to a range of useful products (Bauman *et al.* 1988). These include large-volume applications in production of adhesives, agrichemicals, coatings and paints, engineering thermoplastics, fabric softeners, plastic additives, surfactants, lubricants and cosmetics (Pryde & Rothfus 1989). Most of these derivatizations involve reactions of the carboxyl function of the fatty acid. However, there is increasing interest in the chemical reactions of the unsaturations (Bauman *et al.* 1988).

One of the main limitations to the use of fatty acids as industrial feedstocks is that all natural sources represent mixtures of related compounds. In order to obtain a uniform chemical product, it is necessary to either convert the different components of a mixture to a common derivative by a process such as catalytic hydrogenation, or to separate the various fatty acids. Thus, one of the goals of genetic engineering is to produce oils of high purity. The other goal is to expand the range of fatty acids that are available in field or plantation crops. For instance, many undomesticated plants accumulate mono- or polyhydroxylated or epoxidated fatty acids in which the functional groups are at various locations on the fatty acids. Others contain conjugated unsaturations, triple bonds and other modifications such as cyclopentene or furan groups (van de Loo *et al.* 1993). The availability of any of these unusual fatty acids would create novel possibilities for the oleochemical industry. For instance, because many fatty acids have several functional groups, there may be attractive opportunities to create new biodegradable polyesters or other polymers (Allcock 1992).

A practical problem in the development of new oils by genetic engineering is that since the new oils are not currently available, no market exists for these compounds. In addition, in most cases, because the material has not been available in large quantities, there are no defined industrial uses for the oil. This effectively means that industry is generally not willing to support basic research in this area. Thus, the basic research leading to the development of novel sources of oils will almost certainly need to be carried out in the public sector without regard to current economics. Because it can be difficult or impossible to predict the eventual economic value of a particular fatty acid, the goals of academic research in this area should be to acquire the knowledge required to produce large amounts of any novel fatty acid in agricultural or plantation species.

Because many of the enzymes involved in synthesis and modification of fatty acids and lipids are thought to be membrane proteins, very few of the relevant enzymes are amenable to purification and, until recently, none of the corresponding genes had been cloned. During the past three or four years this situation has improved substantially and several preliminary genetic engineering experiments have demonstrated the feasibility of creating plants which produce modified oils.

3. GENETIC CONTROL OF FATTY ACID UNSATURATION

The most common sources of structural variation in fatty acids are chain length and degree of unsaturation. Thus, the initial goals in gene isolation have included the isolation of the enzymes which control these aspects of structure. The unsaturations in fatty acids are generally introduced after the fatty acid has been elongated to at least 16:0. In the economically important oil crops, the first unsaturation is introduced into 18:0 by the chloroplast enzyme stearoyl-ACP desaturase. Until recently, this was the only known soluble fatty acid desaturase in any organism. This enzyme has been purified and the gene has been cloned from a number of plants (see, for example, Sato *et al.* 1992; Shanklin & Somerville 1991; Thompson *et al.* 1991). When expressed in *E. coli* the protein was fully functional. This has permitted the production of large quantities of active protein which have been used to initiate structural studies by X-ray crystallography (Schneider *et al.* 1992) and Mossbauer spectro-

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metry (Fox *et al.* 1993). The Mossbauer studies have revealed that the enzyme is a diiron-oxo protein. As a similar binuclear iron site has previously been found in methane monooxygenase, this raises the possibility that desaturases and hydroxylases have similar catalytic cycles (van de Loo *et al.* 1993) and may, therefore, have enough sequence homology so that the hydroxylase genes can be identified on this basis.

The cloned stearoyl-ACP desaturase genes from *Brassica napus* and *B. rapa* were used to produce transgenic plants in which the amount of the enzyme was strongly reduced by expression of antisense mRNA (Kuntzon *et al.* 1992). As expected, the transgenic plants had large increases in the amount of stearic acid which ranged from 2% to 40% of total fatty acids in different accessions. Although complete elimination of stearoyl-ACP desaturase in developing seeds was deleterious to normal seed development, seeds with as much as 39% stearate (a 25-fold increase above wild-type levels) had normal amounts of oil and germinated well. Thus, there was no apparent impediment to achieving a significant alteration in seed oil composition. The only currently obvious application for oils with increased 18:0 content seems to be in the production of certain structured lipids with high levels of saturated fats which could be used as cocoa butter replacements or in margarine and related food applications. However, it is likely that as the repertoire of genes encoding stearate modifying enzymes is expanded, the availability of the stearoyl-ACP desaturase gene may be useful in diverting 18:0 away from the desaturation pathway.

Although stearate is a relatively minor constituent of most plant oils, it seems likely that stearate levels could be further reduced in some species by overexpression of the cloned stearoyl-ACP desaturase gene. In addition, it has been shown that expression of the rat stearoyl-CoA desaturase gene in transgenic tobacco plants caused a decrease in the relative proportion of 18:0 (Grayburn *et al.* 1992). This is of interest because in contrast to the plant enzymes, the animal Δ9 desaturase is located in the endoplasmic reticulum and catalyses desaturation of both 16:0 and 18:0. Thus, if adequate levels of this heterologous enzyme can be obtained in transgenic plants, it might be possible to reduce the level of nutritionally undesirable 16:0 in edible oils by converting substantial proportions to $16{:}1^{\Delta 9}$.

The seeds of certain species of plants in the Umbelliferae, Araliaceae and Garryaceae accumulate large proportions of petroselenic acid ($18{:}1^{\Delta 6}$). This fatty acid is of potential industrial importance because through chemical cleavage of the double bond, petroselenic acid can be converted to lauric acid (12:0), an important component of many detergents, and adipic acid (6:0 dicarboxylic), a monomeric component used in synthesis of nylon 6,6 (Murphy 1992). A desaturase which appears to be responsible for petroselenic acid synthesis in coriander was isolated by screening a cDNA library with an antibody raised against the avocado stearoyl-ACP desaturase (Cahoon *et al.* 1992). The amino acid sequence of the coriander gene product was approximately 70% identical to the castor stearoyl-ACP desaturase. This high degree of sequence similarity may be very useful in assigning a structural basis for the product specificity of the two enzymes, once the tertiary structure of the stearoyl-ACP desaturase is known.

Expression of the coriander cDNA in transgenic tobacco tissues resulted in accumulation of low levels of petroselenic acid. This confirmed a role for the gene in petroselenic acid synthesis but indicated that some other factor was limiting the accumulation of petroselenic acid in transgenic tobacco. Small amounts of $16{:}1^{\Delta 4}$ were also observed, raising the possibility that the enzyme is a Δ4 desaturase which acts on 16:0. According to this scheme, petroselenic acid would be produced by elongation of $16{:}1^{\Delta 4}$. Thus, the low levels of accumulation of petroselenic acid in the transgenic plants may be due to a requirement for the enzymes required to convert $16{:}1^{\Delta 4}$-ACP to petroselenic acid. At present, the problem must be approached indirectly because it has not yet been possible to detect activity of the enzyme in an *in vitro* assay.

Relatively little is known concerning the other desaturases in higher plants. These enzymes are all thought to be integral membrane proteins which utilize lipids as substrates and require additional proteins, such as reduced cytochrome b_5 or ferredoxin, as electron donors (Browse & Somerville 1991). Because of the technical problems associated with purifying integral membrane proteins, a genetic approach was used to clone the genes for several plant desaturases from *Arabidopsis*. A comprehensive collection of mutants with alterations in the levels of leaf, root and seed lipids is available in *Arabidopsis* (Browse & Somerville 1991). One class of mutants, designated *fad3*, is deficient in the desaturation of $18{:}2^{\Delta 9,12}$ to $18{:}3^{\Delta 9,12,15}$ by a pathway of desaturation present in the endoplasmic reticulum. The *fad3* gene was, therefore, presumed to be encode or to specifically control the activity of a Δ15 desaturase (Browse *et al.* 1993; Lemieux *et al.* 1990). The gene was cloned by two independent approaches, map-based cloning and T-DNA tagging. In one instance, a *fad3* mutation was genetically mapped relative to the nearest restriction-fragment-length-polymorphisms (RFLPs). The RFLPs were then used to isolate yeast-artificial-chromosome (YAC) clones which covered the region of the genome where the mutation was located. The YAC clones were then used to probe a cDNA library from developing seeds of *Brassica napus*. One of the clones which hybridized to the YAC exhibited low but detectable sequence homology to a fatty acid desaturase from cyanobacteria. When this cDNA was transformed into the *fad3* mutant, it complemented the mutation indicating that it was the correct gene (Arondel *et al.* 1992).

The *fad3* gene was also cloned by screening a large collection of independently transformed lines of *Arabidopsis* for altered fatty acid composition by gas chromatography (Yadav *et al.* 1993). One line was found in which the *fad3* gene was partially defective due to the insertion of ectopic (i.e. T-DNA) DNA near the 5′ region of the gene. By using T-DNA as a hybridization probe it was possible to isolate the

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flanking regions of DNA and to identify the coding sequence of the *fad3* gene. Expression of the cDNA clones in transgenic plant tissues resulted in strongly increased levels of $18:3^{\Delta 9,12,15}$ and corresponding decreases in the amount of $18:2^{\Delta 9,12}$ (Arondel *et al.* 1992; Yadav *et al.* 1993). Thus, it is apparent that the ratio of these two fatty acids can be changed by altering the expression of this gene. This should create new opportunities to improve edible oils which lack $18:3^{\Delta 9,12,15}$ by using antisense versions of the gene to suppress the activity of endogenous copies of the gene in various crop species. This would reduce or eliminate the need for catalytic hydrogenation of edible oils, a process which leads to the formation of undesirable levels of *trans*-isomers. Conversely, it seems likely that the *fad3* genes may be used to produce plants with very high levels of $18:3^{\Delta 9,12,15}$. At present, flax (*Linum usitatissimum* L.) is the only plant used for production of oils high in $18:3^{\Delta 9,12,15}$. Such oils are called drying oils because the double bonds oxidize readily in air and the resulting compounds polymerize into a soft and flexible film. Linseed oil has been widely used in paints, industrial coatings, printing ink, core oil for sand casting, automobile brake linings, concrete protection and curing and in restructured wood products. It should soon be possible to examine the utility of oils with significantly higher levels of $18:3^{\Delta 9,12,15}$ than that found normally in flax (approximately 50% by mass $18:3^{\Delta 9,12,15}$).

In addition to the *fad3* gene, *Arabidopsis* is known from mutant studies to contain two additional Δ15 desaturases which act on fatty acids esterified to chloroplast lipids (Browse & Somerville 1991). One of these, originally designated *fadD* but renamed *fad7*, has recently been cloned by using the *fad3* gene as a heterologous hybridization probe (Iba *et al.* 1993; Yadav *et al.* 1993). The *fad7* gene shows strong sequence identity to the *fad3* gene except near the amino terminus where the two gene products diverge because of the different targeting signals required to deliver *fad3* to the endoplasmic reticulum and *fad7* to the chloroplast. Although the plastid genes are not of primary importance in controlling the degree of unsaturation of storage oils, the availability of the pair of highly related genes may be very useful in examining the mechanisms involved in insertion of the proteins into membranes, and the mechanisms which regulate the substrate specificity, activity and turnover of these important enzymes.

Altogether, *Arabidopsis* has six different plastid-localized desaturases and two endoplasmic reticulum-localized desaturases. Genes for three of the eight different desaturases have recently been cloned and used to create alterations in the fatty acid composition of transgenic plants. Because the methods used to clone the *fad3* gene can also be applied to any of the other desaturases, it seems likely that genes will soon be available to create a wide range of modifications of fatty acid desaturation. Although virtually nothing is known at present about the mechanisms which regulate desaturation, the genetic materials are now available to permit a detailed analysis of this aspect of cellular regulation.

4. GENETIC CONTROL OF FATTY ACID LENGTH

The most important edible oils contain almost exclusively C18 and C16 fatty acids. Substantial quantities of oils containing longer or shorter fatty acids are used for industrial purposes. Long-chain fatty acids (C20 to C24) are used as lubricants and plasticizers. The most important long-chain fatty acid is erucic acid ($22:1^{\Delta 13}$) which is largely obtained from certain cultivars of rapeseed. Refined erucic acid is used for the synthesis of brassylic acid, erucamide and for the production of precursors for the synthesis of nylon 13,13. Murphy (1992) reported that the demand for engineering plastics such as nylon 13,13 is expected to exceed one million tonnes by the end of the decade.

The value of erucic acid as an industrial material is limited by the fact that the oil obtained from the most advanced cultivars of rapeseed contain only about 56% erucic acid. To reduce or eliminate a significant processing cost associated with refining the oil, it would be desirable to produce cultivars in which the storage triacylglycerols contained essentially pure erucic acid. In the oil from existing cultivars of rape, erucic acid is not found on the sn-2 position of the triacylglycerol (Bafor *et al.* 1990). By contrast, oil from other species such as meadowfoam (*Limnanthes alba*) contains erucic acid on all three positions. Although the reason for the absence of erucic on the sn-2 position in rape is not known, an attractively simple hypothesis is that the sn-2 acyltransferase in rape may discriminate against erucyl-CoA, whereas the meadowfoam enzyme does not discriminate. Thus, efforts to isolate a gene for an sn-2 acyltransferase are currently underway in a number of laboratories. It may also be necessary to increase the activity of the fatty acyl elongase which converts $18:1^{\Delta 9}$ to $22:1^{\Delta 5}$. Although very little is known about the properties of the relevant enzymes, mutants of *Arabidopsis* deficient in erucic acid accumulation are available (Lemieux *et al.* 1990). Therefore, at least some of the genes involved in elongation can be isolated by exploiting the map-based cloning methods used to isolate desaturase clones (Arondel *et al.* 1992).

Approximately one million tonnes of waxes are produced each year from petroleum. Plant waxes are utilized on a much smaller scale for certain applications which require properties not available in petroleum based waxes. Plant waxes have relatively high unit value, compared to other plant oleochemicals, because of the high costs of production from species which have low yield or are not well adapted to mechanized agricultural practices. Most plant waxes are produced as epidermal secretions which are harvested by boiling plant tissues in water or, in the case of carnauba, by threshing the wax from detached fronds. Jojoba produces liquid wax esters which accumulate as intracellular inclusions in much the same way as triacylglycerols in oilseeds. Waxes such as carnauba and jojoba are primarily composed of simple esters of long chain fatty acids and alcohols. Approximately 90% of the acyl groups in jojoba oil are unsaturated C20 and C22 fatty acids and alcohols.

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Liquid esters from jojoba are used in more than 300 cosmetic applications, as lubricant additives, in machine cutting oils and many other miscellaneous applications. Catalytic hydrogenation converts the liquid wax to a hard wax, similar in properties to carnauba, which has additional applications in coatings. Because of the high unit value, it may be useful to transfer the capability to synthesize wax esters into plant species other than jojoba. In a species which normally produces large quantities of long chain fatty acids, as few as two additional enzymes may be required to synthesize intracellular wax esters; a dehydrogenase which converts acyl-CoAs to alcohols, and a fatty alcohol:acyl CoA transferase which condenses an acyl-CoA and an alcohol to form a wax ester.

Medium chain fatty acids are used extensively for synthesis of detergents. The US market for lauric acid (12:0) has been estimated to be on the order of 640×10^6 kg per year (Battey *et al.* 1989). Lauric acid is produced on a commercial scale from coconut and palm kernel, but several species of herbaceous plants also accumulate relatively large proportions of their storage oils as medium chain fatty acids (Murphy 1992; van de Loo *et al.* 1993). Because of large fluctuations in the value of tropical oils, there has been industrial interest in the possibility of producing lauric acid in a temperate zone field crop. A long-term effort to domesticate laurate-producing herbaceous plants, such as certain species in the genus *Cuphea*, have not yet been successful. Therefore, there has been sustained interest in the mechanisms by which plants accumulate oils containing lauric acid. Recently, it has been demonstrated that in at least some species, the accumulation of medium chain fatty acids in storage oils is due to the presence of a medium-chain specific thioesterase which cleaves elongating medium chain fatty acids from ACP and thereby prevents the further elongation by the fatty acid synthase complex (Davies & Voelker 1993). The production of transgenic *Arabidopsis* and *B. napus* plants which accumulated high levels of lauric acid was accomplished by the introduction of a medium-chain specific thioesterase gene from California bay (Voelker *et al.* 1992). Even though these species do not normally accumulate significant amounts of lauric acid, the C12 fatty acids appeared to be specifically incorporated into storage triacylglycerols but not into membrane lipids. The mechanism by which such 'abnormal' fatty acids are excluded from membrane lipids is not known (Bafor *et al.* 1990; Battey *et al.* 1989). However, this encouraging result suggests that no additional cellular functions are required in order to specifically direct novel non-membrane fatty acids into storage oils. Thus, it may also be possible to produce other unusual fatty acids in transgenic plants without potentially deleterious accumulation of unusual fatty acids in membrane lipids.

5. PRODUCTION OF NOVEL POLYMERS

Many species of bacteria accumulate excess carbon as small intracellular granules composed of aliphatic polyesters which are collectively designated polyalkanoates (PHAs). Depending upon the species of bacteria and the composition of the growth medium, the polymers may be composed of various monomers ranging from 3-hydroxypropionate to 3-hydroxylaurate (Poirier *et al.* 1992*b*). Typically, each granule is composed of about 1000 individual PHA molecules, each of which contains approximately 8000 monomers. These compounds are thermoplastics with melting temperatures which are in the same range as petroleum-based thermoplastics such as polypropylene. Because many species of soil bacteria secrete an enzyme called PHA-depolymerase, plastic films produced from PHAs are rapidly biodegraded when placed in soil. A copolymer of polyhydroxybutyrate and polyhydroxyvalerate is commercially produced by ICI plc under the trade name Biopol which is used to produce biodegradable containers. However, even though the bacterial cultures accumulate as much as 90% of their dry mass as PHA, the most optimistic projections indicate that it will not be possible to produce PHA by fermentation for less than about four times the current cost of comparable petroleum based plastics. By contrast, the unit value of starch from higher plants such as maize is substantially less than the unit value of petroleum-based plastics. Therefore, if the carbon which is normally present in starch or sucrose were quantitatively diverted to PHAs or related compounds, it may be possible to produce these materials in large quantities and at low cost in higher plants.

The feasibility of producing small amounts of polyhydroxybutyrate (PHB) in plants was recently demonstrated by introducing genes for the enzymes acetoacetyl-CoA reductase and PHB-synthase from the bacterium *Alcaligenes eutrophus* into *Arabidopsis* (Poirier *et al.* 1992*a*). These two enzymes catalyse the reduction of acetoacetyl-CoA to 3-hydroxybutyrl-CoA and the polymerization of hydroxybutyrl monomers into PHB. Transgenic plant lines that contained both genes accumulated PHB as small granules which were primarily localized in the nucleus for some unknown reason. Since there is no precedent for this observation, it is not possible to evaluate whether this may prove to be a significant hurdle in the large-scale production of the materials in plants. The size and appearance of these granules were similar to the PHB granules that accumulate in bacteria. The amount of production was only about 0.1 mg per gram of fresh tissue. However, in these preliminary experiments no effort was made to maximize production of the polymers. Thus, further increases in production may be possible. If further studies demonstrate the feasibility of producing economically significant quantities of PHB in transgenic plants, additional opportunities to produce novel polymers will depend upon expanding the range of hydroxyalkyl-CoA monomers produced by higher plants.

6. FUTURE DIRECTIONS

It has been almost a decade since the first reports of the production of fertile transgenic plants were published (DeBlock *et al.* 1984; Horsch *et al.* 1984).

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Although great strides have been made since that time, progress in exploiting genetic engineering technology for the production of new materials remains limited by the availability of genes encoding useful functions, and by lack of knowledge about the basic biochemical mechanisms underlying many aspects of plant metabolism. High priority goals for gene cloning should include the isolation of genes which catalyse the hydroxylation and epoxidation of fatty acids, and representatives of the various acyltransferases involved in the synthesis of storage lipids. Basic biochemical information about the reaction mechanisms involved in the biosynthesis and metabolism of alkynes, furanoic acids, cyclopentanes, cyclopentenes and conjugated unsaturations is also required to ensure future progress. At present, there is virtually no research activity concerning these topics. A central problem of broad importance concerns the mechanisms by which unusual fatty acids appear to be specifically targeted to oil bodies and excluded from membranes. Until this is understood, it will not be possible to evaluate the potential problems associated with producing unusual fatty acids in crop species. It is also essential to have a detailed knowledge of the mechanisms which regulate the allocation of C2 groups between membrane and storage lipid synthesis.

If the knowledge and genetic materials were available to produce large quantities of a novel fatty acid or polyalkanoate in transgenic plants, several ancillary problems would remain. At present, the value of an oilseed is determined by the combined value of the oil and the storage protein. However, if the protein were contaminated by an inedible oil or other compound, it will not be useful as animal feed and will, therefore, have no value. One possible solution to this problem may be to develop parallel non-food applications for the storage proteins. As noted earlier, it may be worthwhile to produce analogs of certain structural proteins such as silk or wool. Also, the recent development of interest in paper production using an enzymic process may create markets for very large quantities of inexpensive (i.e. impure) hydrolytic enzymes such as xylanases. Alternatively, perhaps it may be possible to produce storage oil or polyalkanoates in a tissue other than seeds: potato tubers for example. The ability to produce unusual biomaterials in a tuber or root may also be desirable as a means of preventing birds and other wildlife from ingesting biomaterials with poor nutritional qualities. Achieving this would probably require major alterations in the metabolism of tuber cells. However, in view of recent evidence indicating that at least some multistep pathways are under transcriptional control of a single regulatory factor (Ludwig *et al.* 1990), it might be possible to effect such changes if the transcriptional factors controlling the pathways of fatty acid and lipid synthesis were available.

In conclusion, it is possible to envision many interesting new opportunities to utilize genetic engineering for the development of renewable materials. However, a great deal of basic research remains to be done before we will have the broad base of detailed knowledge required to undertake many desirable modifications with the same degree of predictability that non-biological engineers design new devices, structures and processes.

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