The non-mevalonate pathway of isoprenoids: genes, enzymes and intermediates

Felix Rohdich*, Klaus Kis, Adelbert Bacher and Wolfgang Eisenreich

Although the mevalonate pathway had been considered for a long time as the unique source of biosynthetic isoprenoids, an alternative pathway has recently been discovered. The first intermediate, 1-deoxy-D-xylulose 5-phosphate, is assembled by condensation of glyceraldehyde 3-phosphate and pyruvate. A skeletal rearrangement coupled with a reduction step affords the branched-chain polyol, 2*C*-methyl-D-erythritol 4-phosphate, which is subsequently converted into a cyclic 2,4-diphosphate by the consecutive action of three enzymes via nucleotide diphosphate intermediates. The genes specifying these enzymes have been cloned from bacteria, plants and protozoa. Their expression in recombinant bacterial hosts has opened the way to the identification of several novel pathway intermediates.

Addresses
Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, D-85747 Garching, Germany
*e-mail: felix.rohdich@ch.tum.de

Current Opinion in Chemical Biology 2001, 5:535–540

1367-5931/01/$ – see front matter

Abbreviations

CDP	cytidine 5′-triphosphate
CTP	cytidine 5′-diphosphate
DMAPP	dimethylallyl pyrophosphate
IPP	isopentenyl pyrophosphate

Introduction

Terpenes are one of the largest groups of natural products. More than 30,000 representatives including medically important compounds such as vitamins, hormones and cytostatic agents have been described. They are all assembled from two precursors, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP).

During a period of several decades, the mevalonate pathway was considered as the universal source of biosynthetic DMAPP and IPP. The existence of a second isoprenoid pathway was discovered relatively recently by the research groups of Rohmer and Arigoni in the course of stable isotope incorporation studies using various eubacteria and plants [1–3]. These data suggested that pyruvate and a triose phosphate can serve as precursors for the formation of IPP and DMAPP by an alternative pathway (for reviews, see [4–6]). Arigoni and co-workers also showed that 1-deoxy-D-xylulose, a known precursor of thiamine and pyridoxal [7–9], can be diverted very efficiently to terpenoids [2,6]. Subsequent work by several research groups identified 1-deoxy-D-xylulose 5-phosphate as the first intermediate of the alternative terpenoid pathway [10–12].

Enzymes specified by the *dxs* gene of *Escherichia coli* and its orthologs from various eubacteria and plants were shown to catalyze the formation of 1-deoxy-D-xylulose 5-phosphate (**3**) from pyruvate (**1**) and glyceraldehyde 3-phosphate (**2**) as shown in Figure 1 [11–19]. In a second step, the enzyme product is converted into the branched-chain polyol 2*C*-methyl-D-erythritol 4-phosphate (**4**) by 2*C*-methyl-D-erythritol 4-phosphate synthases specified by the *ispC* genes of *E. coli* and its respective orthologs from *Mentha piperita*, *Arabidopsis thaliana*, *Synechocystis* sp. and *Plasmodium falciparum* (Figure 1) [20–25].

Recently, additional genes and cognate enzymes of this novel pathway have been discovered and the corresponding intermediates have been identified in rapid sequence [26–28]. They are described in the following sections.

The orthologous *ispD* group

Initially, cell extract of *E. coli* was found to convert 2*C*-methyl-D-erythritol 4-phosphate (**4**) into a novel product in a cytidine 5′-triphosphate (CTP)-dependent reaction [26]. A database search for CDP (cytidine 5′-diphosphate) pyrophosphorylases retrieved the *asc1* gene of a serotype-specific DNA region [29] of *Haemophilus influenzae*. Subsequent similarity searches using that gene as a search motif retrieved the orthologous *ygbP* group (now designated *ispD*) represented in many eubacteria and in *A. thaliana*.

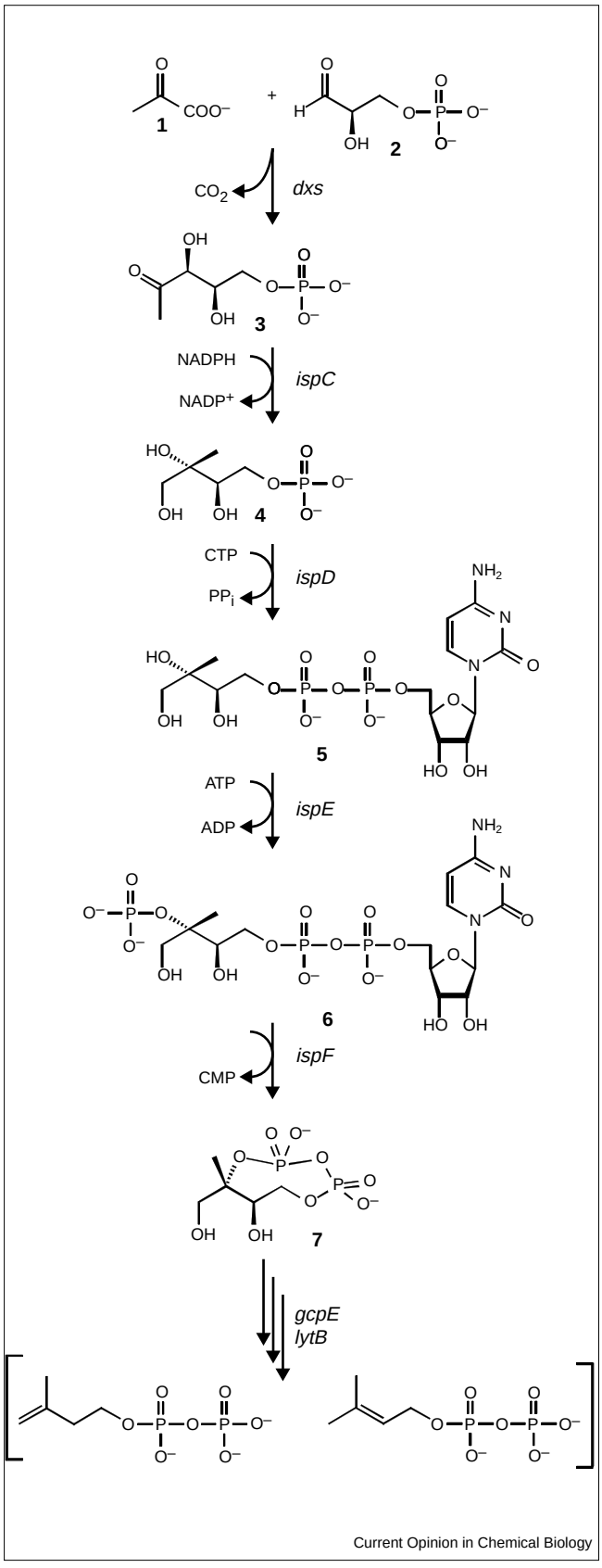
The *ispD* gene of *E. coli* was hyperexpressed in a recombinant *E. coli* strain [26]. The recombinant protein was shown to catalyze the formation of 4-diphosphocytidyl-2*C*-methyl-D-erythritol (**5**) from 2*C*-methyl-D-erythritol 4-phosphate and CTP (Figure 1; Table 1) [26,30]. The chromosomal *A. thaliana* gene comprises 11 introns and specifies a putative plastid-targeting sequence preceding the catalytic domain sequence [31]. 4-Diphosphocytidyl-2*C*-methyl-D-erythritol synthases require divalent cations, preferably Mg^{2+}, and could use several nucleotide triphosphates as substrates.

The product of the IspD proteins, 4-diphosphocytidyl-2*C*-methyl-D-erythritol, is incorporated efficiently into carotenoids of red pepper [26]. More recently, studies with an *ispD* deletion mutant of *E. coli*, engineered for utilization of a part of the mevalonate pathway, confirmed that the enzyme is required for IPP biosynthesis [32]. The phenotype of this strain is conditional lethal, and the recombinant bacteria can be rescued by addition of exogenous mevalonate.

The orthologous *ispE* group

A search for orthologous groups with the same distribution pattern as *dxs*, *ispC* and *ispD* genes retrieved the *ychB* (*ispE*) and *ygbB* (*ispF*) genes as potential members of the

Figure 1



The non-mevalonate pathway of isoprenoid biosynthesis.

non-mevalonate pathway (Table 2) [26,27]. The tomato ortholog of *ispE* had been shown earlier to be involved in fruit ripening [33]. In retrospect, it is clear that the phenotype of the tomato mutant was caused by the deficiency of carotenoid precursors caused by the mutation.

The *ispE* gene of *E. coli* was expressed in a recombinant *E. coli* strain where it directed the synthesis of a 30 kDa peptide. The purified recombinant enzyme from *E. coli* was shown to catalyze the ATP-dependent phosphorylation of 4-diphosphocytidyl-2*C*-methyl-D-erythritol (**5**) at the C-2 hydroxy group yielding 4-diphosphocytidyl-2*C*-methyl-D-erythritol 2-phosphate (**6**) [27]. Again, the use of ^{13}C-labeled enzyme substrates was crucial for the elucidation of the enzyme product by NMR spectroscopy. More recently, these data were confirmed by other authors [34].

Orthologous *ispE* genes were also cloned from tomato and peppermint [35,36]. A gene cassette specifying the putative catalytic domain of the tomato 4-diphosphocytidyl-2*C*-methyl-D-erythritol kinase was engineered for high expression in enzymatically active form in a recombinant strain of *E. coli* [35]. Some properties are summarized in Table 1. Whereas the IspE orthologs of tomato, peppermint and *Arabidopsis* are highly similar over their entire length (about 74% identity), their similarity with the microbial enzyme is much lower (30% identity). The plant enzymes carry putative plastid-targeting sequences.

The IspE proteins of *E. coli* and peppermint have been reported to convert isopentenyl monophosphate into isopentenyl pyrophosphate [36], but the reported rates (178 ± 81 and 1.43 pmol g^{-1} s^{-1} for the *E. coli* and peppermint enzymes, respectively) were so low that any physiological relevance appears doubtful.

The orthologous *ispF* group

The members of the *ispF* (formerly *ygbB*) orthologous group follow the distribution pattern of the genes described above in completely sequenced genomes (Table 2). Moreover, *ispF* orthologs are closely linked to or fused with *ispD* orthologs in numerous microorganisms [26,28].

The *E. coli ispF* gene was expressed in a recombinant host where it directed the synthesis of a 16 kDa peptide. The recombinant protein was shown to catalyze the formation of 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate (**7**) from 4-diphosphocytidyl-2*C*-methyl-D-erythritol 2-phosphate (**6**) [28,37]. At a much lower rate, the enzyme also catalyzes the formation of 2*C*-methyl-D-erythritol 3,4-cyclophosphate from 4-diphosphocytidyl-2*C*-methyl-D-erythritol (**5**) [28]. Orthologous *ispF* sequences from *A. thaliana* and *Catharanthus roseus* carry putative plastid-targeting sequences and are highly similar over their entire length (88% identity).

The amino-terminal segment of the IspF protein from *P. falciparum* is likely to act as an apicoplexan-targeting sequence. The similarity of the plant enzymes with the

Table 1

Recombinant enzymes of the non-mevalonate pathway.

Enzyme Organism	Characteristics			References
	v_{max} µmol min^{-1} mg^{-1}	Cofactor	Metal ion	
1-Deoxy-D-xylulose 5-phosphate synthase				
Arabidopsis thaliana		TPP	Mg^{2+}	[18]
Bacillus subtilis		TPP	Mg^{2+}	[16]
Capsicum annuum	500	TPP	Mg^{2+}	[14]
Escherichia coli	300	TPP	Mg^{2+}	[11,12]
Lycopersicon esculentum		TPP	Mg^{2+}	[17]
Mentha piperita		TPP	Mg^{2+}	[13]
Streptomyces sp.	370	TPP	Mg^{2+}	[19]
Synechococcus leopoliensis		TPP	Mg^{2+}	[15]
Synechocystis sp.		TPP	Mg^{2+}	[16]
2***C***-methyl-D-erythritol 4-phosphate synthase				
Arabidopsis thaliana		NADPH	Mn^{2+}, Mg^{2+}	[22]
Escherichia coli	11.8	NADPH	Mn^{2+}, Mg^{2+}	[20]
Mentha piperita		NADPH	Mn^{2+}, Mg^{2+}	[21]
Plasmodium falciparum		NADPH	Mn^{2+}, Mg^{2+}	[25]
Synechocystis sp.		NADPH	Mn^{2+}, Mg^{2+}	[23]
Synechococcus leopoliensis		NADPH	Mn^{2+}, Mg^{2+}	[26]
4-Diphosphocytidyl-2***C***-methyl-D-erythritol synthase				
Escherichia coli	23	CTP	Mg^{2+}, Mn^{2+}	[26,32]
Arabidopsis thaliana	67	CTP	Mg^{2+}, Ni^{2+}	[31]
4-Diphosphocytidyl-2***C***-methyl-D-erythritol kinase				
Escherichia coli	34	ATP	Mg^{2+}	[27,34]
Lycopersicon esculentum	33	ATP	Mg^{2+}	[35]
2***C***-Methyl-D-erythritol 2,4-cyclodiphosphate synthase				
Escherichia coli			Mg^{2+}, Mn^{2+}	[28,37]
Plasmodium falciparum	4.3		Mg^{2+}, Mn^{2+}	[38]

microbial and/or protozoal enzymes is low (45% and 38% identity, respectively).

The pseudo-mature recombinant *Plasmodium* IspF protein was shown to catalyze the same reactions as described above for the *E. coli* IspF protein [38]. The enzyme also catalyzed the formation of 2-phospho-*2C*-methyl-D-erythritol 3,4-cyclophosphate from 4-diphosphocytidyl-*2C*-methyl-D-erythritol 2-phosphate, albeit at a relatively low rate [38]. Kinetic properties of the enzymes from *E. coli* and *Plasmodium* are summarized in Table 1.

2C-methyl-D-erythritol 2,4-cyclodiphosphate (**7**) was shown to be transformed into carotenoids by isolated chromoplasts from *Capsicum annuum* [28]. This finding confirms that the cyclic diphosphate is a committed intermediate in the non-mevalonate pathway of terpenoid biosynthesis. In earlier reports, **7** had been reported to be formed in certain microorganisms under stress conditions [39,40].

The orthologous *lytB* and *gcpE* groups

The distribution patterns of the orthologous *lytB* and *gcpE* groups are identical with those of the other orthologous groups of the non-mevalonate pathway genes (Table 2). Insertions into the coding region of *lytB* were lethal for *Synechocystis* sp. Supplementation with isopentenol and dimethylallyl alcohol completely alleviated this growth impairment [41]. Studies with an *E. coli gcpE* deletion mutant that was engineered for utilization of a partial mevalonate pathway confirmed that the enzyme is required for IPP biosynthesis in *E. coli* [42]. The phenotype of this strain is conditional lethal; the mutant bacteria can be rescued by addition of exogenous mevalonate. The reactions catalyzed by LytB and GcpE proteins are still unknown.

Conclusions

The occurrence of the two isoprenoid pathways in different species can be gleaned from recent isotope incorporation studies in conjunction with comparative genomics [4,5,27,43]. Archaea, fungi and animals use exclusively the mevalonate pathway. Plants invariably utilize the mevalonate pathway in the cytosolic compartment and the non-mevalonate pathway in plastids. The translocation into plastids was demonstrated for the tomato 1-deoxy-D-xylulose 5-phosphate synthase [17]. The majority of eubacteria and certain algae use the non-mevalonate pathway. Most human pathogens are in the non-mevalonate pathway group, and the pathway enzymes are attractive targets for the development of novel antibiotics. *Streptomycetes* and possibly Gram-positive cocci may use

Table 2

Terpenoid genes in completely sequenced organisms.

Organism	Non-mevalonate pathway							Mevalonate pathway						
	dxs	ispC	ispD	ispE	ispF	lytB	gcpE	mvaS	mvaA	mvaK1	mvaK2	mvaD	ipiI	ipiII
Bacteria														
Aquifex aeolicus	+	+	+	+	+	+	+							
Bacillus subtilis	+	+	+	+	+	+	+							+
Buchnera sp.	+	+	+	+	+	+	+							
Campylobacter jejuni	+	+	+	+	+	+	+							
Chlamydia sp.	+	+	+	+	+	+	+							
Deinococcus radiodurans	+	+	+	+	+	+	+							
Escherichia coli	+	+	+	+	+	+	+						+	
Haemophilus influenzae	+	+	+	+	+	+	+							
Helicobacter pylori	+	+	+	+	+	+	+							
Mycobacterium tuberculosis	+	+	+	+	+	+	+						+	
Neisseria meningitidis	+	+	+	+	+	+	+							
Pseudomonas aeruginosa	+	+	+	+	+	+	+							
Synechocystis sp.	+	+	+	+	+	+	+							+
Thermotoga maritima	+	+	+	+	+	+	+							
Trepomema pallidum	+	+	+	+	+	+	+							
Vibrio cholerae	+	+	+	+	+	+	+		+					
Xylella fastidiosa	+	+	+	+	+	+	+							
Borrelia burgdorferi								+	+	+	+	+		+
Archaea														
Aeropyrum pernix								+	+	+				+
Archeoglobus fulgidus								+	+	+				+
Halobcaterium sp.								+	+	+				+
M. thermoautotrophicum								+	+	+				+
Methanococcus jannaschii								+	+	+				+
Pyrococcus abyssii								+	+	+				+
Pyrococcus horikoshii			+					+	+	+				+
Thermoplasma acidophilum								+	+	+				+
Eukarya														
Arabidopsis thaliana	+	+	+	+	+	+	+	+	+	+	+	+	+	
Caenorhabditis elegans								+	+	+	+	+	+	
Drosophila melanogaster								+	+	+	+	+	+	
Homo sapiens								+	+	+	+	+	+	
Saccharomyces cerevisiae								+	+	+	+	+	+	

dxs, 1-deoxy-D-xylulose 5-phosphate synthase; *ipiI*, isopentenyl pyrophosphate isomerase type I; *ipiII*, Isopentenyl pyrophosphate isomerase type II; *ispC*, 2*C*-methyl-D-erythritol 4-phosphate synthase; *ispD*, 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase; *ispE*, 4-diphosphocytidyl-2*C*-methyl-D-erythritol kinase; *ispF*, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; *mvaA*, HMG-CoA reductase; *mvaD*, mevalonate diphosphate decarboxylase; *mvaK1*, mevalonate kinase; *mvaK2*, phosphomevalonate kinase; *mvaS*, HMG-CoA synthase.

both pathways [19,44]. *Mycoplasma*-type bacteria appear not to biosynthesize isoprenoids. It is unknown whether they can obtain required terpenoids from their hosts. The situation in protozoa is incompletely known. For *Leishmania* and *Trypanosoma* sp. there is strong evidence for the mevalonate pathway [45,46]. In *Plasmodium falciparum*, only enzymes of the non-mevalonate pathway have been found up to now [25].

The genes, enzymes and reactions involved in the conversion of 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate (**7**) into IPP and DMAPP are still unknown. A ring-opening reaction, two dehydration steps and two reduction steps are required for transformation of **7** into IPP and DMAPP, which both appear to be obtained via a common precursor as opposed to isomerization [47]. Putative orthologs of the classical (type 1) isomerase or of the recently discovered type II isomerase [48] are present in the genomes of some eubacteria using the non-mevalonate pathway (Table 2) but their metabolic role is unknown.

The non-mevalonate pathway is absent in vertebrates (Table 2). Isoprenoid biosynthesis is essential, and the blocking of genes or enzymes involved in an isoprenoid pathway is lethal. Therefore, the enzymes of the non-mevalonate pathway are attractive targets for the development of herbicides and antibiotics. The 2*C*-methyl-D-erythritol 4-phosphate synthase inhibitor fosmidomycin has been shown to have bactericidal, herbicidal and antimalarial activities [25]. The detailed knowledge of the mechanisms and regulation processes of the non-mevalonate pathway may also benefit the biotechnological production of commercially interesting isoprenoids.

Acknowledgements

This work was supported by grants from the Fonds der Chemischen Industrie, the Deutsche Forschungsgemeinschaft and the Hans-Fischer Gesellschaft.

References

1. Rohmer M, Knani M, Simonin P, Sutter B, Sahm H: Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. *Biochem J* 1993, 295:517-524.

2. Broers STJ: Über die frühen Stufen der Biosynthese von Isoprenoiden in ***Escherichia coli***. [PhD Thesis]. Nr 10978, ETH Zürich, Switzerland; 1994.

3. Schwarz MK: Terpen-Biosynthese in ***Ginkgo biloba***: eine überraschende Geschichte. [PhD Thesis]. Nr 10951, ETH Zürich, Switzerland; 1994.

4. Eisenreich W, Schwarz M, Cartayrade A, Arigoni D, Zenk M, Bacher A: The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. *Chem Biol* 1998, 5:R221-R233.

5. Rohmer M: A mevalonate-independent route to isopentenyl diphosphate. In *Comprehensive Natural Product Chemistry*, vol 2. Edited by Barton D, Nakanishi K. Oxford: Pergamon; 1999:45-68.

6. Arigoni D, Schwarz MK: Ginkgolide biosynthesis. In *Comprehensive Natural Product Chemistry*, vol 2. Edited by Cane D. Oxford: Pergamon; 1999:367-399.

7. White RH: Stable isotope studies on the biosynthesis of the thiazole moiety of thiamin in ***Escherichia coli***. *Biochemistry* 1978, 17:3833-3840.

8. David S, Estramareix B, Fischer J-C, Therisod M: 1-Deoxy-D-threo-2-pentulose: the precursor of the five-carbon chain of the thiazole of thiamine. *J Am Chem Soc* 1981, 103:7341-7342.

9. Hill RE, Sayer BG, Spenser JD: Biosynthesis of vitamin B_6: incorporation of D-1-deoxyxylulose. *J Am Chem Soc* 1989, 111:1916-1917.

10. Rohmer M, Seemann M, Horbach S, Bringer-Meyer S, Sahm H: Glyceraldehyde 3-phosphate and pyruvate as precursors of isoprenic units in an alternative non-mevalonate pathway for terpenoid biosynthesis. *J Am Chem Soc* 1996, 118:2564-2566.

11. Sprenger GA, Schörken U, Wiegert T, Grolle S, deGraaf AA, Taylor SV, Begley TP, Bringer-Meyer S, Sahm H: 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* 1997, 94:12857-12862.

12. Lois LM, Campos N, Putra SR, Danielsen K, Rohmer M, Boronat A: Cloning and characterization of a gene from ***Escherichia coli*** encoding a transketolase-like enzyme that catalyzes the synthesis of D-1-deoxyxylulose 5-phosphate, a common precursor for isoprenoid, thiamine, and pyridoxol biosynthesis. *Proc Natl Acad Sci USA* 1998, 95:2105-2110.

13. Lange BM, Wildung MR, McCaskill D, Croteau R: A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-independent pathway. *Proc Natl Acad Sci USA* 1998, 95:2100-2104.

14. Bouvier F, d'Harlingue A, Suire C, Backhaus RA, Camara B: Dedicated roles of plastid transketolases during the early onset of isoprenoid biogenesis in pepper fruits. *Plant Physiol* 1998, 117:1423-1431.

15. Miller B, Heuser T, Zimmer W: A ***Synechococcus leopoliensis*** SAUG 1402-1 operon harboring the 1-deoxy-D-xylulose 5-phosphate synthase gene and two additional open reading frames is functionally involved in the dimethylallyl diphosphate synthesis. *FEBS Letters* 1999, 460:485-490.

16. Harker M, Bramley PM: Expression of prokaryotic 1-deoxy-D-xylulose-5-phosphatases in ***Escherichia coli*** increases carotenoid and ubiquinone biosynthesis. *FEBS Lett* 1999, 448:115-119.

17. Lois LM, Rodriguez-Concepcion M, Gallego F, Campos N, Boronat A: Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. *Plant J* 2000, 22:503-513.

18. Araki N, Kesumi K, Masamoto K, Niwa Y, Iba K: Temperature-sensitive ***Arabidopsis*** mutant defective in 1-deoxy-D-xylulose 5-phosphate synthase within the plastid non-mevalonate pathway of isoprenoid biosynthesis. *Physiol Plant* 2000, 108:19-24.

19. Kuzuyama T, Takagi M, Takahashi S, Seto H: Cloning and characterization of 1-deoxy-D-xylulose 5-phosphate synthase from ***Streptomyces sp.*** Strain CL190, which uses both the mevalonate and nonmevalonate pathways for isopentenyl diphosphate biosynthesis. *J Bacteriol* 2000, 182:891-897.

20. Takahashi S, Kuzuyama T, Watanabe H, Seto H: 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 Natl Acad Sci USA* 1998, 95:9879-9884.

21. Lange BM, Croteau R: Isoprenoid biosynthesis via a mevalonate-independent pathway in plants: cloning and heterologous expression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase from peppermint. *Arch Biochem Biophys* 1999, 365:170-174.

22. Schwender J, Müller C, Zeidler J, Lichtenthaler HK: Cloning and heterologous expression of a cDNA encoding 1-deoxy-D-xylulose-5-phosphate reductoisomerase of ***Arabidopsis thaliana***. *FEBS Lett* 1999, 455:140-144.

23. Proteau PJ, Woo Y-H, Williamson T, Phaosiri C: Stereochemistry of the reduction step mediated by recombinant 1-deoxy-D-xylulose 5-phosphate isomeroreductase. *Org Lett* 1999, 1:921-923.

24. Miller B, Heuser T, Zimmer W: Functional involvement of a deoxy-D-xylulose 5-phosphate reductoisomerase locus of ***Synechococcus leopoliensis*** in isoprenoid biosynthesis. *FEBS Lett* 2000, 481:221-226.

25. Jomaa H, Wiesner J, Sanderbrand S, Altinicicek B, Weidemeyer C, Hintz M, Türbachova I, Eberl M, Zeidler J, Lichtenthaler HK *et al.*: Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science* 1999, 285:1573-1576.

26. Rohdich F, Wungsintaweekul J, Fellermeier M, Sagner S, Herz S, Kis K, Eisenreich W, Bacher A, Zenk MH: Cytidine 5′-triphosphate-dependent biosynthesis of isoprenoids: YgbP protein of ***Escherichia coli*** catalyzes the formation of 4-diphosphocytidyl-2-***C***-methyl-D-erythritol. *Proc Natl Acad Sci USA* 1999, 96:11758-11763.

27. Lüttgen H, Rohdich F, Herz S, Wungsintaweekul J, Hecht S, Schuhr CA, Fellermeier M, Sagner S, Zenk MH, Bacher A, Eisenreich W: Biosynthesis of terpenoids: YchB protein of ***Escherichia coli*** phosphorylates the 2-hydroxy group of 4-diphosphocytidyl-2***C***-methyl-D-erythritol. *Proc Natl Acad Sci USA* 2000, 97:1062-1067.

28. Herz S, Wungsintaweekul J, Schuhr CA, Hecht S, Lüttgen H, Sagner S, Fellermeier M, Eisenreich W, Zenk MH, Bacher A, Rohdich F: Biosynthesis of terpenoids: YgbB protein converts 4-diphosphocytidyl-2***C***-methyl-D-erythritol 2-phosphate to 2***C***-methyl-D-erythritol 2,4-cyclodiphosphate. *Proc Natl Acad Sci USA* 2000, 97:2486-2490.

29. Follens A, Veigha-Da-Cunha M, Merckx R, Van Schaftingen E, Van Eldere J: ***Acs1*** of ***Haemophilus influenzae*** type a capsulation locus region II encodes a bifunctional 5-phosphate reductase-CDP-ribitol pyrophosphorylase. *J Bacteriol* 1999, 181:2001-2207.

30. Rohdich F, Schuhr CA, Hecht S, Herz S, Wungsintaweekul J, Eisenreich W, Zenk MH, Bacher A: Biosynthesis of isoprenoids. A rapid method for the preparation of isotope-labeled 4-diphosphocytidyl-2***C***-methyl-D-erythritol. *J Am Chem Soc* 2000, 122:9571-9594.

31. Rohdich F, Wungsintaweekul J, Eisenreich W, Richter G, Schuhr CA, Hecht S, Zenk MH, Bacher A: Biosynthesis of terpenoids: 4-diphosphocytidyl-2***C***-methyl-D-erythritol synthase of ***Arabidopsis thaliana***. *Proc Natl Acad Sci USA* 2000, 97:6451-6456.

32. Kuzuyama T, Takagi M, Kaneda K, Dairi T, Seto H: Formation of 4-(cytidine 5′-diphospho)-2-***C***-methyl-D-erythritol from 2-***C***-methyl-D-erythritol 4-phosphate by 2-***C***-methyl-D-erythritol 4-phosphate cytidylyltransferase. A new enzyme in the non-mevalonate pathway. *Tetrahedron Lett* 2000, 41:703-706.

33. Lawrence DL, Cline K, Moore GA: Chromoplast development in ripening tomato fruit: identification of cDNAs for chromoplast-targeted proteins and characterization of a cDNA encoding a plastid-localized low-molecular-weight heat shock protein. *Plant Mol Biol* 1997, 33:483-492.

34. Kuzuyama T, Takagi M, Kaneda K, Watanabe H, Dairi T, Seto H: Studies on the nonmevalonate pathway: conversion of 4-(cytidine 5′-diphospho)-2-***C***-methyl-D-erythritol to its 2-phospho derivative by 4-(cytidine 5′-diphospho)-2-***C***-methyl-D-erythritol kinase. *Tetrahedron Lett* 2000, 41:2925-2928.

35. Rohdich F, Wungsintaweekul J, Lüttgen H, Fischer M, Eisenreich W, Schuhr CA, Fellermeier M, Schramek N, Zenk MH, Bacher A: Biosynthesis of terpenoids: 4-diphosphocytidyl-2-***C***-methyl-D-erythritol kinase from tomato. *Proc Natl Acad Sci USA* 2000, 97:8251-8256.

36. Lange M, Croteau R: Isopentenyl diphosphate biosynthesis via a mevalonate-independent pathway: isopentenyl monophosphate kinase catalyzes the terminal enzymatic step. *Proc Natl Acad Sci USA* 1999, 96:13714-13719.

37. Takagi M, Kuzuyama T, Kaneda K, Watanabe H, Dairi T, Seto H: Studies on the nonmevalonate pathway: formation 2-***C***-methyl-D-erythritol 2,4-cyclodiphosphate from 2-phospho-4-(cytidine 5′-diphospho)-2-***C***-methyl-D-erythritol. *Tetrahedron Lett* 2000, 41:3395-3398.

38. Rohdich F, Eisenreich W, Wungsintaweekul J, Hecht S, Schuhr CA, Zenk MH, Bacher A: Biosynthesis of terpenoids. 2***C***-methyl-D-erythritol 2,4-cyclodiphosphate synthase (IspF) from ***Plasmodium falciparum***. *Eur J Biochem* 2001, 268:3190-3197.

39. Turner D, Santos H, Fareleira P, Pacheco I, LeGall Y, Xavier AV: Structure determination of a novel cyclic phosphocompound isolated from ***Desulfovibrio desulfuricans***. *Biochem J* 1992, 285:387-390.

40. Ostrovsky D, Kharatian E, Dubrovsky T, Ogrel O, Shipanova I, Sibeldina L: The ability of bacteria to synthesize a new cyclopyrophosphate correlates with their tolerance to redox-cycling drugs: on a crossroad of chemotherapy, environmental toxicology and immunobiochemical problems. *Biofactors* 1992, 4:63-68.

41. Cunningham FX Jr, Lafond TP, Gantt E: Evidence of a role for LytB in the nonmevalonate pathway of isoprenoid biosynthesis. *J Bacteriol* 2000, 182:5841-5848.

42. Campos N, Rodriguez-Concepción M, Seemann M, Rohmer M, Boronat A: Identification of ***gcpE*** as a novel gene of the 2-***C***-methyl-D-erythritol 4-phosphate pathway for isoprenoid biosynthesis. *FEBS Lett* 2001, 488:170-173.

43. Boucher Y, Doolittle WF: MicroReview: the role of lateral gene transfer in the evolution of isoprenoid biosynthesis pathways. *Mol Microbiol* 2000, 37:703-716.

44. Wilding EI, Brown JR, Bryant AP, Chalker AF, Holmes DJ, Ingraham KA, Iordanescu S, So CY, Rosenberg M, Gwynn MN: Identification, evolution, and essentiality of the mevalonate pathway for isopentenyl diphosphate biosynthesis in Gram-positive cocci. *J Bacteriol* 2000, 182:4319-4327.

45. Ginger ML, Chance ML, Goad LJ: Elucidation of carbon sources used for the biosynthesis of fatty acids and sterols in the trypanosomatid ***Leishmania mexicana***. *Biochem J* 1999, 342:397-897.

46. Concepcion JL, Gonzales-Pacanowska D, Urbina JA: 3-Hydroxy-3-methyl-glutaryl-CoA reductase in ***Trypanosoma*** (***Schizotrypanosoma***) ***cruzi***: subcellular localization and kinetic properties. *Arch Biochem Biophys* 1998, 352:114-120.

47. Rodriguez-Concepción M, Campos N, Lois LM, Maldonado C, Hoeffler J-F, Grosdemange-Billiard C, Rohmer M, Boronat A: Genetic evidence of branching in the isoprenoid pathway for the production of isopentenyl diphosphate and dimethylallyl diphosphate in ***Escherichia coli***. *FEBS Lett* 2000, 473:328-332.

48. Kaneda, K, Kuzuyama T, Takagi M, Hayakawa Y, Seto H: An unusual isopentenyl diphosphate isomerase found in the mevalonate gene cluster from ***Streptomyces*** sp. Strain CL190. *Proc Natl Acad Sci USA* 2001, 98:932-937.