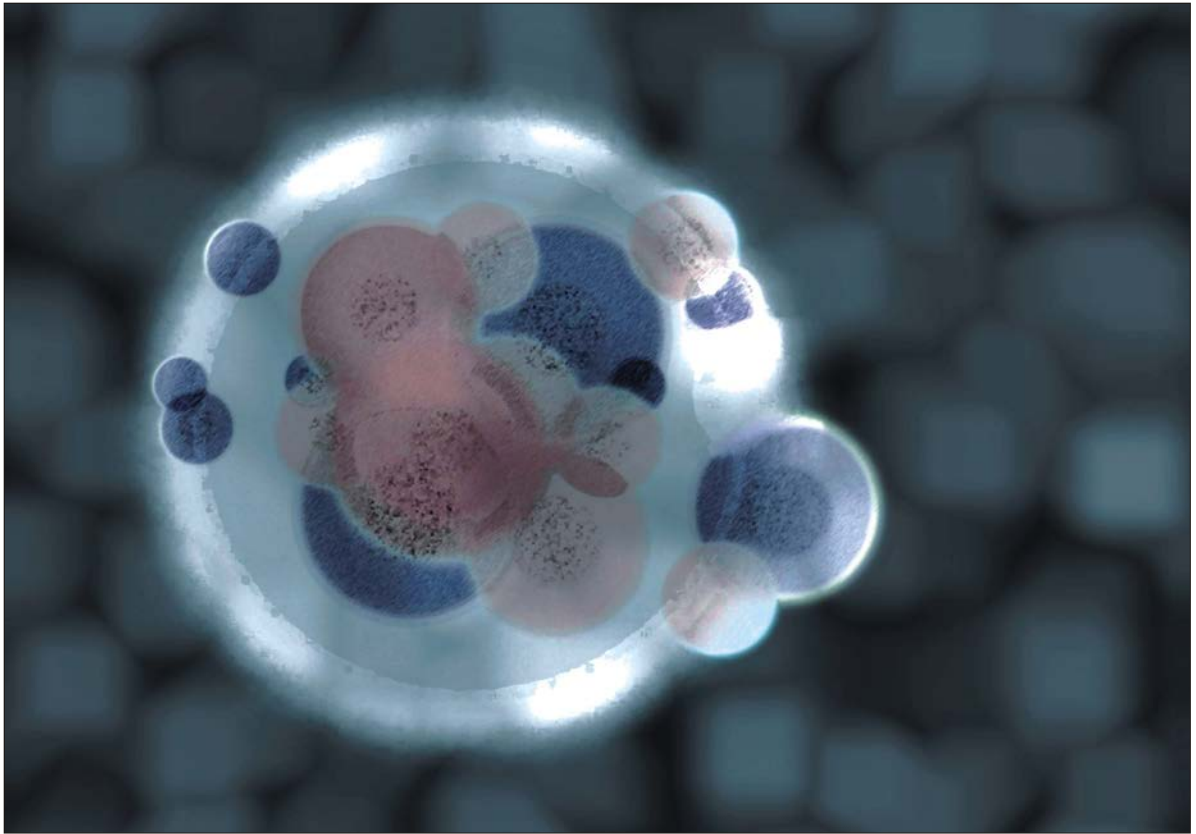

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 6 (1): 209-219, 2006
ISSN 1727-3048
© 2006 Asian Network for Scientific Information

Isoprenoid Biosynthesis in Plants: Pathways, Genes, Regulation and Metabolic Engineering

[1,2]Zhi-Hua Liao,[2]Min Chen, [3,4]Yi-Fu Gong, [3]Zhi-Qi Miao, [1]Xiao-Fen Sun and [1,3]Ke-Xuan Tang
[1]State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Morgan-Tan International Center for Life Sciences, Fudan-SJTU-Nottingham Plant Biotechnology R and D Center, Fudan University, Shanghai 200433, China
[2]School of Life Sciences, Laboratory of Natural Products and Metabolic Engineering, Institute of Biotechnology, Institute of Modern Biopharmaceuticals, Chongqing Sweet potato Research Center, Southwest China Normal University, Chongqing 400715, China
[3]Plant Biotechnology Research Center, Fudan-SJTU-Nottingham Plant Biotechnology R and D Center, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200030, China
[4]Faculty of Life Science and Biotechnology, Ningbo University, Ningbo 315211, China

Abstract: Isoprenoids, namely terpenoids, are the largest and the most structurally varied groups of natural products, which contain more than 30,000 known compounds. All the isoprenoids are biosynthesized from only two C_5 precursors in plants, isopentenyl diphosphate and its isomer, dimethylallyl diphosphate. Two distinct pathways of isopentenyl diphosphate biosynthesis exist in plants: the classical mevalonate pathway and the recently unveiled deoxyxylulose 5-phosphate pathway. In this study, we summarize the recent progress on the molecular genetics of the two pathways, specializing in pathways, genes, enzymes, intermediates, subcellular compartments of isoprenoid biosynthesis, crosstalk of the two pathways and metabolic regulation and engineering.

Key words: Biosynthesis, gene, isoprenoid, metabolic engineering, pathway, regulation

INTRODUCTION

There are enormous varieties of metabolites in plants that can be classified into two basic groups according to their functions: the primary metabolites and secondary metabolites[1]. The primary metabolites include nucleic acids, proteins, fatty acids and others that participate in plant basic functions. The secondary metabolites influence ecological interactions between plants and environment[2]. The structurally and functionally diversified isoprenoids (also called terpenoids) are among the largest and the most structurally varied groups of the natural products with over 30,000 known compounds, which have many essentially biological functions in plants, i.e. sterols as the essential components of bio-membranes, carotenoids and chlorophyllins as photosynthesis pigments, plant hormones as the regulators of plant growth, development and defense isoprenoids. Additionally, some of isoprenoids are important economic chemicals including flavors, pigments, waxes, rubbers, vitamins, taxol[3], artemisinin[4], ginkgolides[5]. Currently there are very active studies on isoprenoid biosynthesis worldwide, and the knowledge and interests in isoprenoid biosynthesis pathways and bioengineering, the important economic isoprenoids, have been recently increased tremendously. This article focuses on the molecular genetics of the two pathways of isoprenoid biosynthesis, including genes, enzymes, intermediates, subcellular compartments and metabolic regulation.

ISOPRENOID BIOSYNTHESIS

All isoprenoids are biosynthesized from just two universal C_5 precursors: IPP and its isomer DMAPP[6]. The isoprenoids can be classified into different groups according to the number of C_5 units that are used to build the skeletons of isoprenoids. The known simplest isoprenoid, isoprene, contains only a single C_5 unit which is named hemiterpenes. The complex terpenes consist of different number of C_5 units. Monoterpenes consist of two C_5 units. Sesquiterpenes have three C_5 units. Diterpenes are C_{20} terpenes built by four C_5units. Triterpenes are C_{30} terpenes utilizing six C_5 units and polyterpenes contain

Corresponding Author: Ke-Xuan Tang,State Key Laboratory of Genetic Engineering, Institute of Genetics, School of Life Sciences, Morgan-Tan International Center for Life Sciences, Fudan-SJTU-Nottingham Plant Biotechnology R and D Center, Fudan University, Shanghai 200433, China Tel: 021-65642772 Fax: 021-65643552

more than eight C_5 units. Although IPP and DMAPP are the universal precursors for isoprenoid biosynthesis, there are two distinct pathways to biosynthesize isoprenoid in plant kingdom: the well-studied mevalonate (MVA) pathway and the recently unveiled deoxyxylulose 5-phosphate/2-C-methyl-D-erythritol 4-phosphate (DXP/MEP) pathway (also called DXP pathway), and the later is mevalonate-independent pathway (Fig.1). Furthermore, the two distinct pathways are localized in different compartments at the subcellular level: the MVA pathway predominates in cytosol and the DXP pathway in plastid[7].

Isoprenoid biosynthesis in cytosol through the MVA pathway: Cytosolic and mitochondrial isoprenoids are derived from acety-CoA which is obtained from CO_2 fixation. Two acetyl-CoA molecules are condensed to acetoacetyl-CoA catalyzed by acetyl-CoA: acetyl-CoA C-acetyltransferase (AACT, EC 2.3.1.9)[8], and acetoacetyl-CoA and acetyl-CoA are condensed to HMG-CoA by 3-hydroxy-3-methylglutaryl-CoA synthase (HMGS, EC 4.1.3.5)[9]. HMG-CoA is finally reduced to MVA catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR, EC 1.1.1.34)[10]. MVA biosynthesis is the first committed step of the pathway and mevalonate 5-diphosphate from mevalonate is biosynthesized by two-step phosphorylation respectively catalyzed by mevalonate kinase (MK, EC 2.7.1.36)[11] and phosphomevalonate kinase (PMK, EC 2.7.4.2)[12] in order. Finally, mevalonate 5-diphosphate decarboxylase (MDC, EC 4.1.1.33)[13] leads to the conversion of mevalonate 5-diphosphate to IPP, which is the end product of MVA pathway for isoprenoid biosynthesis in cytosol and mitochondria. IPP and DMAPP can be converted into each other and this reaction is catalyzed by IPP isomerase (IPI, EC 5.3.3.2)[14]. IPP and DMAPP form the basic skeleton of some sesquiterpenes, sterols and the side-chain of ubiquinone[15].

Isoprenoid biosynthesis in plastid through DXP pathway: In the past few decades, MVA pathway was considered as the universal pathway of isoprenoid biosynthesis. The other distinct isoprenoid biosynthetic pathway, that is mevalonate-independent, was discovered recently in eubacteria[16] and plants[17]. Pyruvate and glyceraldehyde 3-phosphate (G3P) are the precursors for synthesizing isoprenoids on the newly-found isoprenoid biosynthetic pathway. In plants, the pathway is localized in plastid. Pyruvate and glyceraldehyde are condensed to 1-deoxy-D-xylulose 5-phosphate (DXP) by 1-deoxy-D-xylulose 5-phosphate synthase (DXPS, EC 4.1.3.37)[18] and this is the first committed step on the plastid isoprenoid biosynthesis[19]. Thus, the mevalonate-independent isoprenoid biosynthetic pathway is also called DXP pathway. DXP is subsequently rearranged and reduced to 2-C-methyl-D-erythritol 4-phosphate (MEP) catalyzed by 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR, EC 1.1.1.267)[20]. Then MEP is conjugated with CDP by MEP cytidyltransferase (MCT, EC 2.7.7.60)[21] to form 4-(cytidine 5-diphospho)-2-C-methylerythritol (CDP-ME). 4-(Cytidine 5-diphospho)-2-*C*-methylerythritol kinase (CMK, EC 2.7.1.148)[22] catalyzed the phosphorylation of CDP-ME to form CDP-MEP. CDP-MEP is subsequently converted to 2-C-methylerythritol 2,4-cyclodiphosphate (ME-cPP) by 2-C-methylerythritol 2,4-cyclodiphosphate synthase (MECPS, EC 4.6.1.12)[23]. The final two steps of DXP pathway include the formation of hydroxymethylbutenyl 4-diphosphate (HMBPP) from ME-cPP catalyzed by hydroxymethylbutenyl 4-diphosphate synthase (HDS)[24], and the direct conversion of HMBPP into a 5:1 mixture of IPP and DMAPP by IPP and DMAPP synthase (IDS)[25].

THE GENES AND ENZYMES INVOLVED IN THE MVA PATHWAY

The MVA pathway has been discovered over half a century and the molecular genetics of MVA pathway has been elucidated. The genes involved in MVA pathway have been characterized by molecular genetics and biochemistry approaches.

Acetyl-CoA: Acetyl-CoA C-acetyltransferase (AACT): AACT catalyses the first step, the condensation of two acety-CoA molecules to form acetoacetyl-CoA involved in the MVA biosynthetic pathway. AACT belongs to the thiolase family that has two conserved cysteine residues, which is important for thiolase activity. The first one is located in the N-terminal section of the enzymes and involved in the formation of an acetyl-enzyme intermediate; the second is located in the C-terminal extremity and it is the active site involved in deprotonation in the condensation reaction[26]. The full-length cDNA of *ATCC* have been characterized from *Arabipsis thaliana*, para rubber tree, rice and radish[27]. The genomic organization of *ATCC* is different among plants. According to the genomic database, *AACT* belongs to a small gene family in *Arabidopsis thaliana*, but there is only a single *ATCC* copy in radish and it is regulated by light[27].

3-hydroxy-3-methylglutaryl-CoA synthase (HMGS): HMGS catalyzes the condensation of acetyl-CoA with acetoacetyl-CoA to produce HMG-CoA and CoA. *HMGS*

has the consensus pattern of amino acids as the following: N-x-[DN]-[IV]-E-G-[IV]-D-x(2)-N-A-C-[FY]-x-G, in which cysteine acts as the catalytic nucleophile in the first step of the reaction as well as the acetylation of the enzyme by acetyl-CoA[28]. F244, a specific inhibitor of HMGS, can break the MVA pathway and is a useful tool for HMGS research[29]. In plants, *HMGS* expression shows correlation with rapid cell division and growth like *HMGR*, which is regulated by many factors. Wound, methyl jasmonate, salicylic acid[29] and ozone induce *HMGS* expression suggesting that HMGS is involved in the activities of plant defenses[30]. In para rubber tree, the mRNA expression level and enzymatic activity of HMGS showed positive relationship with the accumulation of rubber[31]. These results suggest that HMGS is a highly regulated gene in the beginning of the MVA pathway.

3-hydroxy-3-methylglutaryl-CoA reductase (HMGR): HMGR, a rate-limiting enzyme that can be inhibited specifically by lovastanin, catalyzes the NADP-dependent synthesis of mevalonate from HMG-CoA, which is the most important committed step of the MVA biosynthetic pathway[32]. HMGR is well studied in archeobacteria, bacteria, fungi, plants and animals. Structurally, it consists of 3 domains: the N-terminal region that contains a variable number of transmembrane segments (7 in mammals, insects and fungi; 2 in plants), a linker region and a C-terminal catalytic domain of approximately 400 amino-acid residues. Although little sequence similarity is found among the transmembrane domains of HMG-CoA reductases from different species, the C-terminal catalytic domain is highly conserved. The structure of this region is predicted to consist of amphipathic helices flanking an extended -pleated sheet[33]. The catalytic domains of plant HMGR consist of three domains: the small helical amino-terminal N-domain; the large, central L-domain harboring two HMG-CoA binding motifs (EMPIGYVQIP and TTEGCLVA) and a NADP(H)-binding motif (GTVGGGT). Moreover, its architecture resembled a prism with an alpha helix forming the central structural element; the small helical s-domain harboring a NADP(H)-binding motif characterized by the sequence DAMGMN[34]. The nucleotides correlated to these motifs are well conserved which can usually be used for designing degenerate primers for isolating the members of *hmgr* gene family.

In plants, the mevalonate is the general precursor of many identified isoprenoid compounds, many of which are vital for plant normal growth, development and variety of other normal physiological activities. Loss of function of 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (HMG1) in *Arabidopsis* leaded to dwarfing, early senescence and male sterility, and reduced sterol levels. The results suggested that HMG1 plays a critical role in triterpene biosynthesis, and that sterols and/or triterpenoids contribute to cell elongation, senescence, and fertility[35]. The genes encoding HMGRs have been cloned and characterized from angiosperm species such as *Camptotheca acuminata*[36], *Catharanthus roseus*[37], melon[38], tomato[39] and *Arabidopsis thaliana*[40]. We reported for the first time on cloning and characterization of a *HMGR* gene from gymnosperm plant, *Taxus media* (*Tm-HMGR*) and found *Tm-HMGR* was more ancient than other plant HMGRs because it was from gymnosperm[41]. In animals, *HMGR* is a single copy gene, but in plant there is a small *hmgr* gene family with at least two members[42] and different expression patterns. Many research results show that HMGRs in plants determine the flux to isoprenoid pathway and HMGRs are regulated by a variety of developmental and environmental signals such as light, wound, infection, hormones, herbicides and sterols[43].

The two kinases of the MVA pathway: mevalonate kinase (MK) and mevalonate 5-diphosphate kinase (PMK): Mevalonate 5-diphosphate is biosynthesized from mevalonate by two-step phosphorylation respectively catalyzed by mevalonate MK and PMK in order. Both MK and PMK belong to GHMP kinase ATP-binding protein family containing, in their N-terminal section, a conserved Gly/Ser-rich region which is probably involved in the binding of ATP[44]. MK and PMK have a conserved motif named GHMO motif with the pattern as below: [LIVM]-[PK]-x-[GSTA]-x(0,1)-G-[LM]-[GS]-S-S-[GSA]-[GSTAC]. In *A. thaliana*, the expression pattern of the MVK gene suggests that the role of MK is the production of a general pool of mevalonate 5-phosphate for the synthesis of different classes of isoprenoids involved in both basic and specialized plant cell functions[11]. Till now, only two full-length cDNAs encoding PMK, from *A. thaliana* and *H. brasiliensis*, have been registered in Gen Bank without functional information details.

Mevalonate 5-diphosphate decarboxylase (MDC): The last step of the MVA pathway leads to the conversion of MVPP to IPP catalyzed by MDC. In *A. thaliana*, there is a small MDC gene family and two-hybrid assay in yeast demonstrated that MDC functioned either as homodimer or heterodimer[45].

IPP isomerase (IPI): IPP and its isomer DMAPP are the universal blocks for building isoprenoids. IPP and DMAPP are converted into each other catalyzed by IPP isomerase. In plants, there are multiple-copy *IPI* genes[46]. Unlike the relatively restricted cytosolic compartment of other genes/enzymes involved in the MVA pathway, IPI is not only located in cytosol but also in mitochondia, peroxisomes[47] and plastids[48].

THE GENES AND ENZYMES INVOLVED IN THE DXP PATHWAY

The DXP pathway is firstly discovered in plants in 1994[17] when Dr. Schwarz focused on the biosynthesis of the ginkgolides in *Ginkgo biloba*. Due to the rapid development of bioinformatics and comparative genomics that are revolutionizing the study of plant metabolism, the genes involved in the DXP pathway have been identified in different organisms, including the model plant, *A. thaliana*.

1-Deoxy-D-xylulose 5-phosphate synthase (DXPS): DXPS has been found in bacteria and plants which catalyzes the thiamine pyrophosphoate-dependent acyloin condensation between carbon atoms 2 and 3 of pyruvate and glyceraldehyde 3-phosphate to yield 1-deoxy-D-xylulose-5-phosphate[18]. In plants, DXPS is the first enzyme and a rate-limiting enzyme of the DXP pathway[19], and a potential target for metabolic engineering. In plants, DXPS may be encoded by more than one gene. In *A. thaliana*, there are three genes encoding DXPS, whereas the rest enzymes in DXP pathway in *Arabidopsis* appear to be encoded by a single gene according to the database search. In tomato, DXPS gene shows developmental and organ-specific regulation of mRNA accumulation, and a strong correlation with carotenoid synthesis during fruit development[49]. In pepper, DXPS gene over-expression during the chloroplast-to-chromoplast transition, and the reason probably is to furnish the IPP necessary for increased carotenoid biosynthesis[50]. The transformed root cultures of *Artemisia annua* grown in continuous light showed substantial increases in DXPS transcript levels compared to dark-grown transformed root cultures[51]. These studies strongly demonstrate that DXPS gene is regulated developmentally to synthesize the universal precursor for isoprenoids that are essential for plant growth and development.

1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR): DXR catalyzes the rearrangement and subsequent reduction of DXP to form 2-C-methyl-D-erythritol 4-phosphate (MEP). The herbicide fosmidomycin specifically blocks the activity of DXR[52], so DXR is a new target protein for developing new herbicides[53]. The nuclear-encoded DXRs in plants share the highest similarity with the homologues from *Synechocystis*, which may suggest DXR genes in plants were acquired from *Synechocystis* by gene transfer to the nucleus in the process of the endosymbiotic origin of plastid[54]. Unlike the microbial reductoisomerase, DXR orthologs in plans encode a precursor harhoring the N-terminal plastidial transit peptide which directs the enzyme to plastids where the mevalonate-independent pathway operates in plants. In *A. thaliana*, DXR, encoded by a single gene, is also a committed enzyme of the DXP pathway proven by the transgenic method and analysis of the metabolites[55]. In antitumor-alkaloid-producing *Catharansus roseus*, DXP pathway provides isoprene blocks for building the monotepenoid indole alkaloids; the expression of *DXR* gene isolated from *C. roseus* was up-regulated in cells in parallel with the production of monoterpenoid indole alkaloids[56]. As an important rate-limiting enzyme on the DXP pathway, DXR is an ideal target for engineering the isoprenoid biosynthesis, developing new herbicides and drugs. There are five enzymatic steps in the DXP pathway below, of which molecular genetics knowledge is limited but absolutely worth discovering.

2-C-Methyl-D-erythritol 4-phosphate cytidyltransferase (MCT): MCT catalyzes the third step of the DXP pathway to form CDP-ME by conjugating CDP and MEP. In plants, the gene encoding MCT and the MCT enzyme have been isolated and characterized from *A. thaliana*. The *AtCMT* gene has an ORF of 909 bp specifying a protein of 302 amino acid residues. The amino acid sequence of MCT of *A. thaliana* comprises a putative plastid transit leader sequence, well in line with the assumed plastid location of the deoxyxylulose phosphate pathway[21]. The catalytic domain of enzyme in plants is about 30% identical to the enzyme in *E. coli*.

4-(Cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (CMK): CMK, a member of the GHMP protein family like MK and PMK on the MVA pathway, catalyzes the phosphorylation of CDP-ME to form CDP-MEP. There are only a few *CMK* cDNAs reported in plants. Generally, the studies on CMK are very preliminary in plants. Rohdich *et al.*[57] reported a putative catalytic domain (residues 81–401) of a predicted protein from tomato similar to CMK of *E. coli*, when expressed in *E. coli*, the recombinant protein showed the activity to catalyze the phosphorylation of the position 2-hydroxy group of CDP-ME. Moreover, divalent metal ions, preferably Mg^{2+}, were required for its activity.

2-C-Methylerythritol 2,4-cyclodiphosphate synthase (MECPS): MECPS catalyzes the conversion of CDP-MEP to 2-C-methylerythritol 2,4-cyclodiphosphate (ME-cPP). In plants, only one functional MECPS orthologs from *C. roseus* was reported and the paper reported that the expression level of MECPS was positively related to the accumulation of terpenoid indole alkaloids[56]. In

microorganisms, there are relatively rich knowledge about genomic organization and functional identification by functional genomic tools[23], structure resolution and catalytic mechanism of MECPS[58].

Hydroxymethylbutenyl 4-diphosphate synthase (HDS): The final two steps on the DXP pathway include the formation of hydroxymethylbutenyl 4-diphosphate (HMBPP) from ME-cPP catalyzed by HDS, and the direct conversion of HMBPP into a 5:1 mixture of IPP and DMAPP by IPP/DMAPP synthase (IDS). HDS belongs to GCPE protein family whereas IDS to LytB protein family respectively. These two genes have been characterized in bacteria[25,59]. According to the genome sequence of *Arabidopsis*, some full-length cDNAs of HDS and IDS can be found in GenBank[60]. Querol *et al.*[60] identified a protein from *A. thaliana* that is homology to the product of *gcpE*. A polypeptide from plants, GCPE, contains two structural domains that are absent in *E. coli*: the N-terminal extension and the central domain of 30 kDa. They demonstrate that the N-terminal region targets the protein to chloroplasts *in vivo* in *Arabidopsis*, which is consistent with its role in plastid isoprenoid biosynthesis. Although the presence of the internal extra domain may have an effect on activity, the mature GCPE protein in *Arabidopsis* is able to complement a *gcpE* defective *E. coli* strain, indicating the HDS protein in plants is truly a functional homologue of the bacterial *gcpE* gene product mediating HMBPP biosynthesis. It has been concluded that HDS is not a rate-limiting enzyme but an important house-keeping gene in planta in the non-mevalonate pathway (Bioinformatic and molecular analysis of hydroxymethylbutenyl diphosphate synthase (GCPE) gene expression during carotenoid accumulation in ripening tomato fruit[61].

The last enzyme on the DXP pathways: IPP/DMAPP synthase (IDS): IDS directly converts HMBPP into a mixture of IPP and DMAPP with the ratio of 5:1[62]. Only a few full-length cDNAs encoding IDS were cloned from plants. *lytB* gene from the flowering plant *Adonis aestivalis* expressed in *E. coli* was found to act as the function of IDS[25]. When the gene encoding HDS was posttranscriptionally silenced in tobacco, the IDS-silenced plants had albino leaves that contained less than 4% of the chlorophyll and carotenoid pigments of control leaves; and Albino leaves from IDS-silenced plants displayed a disorganized palisade mesophyll, reduced cuticle, fewer plastids, and disrupted thylakoid membranes. These findings demonstrated the participation of IDS in the DXP pathway in plants, and supported the view that plastid isoprenoid biosynthesis is metabolically and physically segregated from the mevalonate pathway (Functional analysis of the final steps of the 1-Deoxy-D-xylulose 5-phosphate (DXP) pathway to isoprenoids in plants using virus-induced gene silencing[63].

THE CROSSTALK BETWEEN THE MVA AND THE DXP PATHWAYS

The MVA and the DXP pathways are localized in different compartments in plants. The MVA pathway operates in the cytoplasm and mitochondria to predominantly synthesize sterols, some certain sesquiterpenes, ubiquinones and etc. The DXP pathway is employed in plastid to predominantly synthesize hemiterpenes, monoterpenes, diterpenes and some polyterpenes. However, the two distinct pathways are not absolutely separated spatially. Actually, there exists the crosstalk between them. Chamomile sesquiterpenes derived from both of the pathways[64] and it is predicted that the taxol might be also from both of the pathways[65]. In 2003, it is a milestone to clarify the crosstalk between cytosol and plastid reported by several original research papers (Fig. 1). Laule *et al.*[66] proved that there was a crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in the model plant, *A. thaliana*[66]; Bick and Lange[7] provided evidences for the presence of a unidirectional proton symport system for the export of specific isoprenoid intermediates involved in the metabolic crosstalk between cytosolic and plastidial pathways, which is Ca^{2+} gated. The results of high-through-put GeneChip (AffyMatrix) suggest that there must be some limiting plastidial membrane transporters acting for the exchange of isoprenoid intermediates' exchange between plastid and cytosol[66]. Clearly, cloning and functionally identifying these putative transporter genes will be a very interesting, hot and meaningful goal to pursuit.

METABOLIC ENGINEERING OF ISOPRENOIDS

Metabolic engineering of natural products has become increasingly available recently due to fruitful genomic research, bioinformatic discoveries and biosynthetic genes. In this article, we review the recent advances in metabolic engineering of isoprenoids, focusing on the molecular genetics that affects pathway engineering mostly. Examples in different types of isoprenoids synthesis serve a number of different functions. Steroids are present in most eukaryotes and they fulfill roles such as maintaining membrane fluidity and acting as hormones and bile acids. Carotenoids are necessary for photosynthetic organisms and they also act as

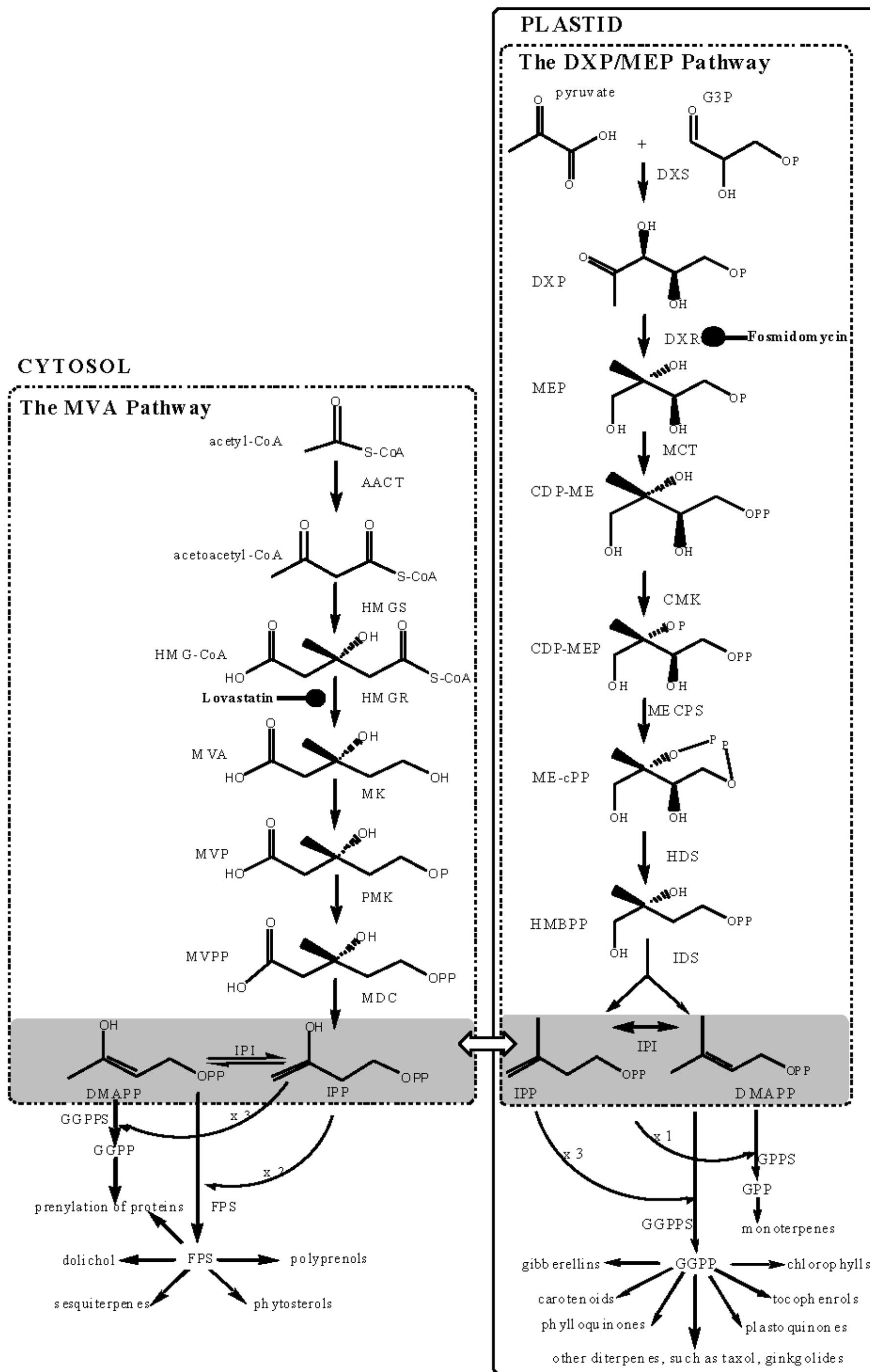

Fig. 1: Isoprenoid biosynthesis pathways in plant cells. The MVA pathway is localized in cytosol and the DXP/MEP pathway in the plastid. There is a crosstalk of IPP between cytosol and plastid. All the genes/enzymes and the intermediates involved in the pathways are indicated. Additionally, the reactions that can be specially inhibited by lovastatin and fosmidomycin, are indicated. The subsequent plant terpenes biosynthetic pathways are also indicated. The crosstalk between the MVA and the DXP pathways is shown by a double-headed arrow

antioxidants. Ubiquinone, menaquinone and plastoquinone are involved in electron transport and dolichol is involved in protein glycosylation. Isoprenoids attached to regulatory proteins have been shown to regulate cellular development. As is the case\ with many alkaloids and the quinones involved in electron transport, isoprenoids form a number of different classes of metabolites constructed by mixed biosynthesis. The other isoprenoid-deviated terpenes (mono-, di-, tri-, etc.) play less essential roles, but they can be found in many organism, particularly in plants and marine invertebrates, many of which have been found to have significant pharmaceutical importance, such as taxol and ginkgolides (diterpenes).

The biosynthesis pathway of isoprenoid in plants has been known for many years, meanwhile, new pathways have been discovered and have been further investigated. The encoding enzymes genes involved in both the MVA and DXP pathways have been identified. These may lead to the possibilities for bioengineering the isoprenoid biosynthetic pathways in plants. Various novel strategies are likely to further accelerate the progress of isoprenoid metabolic engineering[67-70]. Recently, bioinformatic and genomic tools have revolutionized the studies of plant metabolism. All above and other novel technologies, including engineering the isoprenoid-based plant defenses, improving the quality of crop, such as the golden rice[67], breaking the isoprenoid biosynthetic bottleneck in plants to produce a higher amount of essential oils in peppermint[71], rebuilding a pathway in bacteria to produce pharmaceuticals such as artemisinin[72], altering the metabolic flux to the desirable direction to yield high-content pharmaceutical agents such as terpenoid indole alkaloids[73], blocking the checkpoint along the pathways to develop new herbicides[74] and anti-parasite drugs [75] Furthermore, isoprenoid biosynthesis is also important for plant growth and biomass properties[76].

Metabolic engineering of natural products has been built to aim at medical plant improvement with the availability of modern molecular biological technologies. The overproduction or enhancement of isoprenoid might be addressed by increasing the critical precursor, by either adding, modifying or deleting regulatory genes, by altering promoter, terminator or regulatory sequences, increasing the number of gene copies for (a) bottleneck reaction(s) or removing competing, unneeded pathways. The control of flux through each pathway of isoprenoid biosynthesis in plants, in which both MVA and DXP pathways operate, and the level and means of interaction between the two pathways are of considerable interest in the context of both primary and secondary plant metabolism. It is interesting to determine whether flux through the DXP pathway is limiting during the period of rapid terpenoid biosynthesis by manipulating this route for precursor supply. Such a finding could have important implications for production of the essential oils and other terpenoids of commercial significance. In plants, the inhibition of the DXP pathway might be the basis for the development of novel herbicides. Detailed knowledge of the mechanisms and regulation of the pathway will also benefit the biotechnological production of commercially interesting isoprenoids, such as carotenoids[76].

With the availability of genes, host organisms, vector systems and standard molecular biological tools, it is expected that metabolic engineering of isoprenoid will be translated into industrial reality. Moreover, the field of metabolic engineering of natural products has spawned a number of biotechnology companies whose business plans include, or are centered around, the ability to genetically engineering natural products as drug development leads. However, up to date few genes have been introduced into plants for commercial purposes.

Even though good progress has been made in the metabolic engineering of isoprenoids, it is still a largely undiscovered field[77,78]. Information on this pathway could provide the basis for the development of new antibiotics, herbicides and anti-malaria agent. As more genes are characterized and the novel technologies become available, the field of isoprenoid metabolic engineering holds great prospects.

ACKNOWLEDGMENTS

This research is financially supported by China National 863 High-Tech Program (No. 2005AA212191), China Ministry of Education, Shanghai Science and Technology Committee, and Southwest China Normal University (NO. 240-432108). We are very thankful to Dr. Schwarz for kindly providing the CD-ROM of his Ph. D Thesis.

REFERENCES

1. Hartmann, T., 2000. Diversity and variability of plant secondary metabolism: a mechanistic view. Entomol. Gen. Application, 80: 177-188.
2. Croteau, R., T.M. Kutchan and N.G. Lewis, 2000. Biochemistry and molecular biology of plants. Am. Soc. Plant Physiol., Rockville, pp:1250-1318.
3. Wani, M.C., H.L. Taylor, M.E. Wall, P. Coggon and A.T. McPhail, 1971. Plant antitumor agents. VI. The isolation and structure of *taxol*, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc., 93: 2325-2327.

4. Chen, X.Y. and Z.H. Xu, 1996. Recent progress in biotechnology and genetic engineering of medicinal plants in China. Med. Chem. Res., 6: 215-224.
5. Cieza, A., P. Maier and E. Poppel, 2003. The effect of Ginkgo Biloba on healthy elderly subjects. Fortschritte Der Medzin, 1: 10-15.
6. Dewick, P.M., 2002. The biosynthesis of C_5–C_{25} terpenoid compounds. Nat. Prod. Rep., 19: 181-222.
7. Bick, J.A. and B.M. Lange, 2003. Metabolic crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis: Unidirectional transport of intermediates across the chloroplast envelope membrane. Arch. Biochem. Biophys., 415: 146-154.
8. Igual, J.C., C. Gonzalez-Bosch, J. Dopazo and J.E. Perez-Ortin, 1992. Phylogenetic analysis of the thiolase family: Implications for the evolutionary origin of peroxisomes. J. Mol. Evol., 35: 147-155.
9. Luskey, K.L. and B. Stevens, 1985. Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. Conserved domains responsible for catalytic activity and sterol-regulated degradation. J. Biol. Chem., 260: 10271-10277.
10. Basson, M.E., M. Thorsness, J. Finer-Moore, R.M. Stroud and J. Rine, 1988. Structural and functional conservation between yeast and human 3-hydroxy-3-methylglutaryl coenzyme A reductases, the rate-limiting enzyme of sterol biosynthesis. Mol. Cell. Biol., 8: 3797-3808.
11. Lluch, M.A., A. Masferrer, M. Arro, A. Boronat and A. Ferrer, 2000. Molecular cloning and expression analysis of the mevalonate kinase gene from *Arabidopsis thaliana*. Plant Mol. Biol., 42: 365-376.
12. Tsay, Y.H. and G.W. Robinson, 1991. Cloning and characterization of ERG8, an essential gene of *Saccharomyces cerevisiae* that encodes phosphomevalonate kinase. Mol. Cell Biol., 11: 620-631.
13. Dhe-Paganon, S., J. Magrath and R.H. Abeles, 1994. Mechanism of mevalonate pyrophosphate decarboxylase: evidence for a carbocationic transition state. Biochemistry, 33: 133551-133562.
14. Wouters, J., Y. Oudjama, S. Ghosh, V. Stalon, L. Droogmans and E. Oldfield, 2003. Structure and mechanism of action of isopentenylpyrophosphate-dimethylallylpyrophosphate isomerase. J. Am. Chem. Soc., 125: 3198-3199.
15. Disch, A., A. Hemmerlin, T.J. Bach and M. Rohmer, 1998. Mevalonate-derived isopentenyl diphosphate is the biosynthetic precursor of ubiquinone prenyl side chain in tobacco BY-2 cells. Biochem. J., 331: 615-621.
16. Rohmer, M., M. Knani, P. Simonin, B. Sutter and H. Sahm, 1993. Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem. J., 295: 517-524.
17. Schwarz, M.K., 1994. Terpen-Biosynthese in *Ginkgo biloba*: eine überraschende Geschichte. Ph.D Thesis Nr 10951, ETH, Zürich, Switzerland.
18. Sprenger, G.A., U. Schörken, T. Wiegert, S. Grolle, A.A.D. Graaf, S.V. Taylor, T.P. Begley, S. Bringer-Meyer and H. Sahm, 1997. Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to isoprenoids, thiamin, and pyridoxol. Proc. Natl. Acad. Sci. USA, 94: 12857-12862.
19. Juan, M., E.A. Cantero, A. Reindl, S. Reichler and P. León, 2001. 1-deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. J. Biol. Chem., 276: 22901-22909.
20. Takahashi, S., T. Kuzuyama, H. Watanabe and H. Seto, 1998. A 1-deoxy-D-xylulose 5-phosphate reductoisomerase catalyzing the formation of 2-C-methyl-D-erythritol 4-phosphate in an alternative nonmevalonate pathway for terpenoid biosynthesis. Proc. Nat. Acad. Sci. USA, 95: 9879-9884.
21. Rohdich, F., J. Wungsintaweekul, W. Eisenreich, G. Richter, C.A. Schuhr, S. Hecht, M.H. Zenk and A. Bacher, 2000. Biosynthesis of terpenoids: 4-diphosphocytidyl-2C-methyl-D-erythritol synthase of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA, 97: 6451-6456.
22. Steinbacher, S., J. Kaiser, W. Eisenreich, R. Huber, A. Bacher and F. Rohdich, 2003. Structural basis of fosmidomycin action revealed by the complex with 2-C-methyl-D-erythritol 4-phosphate synthase (IspC). Implications for the catalytic mechanism and anti-malaria drug development. J. Biol. Chem., 278: 18401-18407.
23. Herz, S., J. Wungsintaweekul, C.A. Schuhr, S. Hecht, H. Luttgen, S. Sagner, M. Fellermeier, W. Eisenreich, M.H. Zenk, A. Bacher and F. Rohdich, 2000. Biosynthesis of terpenoids: YgbB protein converts 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate to 2C-methyl-D-erythritol 2,4-cyclodiphosphate. Proc. Natl. Acad. Sci. USA, 97: 2486-2490.
24. Baker, J., D.B. Franklin and J. Parker, 1992. Sequence and characterization of the gcpE gene of *Escherichia coli*. FEMS Microbiol. Lett., 73: 175-180.

25. Cunningham, F.X., T.P. Lafond and E. Gantt, 2000. Evidence of a role for LytB in the nonmevalonate pathway of isoprenoid biosynthesis. J. Bacteriol., 182: 5841-5848.
26. Montamat, F., M. Guilloton, F. Karst and S. Delrot, 1995. Isolation and characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl-coenzyme A synthase. Gene, 167: 197-201.
27. Vollack, K.U. and T.J. Bach, 1996. Cloning of a cDNA encoding cytosolic acetoacetyl-coenzyme a thiolase from radish by functional expression in *Saccharomyces cerevisiae*. Plant Physiol., 111: 1097-1107.
28. Rokosz, L.L., D.A. Boulton, E.A. Butkiewicz, G. Sanyal, M.A. Cueto, P.A. Lachance and J.D. Hermes, 1994. Human cytoplasmic 3-hydroxy-3-methylglutaryl coenzyme-A synthase-expression, purification, and characterization of recombinant wild-type and Cys(129) mutant enzymes. Arch. Biochem. Biophys., 312: 1-13.
29. Alex, D., T.J. Bach and M.L. Chye, 2000. Expression of *Brassica juncea* 3-hydroxy-3-methylglutaryl CoA synthase is developmentally regulated and stress-responsive. Plant J., 22: 415-426.
30. Wegener, A., W. Gimbel, T. Werner, J. Hani, D. Ernst and H.J. Sandermann, 1997. Molecular cloning of ozone-inducible protein from *Pinus sylvestris* L. with high sequence similarity to vertebrate 3-hydroxy-3-methylglutaryl-CoA-synthase. Biochem. Biophys. Acta, 1350: 247-252.
31. Suwanmanee, P., N. Sirinupong and W. Suvachittanont, 2004. Regulation of the expression of 3-hydroxy-3-methylglutaryl-CoA synthase gene in *Hevea brasiliensis* (BHK) Mull. Arg. Plant Sci., 166: 531-537.
32. Caelles, C., A. Ferrer, L. Balcells, F.G. Hegardt and A. Boronat, 1989. Isolation and structural characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Mol. Biol., 13: 627-638.
33. Istvan, E.S. and J. Deisenhofer, 2000. The structure of the catalytic portion of human HMG-CoA reductase. Biochem. Biophys. Acta, 1529: 9-18.
34. Ruiz-albert, J., E. Cerda-Olmedo and L.M. Corrochano, 2001. Genes for mevalonate biosynthesis in *Phycomyces*. Mol. Genet. Gen., 266: 768-777.
35. Suzuki, M., Y. Kamide, N. Nagata, H. Seki, K. Ohyama, H. Kato, K. Masuda, S. Sato, T. Kato, S. Tabata, S. Yoshida and T. Muranaka, 2004. Loss of function of 3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (*HMG1*) in *Arabidopsis* leads to dwarfing, early senescence and male sterility and reduced sterol levels. Plant J., 37: 750.
36. Maldonado-Mendoza, I.E., R.M. Vincent and C.L. Nessler, 1997. Molecular characterization of three differentially expressed members of the *Camptotheca acuminata* 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) gene family. Plant Mol. Biol., 34: 781-790.
37. Maldenado-Mendoza, I.E., R.J. Burnett and C.L. Nessler, 1992. Nucleotide sequence of a cDNA encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase from *Catharanthus roseus*. Plant Physiol., 100: 1613-1614.
38. Kato-Emori, S., K. Higashi, K. Hosoya, T. Kobayashi and H. Ezura, 2001. Cloning and characterization of the gene encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase in melon (*Cucumis melo* L. *reticulatus*). Mol. Genet. Gen., 265: 135-142.
39. Park, H., C.J. Denbow and C.L. Cramer, 1992. Structure and nucleotide sequence of tomato HMG2 encoding 3-hydroxy-3-methyl-glutaryl coenzyme A reductase. Plant Mol. Biol., 20: 327-331.
40. Learned, R.M. and G.R. Fink, 1989. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase from *Arabidopsis thaliana* is structurally distinct from the yeast and animal enzymes. Proc. Nat. Acad. Sci. USA, 86: 2779-2783.
41. Liao, Z.H., Q.M. Tan, Y.R. Chai, K.J. Zuo, M. Chen, Y.F. Gong, P. Wang, Y. Pi, F. Tan, X.F. Sun and K.X. Tang, 2003. Cloning and characterization of the gene encoding HMG-CoA reductase from *Taxus media* and its functional identification in yeast. Funct. Plant Biol., 31: 1-9.
42. Chye M.L., C.T. Tan and H.H. Chua, 1992. Three genes encode 3-hydroxy-3- methylglutaryl-coenzyme A reductase in *Hevea brasiliensis*: *hmg1* and *hmg3* are differentially expressed. Plant Mol. Biol., 19: 473-484.
43. Bach, T.J., 1995. Some new aspects of isoprenoid biosynthesis in plants-a review. Lipids, 30: 191-202.
44. Lee, M. and T. Leustek, 1999. Identification of the gene encoding homoserine kinase from *Arabidopsis thaliana* and characterization of the recombinant enzyme derived from the gene. Arch. Biochem. Biophys., 372: 135-142.
45. Cordier, H., F. Karst and T. Berges, 1999. Heterologous expression in Saccharomyces cerevisiae of an *Arabidopsis thaliana* cDNA encoding mevalonate diphosphate decarboxylase. Plant Mol. Biol., 39: 953-967.
46. Cunningham, F.X.J. and E. Gantt, 2000. Identification of multi-gene families encoding isopentenyl diphosphate isomerase in plants by heterologous complementation in *Escherichia coli*. Plant Cell. Physiol., 41: 119-123.

47. Aboushadi, N., W.H. Engfelt, V.G. Paton and S.K. Krisans, 1999. Role of peroxisomes in isoprenoid biosynthesis. J. Histochem. Cytochem., 47: 1127-1132.
48. Ramos-Valdivia, A.C., R. Van der Heijden and R. Verpoorte, 1997. Isopentenyl diphosphate isomerase: a core enzyme in isoprenoid biosynthesis. A review of its biochemistry and function. Natl. Prod. Rep., 14: 591-603.
49. Lois, L.M., M. Rodriguez-Concepcion, F. Gallego, N. Campos and A. Boronat, 2000. Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. Plant J., 22: 503-513.
50. Bouvier, F., A.D. Harlingue, C. Suire, R.A. Backhaus and B. Camara, 1998. Dedicated roles of plastid transketolases during the early onset of isoprenoid biogenesis in pepper fruits. Plant Physiol., 117: 1423-1431.
51. Souret, F.D.F., P.J. Weathers and K.K. Wobbe, 2002. The mevalonate-independent pathway is expressed in transformed roots of artemisia annua and regulated by light and culture age. *In vitro* Cell. Dev. Biol. Plant, 38: 581-588.
52. Okuhara, M., Y. Kuroda, T. Goto, M. Okamoto, H. Terano, M. Kohsaka, H. Aoki and H. Imanaka, 1980. Studies on new phosphonic acid antibiotics III. Isolation and characterization of FR-31564, FR-32863 and FR-33289, So DXR is the new target protein for developing new herbicides. J. Antibiot., 33: 24-28.
53. Markus L., B., E.B. Raymond, L. Ketchum and R.B. Croteau, 2001. Metabolite profiling of peppermint oil gland secretory cells and application to herbicide target analysis. Plant Physiol., 127: 305-314.
54. Markus L., B., T. Rujan, W. Martin and R. Croteau, 2000. Isoprenoid biosynthesis: The evolution of two ancient and distinct pathways across genomes. Proc. Nat. Acad. Sci. USA, 97: 13172-13177.
55. Carretero-Paulet, L., I. Ahumada, N. Cunillera, M. Rodriguez-Concepcion, A. Ferrer, A. Boronat and N. Campos, 2002. Expression and molecular analysis of the *Arabidopsis* DXR gene encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase, the first committed enzyme of the 2-C-methyl-D-erythritol 4-phosphate pathway. Plant Physiol., 129: 1581–1591.
56. Veau, B., M. Courtois, A. Oudin, J.C. Chenieux, M. Rideau and M. Clastre, 2000. Cloning and expression of cDNAs encoding two enzymes of the MEP pathway in *Catharanthus roseus*. Biochem. Biophys. Acta Gen. St. Exp., 1517: 159-163.
57. Rohdich, F., J. Wungsintaweekul, H. Luttgen, M. Fischer, W. Eisenreich, C.A. Schuhr, M. Fellermeier, N. Schramek, M.H. Zenk and A. Bacher, 2000. Biosynthesis of isoprenoids: 4-Diphosphocytidyl-2-Cmethyl-D-erythritol kinase from tomato. Proc. Natl. Acad. Sci. USA, 97: 8251-8256.
58. Kishida, H., T. Wada, S. Unzai, T. Kuzuyama, M. Takagi, T. Terada, M. Shirouzu, S. Yokoyama, J.R. Tame and S.Y. Park, 2003. Structure and catalytic mechanism of 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MECDP) synthase, an enzyme in the non-mevalonate pathway of isoprenoid synthesis. Acta Crystallogr. D. Biol. Crystallogr., 59: 23-31.
59. Altincicek, B., A.K. Kollas, S. Sanderbrand, J. Wiesner, M. Hintz, E. Beck and H. Jomaa, 2001. GcpE is involved in the 2-C-methyl-D-erythritol 4-phosphate pathway of isoprenoid biosynthesis in *Escherichia coli*. J. Bacteriol., 183: 2411-2416.
60. Querol, J., N. Campos, S. Imperial, A. Boronat and M. Rodriguez-Concepcion, 2002. Functional analysis of the *Arabidopsis thaliana* GCPE protein involved in plastid isoprenoid biosynthesis. FEBS Lett., 514: 343-346.
61. Rodriguez-Concepeion, M., J. Querol, L.M. Lois, S. Imperial and A. Boronat, 2003. Bioinformatic and molecular analysis of hydroxymethylbutenyl diphosphate synthase (GCPE) gene expression during carotenoid accumulation in ripening tomato fruit. Planta, 217: 476-482.
62. Rohdich, F., S. Hecht, K. Gartner, P. Adam, C. Krieger, S. Amslinger, D. Arigoni, A. Bacher and W. Eisenreich, 2002. Studies on the nonmevalonate terpene biosynthetic pathway: Metabolic role of IspH (LytB) protein. Proc. Natl. Acad. Sci. USA, 99: 1158-1163.
63. Page, J.E., G. Hause, M. Raschke, W.Y. Gao, J. Schmidt, M.H. Zenk and T.M. Kutchan, 2004. Functional analysis of the final steps of the 1-Deoxy-D-xylulose 5-phosphate (DXP) pathway to isoprenoids in plants using virus-induced gene silencing. Plant Physiol., 134: 1401-1413.
64. Adam, K.P. and J. Zapp, 1998. Biosynthesis of the isoprene units of chamomile sesquiterpenes. Phytochemistry, 48: 953-959.
65. Wang, Y.D., Y.J. Yuan, M. Lu, J.C. Wu and J.L. Jiang, 2003. Inhibitor studies of isopentenyl pyrophosphate biosynthesis in suspension cultures of the new *Taxus chinensis* var. *mairei*. Biotechnol. Applied Biochem., 37: 39-43.

66. Laule, O., A. Fürholz, H.S. Chang, T. Zhu, X. Wang, P.B. Heifetz, W. Gruissem and B. Markus Lange, 2003. Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA, 100: 6866-6871.
67. Ye, X.D., S. Al-Babili, A. Klöti, J. Zhang, P. Lucca, P. Beyer and I. Potrykus, 2000. Engineering provitamin A (β-carotene) biosynthetic pathway into (caroteneoidfree) rice endosperm. Science, 287: 303-305.
68. Romer, S., P.D. Fraser, J.W. Kiano, C.A. Shipton, N. Misawa, W. Schuch and P.M. Bramley, 2000. Elevation of the provitamin A content of transgenic tomato plants. Natl. Biotechnol., 18: 666-669.
69. Mann, V., M. Harker, I. Pecker and J. Hirschberg, 2000. Metabolic engineering of astaxanthin production in tobacco flowers. Natl. Biotechnol., 18: 888-892.
70. Schmidt-Dannert, C., D. Umeno and F.H. Arnold, 2000. Molecular breeding of carotenoid biosynthetic pathways. Natl. Biotechnol., 18: 750-753.
71. Lange, B.M. and R. Croteau, 1999. Genetic engineering of essential oil production in mint. Curr. Opin. Plant Biol., 2: 139-144.
72. Martin, V.J.J., D.J. Pitera, S.T. Withers, J.D. Newman and J.D. Keasling, 2003. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. Nat. Biotech., 21: 796-802.
73. Fits, L.V.D. and J. Memelink, 2000. ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. Science, 289: 295-297.
74. Lange, B.M., R.E.B. Ketchum and R. Croteau, 2001. Isoprenoid biosynthesis: Metabolite profiling of peppermint oil gland secretory cells and application to herbicide target analysis. Plant Physiol., 127: 305-314.
75. Jomaa, H., J. Wiesner, S. Sanderbrand, B. Altincicek, K. Weidemeyer, M. Hintz, I. Tu¨rbachova, M. Eberl, J. Zeidler and H.K. Lichtenthaler, 1999. Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. Science, 285: 1573-1576.
76. Eriksson, M.E., M. Israelsson, O. Olsson and T. Moritz, 2000. Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. Natl. Biotechnol., 18: 784-788.
77. Soriente, A., M.M.C. De Rosa, A. Scettri, G. Sodano, M.C. Terencio, M. Paya and M.J. Alcaraz, 1999. Manoalide. Curr. Med. Chem., 6: 415-431.
78. Matthews, P.D. and E.T. Wurtzel, 2000. Metabolic engineering of carotenoid accumulation in *E. coli* by modulation of the isoprenoid precursor pool with expression of deoxyxylulose phosphate synthase. Applied Microbiol. Biotechnol., 53: 396-400.