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The potential of the mevalonate pathway for enhanced isoprenoid production

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ABSTRACT

The cytosol-localised mevalonic acid (MVA) pathway delivers the basic isoprene unit isopentenyl diphosphate (IPP). In higher plants, this central metabolic intermediate is also synthesised by the plastid-localised methylerythritol phosphate (MEP) pathway. Both MVA and MEP pathways conspire through exchange of intermediates and regulatory interactions. Products downstream of IPP such as phytosterols, carotenoids, vitamin E, artemisinin, tanshinone and paclitaxel demonstrate antioxidant, cholesterol-reducing, anti-ageing, anticancer, antimalarial, anti-inflammatory and antibacterial activities. Other isoprenoid precursors including isoprene, isoprenol, geraniol, farnesene and farnesol are economically valuable. An update on the MVA pathway and its interaction with the MEP pathway is presented, including the improvement in the production of phytosterols and other isoprenoid derivatives. Such attempts are for instance based on the bioengineering of microbes such as *Escherichia coli* and *Saccharomyces cerevisiae*, as well as plants. The function of relevant genes in the MVA pathway that can be utilised in metabolic engineering is reviewed and future perspectives are presented.

Contents

Abbreviations: AACT, acetoacetyl-CoA thiolase; ABA, abscisic acid; ACS2, ACETYL-COA SYNTHASE2; ADH1, ALCOHOL DEHYDROGENASE1; ADS, amorpha-4,11-diene synthase; AFS, α-farnesene synthase; ALDH1, ALDEHYDE DEHYDROGENASE1; atoB, bacterial acetoacetyl-CoA thiolase; BES1, BRI1-EMS-SUPPRESSOR 1; BR, brassinosteroids; BTS1, yeast geranylgeranyl diphosphate synthase; BZR1, BRASSINAZOLE-RESISTANT 1; CaMV, cauliflower mosaic virus; Cas, CRISPR-associated; CK, cytokinins; CMK, 4-diphosphocytidyl-2*C*-methyl-D-erythritol kinase; CRISPRs, clustered regularly interspaced short palindromic repeats; CPS, copalyl diphosphate; crtB, bacterial phytoene synthase; crtE, bacterial geranylgeranyl diphosphate synthase; crtI, bacterial phytoene desaturase; crtY, bacterial lycopene cyclase; crtYB, bacterial a bifunctional phytoene synthase and lycopene cyclase; CPR1, NADPH-cytochrome P450 reductase; CYB5, cytochrome b_5; CYP71AV1, amorphadiene oxidase (cytochrome P450 enzyme); CYP85A1, cytochrome P450 monooxygenase; DIM2, DOES NOT MAKE INFECTIONS2; DMAP, dimethylallyl phosphate; DMAPP, dimethylallyl diphosphate; DWF1, delta-24 sterol reductase; DX, 1-deoxy-D-xylulose; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; ER, endoplasmic reticulum; ERG8, yeast phosphomevalonate kinase; ERG9, yeast squalene synthase; ERG10, yeast acetoacetyl-CoA thiolase; ERG12, yeast mevalonate kinase; ERG13, yeast 3-hydroxy-3-methylglutaryl-CoA synthase; ERG20, yeast farnesyl diphosphate synthase; FPP, farnesyl diphosphate; FPPS, FPP synthase; GA, gibberellin; GA-3-P, glyceraldehyde 3-phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, GGPP synthase; GPP, geranyl diphosphate; GPPS, GPP synthase; HDR, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase; HDS, 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; IDI, isopentenyl diphosphate isomerase; IP, isopentenyl phosphate; ipiHP1, isopentenyl diphosphate:dimethylallyl diphosphate isomerase; IPK, isopentenyl phosphate kinase; IPP, isopentenyl diphosphate; IPS, isoprene synthase; ispA, farnesyl diphosphate synthase; ispS, isoprene synthase; KSL, kaurene synthase-like; *mad, microRNA action deficient*; MCT, 2*C*-methyl-D-erythritol 4-phosphate cytidyl transferase; MDC, mevalonate diphosphate decarboxylase; MDS, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MEP, 2*C*-methyl-D-erythritol 4-phosphate; MIA, monoterpenoid indole alkaloids; miRNAs, microRNAs; MK, mevalonate kinase; MVA, mevalonate; mvaD, bacterial mevalonate 5-diphosphate decarboxylase; mvaE, the bifunctional bacterial AACT and HMGR; MVAP, mevalonate 5-phosphate; MVAPP, mevalonate 5-diphosphate; mvaS, bacterial HMG-CoA synthase; Phos, phosphatase(s); PMK, phosphomevalonate kinase; PPMD, diphospho-mevalonate decarboxylase; S359A, mutant HMGS S359A; SMO1, STEROL METHYL OXIDASE1; SMT, sterol methyltransferase; SQS, squalene synthase; SUD1, SUPPRESSOR OF DRY2 DEFFECTS1; t-HMGR, truncated 3-hydroxy-3-methylglutaryl-CoA reductase; TPS, terpene synthase; UPC2, STEROL UPTAKE CONTROL PROTEIN2; wt, wild-type.

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1. Introduction

Isoprenoids are a group of functionally diverse compounds comprising of at least 50,000 different structures mostly identified from plants and bacteria (Hemmerlin et al., 2012). In animals, the mevalonate (MVA) pathway produces isoprenoids including cholesterol, dolichol, haem A and ubiquinone which are involved in membrane biogenesis, glycoprotein synthesis and electron transport (Goldstein and Brown, 1990). Inhibition of MVA synthesis adversely affects cell growth (DeClue et al., 1991; Fairbanks et al., 1986) and post-translational protein modifications associated with farnesyl or geranylgeranyl moieties (Maltese, 1990; Schaber et al., 1990; Schmidt et al., 1984). Statin-related drugs that manipulate the MVA pathway are used in the treatment of hypercholesterolaemia, cerebrovascular and cardiovascular disease and some cancers in humans (Goldstein and Brown, 1990; Gruenbacher and Thurnher, 2015; Jiang et al., 2014).

In plants, isoprenoids are biosynthesised via the cytosolic MVA pathway (Hemmerlin et al., 2012 and references cited therein) and the plastidial 2*C*-methyl-D-erythritol 4-phosphate (MEP) pathway (Rohmer, 1999). The universal precursor of isoprenoids, isopentenyl diphosphate (IPP), is derived from both pathways. Six enzymes are involved in the MVA pathway (Fig. 1). Acetoacetyl-CoA thiolase (AACT; EC 2.3.1.9) catalyses the production of acetoacetyl-CoA by the condensation of two units of acetyl-CoA (Bach et al., 1990, 1991, and literature cited therein). The second enzyme, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase (HMGS; EC 2.3.3.10) condenses acetoacetyl-CoA and acetyl-CoA to form *S*-HMG-CoA (Balasubramaniam et al., 1977; Ferguson and Rudney, 1959; Lynen, 1967; Rudney and Ferguson, 1959; Stewart and Rudney, 1966). Subsequently, HMG-CoA reductase (HMGR; EC 1.1.1.34) converts HMG-CoA to MVA (Durr and Rudney, 1960). Two consecutive phosphorylation reactions catalysed by mevalonate kinase (MK; EC. 2.7.1.36) and phosphomevalonate kinase (PMK; EC 2.7.4.2) then convert MVA to mevalonate 5-diphosphate (MVAPP). Diphosphomevalonate decarboxylase (PPMD; EC 4.1.1.33) catalyses the conversion of MVAPP to IPP (Henrikson and Smith, 1966), which is used for the biosynthesis of phytosterols that is essential for membrane fluidity and plant growth and development (He et al., 2003), as well as production of cytokinins (CK) and brassinosteroids (BR) (Howell et al., 2003; Li et al., 1996; Shani et al., 2010; Vriet et al., 2012). IPP from the MVA pathway also contributes to the biosynthesis of dolichols, which affect plant growth and development (Zhang et al., 2008) besides isoprenes, monoterpenes and sesquiterpenes that protect against phytopathogens and herbivores (Unsicker et al., 2009); although the MEP pathway has also been reported to play a significant role in the generation of these four compounds (Lange and Ahkami, 2013 and references cited therein; Schnitzler et al., 2005; Skorupinska-Tudek et al., 2008). MVA-related isoprenoids which have economic and pharmaceutical value include rubber for tyres and waterproof-related products (Suwanmanee et al., 2013), phytosterols with antioxidant cholesterol-reducing and anticancer activities (Bradford and Awad, 2007; Moreau et al., 2002; Woyengo et al., 2009) and the anti-malarial artemisinin (Klayman, 1985).

In plants and most bacteria, the seven enzyme-comprised MEP pathway [(1-deoxy-D-xylulose 5-phosphate synthase (DXS); 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR); 2*C*-methyl-D-erythritol 4-phosphate cytidyl transferase (MCT); 4-diphosphocytidyl-2*C*-methyl-erythritol kinase (CMK); 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS); 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate synthase (HDS) and 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-diphosphate reductase (HDR)] initiates with substrates glyceraldehyde 3-phosphate (GA-3-P) and pyruvate, to finally form IPP (Fig. 1). The mainly MEP-derived chlorophylls are essential for photosynthesis (Demming-Adams and Adams, 1996), while gibberellic acid (GA), carotenoid-derived abscisic acid (ABA) and strigolactones represent plant hormones that regulate plant growth and development (Finkelstein et al., 2002; Hedden and Kamiya, 1997; Xie et al., 2010). MEP-derived carotenoids and vitamin E display antioxidant and anti-ageing activities (Brigelius-Flohé and Traber, 1999; Krinsky, 1989), whereas the mainly MEP-derived diterpenoid tanshinone protects against cardiovascular diseases and exhibits antioxidant, anti-inflammatory, antibacterial, cytotoxic and anticancer activities (Shi et al., 2014). The mainly MEP-dependent paclitaxel also displays anticancer activities (Sandler et al., 2006).

It is worth noting that the MVA and MEP pathways are not totally independent as there is some crosstalk between them through common intermediates (IPP, geranyl diphosphate, GPP; farnesyl diphosphate, FPP; or geranylgeranyl diphosphate, GGPP) (Aharoni et al., 2003, 2004; Bick and Lange, 2003; Bouvier et al., 2000; Gutensohn et al., 2013; Hemmerlin et al., 2003, 2012; Kasahara et al., 2002, 2004; Laule et al., 2003; Mendoza-Poudereux et al., 2015; Nagata et al., 2002; Rodríguez-Concepción et al., 2004; Rodríguez-Concepción, 2006; Sakakibara et al., 2005; Schuhr et al., 2003; Skorupinska-Tudek et al., 2008; Wölwer-Rieck et al., 2014) (see details in Section 2.8) or through protein isoprenylation (Courdavault et al., 2005; Gerber et al., 2009; Hedhili et al., 2007; Huchelmann et al., 2014; Imbault et al., 1996). For example, although the MVA pathway does not provide precursors for the biosynthesis of monoterpenoid indole alkaloids (MIA), MVA-derived prenylated proteins regulate the expression of genes in the early steps of monoterpenoid biosynthesis (Courdavault et al., 2005).

Given the significance of isoprenoid compounds, functional analysis of enzymes in both MVA and MEP pathways has been the subject of many studies in the past decades (Hemmerlin et al., 2012 and references cited therein). Strategies to engineer secondary metabolite pathways and guidelines using genomics to enhance secondary metabolites in plants have been well summarised (Oksman-Caldentey and Inzé, 2004). Efforts have been made to improve the MVA and/or MEP routes for overproducing phytosterols, artemisinin, tanshinone, ginsenoside, triacyglycerol, carotenoids and provitamin A in plants (Alam and Abdin, 2011; Chappell et al., 1995; Enfissi et al., 2005; Harker et al., 2003; Holmberg et al., 2003; Kim et al., 2014; Kumar et al., 2012; Liao et al., 2014a; Muñoz-Bertomeu et al., 2007; Nafis et al., 2011; Paddon and Keasling, 2014; Paine et al., 2005; Saxena et al., 2014; Schaller et al., 1995; Singh et al., 2014; van Herpen et al., 2010; Wang et al., 2012; Wu et al., 2006; Ye et al., 2000). Also, many economically and