

REVIEW ARTICLE

Metabolic plasticity for isoprenoid biosynthesis in bacteria

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Isoprenoids are a large family of compounds synthesized by all free-living organisms. In most bacteria, the common precursors of all isoprenoids are produced by the MEP (methylerythritol 4-phosphate) pathway. The MEP pathway is absent from archaea, fungi and animals (including humans), which synthesize their isoprenoid precursors using the completely unrelated MVA (mevalonate) pathway. Because the MEP pathway is essential in most bacterial pathogens (as well as in the malaria parasites), it has been proposed as a promising new target for the development of novel anti-infective agents. However, bacteria show a remarkable plasticity for isoprenoid biosynthesis that should be taken into account when targeting this metabolic pathway for the development of new antibiotics. For example, a few bacteria use the MVA pathway instead of the MEP pathway, whereas others possess the two full pathways, and some parasitic strains lack both the MVA and the MEP pathways

(probably because they obtain their isoprenoids from host cells). Moreover, alternative enzymes and metabolic intermediates to those of the canonical MVA or MEP pathways exist in some organisms. Recent work has also shown that resistance to a block of the first steps of the MEP pathway can easily be developed because several enzymes unrelated to isoprenoid biosynthesis can produce pathway intermediates upon spontaneous mutations. In the present review, we discuss the major advances in our knowledge of the biochemical toolbox exploited by bacteria to synthesize the universal precursors for their essential isoprenoids.

Key words: antibiotic, fosmidomycin, isoprenoid, methylerythritol 4-phosphate pathway (MEP pathway), mevalonate pathway (MVA pathway).

INTRODUCTION

Prokaryotes (bacteria and archaea) flourish in all habitats suitable for life on Earth. In part, this is because they have an astounding biochemical and metabolic diversity, particularly regarding energy-generating metabolism and biosynthesis of secondary metabolites. A remarkable metabolic versatility can be found even within the same organism. For example, *Escherichia coli* can use glucose or lactose as the sole carbon source for the production of all necessary amino acids, vitamins and nucleotides, it can produce energy by fermentation or respiration, and it can grow under aerobic or anaerobic conditions. Endosymbiotic prokaryotes that became mitochondria and plastids provided some of this metabolic diversity to eukaryotic cells. However, there are many biochemical reactions and metabolic pathways that are still exclusive to the prokaryotic world.

Isoprenoids (also known as terpenoids) are one of the groups of metabolites that are essential in all living organisms. Isoprenoids that are vital for the growth and survival of prokaryotes include those playing an indispensable role in cell wall and membrane biosynthesis (bactoprenol and hopanoids), electron transport (ubiquinone and menaquinone) or conversion of light into chemical energy (chlorophylls, bacteriochlorophylls, rhodopsins and carotenoids), among other processes [1]. Despite their remarkable diversity of structures and functions, all isoprenoids derive from a basic five-carbon precursor unit, IPP

(isopentenyl diphosphate), and its isomer DMAPP (dimethylallyl diphosphate). For many years, it was accepted that IPP was synthesized from acetyl-CoA through the well-known MVA (mevalonate) pathway in all organisms. However, an alternative MVA-independent pathway for the biosynthesis of IPP and DMAPP was identified in the mid-1990s in bacteria and plant plastids [2,3]. A detailed description of the discovery and elucidation of this new pathway, currently known as the MEP (methylerythritol 4-phosphate) pathway [4], can be found in other reviews [5–8]. It is now well established that the MEP pathway is the only one present in most bacteria, the apicoplasts of apicomplexan protozoa, and the plastids of green algae and higher plants. By contrast, it is absent from archaea, fungi and animals (including humans), which synthesize their isoprenoids exclusively from MVA-derived precursors [9].

TWO MAJOR PATHWAYS PRODUCE THE UNIVERSAL PRECURSORS OF ALL ISOPRENOIDS

As described above, the universal precursors of all isoprenoids, IPP and DMAPP, can be synthesized by two major pathways [1]: the MVA pathway and the MEP pathway. The canonical reactions of both pathways are described in this section and summarized in Figure 1. In the first steps of the MVA pathway, HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) is produced from

Abbreviations used: DHBP, 3,4-dihydroxy-2-butanone 4-phosphate; DHBPS, DHBP synthase; DMAPP, dimethylallyl diphosphate; DPMD, 5-diphosphomevalonate decarboxylase; DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, DXP reductoisomerase; DXS, DXP synthase; FSM, fosmidomycin; HMBPP, 4-hydroxy-3-methylbut-2-enyl diphosphate; HDR, HMBPP reductase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGR, HMG-CoA reductase; IPK, isopentenyl phosphate kinase; IPP, isopentenyl diphosphate; IDI, IPP/DMAPP isomerase; MEP, methylerythritol 4-phosphate; MCT, MEP cytidyltransferase; MTA, 5-methylthioadenosine; MTRP, 5-methylthio-D-ribulose 1-phosphate; MTXP, 1-methylthio-D-xylulose 5-phosphate; MMS, MTXP methylsulfonylase; MVA, mevalonate; MVP, 5-phosphomevalonate; PDH, pyruvate dehydrogenase; PMVD, MVP decarboxylase; PMVK, MVP kinase; RLP, Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase)-like protein; RP, ribulose 5-phosphate; TPP, thiamine diphosphate; XP, xylulose 5-phosphate.

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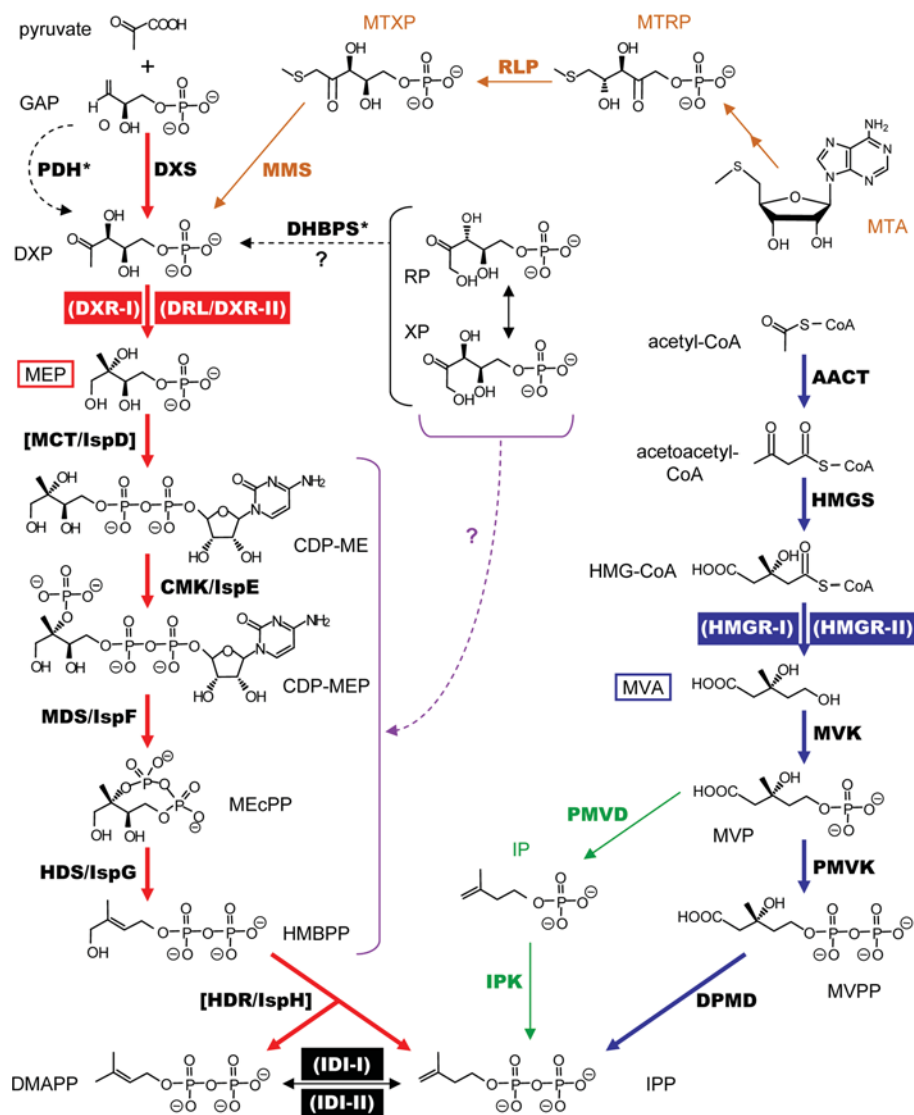


Figure 1 Pathways for the biosynthesis of isoprenoid precursors in bacteria and archaea

The canonical MEP pathway steps are marked in red (GAP, D-glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; MEcPP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP, 4-hydroxy-3-methylbut-2-enyl diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate). The metabolic shunt for DXP biosynthesis discovered in *Rhodospirillum rubrum* is shown in orange (MTA, 5-methylthioadenosine; MTRP, 5-methylthio-D-ribulose 1-phosphate; MTXP, 1-methylthio-D-xylulose 5-phosphate). The alternative pathway for IPP and DMAPP production proposed for *Synechocystis* is shown in purple (RP, D-ribulose 5-phosphate; XP, D-xylulose 5-phosphate). The MVA pathway is represented in blue (HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MVA, mevalonic acid; MVP, 5-phosphomevalonate; MVPP, 5-diphosphomevalonate). The alternative steps described in archaea are shown in green (IP, isopentenyl phosphate). Enzyme acronyms (in bold) are described in the text. Asterisks mark *E. coli* enzymes that produce DXP when mutated. Enzymes catalysing other reactions besides those in the MEP pathway are between brackets. Steps catalysed by different types of enzymes (shown within parentheses) are highlighted.

the sequential condensation of three molecules of acetyl-CoA catalysed by the enzymes AACT (acetoacetyl-CoA thiolase) and HMGS (HMG-CoA synthase). HMGR (HMG-CoA reductase) catalyses the irreversible conversion of HMG-CoA into MVA in the first committed step of the pathway. Then, MVA is sequentially phosphorylated and decarboxylated to generate IPP by the enzymes MVK (mevalonate kinase), PMVK (5-phosphomevalonate kinase) and DPMD (5-diphosphomevalonate decarboxylase). The activity of an IDI (IPP/DMAPP isomerase) enzyme is required to form DMAPP from IPP (Figure 1).

The MEP pathway has been best characterized in *E. coli*, a model bacterium that lacks the MVA pathway [1,8,10]. It starts with the condensation of (hydroxyethyl)thiamin derived from

pyruvate with the C-1 aldehyde group of D-glyceraldehyde 3-phosphate to produce DXP (1-deoxy-D-xylulose 5-phosphate) in a reaction catalysed by the enzyme DXS (DXP synthase). In the second step, DXR (DXP reductoisomerase)/IspC catalyses the intramolecular rearrangement and reduction of DXP to produce MEP. The sequential action of the enzymes MCT (MEP cytidyltransferase)/IspD, CMK [4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase]/IspE, MDS (2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase)/IspF, and HMBPP (4-hydroxy-3-methylbut-2-enyl diphosphate) synthase/IspG transforms MEP into HMBPP. Finally, the enzyme HDR (HMBPP reductase)/IspH catalyses the simultaneous formation of IPP and DMAPP in an approximate 5:1 proportion (Figure 1).

ISOPRENOID METABOLISM AS A TARGET FOR NEW ANTIBIOTICS

The MEP pathway is not present in humans, but it is essential for most pathogenic bacteria and apicomplexan protozoa like the malaria parasite *Plasmodium falciparum* [9]. This phylogenetic distribution makes the MEP pathway a promising new target for the development of desperately needed antibiotics against microbial pathogens that are acquiring resistance to currently available drugs [11–13]. Following the discovery of this pathway, it was proposed that the use of specific inhibitors would result in novel antimicrobial drugs with a broad-spectrum activity and little toxicity to humans, since the MEP pathway enzymes were found to be highly conserved in organisms harbouring the pathway, but showed no homologues in mammals [9]. The specific MEP pathway inhibitor FSM (fosmidomycin) was actually proven to be effective against multidrug-resistant strains of bacteria and malaria parasites [14–16]. However, some results using *E. coli* as a model system have unveiled a diversity of mechanisms that bacteria can develop to bypass a pharmacological blockage of isoprenoid biosynthesis with this inhibitor. For example, the uptake of FSM by *E. coli* cells is an active process involving a cAMP-dependent glycerol 3-phosphate transporter [17]. The absence of this transporter from bacterial pathogens such as *Mycobacterium tuberculosis* or *Brucella abortus* or its mutation in *E. coli* results in FSM resistance [17–19]. On the other hand, resistance was also achieved by up-regulation of proteins that facilitate the efflux of the drug [20].

Antibiotic resistance can result not only from interfering with drug transport or mode of action, but also from the use of alternative pathways or enzymes not affected by the inhibitor. In this context, future strategies to target isoprenoid enzymes for the development of new antibiotics should take into account the astonishing versatility displayed by bacteria to produce their isoprenoid products. In the present review, we summarize our current knowledge on the mechanisms available to bacteria to synthesize their essential isoprenoid precursors, IPP and DMAPP. We have classified the multiple sources of such plasticity into five major blocks: (i) differential distribution of isoprenoid pathways, (ii) alternative pathways for the synthesis of isoprenoid precursors, (iii) alternative enzymes catalysing the same isoprenoid reaction, (iv) isoprenoid enzymes catalysing other reactions, and (v) non-isoprenoid enzymes producing isoprenoid intermediates upon mutation.

(i) Differential distribution of isoprenoid pathways

Although most bacteria only use the MEP pathway for the production of their essential isoprenoid precursors, there are some exceptions [1,9,21,22]. Some bacteria, including the spirochaete *Borrelia burgdorferi* and the Gram-positive cocci *Staphylococcus aureus* and *Streptococcus pneumoniae* have been confirmed to use the MVA pathway instead of the MEP pathway for IPP and DMAPP synthesis. Others, including *Listeria monocytogenes* and some *Streptomyces* strains, possess the two full pathways [6,22–24]. For example, all *Streptomyces* use the MEP pathway to produce their essential isoprenoids, but some strains can additionally use the MVA pathway for the biosynthesis of antibiotics and other secondary metabolites [6]. On the other hand, there are bacteria that lack both isoprenoid pathways. Thus parasitic *Rickettsia* and *Mycoplasma* bacteria have no genes for MVA or MEP pathway enzymes, probably because these obligatory intracellular parasites obtain their isoprenoids (or their precursors) from infected host cells. Most strikingly, related bacteria may use

different pathways for isoprenoid biosynthesis, whereas unrelated bacteria may use the same pathway [1,6,9,21–24].

(ii) Alternative pathways for the synthesis of isoprenoid precursors

The sequenced archaeal genomes only contain genes encoding MVA pathway enzymes. However, with the exception of some *Sulfolobus* species, genomic analyses have failed to identify the full set of MVA pathway genes in other species. In particular, the genes encoding PMVK and DPMD (Figure 1) are absent from most archaea [25,26]. In these organisms, the conversion of MVP (5-phosphomevalonate) into IPP is achieved through the operation of an alternative route involving the formation of isopentenyl phosphate from MVP by PMVD (MVP decarboxylase) and further conversion into IPP by IPK (isopentenyl phosphate kinase) [27]. Although IPK has been characterized at biochemical and structural levels [28,29], the PMVD activity is still speculative and needs biochemical confirmation.

A metabolic shunt linking polyamine metabolism and the MEP pathway has been discovered recently [30,31]. In this novel route (Figure 1), DXP is produced from MTA (5-methylthioadenosine), a by-product of polyamine biosynthesis that originates from S-adenosylmethionine. To recycle sulfur and produce methionine, MTA is cleaved and phosphorylated to yield 5-methylthio-D-ribose 1-phosphate, which is then transformed into MTRP (5-methylthio-D-ribulose 1-phosphate). In the classical methionine salvage pathway, the activity of dehydratase, enolase, phosphatase, dioxygenase and transaminase enzymes subsequently converts MTRP into methionine [32]. However, the photosynthetic proteobacterium *Rhodospirillum rubrum* uses MTRP for DXP biosynthesis [30]. In this organism, an atypical RLP [Rubisco (ribulose 1,5-bisphosphate carboxylase/oxygenase)-like protein] isomerizes MTRP to MTXP (1-methylthio-D-xylulose 5-phosphate). Then, the enzyme MMS (MTXP methylsulfurylase) converts MTXP into DXP with the reductive loss of methanethiol, a compound that can be subsequently used for methionine formation by an alternative salvage pathway [30,31]. Genes encoding RLP, MMS and other enzymes required to convert MTA into DXP are found in several groups of bacteria, suggesting that the MTA–DXP shunt is not restricted to *Rhodospirillum*. Interestingly, genes encoding DXS are also found in these bacteria, indicating that they could potentially use two pathways to synthesize DXP. It has been proposed that the presence of the MTA–DXP route might be a mechanism to cope with the dead-end product MTA and, at the same time, provide extra DXP for the production of carotenoids and other isoprenoids when needed [30].

Another shunt alternative to the canonical MEP pathway has been proposed to operate in the cyanobacterium *Synechocystis* [33,34]. The *Synechocystis* genome contains homologues of all MEP pathway genes [35]. However, the addition of MEP pathway substrates to cells grown photoautotrophically did not stimulate isoprenoid biosynthesis, whereas FSM did not block their growth despite being an effective inhibitor of *Synechocystis* DXR activity *in vitro* [36]. In contrast, some phosphorylated sugars of the pentose phosphate cycle were able to stimulate the incorporation of labelled IPP into isoprenoids in a cell-free system [33]. This process required the activity of the Sll1556 protein [34]. Collectively, these results suggest that alternative substrate pathways are used for isoprenoid biosynthesis when *Synechocystis* grows under photosynthetic conditions. Although further experimental evidence is required, the results available suggest that in this cyanobacterium the canonical (linear) MEP pathway as defined for *E. coli* is not the sole pathway by which isoprenoids are synthesized. Instead, photosynthesis-derived

products of the pentose phosphate cycle serve as substrates for IPP and DMAPP synthesis, probably entering the pathway downstream of MEP via RP (ribulose 5-phosphate) and/or its stereoisomer XP (xylulose 5-phosphate) (Figure 1). An alternative route to IPP and DMAPP production from pentose phosphate cycle substrates can be metabolically advantageous for a photosynthetic organism at optimal growth conditions since it might boost the production of isoprenoids required for photosynthesis.

(iii) Alternative enzymes catalysing the same isoprenoid reaction

There are several examples of reactions related to the biosynthesis of isoprenoid precursors that can be catalysed by different enzymes in bacterial cells. Two types of structurally unrelated IDI enzymes showing no sequence similarity and different reaction mechanisms and cofactor requirements have been reported to catalyse the interconversion of IPP and DMAPP [37–40]. The type I enzyme (IDI-I) found in many bacteria (including *E. coli*) is similar to that found in fungi, plants and animals, and it has been extensively characterized at structural and functional levels [41,42]. The type II enzyme (IDI-II) was discovered in *Streptomyces* and shown to be present in archaea and some bacteria, but not in plants or animals [6,22,37]. There is no correlation between the presence of IDI-I or IDI-II enzymes and the operation of either the MVA or the MEP pathways [1,22]. Interestingly, there are bacteria that possess either IDI-I or IDI-II enzymes, others have both, and a large proportion of bacteria containing the MEP pathway do not contain IDI. Because IDI activity is essential to produce DMAPP in organisms that only contain the MVA pathway, but is not required in those harbouring the MEP pathway, which simultaneously produces both IPP and DMAPP (Figure 1), it may not be surprising that IDI is absent from bacteria using the MEP pathway. In fact, no obvious phenotype is associated with the loss of IDI-encoding genes in bacteria with both the MEP pathway and IDI such as *E. coli* [43,44]. It is likely, however, that the presence of IDI may serve to balance the IPP and DMAPP pools according to particular growth conditions, conferring an advantage in critical situations where optimal isoprenoid biosynthesis is absolutely required.

In the MVA pathway, the first committed step is the irreversible conversion of HMG-CoA into MVA catalysed by HMGR (Figure 1). Two different classes of HMGR have been described on the basis of sequence alignments and phylogenetic analyses and are proposed to have arisen by divergent evolution from a common ancestor [45]. HMGR class I (HMGR-I) enzymes are found predominantly in archaea and eukaryotes, whereas bacteria that use the MVA pathway usually possess HMGR class II (HMGR-II) enzymes. Differences between classes are most apparent around the active site and the preceding region. This feature influences the differential inhibitory effect of statins, which are excellent inhibitors of the HMGR-I enzymes, but relatively poor inhibitors of HMGR-II [23]. Improved resistance to statins might actually explain the progressive substitution of the original HMGR-I enzyme by HMGR-II proteins [21]. Although no significantly effective HMGR-II inhibitors have been reported so far, modelling and structure-based screening strategies appear as promising ways to identify novel lead compounds with high inhibitory activity against HMGR-II, but not HMGR-I [46]. Drugs exclusively targeting HMGR-II could be potentially used as antibiotics targeting bacterial pathogens harbouring these enzymes (such as *S. pneumoniae*) with weak or no side effects for animal cells, which contain a HMGR-I enzyme.

Plasticity in terms of using different enzymes to catalyse the same reaction has also been reported for the first committed

step of the MEP pathway [18,47]. This step, the production of MEP by intramolecular rearrangement and NADPH-dependent reduction of DXP following a retroaldol/aldol mechanism [48,49], is catalysed by the enzyme DXR (Figure 1). The vast majority of bacteria with the MEP pathway have a canonical DXR protein that could be renamed DXR-I. However, a few bacteria lacking DXR-I (including animal and human pathogens such as *Brucella* and *Bartonella*) use a different DXR-like (DRL or, as renamed here, DXR-II) enzyme to catalyse the same biochemical reaction [18]. Both DXR-I and DXR-II enzymes are found in some bacteria. On the other hand, the presence of proteins with homology with DXR-II in bacteria lacking the MEP pathway suggests that they could originally have an activity unrelated to isoprenoid metabolism. The scattered taxonomic distribution of DXR-II enzymes suggests lateral gene transfer and lineage-specific gene duplications [18]. DXR-II belongs to a family of previously uncharacterized proteins with predicted oxidoreductase features and it only shows some sequence similarity to DXR-I at the level of the NADPH-binding domain. Most interestingly, DXR-I and DXR-II enzymes show a different arrangement of their active sites [47]. This feature has led to the identification of FSM derivatives that inhibit DXR-I, but had virtually no effect on DXR-II activity *in vitro*, opening the door for the design of highly specific antibiotics against only one of these two types of DXR enzymes. In particular, the design of antibiotics that selectively target pathogens using DXR-II without affecting beneficial or innocuous bacteria harbouring DXR-I enzymes would be most useful.

(iv) Isoprenoid enzymes catalysing other reactions

The main functional and structural properties of the MEP pathway enzymes have been already established [50]. However, recent results indicate that some of these enzymes have other enzymatic activities unrelated to isoprenoid biosynthesis (Figure 1). HDR/IspH, the iron–sulfur enzyme catalysing the $2\text{H}^+/2\text{e}^-$ reduction and deoxygenation of HMBPP to produce both IPP and DMAPP in the final step of the MEP pathway [51,52], was recently shown to be able to catalyse the addition of water to acetylene groups to produce aldehyde and ketone products [53]. This second class of reaction was only catalysed by the oxidized form of the enzyme. The unforeseen promiscuity of HDR/IspH can be relevant not only for chemical synthesis, but also for inhibitor (antibiotic) design [53].

Another surprising discovery reported recently was that a protein similar to the MEP pathway enzyme MCT/IspD could function as a glycosyltransferase in animals [54,55]. IspD-like proteins belong to a large family of glycosyltransferases conserved from bacteria to mammals, but their function in organisms lacking the MEP pathway remained unexplored. In two recent studies, it was shown that defective function of a human and zebrafish IspD-like protein disrupts glycosylation of α -dystroglycan and causes Walker–Warburg syndrome, a congenital muscular dystrophy accompanied by a variety of brain and eye malformations [54,55]. This discovery has important implications for the use of MEP pathway enzymes as targets for new drugs. The MEP pathway-specific inhibitor FSM has been shown to inhibit the activity of not only DXR [56], but also MCT/IspD [57]. Although MCT/IspD inhibition with FSM was weak *in vitro* and it might require the activity of DXR for efficient inhibition *in vivo* [57], the possibility that FSM and other drugs directed against MCT/IspD enzymes from pathogenic bacteria could also inhibit IspD-like enzymes in human patients and cause undesired side effects should be taken into account.

(v) Non-isoprenoid enzymes producing isoprenoid intermediates upon mutation

All of the previous examples of plasticity illustrate the evolutionary ability of bacteria to develop different solutions to a particular metabolic challenge. But the adaptive capacity of bacteria to situations that compromise their survival can be surprisingly fast. This fact has profound implications for the development of new antibiotics targeting isoprenoid pathways. It has been shown that resistance to a blockage of the first steps of the MEP pathway can easily be developed because several enzymes unrelated to isoprenoid biosynthesis can produce pathway intermediates when mutated [58,59]. The strategy to identify these enzymes was based on the use of *E. coli* strains engineered with a synthetic operon that allows the production of IPP and DMAPP from exogenously supplied MVA [60]. Deletion of MEP pathway genes in this background is lethal, but it can be rescued by growing the engineered cells in the presence of MVA. However, it was observed that MVA auxotrophy was occasionally suppressed by spontaneous mutations in some cells lacking individual MEP pathway genes. In particular, a relatively high frequency of mutations allowing survival on media lacking MVA was observed for cells defective in DXS or DXR (6.4 and 2.4 per 10⁹ cells respectively), whereas no suppressor mutants were found in strains with disruptions of the other MEP pathway genes [58]. These results suggested that bacteria can respond to a block of DXS or DXR activities by using other proteins that deliver DXP or MEP when mutated.

Analysis of MVA auxotrophic mutant strains led to the identification of several mutations in two genes (*aceE* and *ribB*) that could suppress an otherwise lethal loss of DXS activity in *E. coli* [58,59]. The *aceE* gene encodes the catalytic E1 subunit of the PDH (pyruvate dehydrogenase) complex. Like DXS, PDH is a TPP (thiamine diphosphate)-dependent carboxylase that catalyses the decarboxylation of pyruvate with the formation of hydroxyethyl-TPP as an intermediate. Although a wild-type PDH could potentially produce DXP or its dephosphorylated precursor [61–63], only the mutant enzymes were able to produce DXP *in vivo* at levels high enough to rescue growth of DXS-deficient cells [59]. Some of the mutations identified in PDH were shown previously to cause a conformational change on the enzyme structure that facilitates its secondary carboxylase activity, mimicking the DXS reaction [64]. Therefore the mutant PDH enzymes might acquire an improved efficiency to catalyse the same reaction catalysed by DXS (Figure 1). The *ribB* gene encodes DHBPS [DHBP (3,4-dihydroxy-2-butanone 4-phosphate) synthase]. This enzyme converts RP into formate and DHBP (the biosynthetic precursor of the xylene ring of riboflavin), a conversion that involves a complex series of dehydration, intramolecular rearrangement and rehydration steps [65]. Mutant DHBPS, but not the wild-type enzyme, produces DXP (or a metabolic precursor) *in vivo* [59]. Although the mechanism by which a mutant DHBPS produces DXP remains unknown, it is interesting to note that the substrate of this enzyme (RP) was found to be involved in the alternative pathway for IPP and DMAPP biosynthesis in *Synechocystis* [33], as described above (Figure 1). *E. coli* mutants able to grow despite lacking DXR have also been isolated, but not yet analysed, in part because these suppressor mutants show very poor growth [58,59]. Together, the results described demonstrate that bacteria can circumvent a blockage of the MEP pathway at the level of the first and, with a lower efficiency, the second step by recruiting mutant enzymes that are not normally involved in isoprenoid biosynthesis. On the basis of these data, activities other than DXS and even DXR should be targeted to minimize the development of resistance mechanisms

in the context of the MEP pathway as a new target for antibiotic and antimalarial agents.

CONCLUSION

The increasing prevalence of antibiotic resistance is a major threat that will require new targets and strategies. Although the MEP pathway can be a good target for the design of new drugs to fight bacterial pathogens, special attention must be paid to the remarkable plasticity observed in bacteria for isoprenoid biosynthesis. FSM is the only drug targeting the MEP pathway that is being tested in clinical trials. However, the fact that it targets a step that is susceptible to resistance development by relatively frequent spontaneous mutations [58], and the possibility that it may also cause undesired side effects by inhibiting a human enzyme required for proper development [54,55,57], suggest that other enzymes catalysing downstream pathways might be a better choice for antibiotic development. To facilitate effective drug design, genetic, biochemical and crystallographic approaches should identify the most appropriate pathway enzymes to inhibit and the residues that play a relevant structural or catalytic role. Most of this work could be done in a relatively short time by taking advantage of the tools already available in *E. coli*. As an example, the use of strains carrying a synthetic operon for the transformation of MVA into IPP and DMAPP has allowed the identification of point mutations in MEP pathway genes that cause a complete loss of enzyme activity [66].

A major disadvantage of using broad-spectrum antibiotics, like FSM, is their lack of selectivity and the undesired effects on innocuous or beneficial bacteria. Prolonged treatment with such antibiotics may actually result in alteration of the intestinal flora, which causes gastrointestinal and other side effects [67–69]. In this context, the diversity in isoprenoid biosynthesis observed in bacteria represents an opportunity for the development of highly selective narrow-range antibiotics that could specifically inhibit the growth of particular pathogens such as those harbouring HMGR-II or DXR-II enzymes [46,47]. On the other hand, the rich collection of genes and proteins developed by bacteria to synthesize their isoprenoids represents a source of potential biotechnological tools. For example, the availability of unrelated enzymes catalysing the same biochemical reactions should allow the engineering of a particular organism with a heterologous enzyme able to completely elude the endogenous regulation mechanisms limiting the activity of the endogenous enzyme. It is expected that these exciting possibilities will be addressed in the near future.

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REFERENCES

- Rodríguez-Concepción, M. and Boronat, A. (2013) Isoprenoid biosynthesis in prokaryotic organisms. In *Isoprenoid Synthesis in Plants and Microorganisms* (Bach, T. and Rohmer, M., eds), pp. 1–16, Springer, New York.
- Rohmer, M., Knani, M., Simonin, P., Sutter, B. and Sahn, H. (1993) Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. *Biochem. J.* **295**, 517–524.
- Lichtenthaler, H. K. (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 47–65.

- 4 Phillips, M. A., Leon, P., Boronat, A. and Rodríguez-Concepción, M. (2008) The plastidial MEP pathway: unified nomenclature and resources. *Trends Plant Sci.* **13**, 619–623
- 5 Rodríguez-Concepción, M. and Boronat, A. (2002) Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids: a metabolic milestone achieved through genomics. *Plant Physiol.* **130**, 1079–1089
- 6 Kuzuyama, T. and Seto, H. (2003) Diversity of the biosynthesis of the isoprene units. *Nat. Prod. Rep.* **20**, 171–183
- 7 Eisenreich, W., Bacher, A., Arigoni, D. and Rohdich, F. (2004) Biosynthesis of isoprenoids via the non-mevalonate pathway. *Cell. Mol. Life Sci.* **61**, 1401–1426
- 8 Rohmer, M. (2008) From molecular fossils of bacterial hopanoids to the formation of isoprene units: discovery and elucidation of the methylerythritol phosphate pathway. *Lipids* **43**, 1095–1107
- 9 Lange, B. M., Rujan, T., Martin, W. and Croteau, R. (2000) Isoprenoid biosynthesis: the evolution of two ancient and distinct pathways across genomes. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13172–13177
- 10 Eisenreich, W., Rohdich, F. and Bacher, A. (2001) Deoxyxylulose phosphate pathway to terpenoids. *Trends Plant Sci.* **6**, 78–84
- 11 Lichtenthaler, H. K., Zeidler, J., Schwender, J. and Müller, C. (2000) The non-mevalonate isoprenoid biosynthesis of plants as a test system for new herbicides and drugs against pathogenic bacteria and the malaria parasite. *Z. Naturforsch.* **55**, 305–313
- 12 Rodríguez-Concepción, M. (2004) The MEP pathway: a new target for the development of herbicides, antibiotics and antimalarial drugs. *Curr. Pharm. Des.* **10**, 2391–2400
- 13 Rohdich, F., Bacher, A. and Eisenreich, W. (2005) Isoprenoid biosynthetic pathways as anti-infective drug targets. *Biochem. Soc. Trans.* **33**, 785–791
- 14 Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Turbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H. K. et al. (1999) Inhibitors of the nonmevalonate pathway of isoprenoid biosynthesis as antimalarial drugs. *Science* **285**, 1573–1576
- 15 Davey, M. S., Tyrrell, J. M., Howe, R. A., Walsh, T. R., Moser, B., Toleman, M. A. and Eberl, M. (2011) A promising target for treatment of multidrug-resistant bacterial infections. *Antimicrob. Agents Chemother.* **55**, 3635–3636
- 16 Singh, N., Cheve, G., Avery, M. A. and McCurdy, C. R. (2007) Targeting the methyl erythritol phosphate (MEP) pathway for novel antimalarial, antibacterial and herbicidal drug discovery: inhibition of 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) enzyme. *Curr. Pharm. Des.* **13**, 1161–1177
- 17 Sakamoto, Y., Furukawa, S., Ogihara, H. and Yamasaki, M. (2003) Fosmidomycin resistance in adenylate cyclase deficient (*cyt*) mutants of *Escherichia coli*. *Biosci. Biotechnol. Biochem.* **67**, 2030–2033
- 18 Sangari, F. J., Pérez-Gil, J., Carretero-Paulet, L., García-Lobo, J. M. and Rodríguez-Concepción, M. (2010) A new family of enzymes catalyzing the first committed step of the methylerythritol 4-phosphate (MEP) pathway for isoprenoid biosynthesis in bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 14081–14086
- 19 Brown, A. C. and Parish, T. (2008) Dxr is essential in *Mycobacterium tuberculosis* and fosmidomycin resistance is due to a lack of uptake. *BMC Microbiol.* **8**, 78
- 20 Fujisaki, S., Ohnuma, S., Horiuchi, T., Takahashi, I., Tsukui, S., Nishimura, Y., Nishino, T., Kitabatake, M. and Inokuchi, H. (1996) Cloning of a gene from *Escherichia coli* that confers resistance to fosmidomycin as a consequence of amplification. *Gene* **175**, 83–87
- 21 Boucher, Y. and Doolittle, W. F. (2000) The role of lateral gene transfer in the evolution of isoprenoid biosynthesis pathways. *Mol. Microbiol.* **37**, 703–716
- 22 Laupitz, R., Hecht, S., Amslinger, S., Zepeck, F., Kaiser, J., Richter, G., Schramek, N., Steinbacher, S., Huber, R., Arigoni, D. et al. (2004) Biochemical characterization of *Bacillus subtilis* type II isopentenyl diphosphate isomerase, and phylogenetic distribution of isoprenoid biosynthesis pathways. *Eur. J. Biochem.* **271**, 2658–2669
- 23 Boucher, Y., Huber, H., L'Haridon, S., Stetter, K. O. and Doolittle, W. F. (2001) Bacterial origin for the isoprenoid biosynthesis enzyme HMG-CoA reductase of the archaeal orders Thermoplasmatales and Archaeoglobales. *Mol. Biol. Evol.* **18**, 1378–1388
- 24 Begley, M., Gahan, C. G., Kollas, A. K., Hintz, M., Hill, C., Jomaa, H. and Eberl, M. (2004) The interplay between classical and alternative isoprenoid biosynthesis controls $\gamma\delta$ T cell bioactivity of *Listeria monocytogenes*. *FEBS Lett.* **561**, 99–104
- 25 Boucher, Y., Kamekura, M. and Doolittle, W. F. (2004) Origins and evolution of isoprenoid lipid biosynthesis in archaea. *Mol. Microbiol.* **52**, 515–527
- 26 Lombard, J. and Moreira, D. (2011) Origins and early evolution of the mevalonate pathway of isoprenoid biosynthesis in the three domains of life. *Mol. Biol. Evol.* **28**, 87–99
- 27 Grochowski, L. L., Xu, H. and White, R. H. (2006) *Methanocaldococcus jannaschii* uses a modified mevalonate pathway for biosynthesis of isopentenyl diphosphate. *J. Bacteriol.* **188**, 3192–3198
- 28 Chen, M. and Poulter, C. D. (2010) Characterization of thermophilic archaeal isopentenyl phosphate kinases. *Biochemistry* **49**, 207–217
- 29 Mabanglo, M. F., Schubert, H. L., Chen, M., Hill, C. P. and Poulter, C. D. (2010) X-ray structures of isopentenyl phosphate kinase. *ACS Chem. Biol.* **5**, 517–527
- 30 Erb, T. J., Evans, B. S., Cho, K., Warlick, B. P., Sriram, J., Wood, B. M., Imker, H. J., Sweedler, J. V., Tabita, F. R. and Gerlt, J. A. (2012) A RubisCO-like protein links SAM metabolism with isoprenoid biosynthesis. *Nat. Chem. Biol.* **8**, 926–932
- 31 Warlick, B. P., Evans, B. S., Erb, T. J., Ramagopal, U. A., Sriram, J., Imker, H. J., Sauder, J. M., Bonanno, J. B., Burley, S. K., Tabita, F. R. et al. (2012) 1-methylthio-D-xylulose 5-phosphate methylsulfurylase: a novel route to 1-deoxy-D-xylulose 5-phosphate in *Rhodospirillum rubrum*. *Biochemistry* **51**, 8324–8326
- 32 Albers, E. (2009) Metabolic characteristics and importance of the universal methionine salvage pathway recycling methionine from 5'-methylthioadenosine. *IUBMB Life* **61**, 1132–1142
- 33 Ershov, Y. V., Gantt, R. R., Cunningham, Jr, F. X. and Gantt, E. (2002) Isoprenoid biosynthesis in *Synechocystis* sp. strain PCC6803 is stimulated by compounds of the pentose phosphate cycle but not by pyruvate or deoxyxylulose-5-phosphate. *J. Bacteriol.* **184**, 5045–5051
- 34 Poliquin, K., Ershov, Y. V., Cunningham, Jr, F. X., Woreta, T. T., Gantt, R. R. and Gantt, E. (2004) Inactivation of *sl1556* in *Synechocystis* strain PCC 6803 impairs isoprenoid biosynthesis from pentose phosphate cycle substrates *in vitro*. *J. Bacteriol.* **186**, 4685–4693
- 35 Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirose, M., Sugiura, M., Sasamoto, S. et al. (1996) Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res.* **3**, 109–136
- 36 Woo, Y. H., Fernandes, R. P. and Proteau, P. J. (2006) Evaluation of fosmidomycin analogs as inhibitors of the *Synechocystis* sp. PCC6803 1-deoxy-D-xylulose 5-phosphate reductoisomerase. *Bioorg. Med. Chem.* **14**, 2375–2385
- 37 Kaneda, K., Kuzuyama, T., Takagi, M., Hayakawa, Y. and Seto, H. (2001) An unusual isopentenyl diphosphate isomerase found in the mevalonate pathway gene cluster from *Streptomyces* sp. strain CL190. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 932–937
- 38 Rothman, S. C., Johnston, J. B., Lee, S., Walker, J. R. and Poulter, C. D. (2008) Type II isopentenyl diphosphate isomerase: irreversible inactivation by covalent modification of flavin. *J. Am. Chem. Soc.* **130**, 4906–4913
- 39 Unno, H., Yamashita, S., Ikeda, Y., Sekiguchi, S. Y., Yoshida, N., Yoshimura, T., Kusunoki, M., Nakayama, T., Nishino, T. and Hemmi, H. (2009) New role of flavin as a general acid–base catalyst with no redox function in type 2 isopentenyl-diphosphate isomerase. *J. Biol. Chem.* **284**, 9160–9167
- 40 Sharma, N. K., Pan, J. J. and Poulter, C. D. (2010) Type II isopentenyl diphosphate isomerase: probing the mechanism with alkyne/allene diphosphate substrate analogues. *Biochemistry* **49**, 6228–6233
- 41 Durbecq, V., Sainz, G., Oudjama, Y., Clantin, B., Bompard-Gilles, C., Tricot, C., Caillet, J., Stalon, V., Droogmans, L. and Villeret, V. (2001) Crystal structure of isopentenyl diphosphate:dimethylallyl diphosphate isomerase. *EMBO J.* **20**, 1530–1537
- 42 Wouters, J., Oudjama, Y., Ghosh, S., Stalon, V., Droogmans, L. and Oldfield, E. (2003) Structure and mechanism of action of isopentenylpyrophosphate-dimethylallylpyrophosphate isomerase. *J. Am. Chem. Soc.* **125**, 3198–3199
- 43 Hahn, F. M., Hurlburt, A. P. and Poulter, C. D. (1999) *Escherichia coli* open reading frame 696 is *idi*, a nonessential gene encoding isopentenyl diphosphate isomerase. *J. Bacteriol.* **181**, 4499–4504
- 44 Rodríguez-Concepción, M., Campos, N., Lois, L. M., Maldonado, C., Hoeffler, J. F., Grosdemange-Billiard, C., Rohmer, M. and Boronat, A. (2000) Genetic evidence of branching in the isoprenoid pathway for the production of isopentenyl diphosphate and dimethylallyl diphosphate in *Escherichia coli*. *FEBS Lett.* **473**, 328–332
- 45 Bochar, D. A., Stauffacher, C. V. and Rodwell, V. W. (1999) Sequence comparisons reveal two classes of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *Mol. Genet. Metab.* **66**, 122–127
- 46 Li, D., Gui, J., Li, Y., Feng, L., Han, X., Sun, Y., Sun, T., Chen, Z., Cao, Y., Zhang, Y. et al. (2012) Structure-based design and screen of novel inhibitors for class II 3-hydroxy-3-methylglutaryl coenzyme A reductase from *Streptococcus pneumoniae*. *J. Chem. Inf. Model.* **52**, 1833–1841
- 47 Pérez-Gil, J., Calisto, B. M., Behrendt, C., Kurz, T., Fita, I. and Rodríguez-Concepción, M. (2012) Crystal structure of *Brucella abortus* deoxyxylulose-5-phosphate reductoisomerase-like (DRL) enzyme involved in isoprenoid biosynthesis. *J. Biol. Chem.* **287**, 15803–15809
- 48 Munos, J. W., Pu, X., Mansoorabadi, S. O., Kim, H. J. and Liu, H. W. (2009) A secondary kinetic isotope effect study of the 1-deoxy-D-xylulose-5-phosphate reductoisomerase-catalyzed reaction: evidence for a retroaldol-aldol rearrangement. *J. Am. Chem. Soc.* **131**, 2048–2049
- 49 Lauw, S., Illarionova, V., Bacher, A., Rohdich, F. and Eisenreich, W. (2008) Biosynthesis of isoprenoids: studies on the mechanism of 2C-methyl-D-erythritol-4-phosphate synthase. *FEBS J.* **275**, 4060–4073
- 50 Hunter, W. N. (2007) The non-mevalonate pathway of isoprenoid precursor biosynthesis. *J. Biol. Chem.* **282**, 21573–21577

- 51 Rohdich, F., Zepeck, F., Adam, P., Hecht, S., Kaiser, J., Laupitz, R., Grawert, T., Amslinger, S., Eisenreich, W., Bacher, A. and Arigoni, D. (2003) The deoxyxylulose phosphate pathway of isoprenoid biosynthesis: studies on the mechanisms of the reactions catalyzed by IspG and IspH protein. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1586–1591
- 52 Span, I., Gräwert, T., Bacher, A., Eisenreich, W. and Groll, M. (2012) Crystal structures of mutant IspH proteins reveal a rotation of the substrate's hydroxymethyl group during catalysis. *J. Mol. Biol.* **416**, 1–9
- 53 Span, I., Wang, K., Wang, W., Zhang, Y., Bacher, A., Eisenreich, W., Li, K., Schulz, C., Oldfield, E. and Groll, M. (2012) Discovery of acetylene hydratase activity of the iron–sulphur protein IspH. *Nat. Commun.* **3**, 1042
- 54 Roscioli, T., Kamsteeg, E. J., Buysse, K., Maystadt, I., van Reeuwijk, J., van den Elzen, C., van Beusekom, E., Riemersma, M., Pfundt, R., Vissers, L. E. et al. (2012) Mutations in ISPD cause Walker–Warburg syndrome and defective glycosylation of α -dystroglycan. *Nat. Genet.* **44**, 581–585
- 55 Willer, T., Lee, H., Lommel, M., Yoshida-Moriguchi, T., de Bernabe, D. B., Venzke, D., Cirak, S., Schachter, H., Vajsar, J., Voit, T. et al. (2012) ISPD loss-of-function mutations disrupt dystroglycan O-mannosylation and cause Walker–Warburg syndrome. *Nat. Genet.* **44**, 575–580
- 56 Kuzuyama, T., Shimizu, T., Takahashi, S. and Seto, H. (1998) Fosmidomycin, a specific inhibitor of 1-deoxy-D-xylulose 5-phosphate reductoisomerase in the nonmevalonate pathway for terpenoid biosynthesis. *Tetrahedron Lett.* **39**, 7913–7916
- 57 Zhang, B., Watts, K. M., Hodge, D., Kemp, L. M., Hunstad, D. A., Hicks, L. M. and Odom, A. R. (2011) A second target of the antimalarial and antibacterial agent fosmidomycin revealed by cellular metabolic profiling. *Biochemistry* **50**, 3570–3577
- 58 Sauret-Güeto, S., Urós, E. M., Ibáñez, E., Boronat, A. and Rodríguez-Concepción, M. (2006) A mutant pyruvate dehydrogenase E1 subunit allows survival of *Escherichia coli* strains defective in 1-deoxy-D-xylulose 5-phosphate synthase. *FEBS Lett.* **580**, 736–740
- 59 Pérez-Gil, J., Urós, E. M., Sauret-Güeto, S., Lois, L. M., Kirby, J., Nishimoto, M., Baidoo, E. E., Keasling, J. D., Boronat, A. and Rodríguez-Concepción, M. (2012) Mutations in *Escherichia coli* *aceE* and *ribB* genes allow survival of strains defective in the first step of the isoprenoid biosynthesis pathway. *PLoS ONE* **7**, e43775
- 60 Campos, N., Rodríguez-Concepción, M., Sauret-Güeto, S., Gallego, F., Lois, L.-M. and Boronat, A. (2001) *Escherichia coli* engineered to synthesize isopentenyl diphosphate and dimethylallyl diphosphate from mevalonate: a novel system for the genetic analysis of the 2-C-methyl-D-erythritol 4-phosphate pathway for isoprenoid biosynthesis. *Biochem. J.* **353**, 59–67
- 61 Yokota, A. and Sasajima, K. I. (1986) Formation of 1-deoxyketoses by pyruvate dehydrogenase and acetoin dehydrogenase. *Agric. Biol. Chem.* **50**, 2517–2524
- 62 Schoerker, U. and Sprenger, G. A. (1998) Thiamin-dependent enzymes as catalysts in chemoenzymatic syntheses. *Biochim. Biophys. Acta* **1385**, 229–243
- 63 Wungsintaweekul, J., Herz, S., Hecht, S., Eisenreich, W., Feicht, R., Rohdich, F., Bacher, A. and Zenk, M. H. (2001) Phosphorylation of 1-deoxy-D-xylulose by D-xylulokinase of *Escherichia coli*. *Eur. J. Biochem.* **268**, 310–316
- 64 Nemeria, N., Tittmann, K., Joseph, E., Zhou, L., Vazquez-Coll, M. B., Arjunan, P., Hübner, G., Furey, W. and Jordan, F. (2005) Glutamate 636 of the *Escherichia coli* pyruvate dehydrogenase-E1 participates in active center communication and behaves as an engineered acetolactate synthase with unusual stereoselectivity. *J. Biol. Chem.* **280**, 21473–21482
- 65 Bacher, A., Eberhardt, S., Fisher, M., Kis, K. and Richter, G. (2000) Biosynthesis of vitamin B₂ (riboflavin). *Annu. Rev. Nutr.* **20**, 153–167
- 66 Sauret-Güeto, S., Ramos-Valdivia, A., Ibáñez, E., Boronat, A. and Rodríguez-Concepción, M. (2003) Identification of lethal mutations in *Escherichia coli* genes encoding enzymes of the methylerythritol phosphate pathway. *Biochem. Biophys. Res. Commun.* **307**, 408–415
- 67 Missinou, M. A., Borrmann, S., Schindler, A., Issifou, S., Adegnika, A. A., Matsiegui, P. B., Binder, R., Lell, B., Wiesner, J., Baranek, T. et al. (2002) Fosmidomycin for malaria. *Lancet* **360**, 1941–1942
- 68 Kuemmerle, H. P., Murakawa, T., Soneoka, K. and Konishi, T. (1985) Fosmidomycin: a new phosphonic acid antibiotic. Part I. Phase I tolerance studies. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **23**, 515–520
- 69 Wistrom, J., Norrby, S. R., Myhre, E. B., Eriksson, S., Granstrom, G., Lagergren, L., Englund, G., Nord, C. E. and Svenungsson, B. (2001) Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. *J. Antimicrob. Chemother.* **47**, 43–50

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