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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

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Abstract

The conifer *Picea abies* (Norway spruce) employs terpenoid-based oleoresins as part of its constitutive and induced defense responses to herbivores and pathogens. The isoprenyl diphosphate synthases are branch-point enzymes of terpenoid biosynthesis leading to the various terpene classes. We isolated three genes encoding isoprenyl diphosphate synthases from *P. abies* cDNA libraries prepared from the bark and wood of methyl jasmonate-treated saplings and screened via a homology-based PCR approach using degenerate primers. Enzyme assays of the purified recombinant proteins expressed in *Escherichia coli* demonstrated that one gene (*PaIDS* 4) encodes a farnesyl diphosphate synthase and the other two (*PaIDS* 5 and *PaIDS* 6) encode geranylgeranyl diphosphate synthases. The sequences have moderate similarity to those of farnesyl diphosphate and geranylgeranyl diphosphate synthases already known from plants, and the kinetic properties of the enzymes are not unlike those of other isoprenyl diphosphate synthases. Of the three genes, only *PaIDS* 5 displayed a significant increase in transcript level in response to methyl jasmonate spraying, suggesting its involvement in induced oleoresin biosynthesis.

Keywords: *Picea abies*; Pinaceae; Gymnosperm; Conifer defense; Prenyltransferase; Methyl jasmonate; Enzyme kinetics, Phylogenetic analysis

1. Introduction

Conifers of the family Pinaceae frequently come under attack from herbivorous insects, such as the tree-killing bark beetle, *Ips typographus*, and its fungal associate, *Ceratocystis polonica* (Phillips and Croteau, 1999; Trapp and Croteau, 2001; Baier et al., 2002; Franceschi et al., 2005). However, the long life spans of many conifers and their evolutionary persistence indicate the presence of effective defenses. The best known example of conifer defense is oleoresin, a pungent, viscous mixture of terpenoids found in specialized ducts, blisters and cells in stems and foliage. Oleoresin is both a constitutive and an inducible defense. For instance, some species of the Pinaceae, especially in the subfamily Piceoideae and to some degree the Abietoideae, form new (traumatic) resin ducts (TRDs) in response to attack by stem-boring insects and their associated fungi. These oleoresin-filled structures are found mainly in the sapwood and bark of coniferous trees, and are believed to help resist attack by augmenting the constitutive resin flow, thus providing a strong barrier against most herbivores and pathogens (Nagy et al., 2000; Martin et al., 2002; Hudgins et al., 2004; Franceschi et al., 2005; Byun-McKay et al., 2006; Keeling and Bohlmann, 2006).

Abbreviations: TRD, traumatic resin duct; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; IDS, isoprenyl diphosphate synthase; FPPS, farnesyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase; MJ, methyl jasmonate.

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With more than 30,000 structural variants; terpenes are the largest class of plant secondary metabolites. Oleoresin consists mainly of monoterpenes (C_{10}) and diterpene resin acids (C_{20}) as well as smaller amounts of sesquiterpenes (C_{15}) (Langenheim, 2003). The biosynthesis of all terpenoids is initiated by the synthesis of isopentenyl diphosphate (IPP) via the mevalonic acid pathway or the methylerythritol phosphate pathway (Gershenzon and Kreis, 1999) (Fig. 1). IPP and its isomer, dimethylallyl diphosphate (DMAPP), are the five-carbon building blocks that undergo sequential condensation reactions to form geranyl diphosphate (GPP, C_{10}), farnesyl diphosphate (FPP, C_{15}), and geranylgeranyl diphosphate (GGPP, C_{20}), the precursors of monoterpenes, sesquiterpenes and diterpenes, respectively. The enzymes catalyzing these sequential condensations are a group of prenyltransferases referred to collectively as isoprenyl diphosphate synthases. GPP, FPP, and GGPP are each believed to be formed by a specific isoprenyl diphosphate synthase (IDS) named for its product: geranyl diphosphate synthase (GPPS), FPP synthase (FPPS), and GGPP synthase (GGPPS) (Gershenzon and Kreis, 1999) (Fig. 2). In subsequent steps, these linear diphosphates serve as substrates for terpene synthases, which form a complex variety of mono-, sesqui-, and diterpene carbon skeletons (Martin et al., 2004; Keeling and Bohlmann, 2006). An important feature of terpenoid biosynthesis is the way this process is partitioned among different cell compartments. The formation of GPP and GGPP and their subsequent conversion to mono- and diterpenes occur in the plastids, whereas the biosynthesis of FPP and its conversion to sesquiterpenes are localized in the cytosol (Fig. 2).

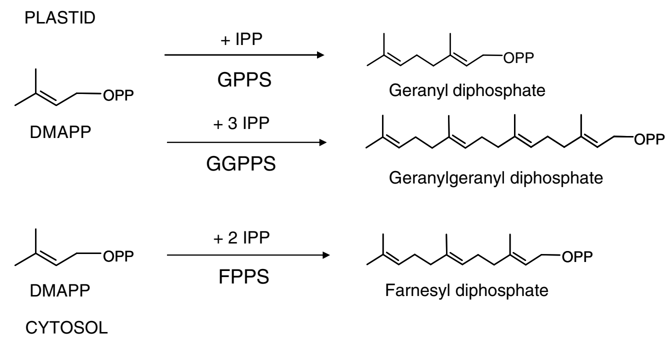

Fig. 2. Scheme for IDS catalysis in terpenoid biosynthesis. Each enzyme catalyzes 1, 2 or 3 sequential condensations to give a single product without significant release of intermediates. GPPS and GGPPS occur in the plastid, whereas FPPS is localized in the cytosol.

We are currently attempting to characterize the molecular and biochemical basis of conifer defenses using Norway spruce (*Picea abies*) L. Karst as a model. Norway spruce stems are constitutively defended by oleoresin, but also possess a suite of induced defenses. The induced defense response of this species to bark beetle attack can be mimicked by spraying trees with methyl jasmonate (MJ) (Martin et al., 2002; Byun-McKay et al., 2006; Erbilgin et al., 2006; Zeneli et al., 2006). This treatment triggers the formation of

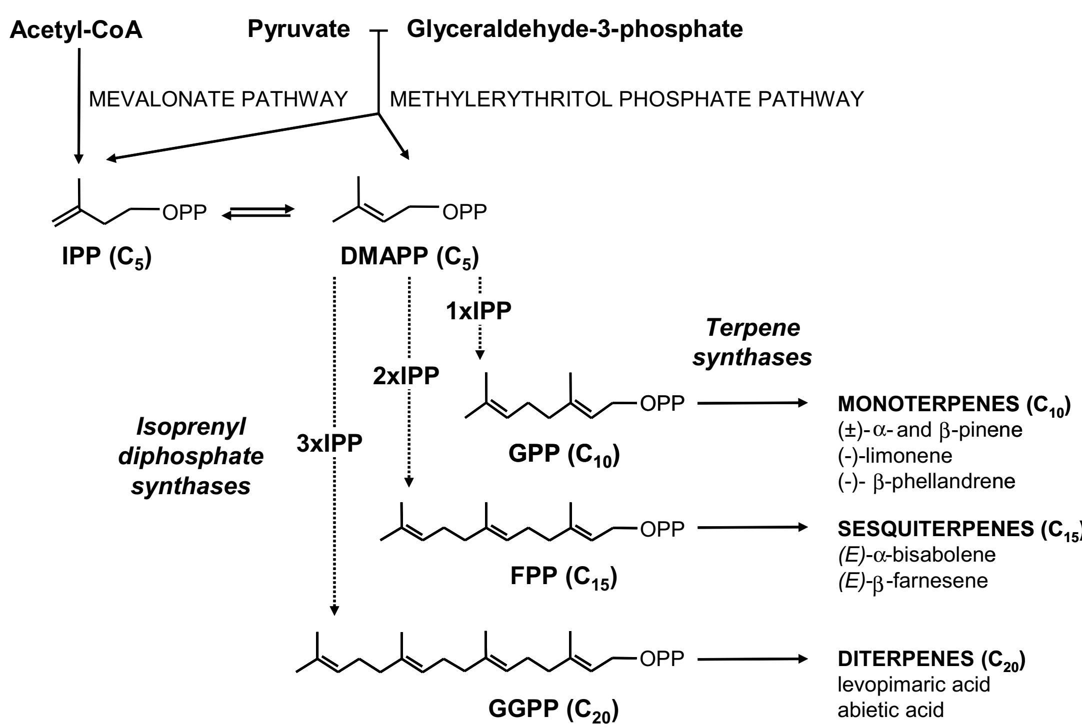

Fig. 1. Outline of terpenoid oleoresin biosynthesis in conifers showing the formation of IPP and DMAPP, the condensations to make larger prenyl diphosphates and the subsequent cyclizations to form the major products in *P. abies*. The isoprenyl diphosphate synthases catalyze the branch-point reactions leading to the different terpene classes.