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Mini-review

Perspectives in anti-infective drug design. The late steps in the biosynthesis of the universal terpenoid precursors, isopentenyl diphosphate and dimethylallyl diphosphate

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Abstract

A mevalonate-independent pathway for the biosynthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) that has been elucidated during the last decade is essential in plants, many eubacteria and apicomplexan parasites, but is absent in Archaea and animals. The enzymes of the pathway are potential targets for the development of novel antibiotic, antimalarial and herbicidal agents. This review is focused on the late steps of this pathway. The intermediate 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate is converted into IPP and DMAPP via 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate by the consecutive action of the iron–sulfur proteins IspG and IspH. IPP and DMAPP can be interconverted by IPP isomerase which is essential in microorganisms using the mevalonate pathway, whereas its presence is optional in microorganisms using the non-mevalonate pathway. A hitherto unknown family of IPP isomerases using FMN as coenzyme has been discovered recently in Archaea and certain eubacteria.

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1. Introduction

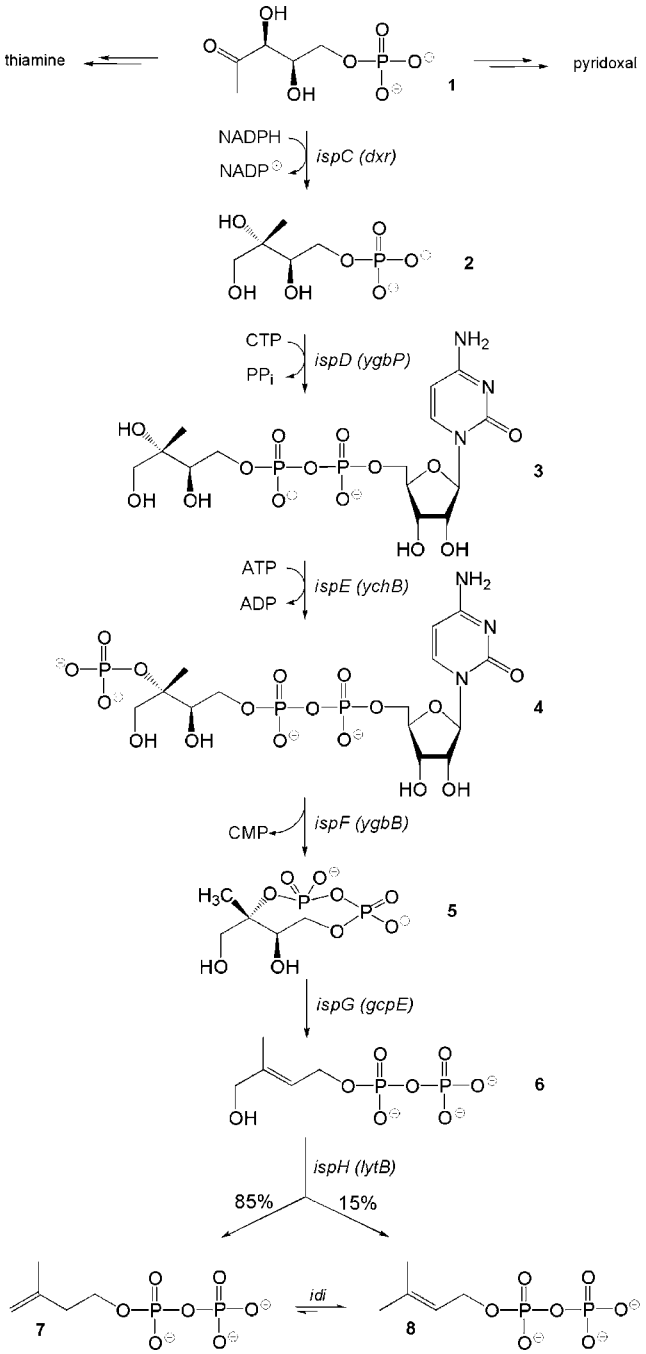
The mevalonate pathway for the biosynthesis of the universal terpenoid precursors, isopentenyl diphosphate (IPP) (**7**, Scheme 1) and dimethylallyl diphosphate (DMAPP) (**8**), has been elucidated half a century ago by Bloch, Cornforth, and Lynen [for review, see 1–4]. These pioneering studies culminated in the development of the statin type drugs for the treatment and prevention of cardiovascular disease by the down-tuning of endogenous cholesterol biosynthesis and of inflammatory processes [5,6]; certain drugs of the statin family such as atorvastatin (Lipitor)) and simvastatin (Zocor) are record holders by revenue per year.

For several decades, the dogma reigned supreme that all of the more than 35,000 reported terpenoids are biosynthesized via mevalonate. However, a second, independent pathway for the biosynthesis of **7** and **8** has been discovered during the last decade and is now known to provide the precursors for the vast majority of plant terpenoids [for review, see 7–11]. Moreover, this non-mevalonate pathway (also designated deoxyxylulose phosphate or methylerythritol phosphate pathway) has been identified as the unique source of terpenoids for numerous eubacteria. More specifically, animals, fungi, and Archaea invariably use the mevalonate pathway, plants use both pathways in different cellular compartments, and eubacteria, with few exceptions, use either the mevalonate or the non-mevalonate pathway on an exclusive basis. Notably, the group of non-mevalonate pathway users among eubacteria includes numerous human pathogens such as *Mycobacterium tuberculosis* [for review, see 7]. It has also been shown that apicoplast type protozoa, most importantly *Plasmodium* spp., depend on the non-mevalonate pathway for terpenoid biosynthesis [12–14].

The non-mevalonate pathway starts with the condensation of D-glyceraldehyde 3-phosphate with pyruvate affording 1-deoxy-D-xylulose 5-phosphate (**1**) [15,16] which has also been shown to serve as precursor for the biosynthesis of the vitamins B_1 (thiamine) [17] and B_6 (pyridoxine) [18,19] (Scheme 1). A sigmatropic rearrangement of the carbohydrate derivative followed by a reduction step affords the branched polyol, 2*C*-methyl-D-erythritol 4-phosphate (**2**) [20]. Both reactions are catalyzed by IspC protein; the enzyme-catalyzed reaction requires NADPH as cofactor.

The branched polyol derivative **2** is converted into a cyclic 2,4-diphosphate **5** by the consecutive action of three enzymes specified by the *ispDEF* genes [for review, see 11]. More specifically, a cytidyl phosphate residue is introduced into **2** by IspD protein [21]. The product **3** is phosphorylated at the position 2 hydroxyl group by the action of IspE protein affording **4** [22]. An intramolecular attack of the position 2 phosphate group of **4** on the diphosphate motif of **4** affords the cyclic diphosphate **5** under the catalytic action of IspF protein [23].

The elucidation of the *ispDEF* genes made extensive use of bioinformatics approaches based on the completely sequenced genomes of numerous bacteria which had become available during the past decade [for review, see 11]. Specifically, the taxonomic association of the *ispDEF* genes with the *ispC* gene and the complementarity of their taxonomic distribution as compared to the mevalonate pathway genes in completely sequenced eubacterial genomes were crucial for their assignment to the non-mevalonate pathway [21–23].



Scheme 1. The non-mevalonate pathway of IPP (7) and DMAPP (8) biosynthesis.

2. The late steps of the non-mevalonate pathway

2.1. The functions of IspG and IspH proteins

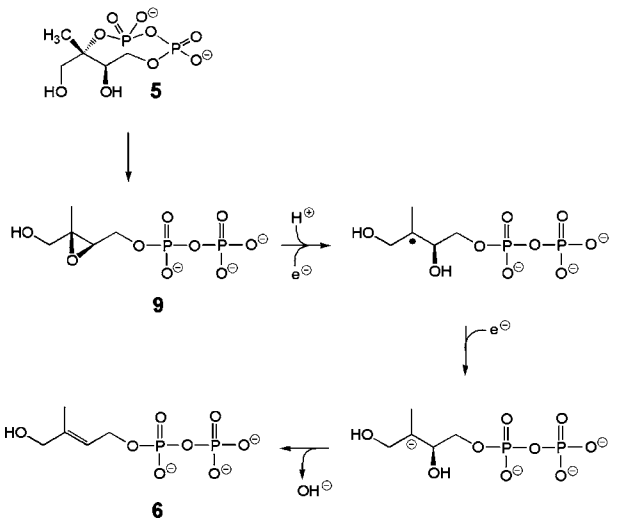
By extension of the comparative genome analysis which had resulted in the identification of the *ispDEF* genes, it was found that the distribution of *ispG* and *ispH* genes (previously designated *gcpE* and *lytB*) in completely sequenced microorganisms faithfully duplicates the distribution of the *ispCDEF* gene family, whereas the genes of the mevalonate pathway had an orthogonal distribution [for review, see 11]. As further evidence, *ispG* and *ispH* genes were shown to be associated with IPP biosynthesis in *Escherichia coli*; bacteria carrying deletions of any of these genes could be rescued by exogenous mevalonate after implementation of recombinant mevalonate pathway genes [24–26].

The function of the protein specified by the *ispG* gene was then elucidated with the aid of genetic engineering techniques. More specifically, the construction of an *ispH* mutant of *E. coli* by reverse genetics enabled the isolation and structural elucidation of 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate (**6**) [27]. The compound was also identified by in vivo studies where ^{13}C-labeled 1-deoxy-D-xylulose was supplied to an *E. coli* strain engineered for the overexpression of the *xylB* gene in conjunction with the *ispCDEFG* genes [28]. In that experimental setup, exogenous 1-deoxy-D-xylulose is converted into its 5-phosphate **1** by the XylB protein; the product **1** is then converted in vivo into **6** by the non-mevalonate pathway enzymes and **6** can be diagnosed in crude cell extract by ^{13}C NMR spectroscopy [28].

This experimental concept was extended further by the inclusion of the *ispH* gene (formerly designated *lytB*) [29]. More specifically, an *E. coli* strain was engineered to overexpress the *ispH* gene implemented in the artificial *xylBispCDEFG* operon. When the recombinant microorganism was supplied with exogenous ^{13}C-labeled 1-deoxy-D-xylulose, the proffered precursor could be shown to be transformed in vivo into a mixture of **6**, **7**, and **8**; the ratio of **7** and **8** was approximately 5:1 [29]. The yield of **7** and **8** could be further enhanced by the co-expression of genes specifying the redox shuttle proteins flavodoxin reductase (Fpr) and flavodoxin (FldA) (Rohdich et al., unpublished).

2.2. Characterization of IspG protein

In vitro studies on IspG protein were complicated by its oxygen sensitivity. The transformation of the cyclic diphosphate **5** into **6** formally implicates a 2-electron reduction step (Scheme 2). Initial experiments with recombinant IspG protein from *E. coli* used photoreduced 5-deaza-7,8,10-trimethylisoalloxazine (deazaflavin) as an artificial source of redox equivalents [30,31]. Subsequent studies showed that NADPH together with Fpr and FldA could serve as more physiological electron transponders under anaerobic conditions, although the observed catalytic activities were low, in the range of few nmol mg^{-1} min^{-1} [30]. The conversion of **5** into **6** catalyzed by the IspG protein of the hyperthermophilic eubacterium *Thermus thermophi-*



Scheme 2. Hypothetical mechanism of the IspG-catalyzed reaction.

lus has been reported to proceed also with dithionite as an artificial reducing agent [32].

Putative orthologs of the IspG protein can be found in 80 out of 124 eubacterial families whose genomes are currently available in the GenBank database (Fig. 1) [see also 7,33]. Additionally, *ispG* genes from 4 plant species (GenBank Accession No. AAO15447, AAO24774, AAO15446, and BAD19354) and from the malaria parasite *P. falciparum* (GenBank Accession No. AF323928) have been sequenced (Fig. 1). An amino acid sequence comparison of the *E. coli* protein with the orthologous proteins form the two pathogens *M. tuberculosis* and *P. falciparum* shows a high degree of homology between the three sequences (Fig. 2). Invariably, these genes specify a cluster of three absolutely conserved cysteines (residues 270, 273, and 305 in case of the *E. coli* protein) [28] typically for iron–sulfur proteins such as the SAM-dependent radical enzyme superfamily [34]. Optical spectra show a shallow absorption maximum at about 410 nm which has been interpreted as evidence for an iron–sulfur cluster [30–32]; the absolutely conserved cysteine residues are believed to be involved in the binding of that cluster [28]. The extraordinary sensitivity of the enzyme against molecular oxygen is well in line with the hypothesis that the iron–sulfur cluster is essential for the reaction mechanism.

Whereas the evidence is as yet not conclusive, the available data are all consistent with the hypothetical mechanism shown in Scheme 2. Briefly, it has been proposed that the cyclic diphosphate **5** is initially converted into the epoxide **9** which is then opened by the insertion of an electron [31]. However, the proposed involvement of an epoxide intermediate was claimed to be impossible on the basis of semi-empirical in vacuo calculations and a homology-based structural model of the enzyme [35].

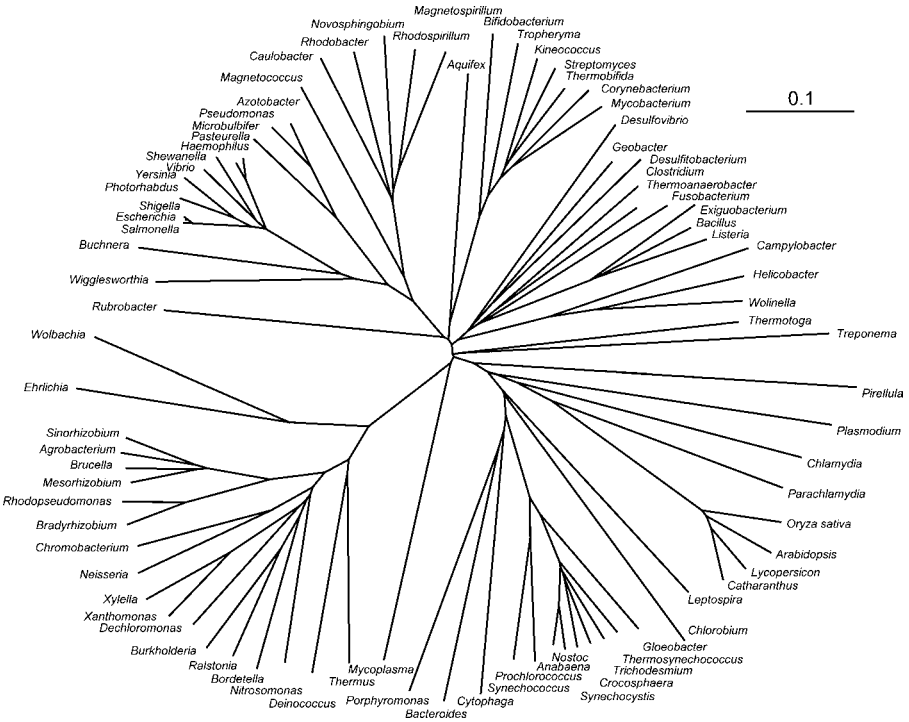


Fig. 1. Phylogenetic tree of IspG proteins. The tree was constructed from 85 IspG amino acid sequences with the ClustalW server from the European Informatic Institute (http://www.ebi.ac.uk/clustalw) using the standard settings.

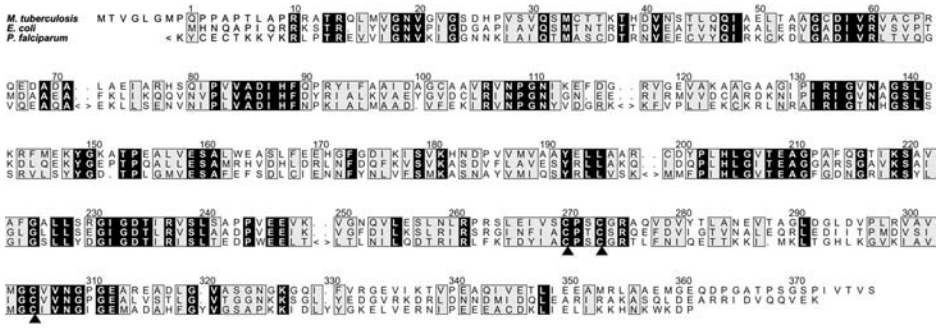


Fig. 2. Alignment of the IspG proteins from *E. coli*, *M. tuberculosis*, and *P. falciparum*. Identical residues are shown in inverse contrast, similar residues are colored in grey. Symbols: <, N-terminal extension; < >, extra-loop; ▲, conserved cysteine residue; gap introduced for proper alignment.

2.3. Characterization of IspH protein

In close parallel to *ispG* genes, a large number of putative *ispH* orthologs have been sequenced from eubacteria and entire plants [see also 7,33]. As shown in Fig. 3,

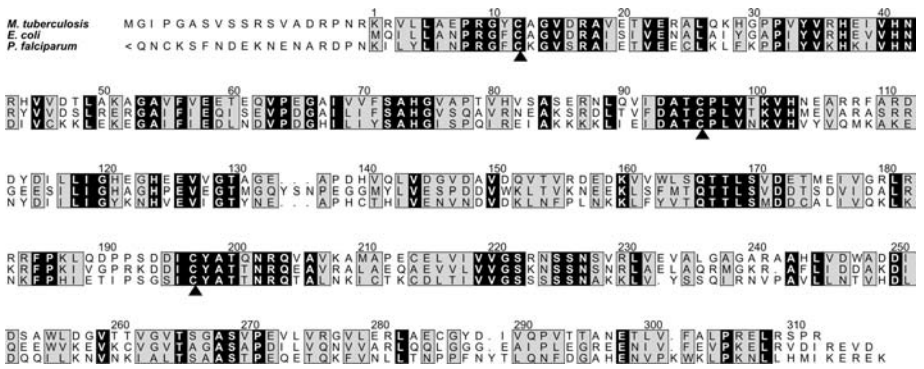


Fig. 3. Alignment of the IspH proteins from *E. coli*, *M. tuberculosis*, and *P. falciparum*. Identical residues are shown in inverse contrast, similar residues are colored in gray. Symbols: <, N-extension; ▲, conserved cysteine residue; gap introduced for proper alignment.

putative orthologs of the IspG protein of *E. coli* share high sequence similarity and invariably comprise three absolutely conserved cysteines (residues 12, 96, and 197 in case of the *E. coli* protein) which are again implicated as ligands for an iron–sulfur cluster [36]. Replacement of any of the conserved cysteine residues 12, 96 or 197 of IspH protein reduced the catalytic activity by a factor of more than 70,000 [37].

The brown-colored protein shows a shallow absorption maximum around 410 nm. The treatment of catalytically inactive protein that had been isolated under aerobic conditions with a mixture of ferric chloride and sulfide has been reported to restore at least some catalytic activity (44 nmol mg^{-1} min^{-1}) [38]. An EPR spectrum obtained with the reconstituted protein was interpreted in terms of a [4Fe–4S] cluster [38].

IspH protein has been expressed in the form of fusion proteins designed for rapid affinity purification. However, it became obvious that the supply of iron–sulfur clusters could not keep pace with the massif overexpression of the protein in recombinant *E. coli* strains. That problem could be solved by the co-expression of genes of the *isc* cluster of *E. coli* [37] which is involved in the biosynthesis of iron–sulfur clusters [39]. More specifically, the co-expression of the *iscA*, *iscS*, *iscU*, and *hscA* genes was found to increase the catalytic activity of recombinant IspH protein by more than two orders of magnitude. In parallel, the expression of the *isc* genes enhanced the absorbance at 410 nm. The iron content increased to 2.6 iron ions per protein subunits under these conditions, without any in vitro treatment of the recombinant enzyme [37].

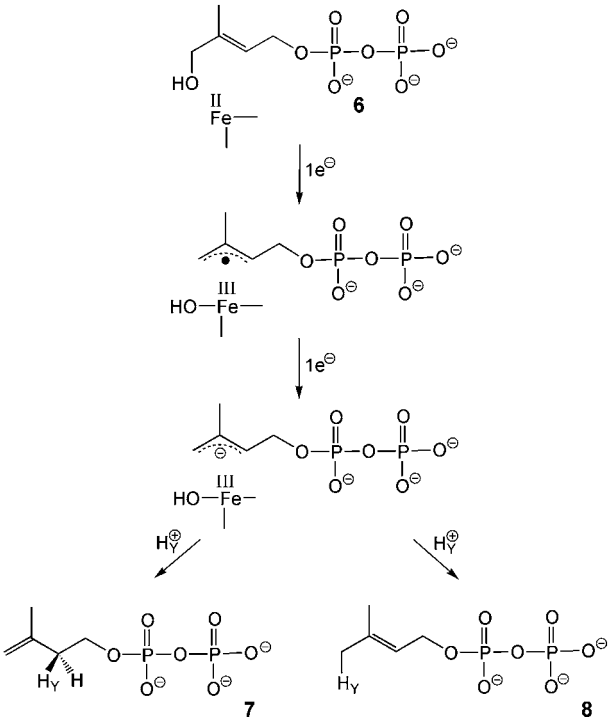
Using these enzyme samples, it could be shown that electrons required for the reduction of the substrate **6** could be supplied efficiently by a mixture of NADPH, Fpr, and FldA [37]. The redox shuttle proteins could be replaced by ferredoxin reductase and ferredoxin. The highest activities in the range of 0.7 μmol mg^{-1} min^{-1} were obtained with a large excess of flavodoxin and flavodoxin reductase over IspH protein. Even higher catalytic rates (3.4 μmol min^{-1} mg^{-1}) were observed with photoreduced deazaflavin as reducing agent [37]. However, it could be shown that

this was not due to an activation of the enzyme per se; on the contrary, IspH protein that had been subjected to ultraviolet irradiation in the presence of deazaflavin could not subsequently catalyze the formation of **7** and **8** with redox shuttle proteins as electron donors.

In close similarity to the in vivo reaction, a ratio of 6.3:1 for **7**:**8** has been observed in vitro with flavodoxin and flavodoxin reductase as electron shuttle proteins, whereas a ratio of 4.5:1 was observed during in vitro studies of IspH protein using photoreduced deazaflavin as a non-natural electron donor [37].

EPR analysis of the recombinant enzyme that had been purified under anaerobic conditions but had not been subjected to any additional treatment (such as reconstitution with iron and/or sulfide ions) showed a signal centered at 345 mT indicating the presence of a $[3Fe–4S]^{+}$ cluster [37]. The presence of a redox-active [3Fe–4S] cluster suggests the involvement of radical intermediates [31] in the IspH catalyzed reaction (Scheme 3).

The enzyme-catalyzed transformation of **6** into a mixture of **7** and **8** involves the insertion of a hydrogen atom at position 2 of IPP (Scheme 3). Studies with recombinant IspH protein from *E. coli* in D_2O as solvent show that the added atom (H_Y in Scheme 3) is efficiently derived from solvent water [40]. Moreover, using IPP isomerase as an auxiliary enzyme, it could be shown that the insertion occurs stereo-



Scheme 3. Hypothetical mechanism of the IspH-catalyzed reaction.

specifically in the (Si) position at C-2 of **7**. The same stereochemical course of the reaction was reconstructed from the labelling patterns observed in the prenyl side chain of ubiquinones obtained from *E. coli* supplied with specifically deuterated 1-deoxy-D-xylulose [41] or 2*C*-methyl-D-erythritol [42] (for a detailed discussion, see also [7]).

2.4. Assembly of iron–sulfur clusters in IspH proteins

The present data suggest that the formation of functional IspH protein requires auxiliary proteins for iron–sulfur cluster assembly. These proteins are widely disseminated in eubacteria. *E. coli* has two iron–sulfur cluster assembly lines specified by the *isc* and *suf* operons [43]; hence, the deletion of either operon can be tolerated without loss of function. In organisms which use only a single assembly pipeline, the genes for iron–sulfur cluster production are essential.

The functions of proteins for iron–sulfur cluster assembly have been reviewed elsewhere [44]. In the context on the efficient expression of IspH protein, it is relevant that the cysteine desulfurase specified by the *iscS* gene of the *isc* operon catalyzes the release of sulfur from cysteine yielding alanine and IscS-bound persulfide which is believed to be the activated form of sulfur [45]. The sulfur is subsequently transferred to IscU and IscA on which unstable iron–sulfur clusters are constructed which are delivered to other apo-iron–sulfur proteins.

The *ispG* and *ispH* genes of plants specify putative N-terminal plastid targeting sequences [10]. A plastid location of these enzymes is well in line with numerous observations indicating that the non-mevalonate pathway proceeds in the plastid compartment, whereas the mevalonate pathway proceeds in the cytoplasmic compartment of higher plants [for review, see 10]. In eukaryotic cells, iron–sulfur clusters have been shown to be assembled inside mitochondria and to be translocated into the cytoplasmic space [46]. The mechanism and topology of the assembly of functional IspG and IspH protein in plants remains to be elucidated.

3. IspG and IspH as potential anti-infective drug targets

3.1. Antimalarial targets

Malaria takes an enormous toll on human health as the cause of 1–3 million deaths per year. The frequency of novel infections is estimated to be in the range of hundreds of millions per year [47]. Since malaria can be cured, in principle, by chemotherapy, this outcome must be classified as a huge toll of preventable death. By comparison, cigarette smoking, the most frequent cause of preventable death, causes approximately 4.8 million deaths per year [48].

Despite the heavy toll of malaria morbidity and mortality, the development of novel tools for its therapy and prevention has been slow for an extended period. At the same time, the available chemotherapeutic agents have been subject to a progressively rapid process of attrition by resistance development [49]. It can be added that

the chemical control of insect vectors is also faced with diminishing returns in consequence of progressive resistance [50].

The malaria parasites are absolutely dependent on the non-mevalonate pathway for the biosynthesis of terpenoids, and the pathway has already been validated as a chemotherapeutic target [14,51]. More specifically, the antibiotic fosmidomycin which has been discovered in the 1980s [52] and reportedly passed through initial development as an antibacterial agent but was subsequently abandoned, possibly in consequence of pharmacodynamic problems, was recently shown to inhibit the enzyme catalyzing the first committed step of the non-mevalonate pathway specified by the *ispC* gene [53]. In initial preclinical studies, the antibiotic has been shown to cure mice infected with *Plasmodium vinckei* [54]. More recent clinical studies established the successful treatment of human malaria patients [55]. Notably, the antibiotic has the demonstrated potential to cure infections by drug-resistant *Plasmodium* strains. To increase the security of treatment, the combination of fosmidomycin with a second established antimalarial agent, the antibiotic clindamycin, has been advocated [14]. Since the non-mevalonate pathway is absent in mammalian hosts, the development of drugs directed against enzymes of the pathway does not have to aim for selectivity between host and parasite metabolism.

The non-mevalonate pathway may offer many additional opportunities for the development of novel antimalarial agents. Notably, several cDNA orthologs from *Plasmodium* spp. have been cloned and sequenced (including genes encoding for IspG and IspH proteins; cf. Figs. 2 and 3), and the IspC and IspF proteins have been expressed in recombinant *E. coli* strains [54,56]. These developments could serve as the basis for drug screening systems.

3.2. Tuberculostatic targets

In parallel to malaria, tuberculosis has a death toll in the range of one million, predominantly in developing countries. The level of resistance to currently available tuberculostatic drugs is rising rapidly [57]. Interestingly, all genes of the non-mevalonate pathway are present in *Mycobacterium leprae* (cf. [33]), despite the fact that about one-half of the typical mycobacterial genes have undergone deletion during the transition of the microorganisms to an obligatorily intracellular lifestyle which is believed to have occurred relatively recently. The fact that this development has left the non-mevalonate pathway intact indicates its indispensability.

Putative orthologs of IspG and IspH from *M. tuberculosis* are quite similar to those of *E. coli* (cf. Figs. 2 and 3). Hence, it can be expected that IspG and IspH proteins are targets for novel tuberculostatic drugs.

4. IspG and IspH as herbicidal targets

It has been shown that the the non-mevalonate pathway supplies the C_5 precursors **7** and **8** for the biosynthesis of essential components of the light-harvesting apparatus and for photoprotection in plants [58–60]. Homozygous mutants of

Arabidopsis thaliana lacking 1-deoxyxylulose 5-phosphate synthase could not synthesize carotenoids and chlorophylls in sufficient amounts for normal growth [61]. Plant growth was also inhibited by the IspC inhibitor, fosmidomycin [62–64]. These data can be taken as clear evidence that all enzymes of the non-mevalonate pathway (including IspG and IspH proteins) are promising targets for the development of novel herbicides. Indeed, IspG and IspH proteins were found to be indispensable for normal growth of *A. thaliana* [65].

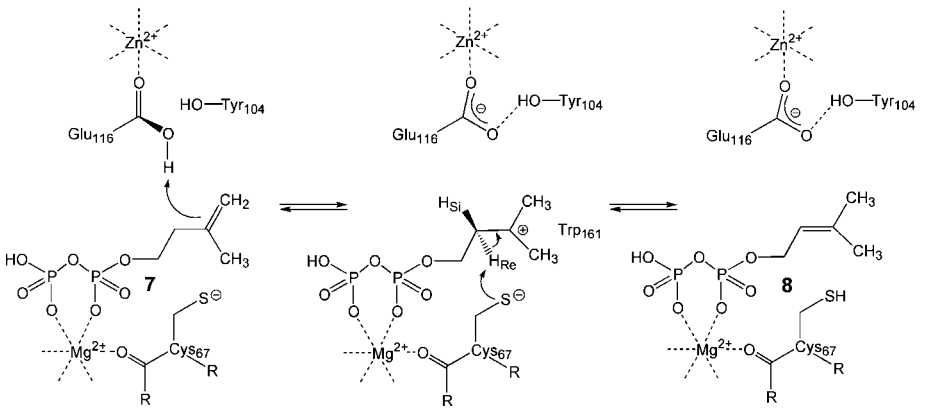
5. IPP isomerases

5.1. IPP isomerase (type-I)

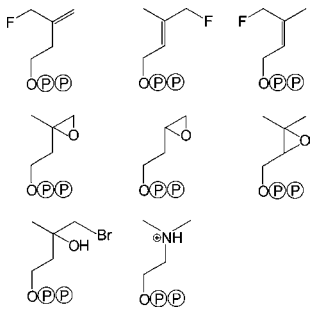
The primary product of the mevalonate pathway is IPP (**7**), and an isomerase is required for its partial conversion into DMAPP (**8**). An enzyme catalyzing the conversion of **7** into **8** has been discovered in yeast by Lynen and co-workers [66]. In the meantime, orthologs of the enzyme have been characterized from certain bacteria, plants and animals [for review, see 67,68]. The enzymes have an absolute requirement for divalent cations (Mg^{2+} or Mn^{2+}).

The enzyme-catalyzed reaction involves protonation of the *Re*-face of the double bond in **7** and deprotonation of the H-*Re* atom at C-2 of **7** [for review, see 67,68] (Scheme 4). The (*E*)-methyl group in **8** is derived from the 4-methylene carbon of **7**. The structure of the enzyme from *E. coli* has been analyzed by X-ray crystallography [69–73]. Cys-67, Glu-116, and Tyr-104 have been proposed to be involved in the protonation/deprotonation process (cf. Scheme 4). A Zn^{2+} ion appears to activate protonation of **7** [71]. A putative cationic intermediate or transition state could be stabilized by the neighboring Trp-161.

Potent inhibitors of the enzyme have been known for several decades, among them several fluorinated substrate analogs, epoxides, a bromohydrine compound,



Scheme 4. Hypothetical mechanism of the IPP isomerase type-I catalyzed reaction. Amino acid residues of the *E. coli* protein are indicated.



Scheme 5. Structures of IPP isomerase type-I inhibitors.

and an ammonium derivative which has been proposed to act as a transition state analog (Scheme 5). In analogy to HMG-CoA reductase, IPP isomerase is essential for the synthesis of terpenoid biosynthesis (e.g., cholesterol). Therefore, IPP isomerase inhibitors could serve as drugs mimicking the action of statins (see above).

5.2. Discovery of a new class of IPP isomerase (type-II)

Whereas Archaea were known for quite a while to invariably use the mevalonate pathway, the rapidly emerging genomic data failed to yield any archaeal orthologs of the known mammalian IPP isomerases [74]. The apparent paradox was resolved by the recent discovery of novel IPP isomerases initially isolated from a *Streptomyces* sp. [75]. Sequence comparison showed that orthologs of the *Streptomyces* enzyme (which is devoid of similarity with the previously known IPP isomerases from mammals and yeasts) are present in Archaea and a variety of eubacteria; so far orthologs can be found in the genomes of each archaeal family (in total 11) and in the genomes of 35 out of 124 eubacterial families (Fig. 4). The enzymes from *B. subtilis* [33,76] and two archaeal species [77,78] were subsequently characterized in some detail. They are now designated type II, whereas the mammalian, plant and yeast isomerases are now designated type I.

Archaea appear to invariably use the type II enzymes (Fig. 4). In eubacteria, type I and type II isomerases occur in every possible combination with each of the two different isoprenoid pathways (Fig. 5). Current evidence suggests that no isomerase at all is present in about 70% of completely sequenced eubacteria which utilize exclusively the non-mevalonate pathway (Fig. 5). In other eubacterial non-mevalonate pathway users including *E. coli*, isomerases accompany the non-mevalonate pathway enzymes and may serve as salvage enzymes in order to adjust the ratio of **7** and **8** to the specific needs of the downstream consumer processes.

5.3. Characterization of IPP isomerase type-II

By comparison with the well-studied type I enzymes, the type II enzymes have numerous peculiarities. When purified and assayed under strictly anaerobic conditions, the enzyme requires FMN in addition to divalent cations (Mg^{2+} or Ca^{2+}) for

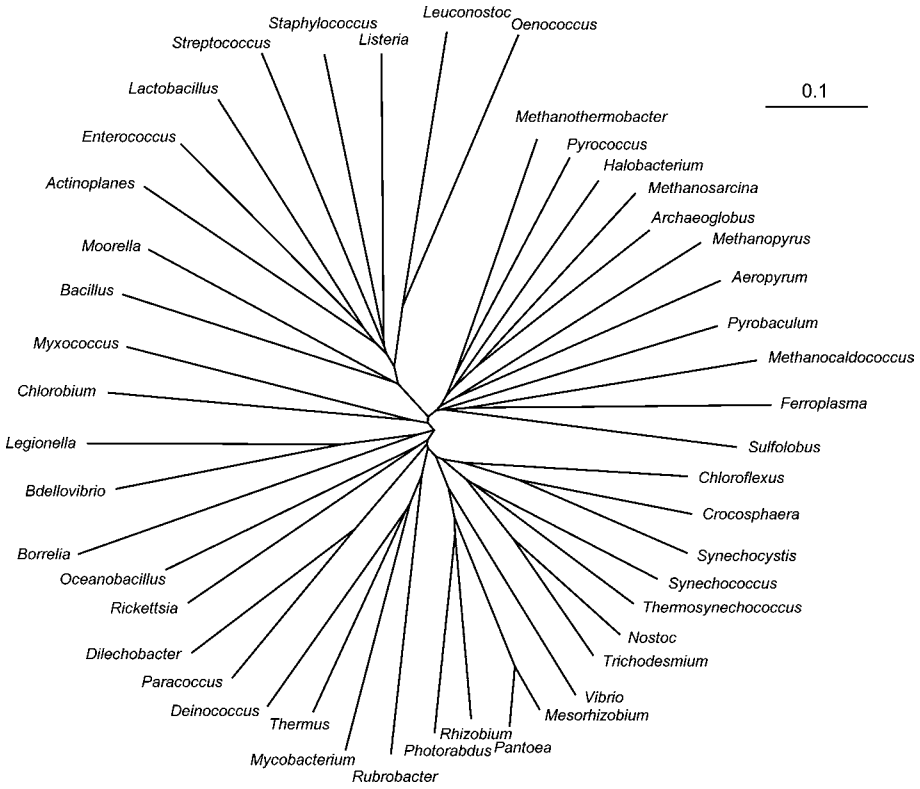


Fig. 4. Phylogenetic tree of Idi-2 proteins. The tree was constructed from 46 Idi-2 amino acid sequences with the ClustalW server from the European Informatic Institute (http://www.ebi.ac.uk/clustalw) using the standard settings.

catalytic activity [33]. Under aerobic conditions, NADPH is additionally required for activity [33,75–78]. The enzyme has no sequence similarity with the type I isomerases but is a member of a superfamily of (*S*)–hydroxyacid dehydrogenases including flavocytochrome b_2 from yeast, long chain hydroxyacid oxidase from mammals, L-lactate dehydrogenases from bacteria, glycolate oxidase from spinach and (*S*)-mandelate dehydrogenase and retains a low lactate dehydrogenase activity in the range of $1\,\mathrm{nmol\,mg^{-1}\,min^{-1}}$ [33]. X-ray structure analysis of the *B. subtilis* protein elucidated the binding site for FMN but failed to reveal the topology of bound substrate (IPP or DMAPP) [79].

Stereochemical features of type II IPP isomerase from *B. subtilis* could be elucidated using IspH protein and type I IPP isomerase from *E. coli* as auxiliary enzymes [44]. More specifically, [1,2-$^{13}C_2$]-**6** was treated with IspH protein in D_2O affording chirally deuterated [1,2-$^{13}C_2$,2-$^{2}H_1$]-**7**. The reaction product was then reacted with type I or type II IPP isomerase in normal water. In both experiments, the isomerases retained the deuterium atom at C-2 of DMAPP (**8**) which had been incorporated previously by the catalytic action of IspH protein. Based on the known stereochemistry

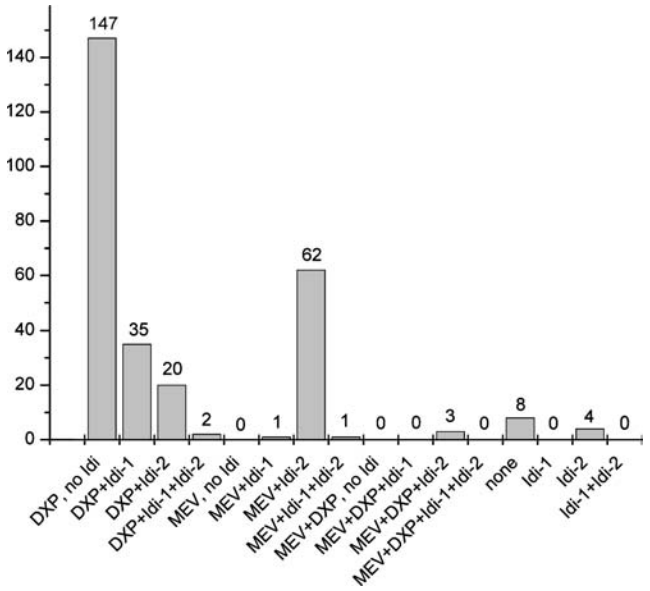


Fig. 5. Distribution of isoprenoid biosynthesis pathways and IPP isomerases in 283 completed and unfinished prokaryotic genomes. MEV, mevalonate pathway genes; DXP, deoxyxylulose pathway genes.

of the type I isomerase [80], it follows that both isomerases release the C-2 H-(*Re*) of IPP. In an additional experiment, unlabeled **7** was incubated in D_2O with the type-II enzyme [44]. ^2H was found at the (*E*)-methyl group of **8** demonstrating that the type II enzyme has the same (*E*)/(*Z*) specificity as the type-1 protein [81].

5.4. IPP isomerase type-II as a potential anti-infective drug target

The taxonomic distribution of IPP isomerases is highly complex as described above (cf. Fig. 5 and [33]). Under chemotherapeutic aspects, it is relevant that IPP isomerases are essential enzymes in microorganisms using exclusively the mevalonate pathway. In *Streptococci*, *Staphylococci*, and *Enterococci*, the mevalonate pathway is associated with type II IPP isomerase. The occurrence of methicillin-resistant *Staphylococci* (MRSA) and vancomycin-resistant *Enterococci* (VRE) is increasing rapidly [82–84], and novel chemotherapeutic strategies are urgently needed. Type II IPP isomerase inhibitors could possibly serve as selective drugs for infections by drug-resistant strains.

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