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Abstract

Main Conclusion

This review summarizes the recent developments in the study of isoprenyl diphosphate synthases with an emphasis on analytical techniques, product length determination, and the physiological consequences of manipulating expression *in planta*.

The highly diverse structures of all terpenes are synthesized from the five carbon precursors dimethylallyl diphosphate and a varying number of isopentenyl diphosphate units through 1'-4 alkylation reactions. These elongation reactions are catalyzed by isoprenyl diphosphate synthases (IDS). IDS are classified depending on the configuration of the ensuing double bond as *trans*- and *cis*-IDS. In addition, IDS are further stratified by the length of their prenyl diphosphate product. This review discusses analytical techniques for the determination of product length and the factors that control product length, with an emphasis on alternative mechanisms. With recent advances in analytics, multiple IDS of *Arabidopsis thaliana* have been recently reinvestigated and demonstrated to yield products of different lengths than originally reported, which is summarized here. As IDS dictate prenyl diphosphate length and thereby which class of terpenes is ultimately produced, another focus of this review is the impact that altering IDS expression has on terpenoid natural product accumulation. Finally, recent findings regarding the ability of a few IDS to not catalyze 1'-4 alkylation reactions, but instead produce irregular products, with unusual connectivity, or act as terpene synthases, are also discussed.

Keywords

Isoprenyl diphosphate synthases, terpenes, isoprenoids, prenyl diphosphates, Arabidopsis

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Comments

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Isoprenyl diphosphates synthases, the chain length determining step in terpene biosynthesis

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Abstract

Main conclusion: This review summarizes recent developments in the study of isoprenyl diphosphate synthases with an emphasis on analytical techniques, product length determination and the physiological consequences of manipulating expression *in planta*.

The highly diverse structures of all terpenes are synthesized from the 5 carbon precursors dimethylallyl diphosphate and a varying number of isopentenyl diphosphate units through 1'-4 alkylation reactions. These elongation reactions are catalyzed by isoprenyl diphosphate synthases (IDS). IDS are classified depending on the configuration of the ensuing double bond as *trans*- and *cis*-IDS. Additionally IDS are further stratified by the length of their prenyl diphosphate product. This review discusses analytical techniques for determination of product length and the factors that control product length, with an emphasis on alternative mechanisms. With recent advances in analytics, multiple IDS of *Arabidopsis thaliana* have been recently reinvestigated and demonstrated to yield products of different lengths than originally reported, which is summarized here. As IDS dictate prenyl diphosphate length and thereby which class of terpenes are ultimately produced, another focus of this review is the impact that altering IDS expression has on terpenoid natural product accumulation. Finally, recent findings regarding the ability of a few IDS to not catalyze 1'-4 alkylation reactions, but instead produce irregular products, with unusual connectivity, or act as terpene synthases, is also discussed.

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Introduction

Terpenes are a structurally diverse class of metabolites, with currently over 60,000 known compounds (Köksal et al. 2011). Despite this large diversity in structures, they follow the so called 'isoprene rule', that all of them are synthesized from C_5 isopentenoid building blocks (Christianson 2008). They are ubiquitously present in every living organism and despite their broad structural and functional diversity, the initial steps of terpene biosynthesis are highly conserved. The basic C_5 building blocks, isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP), are synthesized by two pathways, the mevalonate (MVA) and the methylerythritol phosphate (MEP) pathways. The MVA pathway uses three units of acetyl-CoA, whereas the MEP pathway starts from glyceraldehyde 3-phosphate and pyruvate (Arigoni et al. 1997; Rodríguez-Concepción 2006). In both pathways, specific regulatory enzymes have been identified (Hemmerlin et al. 2012). The MVA pathway is common to all eukaryotes and some bacteria, whereas the MEP pathway is present in most bacteria and photosynthetic eukaryotes (Lombard and Moreira 2011; Hemmerlin et al. 2012). Plants are distinct as both pathways are present in all species, with the MVA pathway localized in the cytosol and the MEP pathway in the plastids. The MVA pathway produces exclusively IDP, later isomerized to DMADP by isopentenyl diphosphate isomerase (IDI). The MEP pathway produces both DMADP and IDP in a fixed ratio of 1:5-6 (Hemmerlin et al. 2012), which can be altered by IDI for the varying requirements of the organism (Berthelot et al. 2012).

After formation of the basic C_5 building blocks, the smallest terpene with only 5 carbon atoms, isoprene, can be produced directly from DMADP (Behnke et al. 2007). For the biosynthesis of terpenes larger than C_5, isoprenyl diphosphate synthases (IDS) are critical. IDS, also called prenyltransferases, catalyze the sequential head-to-tail condensation of IDP with an allylic substrate, usually DMADP, but also geranyl diphosphate (GDP), farnesyl diphosphate (FDP), geranylgeranyl diphosphate (GGDP), geranylfarnesyl diphosphate (GFDP), and so on, in order of increasing chain length (Figure 1). For IDS two naming conventions exist. In one the IDS is named after the main product – e.g. geranyl diphosphate synthase (GDPS) produce GDP, FDPS produce FDP, GGDPS produce GGDP, etc. The alternative convention is naming all IDS followed by a number in order of discovery, e.g. IDS1, IDS2 and so on. The first naming convention has the advantage of identifying the product with the enzyme name, while the other is better if one IDS has multiple products or the main product was initially misidentified, as the enzyme would not have to be renamed in such cases.

From the carbon-carbon double-bond configuration of the product, IDS can be classified as either *cis*- or *trans*-IDS (Liang et al. 2002). Although carrying out the same reaction, these are evolutionarily and

structurally distinct from each other. However both types of IDS use divalent cations as cofactors for catalysis, in most cases Mg^{2+} (Liang et al. 2002; Aaron and Christianson 2010). Other metal cofactors have been tested but are generally found to result in lower catalytic activities (Tholl et al. 2001; Sen et al. 2007a; Sen et al. 2007b; Sen et al. 2007c; Vandermoten et al. 2009b; Barbar et al. 2013). The catalytic activity of *trans*-IDS depends on the first and second aspartate rich motifs (FARM/SARM). The FARM is found as either DDxxD or DDxxxxD sequences, whereas the SARM is only present as DDxxD. Both motifs together bind three divalent cations (Mg^{2+}) which themselves bind to the diphosphate of the allylic substrate (e.g., DMADP/GDP/FDP), while IDP is coordinated by basic residues such as lysine and arginine (Aaron and Christianson 2010). Mutations of the aspartate in either the FARM or SARM decreases catalytic activity (Liang et al. 2002). Surprisingly, there is a functional bacterial IDS from *Mycobacterium tuberculosis* whose FARM sequence is altered to DRxxD and its SARM sequence is altered to RDxxA (Mann et al. 2011). *Cis*-IDS lack such distinct motifs, instead they utilize aspartate and glutamate residues distributed throughout the enzymatic sequence. These also bind Mg^{2+} and mutations thereof similarly decrease catalytic activity (Liang et al. 2002).

In addition to the classification of *cis*- and *trans*-IDS, the enzymes can be classified by the length of their products, either short (C_{10}-C_{25}), medium (C_{30}-C_{45}) or long-chain (≥C_{40}) prenyl diphosphates. *Trans*-IDS are more often involved in the biosynthesis of short-chain prenyl diphosphates, whereas *cis*-IDS generally produce longer prenyl diphosphates, with natural rubber containing chains up to 5000 C_5 units in length (Liang et al. 2002; Wang and Ohnuma 2000). However a medium/long-chain *trans*-IDS is present in *Arabidopsis thaliana* (Hsieh et al. 2011) and a group of short-chain *cis*-IDS is found in several tomato species (Sallaud et al. 2009; Schilmiller et al. 2009; Akhtar et al. 2013; Zi et al. 2014).

This review will discuss recent developments in analytical techniques, how product length is determined and the physiological consequences of manipulating IDS expression *in planta*. The focus is on plant IDS, however non-plant IDS are used to illustrate specific aspects that have not yet been investigated with plant IDS.

Analytical measurement of prenyl diphosphate products *in vitro* and *in vivo*

A recent publication revealed that of the over 5,800 annotated IDS only 46 have been biochemically characterized in detail (Wallrapp et al. 2013). In the same publication 79 additional IDS were biochemically characterized. However even with this significant increase of IDS with known product specificity, assigning the length of prenyl diphosphate products by means of sequence comparison is still not accurate. Thus, there is still a need for reliable methods to identify the product distribution of individual IDS.

There are established assays for detection of prenyl diphosphates that use radioactively-labeled [^{14}C]-IDP as substrate, generally followed by acid or alkaline hydrolysis. The resulting prenyl alcohols are easily extracted and then analyzed via radio-gas chromatography (radio-GC), radio-high-performance liquid chromatography (radio-HPLC), thin layer chromatography (TLC) or liquid scintillation counting (LSC) (Zhang and Poulter 1993; Cunillera et al. 1996; Martin et al. 2002; Masferrer et al. 2002; Gilg et al. 2005; Gilg et al. 2009; Manzano et al. 2006; Schmidt and Gershenzon 2008, 2007; Schmidt et al. 2010; Chang et al. 2010; Kim et al. 2010)(Table 1). The previously mentioned methods involve radioactive isotopes, which in most cases require special permits and dedicated lab spaces for their use. A less sensitive method without radio-labeled substrates is GC-MS, although this also necessitates a dephosphorylation step (Demissie et al. 2013; Mann et al. 2011; Akhtar et al. 2013; Schilmiller et al. 2009). Such hydrolysis of the diphosphate is rather laborious, time-consuming and frequently imprecise. In addition, cleavage of the diphosphate under highly acidic conditions usually generates more than one reaction product, making interpretation and quantification fairly complicated (Schilmiller et al. 2009). If instead of acidic conditions, phosphatases are used to cleave the diphosphate enzymatically, the pH of the enzyme assay needs to be shifted first from the optimum for IDS, towards that of the utilized phosphatase. Moreover prenyl diphosphates have been shown to be suboptimal substrates for commercially available phosphatases (Kurokawa et al. 1971). Nevertheless, GC based methods are well suited to resolving different structural and double bond isomers of the same prenyl length (Demissie et al. 2013; Schilmiller et al. 2009). Radio-HPLC in theory should be able to resolve structural isomers, this method was however never used for such purposes. In contrast the resolution of TLC is able to distinguish chain length (Akhtar et al. 2013), but is not able to reliably resolve structural isomers of the same length. LSC can neither resolve chain length nor isomers (Table 1).

The most recently developed method uses HPLC-MS/MS for quantification and works without prior dephosphorylation, improving the signal to noise ratio by two orders of magnitude in comparison to a similar radio-HPLC based assay. This technique has so far been used for short-chain products up to GFDP (C_{25}) and can also distinguish between different double bond isomers of the same prenyl diphosphate length, although additional structural isomers – i.e. irregular prenyl diphosphates – have not yet been tested (Nagel et al. 2012; Nagel et al. 2014; Richter et al. 2015; Nagel et al. 2015; Beran et al. 2016). For prenyl diphosphates longer than GFDP, radioactive assays followed by thin layer chromatography are still the method of choice (Wallrapp et al. 2013; Wang et al. 2016), as ionization of these larger molecules is suppressed in mass spectrometers. This effect can already be observed with GGDP when compared to FDP and GDP (Nagel et al. 2012). Tuning the LC-MS/MS for the detection of solanesyl diphosphate (C_{45}) was not successful, as no ions were detected even after increasing the sample concentration by 100 fold

relative to GGDP. While IDP and DMADP can in theory also be detected with this method, they seem to flow through the column with little interaction with the matrix, eluting very early, and are not separated, preventing distinct quantification of these. Future directions for the development of LC-MS(/MS) based methods would be to increase the ability to reliably analyze IDP and DMADP and also the longer prenyl diphosphates, while increases in sensitivity will largely depend on advances in the mass spectrometers themselves.

The HPLC-MS/MS based method was successfully used for quantification of prenyl diphosphates *in vivo*, representing the first such quantification of GDP, FDP and GGDP together *in planta*. The most recent method describing quantification of GGDP alone dates back to 1983, requiring 1.5 kg of etiolated oat seedlings as starting material, followed by 6 purification steps (Benz et al. 1983). Use of the recently developed HPLC-MS/MS method reduces the amount of requisite plant mass 2000 times (i.e., to 0.75 g), and the purification to a single step using mixed mode interaction SPE columns (Nagel et al. 2014). The amount of GGDP detected in oat seedlings was 10 times higher than the amount in *P. glauca* needles. This can be rationalized by the etiolated state of these seedlings, which would need larger quantities of GGDP for the synthesis of chlorophyll and carotenoids to initiate photosynthesis. Quantification of GDP has been performed in several publications using acid hydrolysis, whereby geraniol is formed from GDP and rearranges to linalool, with subsequent quantification by proton transfer reaction mass spectrometry (PTR-MS). Using acid hydrolysis, significantly larger amounts of GDP (200-fold) were reported for spruce (20 pmol mg^{-1} FW)(Ghirardo et al. 2010; Nogues et al. 2006) compared to using the HPLC-MS/MS based method (0.1 pmol mg^{-1} FW)(Nagel et al. 2014). This difference is most likely caused by the presence of already formed linalool or the conversion of other terpenes to linalool under the highly acidic conditions, as already discussed by the authors themselves (Ghirardo et al. 2010). Although the extraction protocol for the HPLC-MS/MS based method already incorporated internal standards that are similar to prenyl diphosphates, the most reliable means of quantification would be stable isotope labeled compounds (Stokvis et al. 2005). Fortunately, a procedure for chemical synthesis of hexadeuterated FDP and GGDP was recently published, which would be useful in supplying standards for such more reliable quantification of prenyl diphosphates *in vivo* (Subramanian et al. 2013).

Quantification of GDP, FDP and GGDP allows comparison with the reported concentrations of their direct precursors IDP and DMADP. Prenyl diphosphate amounts in *P. glauca* vary between 0.1-0.5 nmol g^{-1} FW in needle tissue (Nagel et al. 2014), whereas DMADP can be found in amounts more than 100 fold higher in *Populus* x *canescens* leaves (21-36 nmol g^{-1} DW) and in even higher amounts in pine (50-650 nmol g^{-1} FW) and *P. abies* (100 nmol g^{-1} FW) needles (Rosenstiel et al. 2002; Behnke et al. 2007; Ghirardo

et al. 2010). Even though only DMADP was quantified, the equilibrium between DMADP and IDP is established by isopentenyl diphosphate isomerases in the plastid and cytosol (Berthelot et al. 2012). Interestingly the equilibrium is different from the initial ratio produced by hydroxymethylbutenyl diphosphate reductase (HDR) in the plastid (Vickers et al. 2014). The overall DMADP:IDP ratio in kudzu (*Pueraria montana*) was determined to be close to 2 in light adapted plants (Zhou et al. 2013). Though different methods and different species were used for quantifying prenyl diphosphates, the available data indicates that the IDS substrates, DMADP and IDP, are present at higher levels than the IDS products, GDP, FDP and GGDP. Therefore, IDS seem to represent an overall limiting step in terpene biosynthesis, which should be investigated in future research through alteration of IDS expression, even beyond that discussed in detail later in this review.

Determination of Product specificity

The product specificity of short-chain *trans*-IDS is to a large extent regulated by the identity of the amino acids on the N-terminal side of the FARM. Most notably the amino acids in the positions falling 4, 5, 8 and 11 residues before the FARM. These form a pocket that can limit the size of the growing prenyl carbon chain. Increasing the size of those amino acids causes steric hindrance and decreases the length of the final prenyl diphosphate product. The converse is also true, which has led to the molecular ruler theory (Nagel et al. 2015; Wang et al. 2016; Oldfield and Lin 2012; Wang and Ohnuma 2000; Liang et al. 2002; Vandermoten et al. 2009a; Vandermoten et al. 2009b). Recently arginine substitution for two aspartates in the FARM and SARM of IDSs from *Mycobacterium tuberculosis* were identified as another factor that can influence chain length to some degree (Nagel et al. 2018). Some findings further indicate the importance of the interface between IDS subunits, which often form dimers, as this is known to be relevant to product specificity (Ogawa et al. 2011; Chang et al. 2013). In particular, beyond the usual formation of homodimers, certain *trans*-IDS can also form heterodimers/tetramers with non-catalytic small subunits, which can have distinct effects on product outcome or stimulate activity without changing product outcome (Tholl et al. 2004; Orlova et al. 2009; Zhou et al. 2017). For long-chain IDS it has been shown that the purified enzyme requires the presence of detergents or phospholipids for effective turnover. These amphiphilic compounds help sequester the hydrophobic prenyl chain as it is extruded from the enzyme into the aqueous assay buffer. However, in addition to increasing enzymatic activity, use of different detergents can lead to variation in the chain length of the final prenyl diphosphate product (Pan et al. 2013).

Some IDS naturally release prenyl diphosphates of different chain lengths, although this has been most frequently reported with long-chain IDS under *in vitro* conditions (Pan et al. 2013; Hsieh et al. 2011;

Wallrapp et al. 2013). Nevertheless, short-chain IDS also can release multiple products, as demonstrated for IDS from aphids and bark beetles that produce GDP and FDP, and for IDS1 from *Picea abies*, which produces GDP and GGDP, but does not release the intermediately formed FDP (Vandermoten et al. 2008; Vandermoten et al. 2009b; Schmidt et al. 2010). The product specificity of some aphid and bark beetle short chain IDS further appears to be modulated by the ratio of IDP and DMADP (Vandermoten et al. 2008; Vandermoten et al. 2009b; Keeling et al. 2013), which also has been demonstrated with long-chain IDS (Wallrapp et al. 2013).

Besides the factors discussed above, the identity of the divalent metal ion co-factor also has recently been shown to affect product chain length. Such influence was first shown for a long-chain IDS, where exchanging Mg^{2+} to Co^{2+} increased the product size by one C_5 unit (Ohnuma et al. 1993). For short-chain IDS it was reported that Mg^{2+} was the preferred co-factor, with use of other metals usually decreasing enzymatic activity, such that alternative divalent metal ions were typically not investigated, even for the ability to change product output (Tholl et al. 2001; Sen et al. 2007a; Sen et al. 2007b; Sen et al. 2007c; Vandermoten et al. 2009b; Barbar et al. 2013). However, it has recently been reported that two insect IDS, from the leaf beetle *Phaedon cochleariae* and the mosquito *Aedes aegypti,* are able to utilize either Mg^{2+} or Co^{2+} as their metal cofactor without reduced enzyme activity. Instead the identity of the divalent metal co-factor seems to determine product chain length, as FDP is produced with Mg^{2+} and GDP with Co^{2+} (Frick et al. 2013; Rivera-Perez et al. 2015). If both are available, Co^{2+} is preferred for at least the IDS from *P. cochleariae*. These two divalent metal ions have differing atomic radii, which might induce changes in the protein structure that influence the size of the active site cavity. Regardless of exact mechanism, metal co-factor specificity also appears to influence IDS product specificity, although it is unknown if similar mechanisms are used in plants to alter the product specificity of IDS or if this is only relevant in insects.

Given all of the factors described above, it is perhaps not surprising that the length of the prenyl diphosphate produced by *trans*-IDS cannot be accurately predicted through bioinformatics approaches (Wallrapp et al. 2013). In addition, regulation of product length for *cis*-IDS is even less well understood, although it has been reported that the number of amino acids found between conserved regions III and IV of *cis*-IDS varies between those producing long- versus short-chain prenyl diphosphates (Akhtar et al. 2013), and that production of C_{10} versus C_{15} by short-chain *cis*-IDS from tomato is governed by a few amino acids in the active site (Kang et al. 2014).

Trans*-IDS in *Arabidopsis thaliana

Most of the genes involved in terpenoid biosynthesis from the model plant *A. thaliana* have been identified (Tholl and Lee 2011), including the multitude of *trans*-IDS genes found in its genome. However, some of them were initially misidentified. Therefore the latest biochemically characterized activities of the *A. thaliana trans*-IDS are summarized here. The originally designated lone GDPS was recently shown to produce medium/long-chain prenyl diphosphates instead and renamed polyprenyl diphosphate synthase PDPS (Hsieh et al. 2011). The two FDPS, each of which has two splicing variants, all produce FDP as their sole product (Cunillera et al. 1996; Closa et al. 2010; Keim et al. 2012). However, major changes in assigned functionality have been reported for those *trans*-IDS originally all termed GGDPS, of which 11 (renamed to *At*IDS1-11) are present in the genome (Tholl and Lee 2011). An additional nonfunctional small subunit also is present, originally named GGDPS12 and since renamed *At*SSU (Nagel et al. 2015; Wang et al. 2016). IDS1 is a bifunctional enzyme which produces GGDP and GFDP from IDP and DMADP (Nagel et al. 2015). IDS2 and IDS11 both produce GGDP from IDP and DMADP (Nagel et al. 2015; Wang et al. 2016). However, IDS11 interacts with the small subunit *At*SSU, which leads to the release of GDP in addition to the subsequently formed GGDP (Chen et al. 2015; Wang and Dixon 2009). IDS3 and IDS4 also produce GGDP, but only from FDP and IDP, as DMADP does not serve as a substrate for these (Wang et al. 2016). IDS5 is an inactive pseudo-gene (Nagel et al. 2015; Wang et al. 2016). IDS6, IDS7, IDS9 and IDS10 produce GFDP as their sole product from IDP and DMADP (Nagel et al. 2015; Wang et al. 2016). Notably, in the genome the genes for these four IDS are clustered with subsequently acting terpene synthases (TPS) – i.e., these TPS produce cyclized sesterterpene skeletons from GFDP (Shao et al. 2017; Huang et al. 2017). Finally, IDS8 produces polyprenyl diphosphates with chain lengths in excess of C_{30} (Nagel et al. 2015; Wang et al. 2016).

RNAi and knock out of IDS

Limited information is available about knock-down or knock-out of plant IDS. Double knock-out of FDPS1 and FDPS2 in *A. thaliana* led to a lethal developmental arrest at a very early embryonic stage (Closa et al. 2010). In contrast to this, knock-down by viral induced gene silencing (VIGS) of an FDPS in *Nicotiana attenuata* reduced the transcript abundance, FDPS enzyme activity and sterol content, but the plants were still viable (Jassbi et al. 2008). The advantage of knock-down over knock-out approaches becomes evident when the enzyme is involved in primary metabolism like FDPS. In plants, as in most other eukaryotes, FDP and therefore FDPS are needed for *de novo* sterol synthesis. Sterols are essential for regulation of membrane fluidity and permeability, with further processing leading to hormonal steroids, such as the plant brassinosteroids (Schaller 2003), thus a complete knock-out would not

expected to be viable. The remaining activity of the FDPS in knock-down experiments might be sufficient to supply primary metabolism and still yield an observable phenotype without lethal effects. Knock-down of a GGDPS in *N. attenuata*, which was presumably only involved in secondary metabolism, reduced the amount of diterpene glycosides. This impaired defense against the tobacco hornworm, *Manduca sexta*, but had no adverse side effects on general plant viability in the absence of herbivory (Jassbi et al. 2008). Mutations in GGDPS11/IDS11 in *A. thaliana* affect chloroplast development and reduce chlorophyll and carotenoid concentrations, indicating that GGDPS11 is the major IDS involved in production of these compounds, as was also indicated by the finding that this was the hub enzyme in bioinformatics investigations (Ruiz-Sola et al. 2015; Ruppel et al. 2013). Interestingly overexpression of one of the GFDPS in *A. thaliana* also led to a chlorotic phenotype, most likely by depleting the IDP and DMADP pool and/or further conversion of GGDP to GFDP, which can then not be used for chlorophyll and carotenoid biosynthesis (Nagel et al. 2015).

Overexpression of IDS

Overexpression of IDS has only been successfully achieved in plants, as it has not been possible to do this in animals, with the ensuing results depending on the individual IDS and plant species. FDPS1 overexpression causes severe alterations in cytokinin homeostasis and induces senescence in *A. thaliana* (Masferrer et al. 2002; Manzano et al. 2006). By contrast, overexpression of a different FDPS in *Artemisia annua* increases artemisinin levels without the severe effects observed in *A. thaliana*, although some phenotypic variation in leaf morphology were noted (Han et al. 2006; Banyai et al. 2010; Kai et al. 2011). However, artemisinin biosynthesis is somewhat unique, as IDP and DMADP are not exclusively supplied by the MVA pathway as expected for a sesquiterpene. Instead, one IDP unit originates from the MEP pathway (Towler and Weathers 2007; Schramek et al. 2010). Unlike the previously mentioned IDS which are all localized in the cytosol, other IDS are localized to the plastid. Similar to the case in *A. annua,* overexpression of these has successfully targeted secondary (more specialized) metabolism. For example, with the native GGDPS of *Salvia miltiorrhiza* in hairy root cultures, which increased diterpenoid tanshinone levels (Kai et al. 2011), while overexpression of a GDPS in mint increased the total monoterpene content (Lange et al. 2011), and overexpression of a bifunctional IDS producing GDP and GGDP in *Catharanthus roseus* increased secologanin production, which was further enhanced by co-expression of a geraniol synthase (Kumar et al. 2018). Overexpression of the bifunctional GDP/GGDP IDS1 in *P. glauca* increased *in planta* prenyl diphosphates concentrations and the levels of total terpenoids, the later however by production of geranylgeranyl fatty acid ester diversion products (Nagel

et al. 2014). By contrast, IDS overexpression increased levels of the major terpenoids in mint, *C. roseus* and *S. miltiorrhiza*. It appears that overexpression of IDS in plants is strongly dependent on the subcellular compartment and cell type in which the enzyme is expressed and on the proportion of IDP and DMADP supplied by the MVA and MEP pathways. This hypothesis was tested by overexpressing a TPS or a TPS together with an IDS either in the cytosol or plastid of *Nicotiana tabacum* (Wu et al. 2006). The authors chose a monoterpene or sesquiterpene producing TPS together with an IDS thought to produce either GDP or FDP. Overexpression of the IDS producing FDP in combination with a sesquiterpene producing TPS increased the amount of sesquiterpenes compared to overexpression of the TPS alone. Moreover, if both enzymes were localized in the plastid rather than in the cytosol even greater increases were observed, indicating regulatory differences between the two compartments. Unfortunately, for monoterpene analysis these studies utilized the IDS that was then thought to produce GDP as its main product, but that since been shown to actually produce medium/long-chain prenyl diphosphates instead (Hsieh et al. 2011). This then provides a rationale for the reported minimal increase in monoterpenes after combined overexpression, and is consistent with production of longer chain products by this IDS. Accordingly, the effect of IDS overexpression depends on different cellular compartments (Wu et al. 2006), but also on the combination of promoter and investigated tissue. Another similar example arises from use of a fruit ripening specific promotor in tomato with a *cis*-IDS producing neryl diphosphate (NDP). The fruits of these plants showed a major increase in NDP derived monoterpenes coupled to a significant decrease of lycopene. This reduction of lycopene was traced to an inhibitory effect of the *cisoid* NDP on the activity of *trans*-GGDPS (Gutensohn et al. 2014).

Altered expression of non-catalytic small subunits

For some IDS, their activity and/or product specificity are altered upon interaction with non-catalytic small subunits (SSU). There are a few studies that investigate the consequences of terpene metabolism upon alteration of the expression of these SSU *in planta*. For instance overexpression of the *Arabidopsis* SSU slightly increased the amount of monoterpenes emitted by flowers, while SSU T-DNA insertion lines showed a more pronounced decrease of monoterpene emission (Chen et al. 2015). Expression of the SSU from snap dragon (*Antirrhinum majus*) in tobacco with a petal specific promotor from *Clarkia breweri* increased monoterpene emissions, but at the same time significantly reduced the levels of chlorophyll, carotenoids and gibberellins. These plants exhibited leaf chlorosis and a significant dwarfing phenotype. The results indicate that the chosen promotor does not direct petal specific expression in tobacco (Orlova et al. 2009). A more targeted expression of the same SSU in ripening

tomato fruits led to less drastic effects on overall plant physiology and increased monoterpene emissions, albeit with decreased lycopene content, only in fruits (Gutensohn et al. 2013). A special case is the interaction of the SSU from rice (*Oryza sativa*) with its native GGDPS, which does not alter product chain length distribution, but instead increases overall GGDPS activity. Overexpression of this SSU in rice increased chlorophyll content, along with slight reductions in plant height and carotenoid content. RNAi mediated reduction in SSU expression or knock-out mutants exhibited major reductions in carotenoid and chlorophyll content, as well as reduced height (Zhou et al. 2017). Altogether, it appears that alteration of SSU expression can have varying, even drastic effects on plant physiology. The effects strongly depend on the particular SSU (alteration of activity vs. product specificity) and heterologous expression pattern (global vs. tissue specific). Given the small number of publications available, there is need for future research to better understand how SSUs regulate terpene biosynthesis *in planta*.

IDS catalyzing alternate reactions

Some IDS from both the *cis*- and *trans*-IDS families catalyze usual reactions that differ from the usual 1'-4 alkylation reactions between IDP and DMADP, instead carrying out coupling reactions between two molecules of DMADP, or DMADP and another allylic substrate. One *cis*-IDS from *Lavandula x intermedia* produces lavandulyl diphosphate (Demissie et al. 2013) and two IDS from *Streptomyces* spp. produce cyclolavandulyl diphosphate or isosesquilavandulyl diphosphate (Ozaki et al. 2014; Teufel et al. 2014)(Figure 2). Their exact enzymatic mechanism has not yet been investigated in detail. However, mutational analysis of a *cis*-IDS from *Solanum habrochaites* identified the residue at position 103 as important for such irregular coupling, as a H103Y mutant of this produces limonene, lavendulol and an unidentified irregular product in addition to the (*Z,Z*)-FDP produced by the wild type enzyme (Chan et al. 2017). Crystal structures for cyclolavandulyl diphosphate synthase and isosesquilavandulyl diphosphate synthase, each both alone and in substrate bound complexes, were recently reported (Gao et al. 2018; Malwal et al. 2018). However, the critical amino acids responsible for their deviation from the usual 1'-4 alkylation reactions between IDP and DMADP towards irregular product linkages were not identified.

Trans-IDS from *Chrysanthemum cinerariaefolium* and *Artemisia tridentata* ssp. *spiciformis* have also been shown to couple two molecules of DMADP to produce chrysanthemyl diphosphate via an unusual 1'-2-3 alkylation reaction (Rivera et al. 2001; Hemmerlin et al. 2003)(Figure 2). In both enzymes the ability to form irregular products was investigated via side directed mutagenesis, and alterations in the SARM, which takes the form of NDxxD instead of DDxxD, were identified as critical for this unusual reaction (Erickson and Poulter 2003; Thulasiram et al. 2008). More recently the same IDS from *C.*

cinerariaefolium was further shown to also act as a TPS, producing chrysanthemol from chrysanthemyl diphosphate (Yang et al. 2014).

Similar to these findings, insects are now emerging as a rich source of IDS that carry out atypical reactions. Four *trans*-IDS from the flea beetle *Phyllotreta striolata* and one *trans*-IDS each from the harlequin bug *Murgantia histrionica* or Southern green stink bug *Nezara viridula* were shown not to catalyze prenyl elongation steps at all, but to be bona fide TPS (Beran et al. 2016; Lancaster et al. 2018a; Lancaster et al. 2018b). *P. striolata* also has an unusually high number of IDS and IDS-like enzymes in its genome and another *trans*-IDS was shown to produce the *cisoid* products neryl diphosphate from IDP and DMADP and (*Z*,*E*)-FDP from (*E*)-GDP and IDP, making it the first *trans*-IDS to yield *cisoid* products (Beran et al. 2016)(Figure 2).

Conclusion

IDS mediate an essential branch point in terpene biosynthesis, as they dictate the length of terpenes produced by a cell/organism. It is therefore essential to understand the product profile of individual IDS and the factors influencing their product length. This review summarizes recent developments in analytical techniques for such IDS product analysis, along with investigation of the factors that alter the product profile of *trans*-IDS. For *cis*-IDS our understanding of product outcome is more limited and should be further investigated in the future. Similarly, our understanding of the role of IDS in overall regulation of terpene biosynthesis needs to be advanced as well. Major future directions are tissue and cellular compartment specific alterations of IDS activity, instead of the current use of promotors with a global expression pattern. At the same time shifting the focus from the end products of terpene biosynthesis towards investigating alterations of the pathway intermediates would help to identify bottlenecks within such metabolism and specifically determine if and when IDS represent the bottleneck step in these pathways. During the revision of this review a study reported a specific increase of carotenoids in Arabidopsis through expression of a protein fusion between AtIDS11 and phytoene synthase (Camagna et al. 2018). Similar fusions with other downstream enzymes could be a novel strategy to specifically increase individual terpenes or at least a subset of terpenes. As more IDS, both *trans* and *cis*, are identified that catalyze irregular reactions, previously inactive IDS might need to be revisited and tested for their ability to catalyze similar or additional unusual reactions, which is especially applicable to insects, with at least two such reports appearing this year (2018).

References

Aaron JA, Christianson DW (2010) Trinuclear metal clusters in catalysis by terpenoid synthases. Pure and Applied Chemistry 82 (8):1585-1597. doi:10.1351/pac-con-09-09-37

Akhtar TA, Matsuba Y, Schauvinhold I, Yu G, Lees HA, Klein SE, Pichersky E (2013) The tomato cis–prenyltransferase gene family. The Plant Journal 73 (4):640-652. doi:10.1111/tpj.12063

Arigoni D, Sagner S, Latzel C, Eisenreich W, Bacher A, Zenk MH (1997) Terpenoid biosynthesis from 1-deoxy-d-xylulose in higher plants by intramolecular skeletal rearrangement. Proceedings of the National Academy of Sciences 94 (20):10600-10605. doi:10.1073/pnas.94.20.10600

Banyai W, Kirdmanee C, Mii M, Supaibulwatana K (2010) Overexpression of farnesyl pyrophosphate synthase (FPS) gene affected artemisinin content and growth of Artemisia annua L. Plant Cell Tissue and Organ Culture 103 (2):255-265. doi:10.1007/s11240-010-9775-8

Barbar A, Couture M, Sen SE, Beliveau C, Nisole A, Bipfubusa M, Cusson M (2013) Cloning, expression and characterization of an insect geranylgeranyl diphosphate synthase from Choristoneura fumiferana. Insect Biochemistry and Molecular Biology 43 (10):947-958. doi:10.1016/j.ibmb.2013.07.004

Behnke K, Ehlting B, Teuber M, Bauerfeind M, Louis S, Hänsch R, Polle A, Bohlmann J, Schnitzler J-P (2007) Transgenic, non-isoprene emitting poplars don't like it hot. The Plant Journal 51 (3):485-499. doi:10.1111/j.1365-313X.2007.03157.x

Benz J, Fischer I, Rüdiger W (1983) Determination of phytyl diphosphate and geranylgeranyl diphosphate in etiolated oat seedlings. Phytochemistry 22 (12):2801-2804. doi:http://dx.doi.org/10.1016/S0031-9422(00)97700-8

Beran F, Rahfeld P, Luck K, Nagel R, Vogel H, Wielsch N, Irmisch S, Ramasamy S, Gershenzon J, Heckel DG, Kollner TG (2016) Novel family of terpene synthases evolved from *trans*-isoprenyl diphosphate synthases in a flea beetle. Proc Natl Acad Sci U S A 113 (11):2922-2927. doi:10.1073/pnas.1523468113

Berthelot K, Estevez Y, Deffieux A, Peruch F (2012) Isopentenyl diphosphate isomerase: A checkpoint to isoprenoid biosynthesis. Biochimie 94 (8):1621-1634. doi:10.1016/j.biochi.2012.03.021

Camagna M, Grundmann A, Bär C, Koschmieder J, Beyer P, Welsch R (2018) Enzyme fusion removes competition for geranylgeranyl diphosphate in carotenogenesis. Plant Physiology. doi:10.1104/pp.18.01026

Chan Y-T, Ko T-P, Yao S-H, Chen Y-W, Lee C-C, Wang AHJ (2017) Crystal Structure and Potential Head-to-Middle Condensation Function of a *Z,Z*-Farnesyl Diphosphate Synthase. ACS Omega 2 (3):930-936. doi:10.1021/acsomega.6b00562

Chang TH, Hsieh FL, Ko TP, Teng KH, Liang PH, Wang AHJ (2010) Structure of a Heterotetrameric Geranyl Pyrophosphate Synthase from Mint (*Mentha piperita*) Reveals Intersubunit Regulation. Plant Cell 22 (2):454-467. doi:10.1105/tpc.109.071738

Chang WC, Song H, Liu HW, Liu P (2013) Current development in isoprenoid precursor biosynthesis and regulation. Curr Opin Chem Biol 17 (4):571-579. doi:10.1016/j.cbpa.2013.06.020

Chen Q, Fan D, Wang G (2015) Heteromeric Geranyl(geranyl) Diphosphate Synthase Is Involved in Monoterpene Biosynthesis in *Arabidopsis* Flowers. Mol Plant (0). doi:10.1016/j.molp.2015.05.001

Christianson DW (2008) Unearthing the roots of the terpenome. Current Opinion in Chemical Biology 12 (2):141-150. doi:10.1016/j.cbpa.2007.12.008

Closa M, Vranova E, Bortolotti C, Bigler L, Arro M, Ferrer A, Gruissem W (2010) The *Arabidopsis thaliana* FPP synthase isozymes have overlapping and specific functions in isoprenoid biosynthesis, and complete loss of FPP synthase activity causes early

developmental arrest. Plant Journal 63 (3):512-525. doi:10.1111/j.1365-313X.2010.04253.x
Cunillera N, Arro M, Delourme D, Karst F, Boronat A, Ferrer A (1996) *Arabidopsis thaliana* contains two differentially expressed farnesyl-diphosphate synthase genes. Journal of Biological Chemistry 271 (13):7774-7780
Demissie ZA, Erland LAE, Rheault MR, Mahmoud SS (2013) The Biosynthetic Origin of Irregular Monoterpenes in Lavandula ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF A NOVEL *cis*-PRENYL DIPHOSPHATE SYNTHASE GENE, LAVANDULYL DIPHOSPHATE SYNTHASE. Journal of Biological Chemistry 288 (9):6333-6341. doi:10.1074/jbc.M112.431171
Erickson HK, Poulter CD (2003) Chrysanthemyl Diphosphate Synthase. The Relationship among Chain Elongation, Branching, and Cyclopropanation Reactions in the Isoprenoid Biosynthetic Pathway. Journal of the American Chemical Society 125 (23):6886-6888. doi:10.1021/ja034520g
Frick S, Nagel R, Schmidt A, Bodemann RR, Rahfeld P, Pauls G, Brandt W, Gershenzon J, Boland W, Burse A (2013) Metal ions control product specificity of isoprenyl diphosphate synthases in the insect terpenoid pathway. Proceedings of the National Academy of Sciences of the United States of America 110 (11):4194-4199. doi:10.1073/pnas.1221489110
Gao J, Ko T-P, Chen L, Malwal SR, Zhang J, Hu X, Qu F, Liu W, Huang J-W, Cheng Y-S, Chen C-C, Yang Y, Zhang Y, Oldfield E, Guo R-T (2018) “Head-to-Middle” and “Head-to-Tail” *cis*-Prenyl Transferases: Structure of Isosesquilavandulyl Diphosphate Synthase. Angewandte Chemie International Edition 57 (3):683-687. doi:doi:10.1002/anie.201710185
Ghirardo A, Koch K, Taipale R, Zimmer INA, Schnitzler J-P, Rinne J (2010) Determination of de novo and pool emissions of terpenes from four common boreal/alpine trees by $^{13}CO_2$ labelling and PTR-MS analysis. Plant, Cell & Environment 33 (5):781-792. doi:10.1111/j.1365-3040.2009.02104.x
Gilg AB, Bearfield JC, Tittiger C, Welch WH, Blomquist GJ (2005) Isolation and functional expression of an animal geranyl diphosphate synthase and its role in bark beetle pheromone biosynthesis. Proceedings of the National Academy of Sciences of the United States of America 102 (28):9760-9765. doi:10.1073/pnas.0503277102
Gilg AB, Tittiger C, Blomquist GJ (2009) Unique animal prenyltransferase with monoterpene synthase activity. Naturwissenschaften 96 (6):731-735. doi:10.1007/s00114-009-0521-1
Gutensohn M, Nguyen TTH, McMahon RD, Kaplan I, Pichersky E, Dudareva N (2014) Metabolic engineering of monoterpene biosynthesis in tomato fruits via introduction of the non-canonical substrate neryl diphosphate. Metabolic Engineering 24:107-116. doi:https://doi.org/10.1016/j.ymben.2014.05.008
Gutensohn M, Orlova I, Nguyen TTH, Davidovich-Rikanati R, Ferruzzi MG, Sitrit Y, Lewinsohn E, Pichersky E, Dudareva N (2013) Cytosolic monoterpene biosynthesis is supported by plastid-generated geranyl diphosphate substrate in transgenic tomato fruits. Plant Journal 75 (3):351-363. doi:10.1111/tpj.12212
Han JL, Liu BY, Ye HC, Wang H, Li ZQ, Li GF (2006) Effects of overexpression of the endogenous farnesyl diphosphate synthase on the artemisinin content in *Artemisia annua* L. Journal of Integrative Plant Biology 48 (4):482-487. doi:10.1111/j.1744-7909.2006.00208.x
Hemmerlin A, Harwood JL, Bach TJ (2012) A raison d’être for two distinct pathways in the early steps of plant isoprenoid biosynthesis? Progress in Lipid Research 51 (2):95-148. doi:http://dx.doi.org/10.1016/j.plipres.2011.12.001
Hemmerlin A, Rivera SB, Erickson HK, Poulter CD (2003) Enzymes encoded by the farnesyl diphosphate synthase gene family in the big sagebrush *Artemisia tridentata* ssp

spiciformis. Journal of Biological Chemistry 278 (34):32132-32140. doi:10.1074/jbc.M213045200
Hsieh FL, Chang TH, Ko TP, Wang AHJ (2011) Structure and Mechanism of an *Arabidopsis* Medium/Long-Chain-Length Prenyl Pyrophosphate Synthase. Plant Physiology 155 (3):1079-1090. doi:10.1104/pp.110.168799
Huang AC, Kautsar SA, Hong YJ, Medema MH, Bond AD, Tantillo DJ, Osbourn A (2017) Unearthing a sesterterpene biosynthetic repertoire in the Brassicaceae through genome mining reveals convergent evolution. Proceedings of the National Academy of Sciences. doi:10.1073/pnas.1705567114
Jassbi AR, Gase K, Hettenhausen C, Schmidt A, Baldwin IT (2008) Silencing geranylgeranyl diphosphate synthase in *Nicotiana attenuata* dramatically impairs resistance to tobacco hornworm. Plant Physiology 146 (3):974-986. doi:10.1104/pp.107.108811
Kai GY, Xu H, Zhou CC, Liao P, Xiao JB, Luo XQ, You LJ, Zhang L (2011) Metabolic engineering tanshinone biosynthetic pathway in *Salvia miltiorrhiza* hairy root cultures. Metabolic Engineering 13 (3):319-327. doi:10.1016/j.ymben.2011.02.003
Kang JH, Gonzales-Vigil E, Matsuba Y, Pichersky E, Barry CS (2014) Determination of Residues Responsible for Substrate and Product Specificity of *Solanum habrochaites* Short-Chain cis-Prenyltransferases. Plant Physiology 164 (1):80-91. doi:10.1104/pp.113.230466
Keeling CI, Chiu CC, Aw T, Li M, Henderson H, Tittiger C, Weng HB, Blomquist GJ, Bohlmann J (2013) Frontalin pheromone biosynthesis in the mountain pine beetle, *Dendroctonus ponderosae*, and the role of isoprenyl diphosphate synthases. Proceedings of the National Academy of Sciences of the United States of America 110 (47):18838-18843. doi:10.1073/pnas.1316498110
Keim V, Manzano D, Fernandez FJ, Closa M, Andrade P, Caudepon D, Bortolotti C, Vega MC, Arro M, Ferrer A (2012) Characterization of *Arabidopsis* FPS Isozymes and FPS Gene Expression Analysis Provide Insight into the Biosynthesis of Isoprenoid Precursors in Seeds. Plos One 7 (11). doi:10.1371/journal.pone.0049109
Kim OT, Bang KH, Jung SJ, Kim YC, Hyun DY, Kim SH, Cha SW (2010) Molecular characterization of ginseng farnesyl diphosphate synthase gene and its up-regulation by methyl jasmonate. Biologia Plantarum 54 (1):47-53
Köksal M, Hu H, Coates RM, Peters RJ, Christianson DW (2011) Structure and mechanism of the diterpene cyclase *ent*-copalyl diphosphate synthase. Nat Chem Biol 7 (7):431-433. doi:http://www.nature.com/nchembio/journal/v7/n7/abs/nchembio.578.html#supplementary-information
Kumar SR, Shilpashree HB, Nagegowda DA (2018) Terpene Moiety Enhancement by Overexpression of Geranyl(geranyl) Diphosphate Synthase and Geraniol Synthase Elevates Monomeric and Dimeric Monoterpene Indole Alkaloids in Transgenic *Catharanthus roseus*. Frontiers in Plant Science 9 (942). doi:10.3389/fpls.2018.00942
Kurokawa T, Ogura K, Seto S (1971) Formation of polyprenyl phosphates by a cell-free enzyme of. Biochemical and Biophysical Research Communications 45 (1):251-257. doi:10.1016/0006-291x(71)90077-5
Lancaster J, Khrimian A, Young S, Lehner B, Luck K, Wallingford A, Ghosh SKB, Zerbe P, Muchlinski A, Marek PE, Sparks ME, Tokuhisa JG, Tittiger C, Kollner TG, Weber DC, Gundersen-Rindal DE, Kuhar TP, Tholl D (2018a) De novo formation of an aggregation pheromone precursor by an isoprenyl diphosphate synthase-related terpene synthase in the harlequin bug. Proc Natl Acad Sci U S A 115 (37):E8634-e8641. doi:10.1073/pnas.1800008115
Lancaster J, Lehner B, Khrimian A, Muchlinski A, Luck K, Köllner TG, Weber DC, Gundersen-Rindal DE, Tholl D (2018b) An IDS-Type Sesquiterpene Synthase Produces the Pheromone Precursor (Z)-α-Bisabolene in *Nezara viridula*. Journal of Chemical Ecology. doi:10.1007/s10886-018-1019-0

Lange BM, Mahmoud SS, Wildung MR, Turner GW, Davis EM, Lange I, Baker RC, Boydston RA, Croteau RB (2011) Improving peppermint essential oil yield and composition by metabolic engineering. Proceedings of the National Academy of Sciences of the United States of America 108 (41):16944-16949. doi:10.1073/pnas.1111558108

Liang PH, Ko TP, Wang AHJ (2002) Structure, mechanism and function of prenyltransferases. European Journal of Biochemistry 269 (14):3339-3354. doi:10.1046/j.1432-1033.2002.03014.x

Lombard J, Moreira D (2011) Origins and Early Evolution of the Mevalonate Pathway of Isoprenoid Biosynthesis in the Three Domains of Life. Mol Biol Evol 28 (1):87-99. doi:10.1093/molbev/msq177

Malwal SR, Gao J, Hu X, Yang Y, Liu W, Huang J-W, Ko T-P, Li L, Chen C-C, O'Dowd B, Khade RL, Zhang Y, Zhang Y, Oldfield E, Guo R-T (2018) Catalytic Role of Conserved Asparagine, Glutamine, Serine, and Tyrosine Residues in Isoprenoid Biosynthesis Enzymes. ACS Catalysis 8 (5):4299-4312. doi:10.1021/acscatal.8b00543

Mann FM, Thomas JA, Peters RJ (2011) Rv0989c encodes a novel (E)-geranyl diphosphate synthase facilitating decaprenyl diphosphate biosynthesis in *Mycobacterium tuberculosis*. Febs Letters 585 (3):549-554. doi:DOI 10.1016/j.febslet.2011.01.007

Manzano D, Busquets A, Closa M, Hoyerova K, Schaller H, Kaminek M, Arro M, Ferrer A (2006) Overexpression of farnesyl diphosphate synthase in *Arabidopsis* mitochondria triggers light-dependent lesion formation and alters cytokinin homeostasis. Plant Molecular Biology 61 (1-2):195-213. doi:10.1007/s11103-006-6263-y

Martin D, Tholl D, Gershenzon J, Bohlmann J (2002) Methyl Jasmonate Induces Traumatic Resin Ducts, Terpenoid Resin Biosynthesis, and Terpenoid Accumulation in Developing Xylem of Norway Spruce Stems. Plant Physiology 129 (3):1003-1018. doi:10.1104/pp.011001

Masferrer A, Arro M, Manzano D, Schaller H, Fernandez-Busquets X, Moncalean P, Fernandez B, Cunillera N, Boronat A, Ferrer A (2002) Overexpression of *Arabidopsis thaliana* farnesyl diphosphate synthase (FPS1S) in transgenic *Arabidopsis* induces a cell death/senescence-like response and reduced cytokinin levels. Plant Journal 30 (2):123-132. doi:10.1046/j.1365-313X.2002.01273.x

Nagel R, Berasategui A, Paetz C, Gershenzon J, Schmidt A (2014) Overexpression of an Isoprenyl Diphosphate Synthase in Spruce Leads to Unexpected Terpene Diversion Products That Function in Plant Defense. Plant Physiology 164 (2):555-569. doi:DOI 10.1104/pp.113.228940

Nagel R, Bernholz C, Vranova E, Kosuth J, Bergau N, Ludwig S, Wessjohann L, Gershenzon J, Tissier A, Schmidt A (2015) *Arabidopsis thaliana* isoprenyl diphosphate synthases produce the C25 intermediate geranylfarnesyl diphosphate. Plant J 84 (5):847-859. doi:10.1111/tpj.13064

Nagel R, Gershenzon J, Schmidt A (2012) Nonradioactive assay for detecting isoprenyl diphosphate synthase activity in crude plant extracts using liquid chromatography coupled with tandem mass spectrometry. Analytical Biochemistry 422 (1):33-38. doi:10.1016/j.ab.2011.12.037

Nagel R, Thomas J, Adekunle F, Mann F, Peters R (2018) Arginine in the FARM and SARM: A Role in Chain-Length Determination for Arginine in the Aspartate-Rich Motifs of Isoprenyl Diphosphate Synthases from *Mycobacterium tuberculosis*. Molecules 23 (10). doi:10.3390/molecules23102546

Nogues I, Brilli F, Loreto F (2006) Dimethylallyl diphosphate and geranyl diphosphate pools of plant species characterized by different isoprenoid emissions. Plant Physiology 141 (2):721-730. doi:10.1104/pp.105.073213

Ogawa T, Yoshimura T, Hemmi H (2011) Connected cavity structure enables prenyl elongation across the dimer interface in mutated geranylfarnesyl diphosphate synthase from

Methanosarcina mazei. Biochemical and Biophysical Research Communications 409 (2):333-337. doi:10.1016/j.bbrc.2011.05.018
Ohnuma S, Koyama T, Ogura K (1993) ALTERATION OF THE PRODUCT SPECIFICITIES OF PRENYLTRANSFERASES BY METAL-IONS. Biochemical and Biophysical Research Communications 192 (2):407-412. doi:10.1006/bbrc.1993.1430
Oldfield E, Lin F-Y (2012) Terpene Biosynthesis: Modularity Rules. Angewandte Chemie-International Edition 51 (5):1124-1137. doi:10.1002/anie.201103110
Orlova I, Nagegowda DA, Kish CM, Gutensohn M, Maeda H, Varbanova M, Fridman E, Yamaguchi S, Hanada A, Kamiya Y, Krichevsky A, Citovsky V, Pichersky E, Dudareva N (2009) The Small Subunit of Snapdragon Geranyl Diphosphate Synthase Modifies the Chain Length Specificity of Tobacco Geranylgeranyl Diphosphate Synthase in Planta. Plant Cell 21 (12):4002-4017. doi:10.1105/tpc.109.071282
Ozaki T, Zhao P, Shinada T, Nishiyama M, Kuzuyama T (2014) Cyclolavandulyl skeleton biosynthesis via both condensation and cyclization catalyzed by an unprecedented member of the *cis*-isoprenyl diphosphate synthase superfamily. J Am Chem Soc 136 (13):4837-4840. doi:10.1021/ja500270m
Pan JJ, Ramamoorthy G, Poulter CD (2013) Dependence of the Product Chain-Length on Detergents for Long-Chain *E*-Polyprenyl Diphosphate Synthases. Biochemistry 52 (29):5002-5008. doi:10.1021/bi400681d
Richter A, Seidl-Adams I, Köllner T, Schaff C, Tumlinson J, Degenhardt J (2015) A small, differentially regulated family of farnesyl diphosphate synthases in maize (Zea mays) provides farnesyl diphosphate for the biosynthesis of herbivore-induced sesquiterpenes. Planta:1-11. doi:10.1007/s00425-015-2254-z
Rivera-Perez C, Nyati P, Noriega FG (2015) A corpora allata farnesyl diphosphate synthase in mosquitoes displaying a metal ion dependent substrate specificity. Insect Biochemistry and Molecular Biology 64:44-50. doi:http://dx.doi.org/10.1016/j.ibmb.2015.07.010
Rivera SB, Swedlund BD, King GJ, Bell RN, Hussey CE, Shattuck-Eidens DM, Wrobel WM, Peiser GD, Poulter CD (2001) Chrysanthemyl diphosphate synthase: Isolation of the gene and characterization of the recombinant non-head-to-tail monoterpene synthase from *Chrysanthemum cinerariaefolium*. Proceedings of the National Academy of Sciences of the United States of America 98 (8):4373-4378. doi:10.1073/pnas.071543598
Rodríguez-Concepción M (2006) Early Steps in Isoprenoid Biosynthesis: Multilevel Regulation of the Supply of Common Precursors in Plant Cells. Phytochemistry Reviews 5 (1):1-15. doi:10.1007/s11101-005-3130-4
Rosenstiel TN, Fisher AJ, Fall R, Monson RK (2002) Differential Accumulation of Dimethylallyl Diphosphate in Leaves and Needles of Isoprene- and Methylbutenol-Emitting and Nonemitting Species. Plant Physiology 129 (3):1276-1284. doi:10.1104/pp.002717
Ruiz-Sola MÁ, Coman D, Beck G, Barja MV, Colinas M, Graf A, Welsch R, Rütimann P, Bühlmann P, Bigler L, Gruissem W, Rodríguez-Concepción M, Vranová E (2015) *Arabidopsis* GERANYLGERANYL DIPHOSPHATE SYNTHASE 11 is a hub isozyme required for the production of most photosynthesis-related isoprenoids. New Phytologist:n/a-n/a. doi:10.1111/nph.13580
Ruppel NJ, Kropp KN, Davis PA, Martin AE, Luesse DR, Hangarter RP (2013) MUTATIONS IN GERANYLGERANYL DIPHOSPHATE SYNTHASE 1 AFFECT CHLOROPLAST DEVELOPMENT IN *ARABIDOPSIS THALIANA* (BRASSICACEAE). American Journal of Botany 100 (10):2074-2084. doi:10.3732/ajb.1300124
Sallaud C, Rontein D, Onillon S, Jabes F, Duffe P, Giacalone C, Thoraval S, Escoffier C, Herbette G, Leonhardt N, Causse M, Tissier A (2009) A Novel Pathway for Sesquiterpene Biosynthesis from *Z,Z*-Farnesyl Pyrophosphate in the Wild Tomato Solanum habrochaites. Plant Cell 21 (1):301-317. doi:10.1105/tpc.107.057885

Schaller H (2003) The role of sterols in plant growth and development. Progress in Lipid Research 42 (3):163-175. doi:http://dx.doi.org/10.1016/S0163-7827(02)00047-4
Schilmiller AL, Schauvinhold I, Larson M, Xu R, Charbonneau AL, Schmidt A, Wilkerson C, Last RL, Pichersky E (2009) Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. Proceedings of the National Academy of Sciences of the United States of America 106 (26):10865-10870. doi:10.1073/pnas.0904113106
Schmidt A, Gershenzon J (2007) Cloning and characterization of isoprenyl diphosphate synthases with farnesyl diphosphate and geranylgeranyl diphosphate synthase activity from Norway spruce (*Picea abies*) and their relation to induced oleoresin formation. Phytochemistry 68 (21):2649-2659. doi:10.1016/j.phytochem.2007.05.037
Schmidt A, Gershenzon J (2008) Cloning and characterization of two different types of geranyl diphosphate synthases from Norway spruce (*Picea abies*). Phytochemistry 69 (1):49-57. doi:10.1016/j.phytochem.2007.06.022
Schmidt A, Wachtler B, Temp U, Krekling T, Seguin A, Gershenzon J (2010) A Bifunctional Geranyl and Geranylgeranyl Diphosphate Synthase Is Involved in Terpene Oleoresin Formation in *Picea abies*. Plant Physiology 152 (2):639-655. doi:10.1104/pp.109.144691
Schramek N, Wang H, Römisch-Margl W, Keil B, Radykewicz T, Winzenhörlein B, Beerhues L, Bacher A, Rohdich F, Gershenzon J, Liu B, Eisenreich W (2010) Artemisinin biosynthesis in growing plants of *Artemisia annua*. A $^{13}CO_2$ study. Phytochemistry 71 (2–3):179-187. doi:http://dx.doi.org/10.1016/j.phytochem.2009.10.015
Sen SE, Brown DC, Sperry AE, Hitchcock JR (2007a) Prenyltransferase of larval and adult *M-sexta* corpora allata. Insect Biochemistry and Molecular Biology 37 (1):29-40. doi:10.1016/j.ibmb.2006.09.010
Sen SE, Cusson M, Trobaugh C, Beliveau C, Richard T, Graham W, Mimms A, Roberts G (2007b) Purification, properties and heteromeric association of type-1 and type-2 lepidopteran farnesyl diphosphate synthases. Insect Biochemistry and Molecular Biology 37 (8):819-828. doi:10.1016/j.ibmb.2007.05.012
Sen SE, Trobaugh C, Beliveau C, Richard T, Cusson M (2007c) Cloning, expression and characterization of a dipteran farnesyl diphosphate synthase. Insect Biochemistry and Molecular Biology 37 (11):1198-1206. doi:10.1016/j.ibmb.2007.07.011
Shao J, Chen Q-W, Lv H-J, He J, Liu Z-F, Lu Y-N, Liu H-L, Wang G-D, Wang Y (2017) (+)-Thalianatriene and (−)-Retigeranin B Catalyzed by Sesterterpene Synthases from *Arabidopsis thaliana*. Organic Letters 19 (7):1816-1819. doi:10.1021/acs.orglett.7b00586
Stokvis E, Rosing H, Beijnen JH (2005) Stable isotopically labeled internal standards in quantitative bioanalysis using liquid chromatography/mass spectrometry: necessity or not? Rapid Communications in Mass Spectrometry 19 (3):401-407. doi:10.1002/rcm.1790
Subramanian T, Subramanian KL, Sunkara M, Onono FO, Morris AJ, Spielmann HP (2013) Syntheses of deuterium labeled prenyldiphosphate and prenylcysteine analogues for in vivo mass spectrometric quantification. J Labelled Compd Rad 56 (8):370-375. doi:Doi 10.1002/Jlcr.3049
Teufel R, Kaysser L, Villaume MT, Diethelm S, Carbullido MK, Baran PS, Moore BS (2014) One-Pot Enzymatic Synthesis of Merochlorin A and B. Angewandte Chemie International Edition 53 (41):11019-11022. doi:10.1002/anie.201405694
Tholl D, Croteau R, Gershenzon J (2001) Partial purification and characterization of the short-chain prenyltransferases, geranyl diphosphate synthase and farnesyl diphosphate synthase, from *Abies grandis* (grand fir). Archives of Biochemistry and Biophysics 386 (2):233-242. doi:10.1006/abbi.2000.2212
Tholl D, Kish CM, Orlova I, Sherman D, Gershenzon J, Pichersky E, Dudareva N (2004) Formation of monoterpenes in *Antirrhinum majus* and *Clarkia breweri* flowers involves

heterodimeric geranyl diphosphate synthases. Plant Cell 16 (4):977-992. doi:10.1105/tpc.020156
Tholl D, Lee S (2011) Terpene Specialized Metabolism in *Arabidopsis thaliana*. The Arabidopsis book / American Society of Plant Biologists 9:e0143. doi:10.1199/tab.0143
Thulasiram HV, Erickson HK, Poulter CD (2008) A common mechanism for branching, cyclopropanation, and cyclobutanation reactions in the isoprenoid biosynthetic pathway. Journal of the American Chemical Society 130 (6):1966-1971. doi:10.1021/ja0771282
Towler M, Weathers P (2007) Evidence of artemisinin production from IPP stemming from both the mevalonate and the nonmevalonate pathways. Plant Cell Reports 26 (12):2129-2136. doi:10.1007/s00299-007-0420-x
Vandermoten S, Charloteaux B, Santini S, Sen SE, Beliveau C, Vandenbol M, Francis F, Brasseur R, Cusson M, Haubruge E (2008) Characterization of a novel aphid prenyltransferase displaying dual geranyl/farnesyl diphosphate synthase activity (vol 582, pg 1928, 2008). Febs Letters 582 (16):2471-2471. doi:10.1016/j.febslet.2008.06.002
Vandermoten S, Haubruge E, Cusson M (2009a) New insights into short-chain prenyltransferases: structural features, evolutionary history and potential for selective inhibition. Cellular and Molecular Life Sciences 66 (23):3685-3695. doi:10.1007/s00018-009-0100-9
Vandermoten S, Santini S, Haubruge E, Heuze F, Francis F, Brasseur R, Cusson M, Charloteaux B (2009b) Structural features conferring dual Geranyl/Farnesyl diphosphate synthase activity to an aphid prenyltransferase. Insect Biochemistry and Molecular Biology 39 (10):707-716. doi:10.1016/j.ibmb.2009.08.007
Vickers CE, Bongers M, Liu Q, Delatte T, Bouwmeester H (2014) Metabolic engineering of volatile isoprenoids in plants and microbes. Plant, Cell & Environment 37 (8):1753-1775. doi:doi:10.1111/pce.12316
Wallrapp FH, Pan JJ, Ramamoorthy G, Almonacid DE, Hillerich BS, Seidel R, Patskovsky Y, Babbitt PC, Almo SC, Jacobson MP, Poulter CD (2013) Prediction of function for the polyprenyl transferase subgroup in the isoprenoid synthase superfamily. Proceedings of the National Academy of Sciences of the United States of America 110 (13):E1196-E1202. doi:10.1073/pnas.1300632110
Wang C, Chen Q, Fan D, Li J, Wang G, Zhang P (2016) Structural Analyses of Short-Chain Prenyltransferases Identify an Evolutionarily Conserved GFPPS Clade in Brassicaceae Plants. Molecular Plant 9 (2):195-204. doi:10.1016/j.molp.2015.10.010
Wang GD, Dixon RA (2009) Heterodimeric geranyl(geranyl)diphosphate synthase from hop (*Humulus lupulus*) and the evolution of monoterpene biosynthesis. Proceedings of the National Academy of Sciences of the United States of America 106 (24):9914-9919. doi:10.1073/pnas.0904069106
Wang KC, Ohnuma S (2000) Isoprenyl diphosphate synthases. Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids 1529 (1-3):33-48. doi:10.1016/s1388-1981(00)00136-0
Wu SQ, Schalk M, Clark A, Miles RB, Coates R, Chappell J (2006) Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. Nature Biotechnology 24 (11):1441-1447. doi:10.1038/nbt1251
Yang T, Gao L, Hu H, Stoopen G, Wang C, Jongsma MA (2014) Chrysanthemyl Diphosphate Synthase Operates in Planta as a Bifunctional Enzyme with Chrysanthemol Synthase Activity. Journal of Biological Chemistry 289 (52):36325-36335. doi:10.1074/jbc.M114.623348
Zhang DL, Poulter CD (1993) Analysis and Purification of Phosphorylated Isoprenoids by Reversed-Phase HPLC. Analytical Biochemistry 213 (2):356-361. doi:10.1006/abio.1993.1432
Zhou C, Li Z, Wiberley-Bradford AE, Weise SE, Sharkey TD (2013) Isopentenyl diphosphate and dimethylallyl diphosphate/isopentenyl diphosphate ratio measured with recombinant

isopentenyl diphosphate isomerase and isoprene synthase. Analytical Biochemistry 440 (2):130-136. doi:http://dx.doi.org/10.1016/j.ab.2013.05.028
Zhou F, Wang CY, Gutensohn M, Jiang L, Zhang P, Zhang D, Dudareva N, Lu S (2017) A recruiting protein of geranylgeranyl diphosphate synthase controls metabolic flux toward chlorophyll biosynthesis in rice. Proc Natl Acad Sci U S A 114 (26):6866-6871. doi:10.1073/pnas.1705689114
Zi J, Matsuba Y, Hong YJ, Jackson AJ, Tantillo DJ, Pichersky E, Peters RJ (2014) Biosynthesis of Lycosantalonol, a *cis*-Prenyl Derived Diterpenoid. Journal of the American Chemical Society 136 (49):16951-16953. doi:10.1021/ja508477e

Figure 1

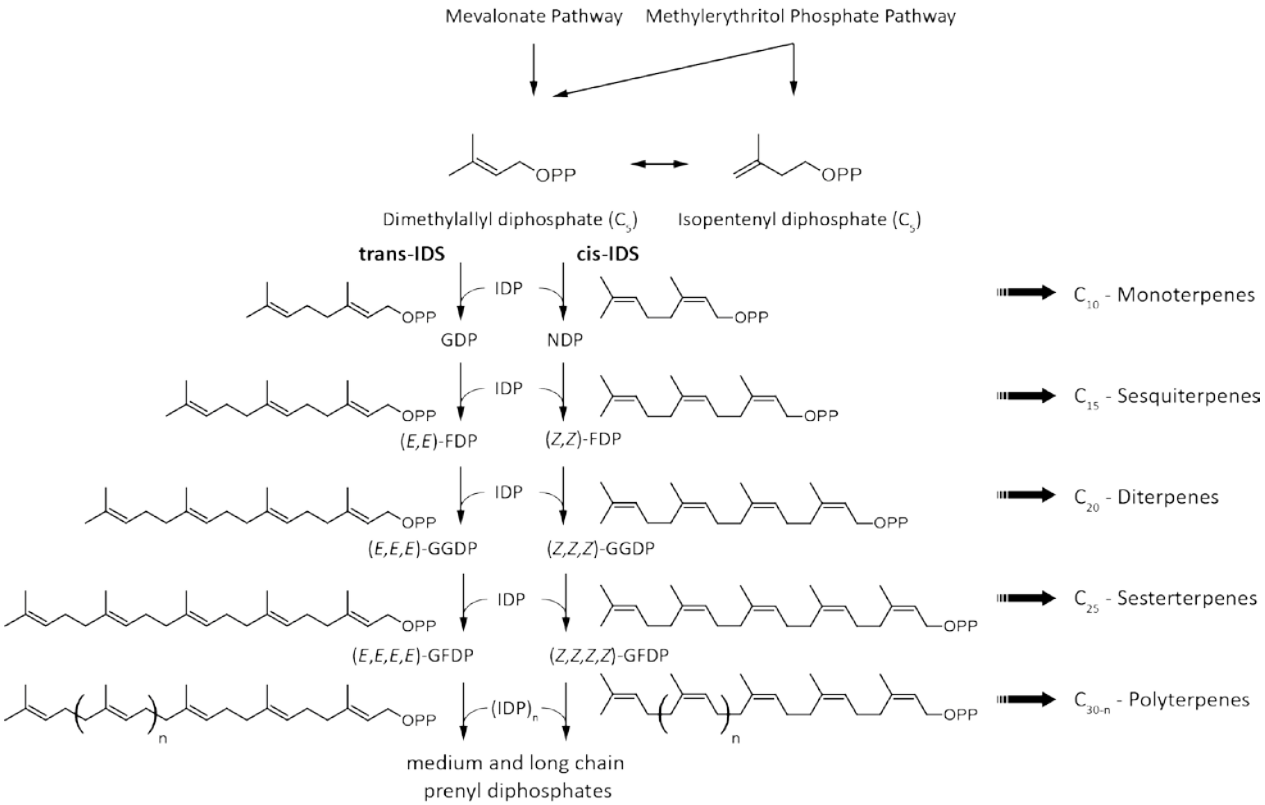

Figure 1: Reactions catalyzed by isoprenyl diphosphates synthases. Dimethylallyl diphosphate (DMADP) and a varying number of isopentenyl diphosphate (IDP) are used as substrates by isoprenyl diphosphate synthases (IDS) to generate longer prenyl diphosphate chains, which are the precursors for the indicated terpene classes. Depending on the stereoisomer of the double bond IDS are classified as either *trans*- or *cis*-IDS. Geranyl diphosphate and neryl diphosphate are the *trans* or *cis*-isomers at the C_{10} stage. Longer prenyl diphosphates share the same name and the double bond isomer is classified by *E* or *Z* nomenclature starting from the double bond closest to the diphosphate.

Figure 2

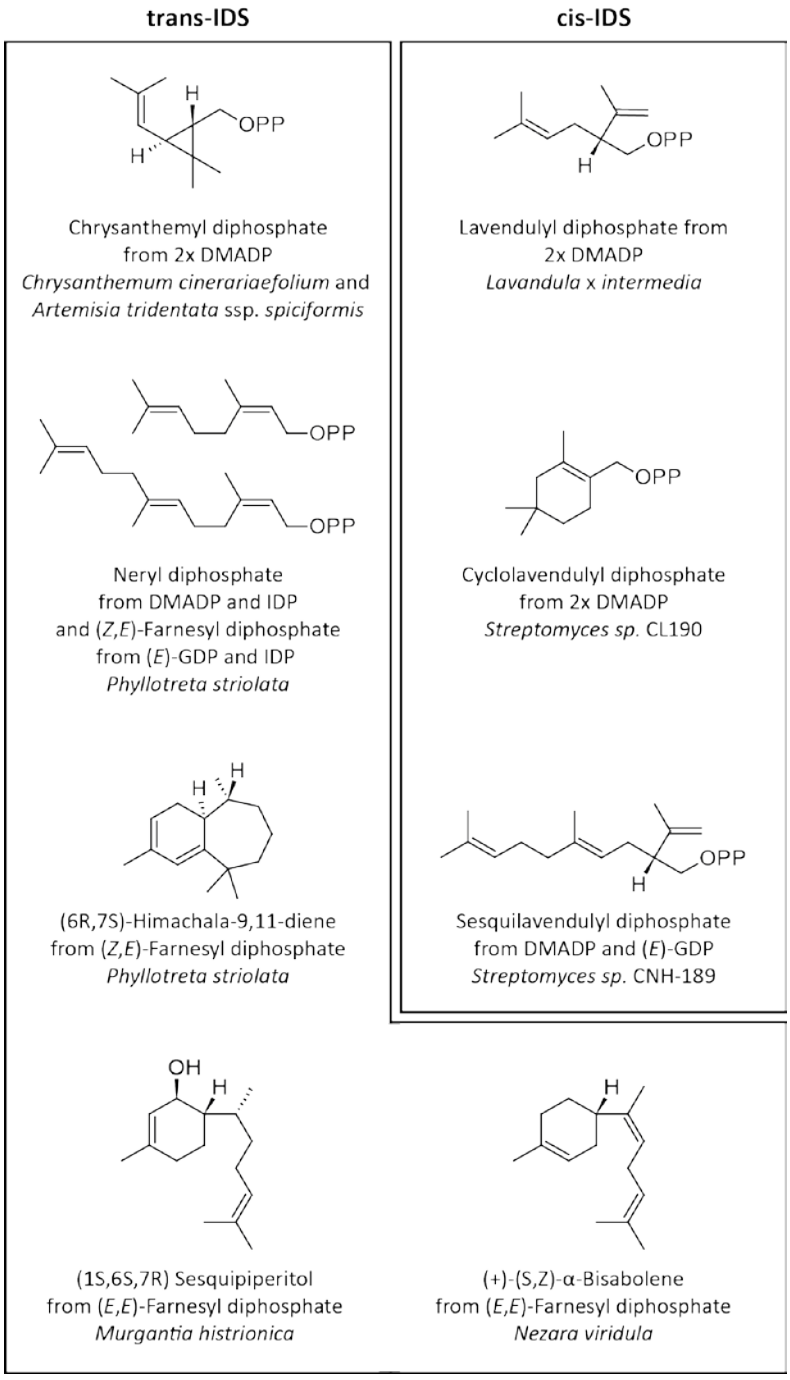

Figure 2: Irregular products produced by IDS. Depiction of products from *trans*- and *cis*-IDS that do not follow the usual 1'-4 alkylation reaction or have unexpected stereochemistry. Besides the structure the organism and the substrates for the product are indicated.