Deoxyxylulose phosphate pathway to terpenoids

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Recently, a mevalonate-independent pathway was discovered in bacteria and plants that leads to the formation of isopentenyl diphosphate and dimethylallyl diphosphate, the two basic precursors of isoprenoids. Although many details of the widely distributed pathway are unknown, some intermediates, mechanisms, enzymes and genes of this novel route have been identified. Information on this pathway could provide the basis for the development of new antibiotics, herbicides and antimalarials.

Terpenoids and isoprenoids are a structurally diverse group of natural products. More than 25 000 representatives with a variety of biological functions have been reported in the plant kingdom[1]. To give just a few examples, carotenoids serve as light-harvesting and light-protecting pigments, sterols play important roles as modulators of membrane properties, the phytol side chain of chlorophyll (the most abundant organic pigment) is of terpenoid origin, and a wide variety of plant terpenoids function as insect attractants or repellents. Various terpenoids have attracted commercial interest as pharmaceuticals or nutraceuticals. Thus, paclitaxel (Taxol), a diterpene from yew, has been established as a major cytostatic agent. Lycopene and lutein were recently registered as oncopreventive agents.

Pathways of terpenoid biosynthesis

All terpenoids are biosynthesized from just two C_5 precursors: isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The biosynthesis of these universal terpene precursors via mevalonate (Fig. 1) has been elucidated by studies of yeast and animal cells (reviewed in Ref. 2). Radioactivity from labelled mevalonate has been reported to be diverted to a wide variety of plant terpenes, albeit at incorporation rates well below 1%, which were explained by poor uptake of mevalonate into plant cells or by compartmentalization.

Several results inconsistent with the mevalonate pathway led to the discovery of a non-mevalonate pathway of terpenoid biosynthesis. For example, unexpected ^{13}C labels were found in hopanoids from certain bacteria that had been grown with ^{13}C-labelled acetates[3]. Furthermore, ^{13}C-labelled glucose applied to *E. coli* and to seedlings of *Ginkgo biloba* caused labelling patterns in ubiquinones (S.T.J. Broers, PhD thesis, ETH Zürich, 1994) and ginkgolides (M.K. Schwarz, PhD thesis, ETH Zürich, 1994) that were clearly inconsistent with the mevalonate route. These labelling patterns suggested that C-3, C-2 and C-1 of a triose could supply C-1, C-2 and C-4 of IPP, and that C-2 and C-3 of pyruvate could supply C-3 and C-5 of IPP in the novel pathway (reviewed in Refs 4–6).

A hypothetical mechanism was suggested with a head-to-head condensation of D-glyceraldehyde 3-phosphate and 'activated acetaldehyde' derived from pyruvate, resulting in the formation of 1-deoxy-D-xylulose 5-phosphate as the first precursor of the novel pathway (S.T.J. Broers, PhD thesis, ETH Zürich, 1994; M.K. Schwarz, PhD thesis, ETH Zürich, 1994) (Fig. 1). This hypothesis was confirmed by efficient incorporation of ^{2}H-labelled 1-deoxy-D-xylulose into the isoprenoid side chain of ubiquinone in *E. coli* (S.T.J. Broers, PhD thesis, ETH Zürich, 1994). This pentulose had earlier been reported to serve as a precursor for the biosynthesis of thiamine[7,8] and pyridoxal[9].

Deoxyxylulose phosphate pathway is widely distributed in nature

Subsequent isotope incorporation studies using bacteria, certain algae, plant cell cultures and plant tissues demonstrated the formation of IPP and DMAPP from exogenous 1-deoxyxylulose. These seminal experiments have been reviewed recently[4–6,10] and are not covered in detail here. In summary (Fig. 2), archaea, certain bacteria, yeasts, fungi, some protozoa and animals appear to use the mevalonate pathway. However, many bacteria (including many human pathogens), green algae and possibly the malaria parasite *Plasmodium falciparum* appear to rely exclusively on the mevalonate-independent pathway. Streptomycetes, some algae, mosses and liverworts, marine diatoms, and higher plants appear to use both pathways. Finally, the capacity to biosynthesize isoprenoid precursors appears to be absent in some parasitic microorganisms that might be able to use terpenoids from their host.

Crosstalk between the mevalonate and deoxyxylulose phosphate pathways in plants

In higher plants, the mevalonate route operates in the cytoplasm and mitochondria; sterols, sesquiterpenes and ubiquinones are formed predominantly via mevalonate. Isoprenoids synthesized in the plastids (hemiterpenes, monoterpenes, diterpenes and carotenoids) are formed predominantly via 1-deoxyxylulose 5-phosphate (reviewed in Refs 4–6). The

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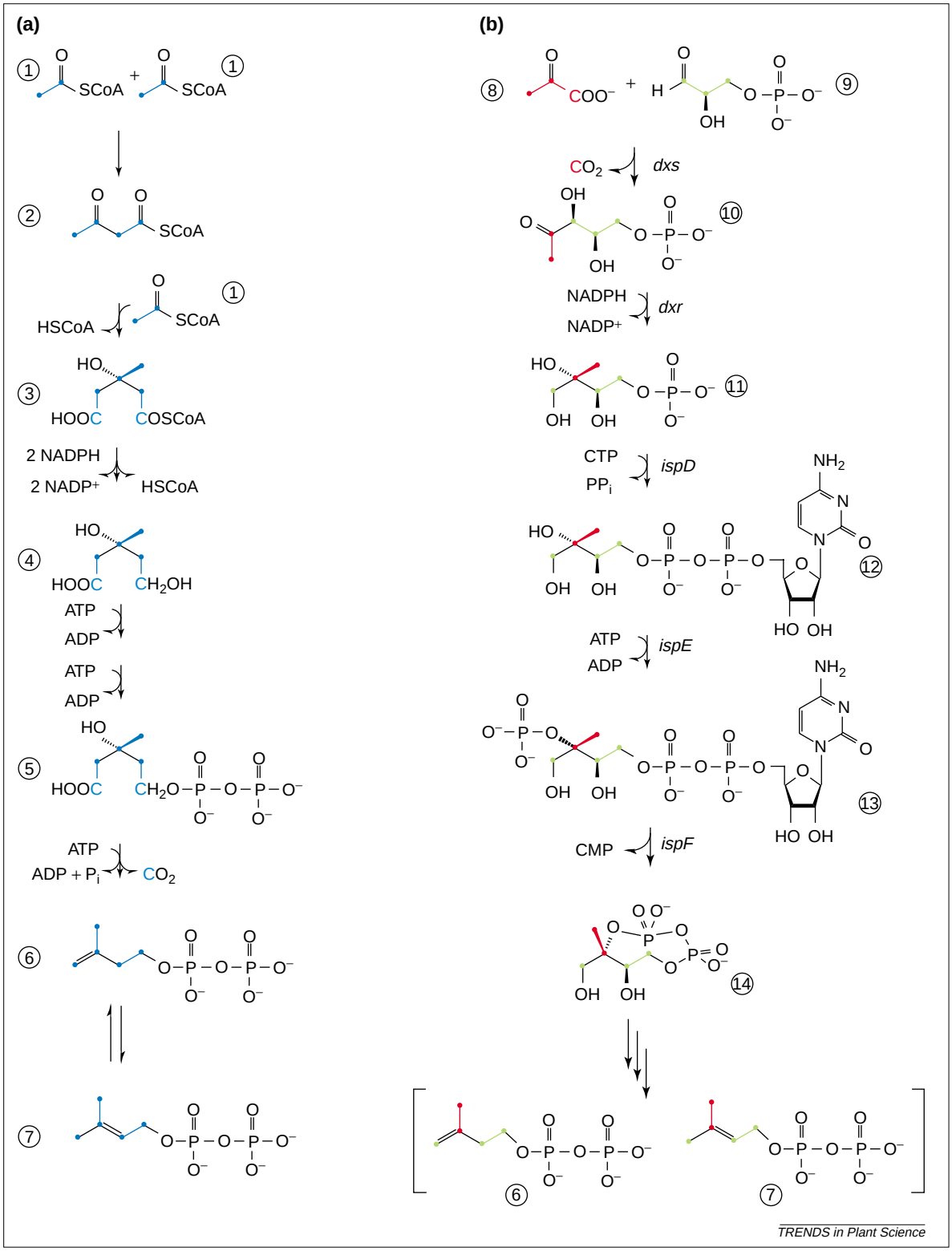

Fig. 1. Pathways of isopentenyl diphosphate (6) and dimethylallyl diphosphate (7) formation. The origins of carbon atoms from acetyl-CoA (1), pyruvate (8) and glyceraldehyde 3-phosphate (9) are indicated in blue, red and green, respectively. (a) In the mevalonate pathway, three molecules of acetyl-CoA (1) are condensed to 3-hydroxy-3-methylglutaryl-CoA (3) via acetoacetyl-CoA (2) by the consecutive action of two enzymes. Subsequent reduction to mevalonate (4), phosphorylation and ATP-dependent decarboxylation produces IPP (6), which can be converted to and from DMAPP (7) by an isomerase. (b) In the deoxyxylulose phosphate pathway, D-glyceraldehyde 3-phosphate (9) and pyruvate (8) are converted into 1-deoxy-D-xylulose 5-phosphate (10) in a decarboxylative reaction. Subsequent rearrangement and reduction leads to 2*C*-methyl-D-erythritol 4-phosphate (11), which is converted into 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate (14) via 4-diphosphocytidyl-2*C*-methyl-D-erythritol (12) and 4-diphosphocytidyl-2*C*-methyl-D-erythritol 2-phosphate (13). The further reactions leading to IPP (6) and DMAPP (7) are unknown.

compartmental separation of the two different IPP biosynthetic pathways is not absolute because at least one metabolite can be exchanged between the compartments[11–13] (M.K. Schwarz, PhD thesis, ETH Zürich, 1994). The extent of this crosstalk depends on the species as well as on the presence and concentration of exogenous precursors. Generally, crosstalk contributions appear to be small in intact plants under physiological conditions (<1%). Higher values have been found in plant cell cultures in the presence of exogenous 1-deoxyxylulose or mevalonate. The nature of the metabolite(s) exchanged between the compartments and the regulation of the process remain to be established.

The crosstalk between the two terpenoid pathways is one of the reasons for the historical failure to

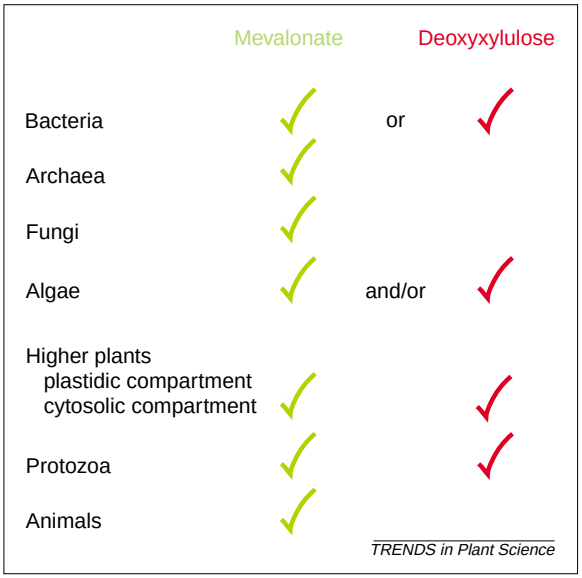

Fig. 2. Distribution in nature of the mevalonate and deoxyxylulose phosphate pathway of isoprenoid biosynthesis.

Table 1. Recombinant enzymes of the deoxyxylulose phosphate pathway

Enzyme / Organism	KM µM		v_{max} µmol min^{-1} mg^{-1}	Refs
1-Deoxy-D-xylulose 5-phosphate synthase				
Arabidopsis				18
Capsicum annuum	500[a]	750.0[b]	500.0	17
Chlamydomonas				19
E. coli	96[a]	250.0[b]	300.0	14,15
Mentha piperita				16
Streptomyces sp.	65[a]	120.0[b]	370.0	20
Synechococcus leopoliensis				21
1-Deoxy-D-xylulose 5-phosphate reductoisomerase				
Arabidopsis				26
E. coli	250[c]	7.4[d]	11.8	23
Mentha piperita				27
Plasmodium falciparum				28
Synechocystis				25
4-Diphosphocytidyl-2*C*-methyl-D-erythritol synthase				
Arabidopsis	500[e]	114[f]	67.0	34
E. coli	131[e]	3.0[f]	23.0	31,33
4-Diphosphocytidyl-2*C*-methyl-D-erythritol kinase				
E. coli			34.0	35,38,39
Lycopersicon esculentum			33.0	37
Mentha piperita				38
2*C*-Methyl-D-erythritol 2,4-cyclodiphosphate synthase				
E. coli				40,41

Substrates are indicated by:

[a]D-glyceraldehyde 3-phosphate.

[b]Pyruvate.

[c]1-deoxy-D-xylulose 5-phosphate.

[d]NADPH.

[e]2C-methyl-D-erythritol 4-phosphate.

[f]CTP.

recognize the deoxyxylulose route. In retrospect, the observed low incorporation rates of mevalonate and acetate into many plant terpenoids were not because of restricted uptake or restricted use of the labelled precursors but rather reflected the small crosstalk contributions of mevalonate to the biosynthesis of terpenoids that are predominantly formed from 1-deoxyxylulose.

Genes and enzymes of the deoxyxylulose phosphate pathway

1-Deoxy-D-xylulose 5-phosphate synthase

In vivo experiments described above suggested that the presumed intermediate 1-deoxy-D-xylulose 5-phosphate is synthesized by a transketolase-like decarboxylation from pyruvate and glyceraldehyde 3-phosphate (Fig. 1). Following this hypothesis, database similarity searches with the E1 subunit (EC 1.2.4.1) of the pyruvate dehydrogenase complex, pyruvate decarboxylase (EC 4.1.1.1), and transketolase (EC 2.2.1.1) as query sequences revealed a homologous unannotated gene at 9.0 min on the *E. coli* genome, directly downstream from the *ispA* gene, that encodes farnesyldiphosphate synthase[14,15]. The unannotated gene and *ispA* were found as part of an operon, which suggested a functional relationship. The unknown gene (now designated as *dxs*) was expressed in *E. coli* and the recombinant protein shown to catalyse the formation of 1-deoxy-D-xylulose 5-phosphate from pyruvate and glyceraldehyde 3-phosphate.

Further genome comparisons revealed putative *dxs* orthologues in many bacteria and plants. 1-Deoxy-D-xylulose 5-phosphate synthases from *Mentha piperita*[16], *Capsicum annuum*[17], *Arabidopsis*[18], *Chlamydomonas*[19], a *Streptomyces* species[20] and *Synechococcus leopoliensis*[21] have been cloned and characterized in some detail. These enzymes require thiamine diphosphate and divalent cations such as Mg^{2+} or Mn^{2+} for activity[14,17,20]. Catalytic properties have been reported for the enzymes from *Streptomyces*, *E. coli* and *C. annuum*[14,15,17,20] (Table 1). The proteins from *M. piperita*, *C. annuum* and *Arabidopsis* are in the nuclear genome and are preceded by putative N-terminal plastid targeting sequences[16–18]. Translocation into chloroplasts was recently shown for 1-deoxy-D-xylulose 5-phosphate synthase in *Arabidopsis*[18] and *Lycopersicon esculentum*[22].

1-Deoxy-D-xylulose 5-phosphate reductoisomerase

A rearrangement is required for the formation of the branched-chain isoprenoid precursors IPP and DMAPP from the linear pentulose phosphate[11] (Fig. 1). The presence of a 1,2-ketol function in 1-deoxy-D-xylulose 5-phosphate fulfils the requirements for a sigmatropic rearrangement reaction as predicted by various *in vivo* labelling studies described above. An unannotated gene (*yaeM*) at 4.2 min on the chromosomal map of *E. coli* was

Table 2. Occurrence of deoxyxylulose pathway genes in 21 completely sequenced bacteria[a]

Organism	Gene				
	dxs	***dxr***	***ispD***	***ispE***	***ispF***
Aquifex aeolicus	AE000712	AE000688	AE000734	AE000713	AE000715
Bacillus subtilis	D84432	Z99112	Z99104	Z99104	Z99104
Campylobacter jejuni	AL139074	AL139078	AL139079	AL139077	AL139079
Chlamydia muridarum	AE002329	AE002301	AE002343	AE002285	AE002340
Chlamydia pneumoniae CWLO29	AE001686	AE001618	AE001642	AE001675	AE001639
Chlamydophila pneumoniae AR39	AE002238	AE002203	AE002178	AE002249	AE002181
Chlamydia trachomatis	AE001306	AE001281	AE001320	AE001352	AE001317
Deinococcus radiodurans	AE001992	AE001994	AE002089	AE002089	AE001885
E. coli	AF035440	AB013300	AF230736	AF216300	AF230738
Haemophilus influenzae	U32822	U32763	U32750	U32834	U32750
Helicobacter pylori 26695	AE000552	AE000541	AE000610	AE000644	AE000610
Helicobacter pylori J99	AE001468	AE000001458	AE001474	AE001556	AE0001474
Mycobacterium tuberculosis	Z96072	Z74024	Z92774	Z92752	Z94774
Neisseria meningitidis Z2491	AL162753	AL162752	AL162756	AL162755	AL162756
Neisseria meningitidis MC58	AE002536	AE002375	AE002501	AE002439	AE002501
Pseudomonas aeruginosa	AE004821	AE004785	AE004783	AE004880	AE004782
Synechocystis sp. PCC6803	D90903	D64000	D90914	D90899	D90906
Thermotoga maritima	AE001815.1	AE001754.1	AE001792	AE001791	AE001738
Treponema pallidum	AE001253	AE001235	AE001227	AE001226	AE001227
Vibrio cholerae	AE004173	AE004297	AE004139	AE004289	AE004139
Xylella fastidiosa	AE004037	AE003942	AE003962	AE004071	AE003962

[a]Putative orthologs of *dxs*, *dxr*, *ispD*, *ispE*, and *ispF* are absent in the genomes of archaea, the parasitic bacteria *Mycoplasma genitalium*, *Borrelia burgdorferi* and *Rickettsia prowazekii*, as well as in the genomes of the eukaryotes *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster*.

identified that complemented mutants requiring 2C-methyl-D-erythritol for growth[23]. The gene, now designated *dxr*, was expressed in *E. coli* and the recombinant protein shown to catalyse the formation of 2*C*-methyl-D-erythritol 4-phosphate from 1-deoxy-D-xylulose 5-phosphate in a NADPH-dependent reaction[23] (Fig. 1).

The *E. coli* enzyme[23,24] and its orthologues from the blue–green alga *Synechocystis*[25], the plants *Arabidopsis*[26] and *M. piperita*[27], and the protist *Plasmodium falciparum*[28] have been characterized in some detail. The *E. coli* enzyme was reported to have a molecular mass of 140 kDa with four subunits. The enzyme requires divalent metal ions such as Mn^{2+}, Co^{2+} or Mg^{2+}, and is strictly dependent on NADPH as a cofactor[23]. The stereochemical features of the reductoisomerase reaction have been studied with the recombinant enzymes from *E. coli* and *Synechocystis*, showing that H_{Si} from C-4 of NADPH is transferred to the H_{Re} position at C-1 of 2*C*-methyl-D-erythritol 4-phosphate. Thus, 1-deoxy-D-xylulose 5-phosphate reductoisomerases from *E. coli*[29] and *Synechocystis*[25] are class B dehydrogenases. The kinetic properties of the *E. coli* enzyme were also studied in some detail (Table 1). Catalytic rates of 11.8 μmol min^{-1} mg^{-1} were measured with Mn^{2+} at a pH optimum of 7.5. The enzyme does not accept 1-deoxy-D-xylulose as a substrate[23].

Site-directed mutagenesis of the recombinant *E. coli* protein revealed that Glu231 is involved in the reaction and that His153, His209 and His257 might be involved in the binding of 1-deoxy-D-xylulose 5-phosphate[24]. The N-terminal region is thought to be the binding site for NADPH (Refs 24,26,27). Fosmidomycin, a long-known antibiotic compound that mimics the structure of the hypothetical reaction intermediate 2*C*-methyl-D-erythrose 4-phosphate, inhibits the reaction with a K_i of 38 nM (Refs 28,30).

4-Diphosphocytidyl-2C-methyl-D-erythritol synthase

Crude enzyme fractions from wild-type *E. coli* convert 2*C*-methyl-D-erythritol 4-phosphate into a novel product in a CTP-dependent reaction[31]. A database search for enzymes transferring a cytidylphosphate residue to a polyol retrieved the *asc1* gene of a serotype-specific DNA region from *Haemophilus influenzae*. The N-terminal domain of the cognate enzyme had been shown to catalyse the formation of CDP–ribitol from ribitol 5-phosphate and CTP (Ref. 32). Subsequent similarity searches starting from that gene retrieved the hitherto unknown *ygbP* gene family (now designated *ispD*), which is present in many bacteria as well as in *Arabidopsis*. More specifically, *ispD* is present in organisms with *dxs* and *dxr* genes but not in organisms devoid of *dxs* and *dxr* genes (Table 2). Thus, it appeared likely that *ispD* had a role in the novel terpenoid pathway.

This hypothesis was confirmed by studies on recombinant IspD protein, which was shown to catalyse the formation of 4-diphosphocytidyl-2*C*-methyl-D-erythritol from 2*C*-methyl-D-erythritol 4-phosphate and CTP (Refs 31,33; Fig. 1). Moreover, it was shown that 4-diphosphocytidyl-2*C*-methyl-D-erythritol is incorporated efficiently into carotenoids

Table 3. Putative *ispD*, *ispE* and *ispF* orthologues in incompletely sequenced organisms

Organism	Gene		
	ispD	***ispE***	***ispF***
Eubacteria			
Actinobacillus actinomycetes	Contig320	Contig344	Contig320
Bacillus anthracis	Banth_1680	Banth_1607	Banth_1680
Bacillus stearothermophilus	Contig541	–	Contig541
Bordetella bronchisepticum	–	Contig2244	–
Bordetella pertussis	Contig301	Contig278	Contig301
Caulobacter crescentus	C.cres._12574	C.cres._12574	C.cres._12574
Clostridium acetobutylicum	C.aceto_gnl	C.aceto_gnl	C.aceto_gnl
Clostridium difficile	Contig935	Contig985	Contig935
Corynebacterium diphteriae	Contig239	Contig14	Contig239
Dehalococcoides ethenogenes	Deth_1507	Deth_1545	Deth_1507
Desulfovibrio vulgaris	Dvulg_gdv23	–	Dvulg_gdv23
Enterococcus faecalis	Gef_10297	Gef_10286	Gef_10286
Geobacter sulfurreducens	Gsulf_ggs98	Gsulf_ggs110	Gsulf_ggs98
Haemophilus ducreyi	HTSC_730	HTSC_730	HTSC_730
Klebsiella pneumoniae	Contig1719	Contig31	Contig1719
Legionella pneumophila	CUGC_446	CUC_446	–
Mycobacterium avium	M.avium_183	M.avium_24	M.avium_183
Mycobacterium bovis	Contig442	Contig365	Contig353
Mycobacterium leprae	Sanger_1769	Sanger_1769	Sanger_1769
Neisseria gonorrhoeae	Contig9	Contig9	Contig9
Pasteurella multicoda	CBCUMN_747	CBCUMN_747	CBCUMN_747
Porphyromonas gingivalis	P.ging._GPG.con	P.ging._GPG.con	P.ging._GPG.con
Pseudomonas putida	Pputida_10705	Pputida_10708	Pputida_10705
Rhodobacter capsulatus	X12382	–	X12382
Salmonella enteritidis	Contig1007	–	–
Salmonella paratyphi	Spara_B_SPA.0.2916	Spara_B_SPA.0.2446	Spara_B_SPA.0.2916
Salmonella typhi	S.typhi_CT18	S.typhi_CT18	S.typhi_CT18
Salmonella typhimurium	Contig997	M77236	Contig997
Shewanella putrefaciens	Sputr_6406	Sputr_6406	Sputr_6406
Streptomyces coelicor	AL160331	AL359989	AL160331
Thiobacillus ferrooxidans	T_ferro._6139	T_ferro._6154	T_ferro._6139
Treponema denticola	Tdent_gtd269	Tdent_gtd51	Tdent_gtd269
Yersinia pestis	Contig854	Contig855	Contig854
Zymomonas mobilis	AF176314	AF088896	AF176314
Plants			
Arabidopsis thaliana	AF230737	AF288615	AC010852
Catharanthus roseus	–	–	AF250236
Mentha × piperita	–	AF179283	–
Solanum esculentum	–	AF263101	–
Protists			
Plasmodium falciparum	–	–	AF279661

– indicates data not available.

of red pepper[31] and that IspD is essential for IPP biosynthesis in *E. coli*[33]. More recently, recombinant IspD from *Arabidopsis* has been shown to catalyse the same reaction[34]. The kinetic properties of the *E. coli* and *Arabidopsis* enzyme are summarized in Table 1. Both enzymes require a divalent cation, preferably Mg^{2+}. The *E. coli* protein appears to be a dimer with 25 kDa subunits[31], but the *Arabidopsis* protein shows a tendency for self-aggregation depending on the buffer system[34]. Sequence analysis of the *Arabidopsis* protein sequence revealed a putative plastid import sequence[34].

4-Diphosphocytidyl-2C-methyl-D-erythritol kinase

A bioinformatics approach has been used to identify more genes following the distribution of the orthologous sets of *dxs*, *dxr* and *ispD* genes. Whole genome comparisons retrieved putative deoxyxylulose pathway genes from the completely sequenced genomes of organisms known to use the deoxyxylulose pathway but not from the genomes of organisms that use the mevalonate pathway[35]. As a hypothetical candidate following this distribution, the *ychB* gene with hitherto unknown function (now designated *ispE*) was identified in the genome of *E. coli* and many other organisms[35]. The involvement of the orthologous gene product from tomato in chromoplast ripening had already been proposed[36]. Both known plant orthologues (tomato and peppermint) carry putative plastid target sequences[37,38]. Sequence analysis revealed putative ATP binding sites[35,37,38].

The purified recombinant enzyme from *E. coli* was shown to catalyse the ATP-dependent phosphorylation of 4-diphosphocytidyl-2*C*-methyl-D-erythritol into 4-diphosphocytidyl-2*C*-methyl-D-erythritol 2-phosphate[35,39] (Fig. 1, Table 1) at a rate[35] of 34 μmol min^{-1} mg^{-1}. The recombinant enzyme from tomato[37] catalysed the same reaction at a rate of 33 μmol min^{-1} mg^{-1} (Table 1). However, phosphorylation of isopentenyl monophosphate by IspE from *E. coli* or *M. piperita* was reported, albeit at extremely low rates at 1.0 nmol min^{-1} mg^{-1} and 0.1 nmol min^{-1} mg^{-1}, respectively[38]. More recent data with the recombinant enzymes from *E. coli* and tomato[37] gave catalytic rates below 0.002 nmol min^{-1} mg^{-1}. Therefore, it is hardly conceivable that phosphorylation of isopentenyl monophosphate catalysed by IspE is metabolically relevant.

2C-Methyl-D-erythritol 2,4-cyclodiphosphate synthase

The next step in the pathway was also identified using bioinformatics. Genome analyses had shown that many putative orthologues of the *E. coli ygbB* gene (now designated *ispF*) were linked or fused to putative orthologues of *ispD* (Refs 31,40). Moreover, the occurrence of *ispF* correlates with the presence of the deoxyxylulose pathway genes (Tables 2,3). Based on these findings, the *E. coli ispF* gene was expressed and the recombinant protein shown to catalyse the conversion of 4-diphosphocytidyl-2*C*-methyl-D-erythritol 2-phosphate into 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate[40,41] (Fig. 1). The enzyme requires Mg^{2+} or Mn^{2+} but no other cofactors. The product, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate, is well known as it accumulates under oxidative stress in some bacteria[42–44]. Results obtained from incorporation experiments with chromoplasts of *C. annuum* suggest that the cyclic diphosphate (Fig. 1) is an intermediate of the deoxyxylulose phosphate pathway[40].

Further downstream reactions

The genes, enzymes and reactions involved in the conversion of 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate into IPP and DMAPP are currently unknown. However, from a structural point of view, a ring-opening reaction, two dehydration steps and two reduction steps are required to biosynthesize IPP and DMAPP. Moreover, there is evidence that IPP and DMAPP are biosynthesized via independent mechanisms in the late steps of the novel pathway[45,46] and that the gene product of *lytB* from *E. coli* is involved in one of the late steps of IPP and DMAPP biosynthesis[47].

Possible applications

The rapidly increasing resistance of many human pathogens to all currently available drugs generates an urgent demand for novel therapeutic approaches. The deoxyxylulose phosphate pathway is used in many pathogenic microorganisms, as well as in *P. falciparum* (Tables 2,3). In addition, the pathway does not occur in humans and animals. This makes the deoxyxylulose pathway of isoprenoid biosynthesis an ideal target for the development of novel antibiotics and antimalarial agents. *P. falciparum* is sensitive to the inhibition of this alternative terpenoid biosynthetic pathway by fosmidomycin[28].

In plants, the inhibition of the deoxyxylulose pathway might be the basis for the development of novel herbicides[19,48]. Detailed knowledge of the mechanisms and regulation of the pathway will also benefit the biotechnological production of commercially interesting isoprenoids, such as carotenoids[49].

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