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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

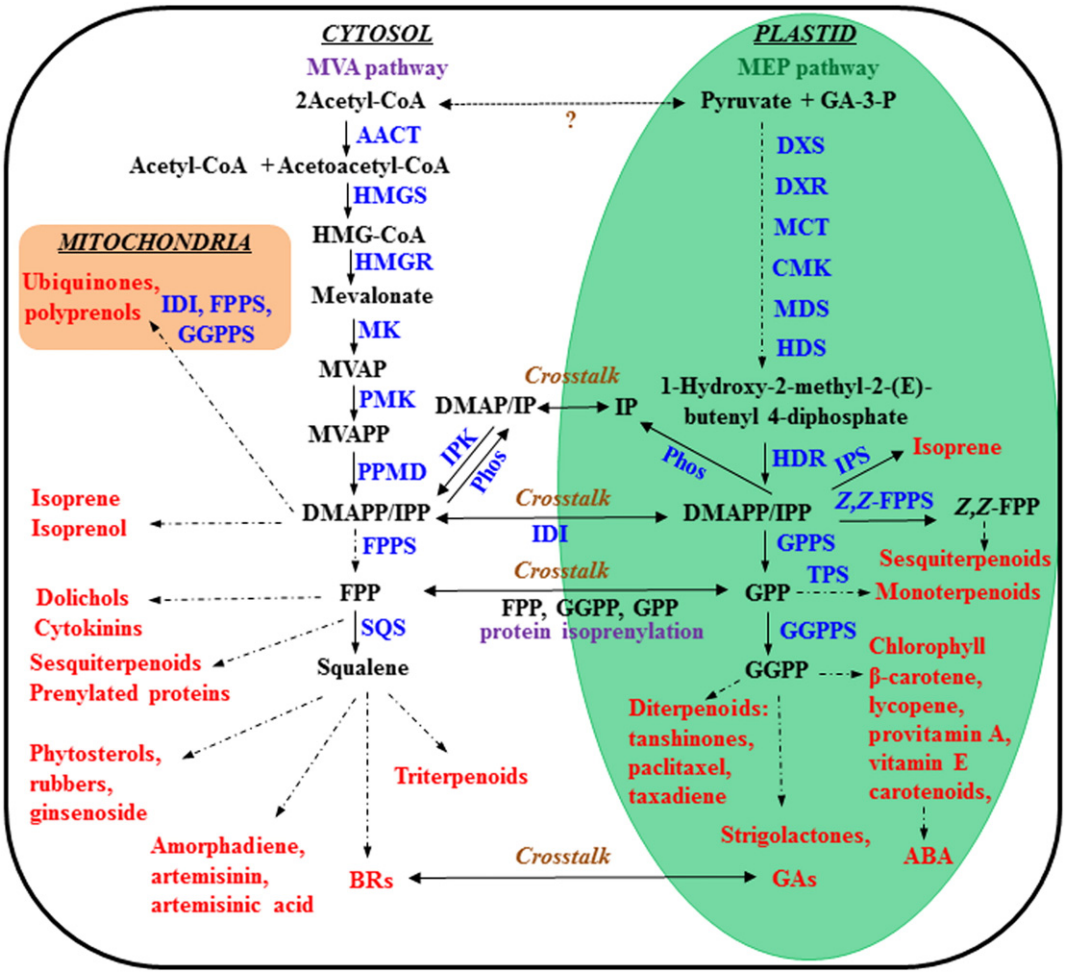

Fig. 1. Isoprenoid biosynthesis pathway in plants. The MVA pathway occurs in the cytosol. First step, acetoacetyl-CoA thiolase catalyses the production of acetoacetyl-CoA by the condensation of two units of acetyl-CoA. Second step, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase condenses acetoacetyl-CoA and acetyl-CoA to form *S*-3-hydroxy-3-methylglutaryl-coenzyme A. Third step, 3-hydroxy-3-methylglutaryl-coenzyme A reductase converts 3-hydroxy-3-methylglutaryl-coenzyme A to MVA. Fourth step, mevalonate kinase catalyses a phosphorylation reaction to convert MVA to mevalonate 5-phosphate. Fifth step, phosphomevalonate kinase catalyses another phosphorylation reaction to convert mevalonate 5-phosphate to mevalonate 5-diphosphate. Then, diphosphomevalonate decarboxylase catalyses the conversion of mevalonate 5-diphosphate to isopentenyl diphosphate. Isopentenyl diphosphate synthesised from the MVA pathway is the universal precursor of isoprenoids including phytosterols, rubbers, ginsenoside, artemisinin, brassinosteroids, triterpenes, dolichols, cytokinins, isoprenes, monoterpenes, sesquiterpenes, diterpenes, prenylated proteins, ubiquinones and polyprenols. The MEP pathway occurs in the plastid (green) with the enzymes in the MEP pathway as indicated. Enzymes are shown in blue. Substrates and intermediates are shown in black. Active components or end-products are shown in red. Arrows between plastid and cytosolic compartments indicate metabolic flow between them. Crosstalk through protein isoprenylation is marked in purple. Abbreviations: AACT, acetoacetyl-CoA thiolase; ABA, abscisic acid; CMK, 4-diphosphocytidyl-2*C*-methyl-D-erythritol kinase; DMAP, dimethylallyl phosphate; DMAPP, dimethylallyl diphosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate 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; IPK, isopentenyl phosphate kinase; IP, isopentenyl phosphate; IPP, isopentenyl diphosphate; IPS, isoprene synthase; MCT, 2*C*-methyl-D-erythritol 4-phosphate cytidyl transferase; MDS, 2*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MEP, 2*C*-methyl-D-erythritol 4-phosphate; MK, mevalonate kinase; MVA, mevalonate; MVAP, mevalonate 5-phosphate; MVAPP, mevalonate 5-diphosphate; Phos, phosphatase(s); PMK, phosphomevalonate kinase; PPMD, diphospho-mevalonate decarboxylase; SQS, squalene synthase; TPS, terpene synthase.

pharmaceutically valuable isoprenoids or their intermediates have been heterologously produced in *Escherichia coli* and/or *Saccharomyces cerevisiae* (yeast) by engineering the MVA pathway, and different strategies have been deployed to improve efficiency in production of MVA (Tabata and Hashimoto, 2004), sesquiterpene amorphadiene and artemisinic acid (precursors of artemisinin) or artemisinin (Anthony et al., 2009; Ma et al., 2011; Martin et al., 2003; Paddon et al., 2013; Pitera et al., 2007; Redding-Johanson et al., 2011), tetraterpene β-carotene and lycopene (Harada and Misawa, 2012; Kim et al., 2009; Nguyen et al., 2012; Yoon et al., 2006, 2007, 2009), diterpene abietadiene (Cyr et al., 2007; Morrone et al., 2010), as well as isoprenes with potential industrial applications for the production of aviation fuel or coatings (Korman et al., 2014; Yang et al., 2012a,b). Furthermore, some MVA-related products including isopentenol (Chou and Keasling, 2012; George et al., 2014; Zheng et al., 2013), geraniol (Zhou et al., 2014), farnesene (Zhu et al., 2014) and bisabolene (Peralta-Yahya et al., 2011), are considered as promising next-generation biofuels, and have been heterologously produced in *E. coli* and *S. cerevisiae*. The status of engineering isoprenoids including monoterpenoids, sesquiterpenoids, triterpenoids, diterpenes and volatile organic compounds in various plants/heterologous hosts has been extensively reviewed (Aharoni et al., 2006; Corsello and Garg, 2015; Dudareva et al., 2013; Grishko et al., 2014; Keasling, 2010; Kirby and Keasling, 2008; Lange, 2015; Lange and Ahkami, 2013; Li and Pfeifer, 2014; Luo et al., 2015; Moses et al., 2013; Paddon and Keasling, 2014; Sawai and Saito, 2011; Schrader and Bohlmann, 2015 and references cited therein; Vickers et al., 2014).

In this review, an updated function of the genes in the MVA pathway and their uses for metabolic engineering in plants, *E. coli* and *S. cerevisiae* are discussed and future perspectives presented.

2. The mevalonate (MVA) pathway in plants

2.1. Importance of acetoacetyl-CoA thiolase in the plant MVA pathway

Two isoforms of AACTs co-exist in *Arabidopsis thaliana* and *Nicotiana tabacum*. Peroxisomal AACT1 likely catalyses the last step of glyoxysomal/peroxisomal fatty acid β-oxidation, while cytosolic and/or nuclear AACT2 plays an essential role in the production of MVA-derived isoprenoids, which are important for pollen tube formation, plant growth and seed production (Ahumada et al., 2008; Jin et al., 2012; Jin and Nikolau, 2007; Wentzinger et al., 2013). AACT may also play a role in abiotic stress by positively regulating squalene production. *Medicago sativa* L. AACT1 (MsAACT1) was induced by cold and salinity stress, and upregulated the biosynthesis of squalene without affecting MsHMGR activity under salinity stress (Soto et al., 2011). AACT has been proposed to detect changes in the ratio of acetyl-CoA to CoA following abiotic stress-triggered inhibition of the tricarboxylic acid cycle (Fox et al., 2014).

2.2. Roles for 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase

Plant HMGS, which was subcellularly localised to the cytosol (Nagegowda et al., 2005), plays a significant role in seed germination, early development, pollen fertility, phytosterol and rubber biosynthesis, stress responses and seed production (Alex et al., 2000; Ishiguro et al., 2010; Liao et al., 2014a,b and references cited therein). Previous in vitro studies on recombinant *Brassica juncea* HMGS1 (BjHMGS1) identified a mutant, S359A, which exhibited about 10-fold elevation in enzyme activity over wild-type (wt) BjHMGS1 (Nagegowda et al., 2004). To test the role of BjHMGS1, particularly S359A, these variants were overexpressed in *Arabidopsis*, which also belongs to the Brassicaceae family (Wang et al., 2012). Transgenic *Arabidopsis* overexpressing wt and mutant BjHMGS1 (S359A) up-regulated several genes in the isoprenoid pathway, increased sterol content and enhanced stress tolerance (Wang et al., 2012). Furthermore, S359A-overexpressors (OE-S359A) displayed elevated total sterol content and tolerance to *Botrytis cinerea* than vector-transformed *Arabidopsis* (Wang et al., 2012). To quickly test the effects of BjHMGS1 overexpression in a more distant species beyond the Brassicaceae, wt and mutant BjHMGS1 (S359A) were overexpressed in tobacco (Liao et al., 2014a). HMGS-OEs showed induction of native *NtHMGR1*, *NtIDI2*, *NtSQS*, *NtSMT1-2*, *NtSMT2-1*, *NtSMT2-2* and *NtCYP85A1* expression in seedlings, improving sterol content, plant growth and seed yield over the vector-transformed control (Liao et al., 2014a). More interestingly, OE-S359A lines displayed enhanced growth and seed yield in comparison to OE-wtBjHNMGS1, consistent with higher *NtSQS* expression and sterol content in OE-S359A (Liao et al., 2014a). Thus, BjHMGS1 (S359A) holds promise in applications aimed at increasing phytosterol content, promoting plant growth and enhancing seed yield in crop plants.

2.3. New roles for HMG-CoA reductase

Plant HMGR is considered as the most important enzyme that regulates a rate-limiting step in the MVA pathway (Bach, 1986; Chappell et al., 1995), it is regulated at the levels of transcription, post-transcription, translation and post-translation (Hemmerlin, 2013 and references cited therein). The function of plant HMGR has been widely reviewed (Bach, 1986; Bach et al., 1991; Hemmerlin, 2013; Hemmerlin et al., 2012 and references cited therein; Stermer et al., 1994; Vranová et al., 2013). Novel insights on the isoform-specific functions of HMGR emerged following verification of interactions between its structural domains and protein partners (Rodríguez-Concepción and Boronat, 2015).

Recently reported roles of HMGR include triterpene biosynthesis, early plant symbiotic signalling, endoplasmic reticulum (ER) membrane proliferation as summarised herein. Ginseng HMGR1 regulates the biosynthesis of triterpene ginsenoside as well as sterols in both transgenic ginseng and *Arabidopsis* (Kim et al., 2014). HMGR activity in *Arabidopsis* was observed to be positively and post-transcriptionally controlled by a putative E3 ubiquitin ligase encoded by SUPPRESSOR OF DRY2 DEFFECTS1 (SUD1), which has homologues in *S. cerevisiae* and animals (Doblas et al., 2013). *Medicago truncatula* HMGR1 interacted with a plasma membrane-localised receptor-like kinase, DOES NOT MAKE INFECTIONS2 (DMI2) in symbiotic and nodule development (Kevei et al., 2007) and such interaction triggered symbiotic signalling between the host plant and rhizobia or mycorrhizal fungi (Oldroyd, 2013; Venkateshwaran et al., 2015). This study demonstrated the importance of the MVA pathway in initial sensing of symbiotic signals at the host plasma membrane, giving it a new role in intracellular signalling (Venkateshwaran et al., 2015).

Also, plant HMGR was recently reported to play a role in ER membrane proliferation through its membrane-spanning domains (Ferrero et al., 2015). Although *S. cerevisiae* HMGR1, hamster or human HMGR possess seven or eight such domains, plant HMGR has only two and they were sufficient to induce ER membrane proliferation (Campos and Boronat, 1995; Ferrero et al., 2015; Liscum et al., 1985; Parrish et al., 1995; Vollack et al., 1994). Therefore, the overexpression of membrane-bound proteins such as native HMGR, or enzymes such as squalene synthase or downstream enzymes in the sterol pathway can yield unexpected effects including ER membrane proliferation arising from their membrane-spanning domains. The use of plant HMGR lacking these domains may overcome any undesirable effects and subsequently enhance HMGR activity in plant metabolic engineering (Harker et al., 2003).

2.4. Roles of acetoacetyl-CoA thiolase, HMG-CoA synthase and HMG-CoA reductase in miRNA activity

MicroRNAs (miRNAs) are short non-coding molecules involved in post-transcriptional eukaryotic gene regulation and affect development and stress responses in plants and animals (Brodersen et al., 2012; Shi and Ruvkun, 2012). Loss of HMGR1 activity in the *microRNA action deficient3* (*mad3*) *Arabidopsis* mutant, or sterol C-8 isomerase activity in the *Arabidopsis microRNA action deficient4* (*mad4*), inhibited sterol-dependent function of plant miRNAs (Brodersen et al., 2012). Furthermore, HMGR1 catalytic activity was shown to be essential for miR171 function, suggesting that sterols and non-sterols are needed for normal miRNA function (Brodersen et al., 2012).

Salvia miltiorrhiza AACT has been identified as a target of miR5072, which is present in several plant species (Xu et al., 2014), but experimental verification of AACT on the miRNA pathway has not been obtained. In contrast, *C. elegans* HMGS1 (CeHMGS1) was reported to regulate miRNAs at various tissues and developmental stages (Shi and Ruvkun, 2012). *C. elegans* which possesses a functional MVA pathway but lacks some enzymes in sterol biosynthesis, has provided a platform to investigate miRNA activity and non-sterol biosynthesis in the MVA pathway (Shi and Ruvkun, 2012). The biosynthesis of non-sterol products such as dolichol, mainly used for protein *N*-linked glycosylation, was essential for miRNA activity in *C. elegans* and fertility in the miRNA *let-7* worms defective in *CeHMGS1* was severely affected (Shi and Ruvkun, 2012).

Taken together, the miRNA-related data on AtHMGR1, SmAACT and CeHMGS1 indicate that MVA-derived sterols and non-sterols are essential in the miRNA pathway (Brodersen et al., 2012; Shi and Ruvkun, 2012).

2.5. Roles for mevalonate kinase, phosphomevalonate kinase and diphosphomevalonate decarboxylase

The roles of MK in cytokinin-mediated growth and development as well as in substrate feedback and diurnal regulation have been reported (Champenoy and Tourte, 1998; Schulte et al., 2000a). The overexpression of *S. cerevisiae* MK encoded by *ERG12* from the CaMV *35S* promoter in transgenic tobacco resulted in stimulation of growth and

development (Champenoy and Tourte, 1998). Integration and expression of the *ERG12* chimeric gene led to an increase by about 60% of apparent MK activity in young plantlets, which was accompanied by an acceleration of regenerating processes, lateral bud growth and a peculiar flowering behaviour (Champenoy and Tourte, 1998). Moreover, a higher chlorophyll content throughout plant development and an unusual starch accumulation in leaves of young plantlets and later in roots of fully-grown plants were also detected, followed by senescence retardation, which strongly argues for a hormonal effect, most likely arising from overproduction of cytokinin (isopentenyl adenine). Also, MK was reported to be sensitive to the downstream product, farnesyl diphosphate (FPP) (Dorsey and Porter, 1968; Fu et al., 2008; Gray and Kekwick, 1972; Schulte et al., 2000b) and was subject to diurnal regulation (Schulte et al., 2000a). This suggests that overproduction of a desirable end product (e.g. FPP) needs the correct filling up of pathways at preceding enzyme steps (e.g. MK), to overcome regulatory circuits by retro-inhibition of enzymes should intermediates accumulate. But such feedback inhibition by prenyl diphosphates does not exist at least for the *S. cerevisiae* phosphomevalonate kinase (PMK), which converts the monophosphate of MVA into its diphosphate form (Garcia and Keasling, 2014). It should be noted that two genes encode PMK in contrast to a single gene encoding MK in *Arabidopsis* (Bhangu-Uhlmann, 2011). It is still unclear whether plant PMK is under feedback regulation.

From the systematic study by Bhangu-Uhlmann (2011) in deciphering interaction networks of enzymes/genes in the MVA (and the MEP) pathways, the enzymes (PMK and PPMD) downstream from MK did not appear to limit the synthesis of IPP, except in special conditions such as during the differentiation of etioplasts into chloroplasts (cf. Albrecht and Sandmann, 1994). Subcellular localization studies suggested that proteins in the last steps of the MVA pathway including PMK and PPMD as well as some IPP isomerases (IDIs) were localised to the peroxisomes (Guirimand et al., 2012; Sapir-Mir et al., 2008; Simkin et al., 2011), implying that biosynthesis of some isoprenoid or non-isoprenoid products is not confined to MVA derivatives in the cytosol. This is further supported by peroxisomal-localised AtAACT1 (Sapir-Mir et al., 2008). Also, a role for PPMD in sterol biosynthesis and triterpene ginsenoside biosynthesis has been reported (Kim et al., 2014).

2.6. Role for isopentenyl phosphate kinase

The cytosolic isopentenyl phosphate kinase (IPK), which is responsible for the phosphorylation of isopentenyl phosphate (IP) and its isomer, dimethylallyl phosphate (DMAP), to corresponding diphosphates (IPP and DMAPP), was recently identified in plants (Henry et al., 2015). *Arabidopsis IPK* was found to be coexpressed with genes in the MVA pathway and those downstream in terpenoid biosynthesis (Henry et al., 2015). AtIPK has a significant role in the regulation of terpenoid compounds from both MVA and MEP pathways by its ability to adjust the ratio of IP to IPP and DMAP to DMAPP (Henry et al., 2015). The overexpression of AtIPK increased not only cytosolic MVA-derived sterol and sesquiterpene production, but also plastidial MEP-derived monoterpenes and sesquiterpenes (Henry et al., 2015). This result implies that both MVA and MEP pathways contribute to the IP/DMAP pool and provides a novel connection between the two pathways through IPK-mediated reactions and suggests a new strategy for engineering of isoprenoid production.

2.7. Roles for isopentenyl diphosphate isomerase and farnesyl diphosphate synthase

The role of cytosolic IDI in squalene and sterol biosynthesis, which affects plant growth and male sterility has been discussed extensively (Okada et al., 2008; Ramos-Valdivia et al., 1997). Two genes encode IDI in *Arabidopsis*, one targeted to the cytosol or plastid following variant splicing, and another to the mitochondria (Phillips et al., 2008). Its presence in plastids is surprising at first thought as the MEP pathway synthesises IPP and its allylic reactive isomer DMAPP in parallel, but it is conceivable to assume that its role is to adjust the ratio of DMAPP (starter molecule for polymerisation) to IPP units in chain elongation, first up to GPP (monoterpenes), then to GGPP, the precursor of chlorophyll, tocopherol and phylloquinone side chains, gibberellic acids, carotenoids from fusion of two GGPP units catalysed by phytoene synthase that yields the C40 phytoene via phytoenePP followed by serried dehydrogenation. Finally, chain elongation of GGPP up to nine isoprene units begets nonaprenylPP which is the precursor of the plastoquinone side chain. The functional role of IDI in multiple organelles (Phillips et al., 2008) is a matter of debate as long as the transport mechanisms for intermediates across membranes are not fully explored. The single mutant lacking AtIDI1 or AtIDI2 did not show any abnormal phenotype, but the double mutant displayed dwarf and male-sterile phenotypes (Okada et al., 2008). This indicated that differentially localised IDIs have overlapping functions and suggested that IPP (or its derivatives, GPP and GGPP) seems to be exported from plastids under certain circumstances and could thus fill up essential biosynthesis routes in the cytoplasm. In this context some older observations (Heintze et al., 1990) may find new interpretations. Prior to the discovery of the MEP pathway, Heintze et al. (1990) had already found that import of extraplastidial IPP gradually increased to overcome the loss of endogenous intermediate supply for plastidial isoprenoid production (more evidence on crosstalk in Section 2.8).

A negative role of FPP synthase (FPPS) in cytokinin-mediated growth and development has been reported (Manzano et al., 2006). When its long form (FPPS1L) was overexpressed in *Arabidopsis* mitochondria, some aberrant growth behaviour was observed: transgenic leaves cultivated in continuous light developed chlorosis and cell death, apparently arising from peroxide accumulation, while detached leaves showed symptoms of rapid senescence accompanied by a decline in leaf *trans*-zeatin and isopentenyladenine-type cytokinin content (Manzano et al., 2006). These physiologically active cytokinins were apparently converted into their inactive glycosides (Manzano et al., 2006). Parallel overexpression of HMGR did not prevent chlorosis in detached leaves, but rescued early senescence, and free cytokinin content was reverted to wt levels (Manzano et al., 2006). The authors interpreted those observations by assuming that overexpression of mitochondrial FPPS led to higher uptake of MVA-derived IPP or DMAPP into the mitochondria, thereby negatively affecting cytokinin homeostasis and even resulted in a mitochondrial dysfunction that increased the sensitivity of the plants to oxidative stress when grown in continuous light (Manzano et al., 2006). When the short form of FPPS (FPPS1S) was overexpressed in the *Arabidopsis* cytosol without a concomitant increase in HMGR, an apparent hypersensitive response ensued (Manzano et al., 2004; Masferrer et al., 2002), most likely due to a reduction in the synthesis of essential isoprenyl derivatives in mitochondria. At this stage it might also be considered that an increase of MVA-derived farnesyl diphosphate in the mitochondria at the expense of its cytosolic equivalent could affect protein farnesylation. In summary, those observations have shed light on the necessity of a correct partitioning between branches of the MVA pathway and sink–source relationships, among different subcellular compartments.

2.8. Crosstalk between the MVA and the 2C-methyl-D-erythritol 4-phosphate (MEP) pathways

Although the MVA pathway and the MEP pathway operate in two different subcellular compartments (cytosol and plastid, respectively), metabolite exchanges or cross-regulation known as crosstalk occurs between them (Hemmerlin et al., 2003; Laule et al., 2003). The *Arabidopsis* MVA pathway can be complemented by the MEP pathway because decrease in sterol after treatment by the HMGR inhibitor lovastatin (mevinolin) was compensated by the MEP pathway (Laule et al., 2003). In tobacco BY-2 cells, mevinolin-induced growth defects could be complemented by 1-deoxy-D-xylulose (DX), which after endogenous

phosphorylation entered the MEP pathway, and caused the synthesis of sterols and isoprenylated proteins (Hemmerlin et al., 2003). The percentage of precursors exchanged from the plastids to the cytosol can reach 100% when required (Hemmerlin et al., 2003). In young cotton seedlings, 20% of the substrates for sterols were MEP-derived (Opitz et al., 2014). Also, the MEP pathway can be complemented by the MVA pathway as the inhibition of plastoquinone biosynthesis in tobacco BY-2 cells by fosmidomycin could be partially complemented by MVA (Hemmerlin et al., 2003). The percentage of precursors exchanged from the cytosol to the plastid can attain 80% when needed (Hemmerlin et al., 2003; Schuhr et al., 2003). In young cotton seedlings, ~50% of the substrate for phytol side chains and carotenoids was apparently MVA-derived (Opitz et al., 2014).

After the discovery of crosstalk between the MVA and MEP pathways, studies to address communication between them indicated that crosstalk occurs through common isoprenoid precursors such as IPP, GPP, FPP or GGPP. IPP is widely accepted as the common intermediate between these two independent pathways (Hemmerlin et al., 2012 and references cited therein; Mendoza-Poudereux et al., 2015). The MVA-derived precursors (IPP and DMAPP) were transported from the cytosol to the plastids in spike lavender leaves (Mendoza-Poudereux et al., 2015). The overexpression of HMGR increased MEP-dependent diterpene tanshinone in *S. miltiorrhiza* hairy roots (Kai et al., 2011; Shi et al., 2014), possibly by exporting more cytosol-synthesised IPP to the plastid. GPP and FPP are also common intermediates in this crosstalk (Aharoni et al., 2003, 2004; Bick and Lange, 2003; Bouvier et al., 2000; Hemmerlin et al., 2003; Kasahara et al., 2002; Laule et al., 2003; Nagata et al., 2002; Rodríguez-Concepción et al., 2004; Rodríguez-Concepción, 2006). Plastid-synthesised GPP increased the cytosolic GPP pool for monoterpene biosynthesis in tomato fruits, likely through the export of plastid-synthesised GPP to the cytosol (Gutensohn et al., 2013). MEP-derived FPP was transported from the plastids to the cytosol in *Stevia rebaudiana* Bertoni (Wölwer-Rieck et al., 2014). GGPP was also shuttled between the cytosol and the plastids in other studies (Bick and Lange, 2003; Gerber et al., 2009; Hemmerlin et al., 2003; Kasahara et al., 2002, 2004; Laule et al., 2003; Sakakibara et al., 2005; Schuhr et al., 2003; Skorupinska-Tudek et al., 2008).

Besides the exchange of upstream metabolites (IPP, GPP, FPP and GGPP), signalling crosstalk between the MVA and MEP pathways can occur through the MVA-derived end product BR and MEP-derived end product GA. Signalling crosstalk between BR and GA occurred through direct interaction between the BR-activated BRASSINAZOLE-RESISTANT 1 (BZR1) transcription regulator and central regulators of GA signalling DELLA proteins, causing GA regulation of BR-responsive gene expression (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012; Schwechheimer, 2011). In the presence of GA, DELLA proteins were degraded and could no longer suppress BZR1 and BES1 activity (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012; Schwechheimer, 2011). A new model for crosstalk between BR and GA has emerged following the interaction between the BR-activated transcription factor BRI1-EMS-SUPPRESSOR 1 (BES1) and a regulatory motif present in the promoters of GA biosynthesis genes: BRs positively regulate GA biosynthetic gene expression and GA biosynthesis in both *Arabidopsis* and rice (Hofmann, 2015; Tong et al., 2014; Unterholzner et al., 2015). In the presence of BR, increased nonphosphorylated BES1 activated GA biosynthetic gene expression, causing enhanced GA production, degradation of DELLA and inhibition of BES1 activity (Unterholzner et al., 2015), demonstrating that crosstalk between BR and GA can occur at the metabolite level.

Other studies on crosstalk at post-translation level indicated that MVA-dependent protein isoprenylation was important in MEP-derived indole alkaloid monoterpene biosynthesis in *Catharanthus roseus* (Courdavault et al., 2005; Hedhili et al., 2007; Imbault et al., 1996). The production of the MVA-derived sesquiterpenoid phytoalexin, capsidiol, was inhibited when MEP-dependent protein geranylgeranylation was blocked in tobacco leaves (Huchelmann et al., 2014). The biosynthesis of the MVA-derived sesquiterpenoid artemisinin in *Artemisia annua* (Schramek et al., 2010) was inhibited by fosmidomycin (Towler and Weathers, 2007) and the generation of MEP-derived diterpene paclitaxel from *Taxus* (Eisenreich et al., 1996) by compactin, an analogue of mevinolin (Soliman et al., 2011). The biosynthesis of MEP-derived monoterpenoid indole alkaloids was completely blocked after treatment with another HMGR inhibitor, pravastatin (Imbault et al., 1996). Such crosstalk requires MVA-dependent protein farnesylation by a CAAX-prenyltransferase (Courdavault et al., 2005; Imbault et al., 1996).

Crosstalk between the MVA and MEP pathways occurs when some intermediates are overproduced within a subcellular compartment, and are subsequently shared. This phenomenon may prove useful in metabolic engineering, i.e. target either pathway to upregulate the production of the other. Furthermore, crosstalk may be affected by development and the environment. In fact, the metabolic precursors of the MVA and MEP pathways such as acetyl-CoA and pyruvate are also precursors of pathways in sugar metabolism, glycolysis, pentose phosphate pathway, amino acid and fatty acid biosyntheses (Hemmerlin et al., 2012).

3. Manipulation of the MVA pathway for isoprenoid accumulation

With advances in the functional analysis of genes of the MVA pathway, further information on their regulatory roles in this pathway and the interaction between the MVA and MEP pathways in different organisms are now available (Hemmerlin et al., 2012 and references cited therein). To enhance the production of valuable secondary metabolites, metabolic engineering of the MVA pathway has been carried out not only in plants, but also in *E. coli* and *S. cerevisiae* (Grishko et al., 2014; Kempinski et al., 2015; Lange, 2015; Moses et al., 2013; Sawai and Saito, 2011; Wu et al., 2006).

3.1. Enhancing isoprenoid production in plants

Activity of HMGR in the MVA pathway has been widely manipulated to upregulate isoprenoid production (all the information mentioned in this section is listed in Table 1). For example, the overexpression of hamster HMGR in tobacco driven by the CaMV *35S* promoter resulted in a 2-fold increase in total end-product sterols including stigmasterol, sitosterol and campesterol with more than a 100-fold enhancement of an intermediate, cycloartenol (Chappell et al., 1995) (Table 1). Cycloartenol and some immediate cyclopropyl sterols downstream in the pathway are usually esterified with fatty acids and accumulate as lipid droplets in the cytosol. As membranes have only a limited capacity to integrate sterols, the cyclopropyl sterols would become “toxic” leading to disruption of membrane architecture and impair its function. Constitutive overexpression of *Hevea brasiliensis* HbHMGR and an *N*-terminally truncated form (t-HbHMGR) in tobacco caused a 2- to 6-fold elevation in total phytosterol content (Table 1) (Harker et al., 2003; Holmberg et al., 2003; Schaller et al., 1995). It appears that if truncated, soluble HMGR is deregulated, its overexpression will result in less drastic side effects. Seed-specific overexpression of (truncated, soluble and deregulated) t-HbHMGR in tobacco resulted in a moderate 1.3-fold (Holmberg et al., 2003) and 3.2-fold (Harker et al., 2003) enhancement in total seed phytosterol (Table 1). Furthermore, the overexpression of *Arabidopsis* t-HMGR1 in *Lavandula latifolia* resulted in a limited 1.8- to 1.9-fold increase in end-product phytosterols (sitosterol and stigmasterol) as well as 1.8 to 2.1-fold increase in essential oil constituents (Table 1) (Muñoz-Bertomeu et al., 2007). Constitutive overexpression of *Arabidopsis* AtHMGR1 in tomato culminated in more than 2.4-fold elevation in fruit total phytosterol content (Table 1) (Enfissi et al., 2005). Kim et al. (2013) reported that the overexpression of the *Panax ginseng* HMGR in *Platycodon grandiflorum* hairy root cultures led to 1.1- to 1.6-fold higher phytosterol and increased triterpenoids including 1.6 mg/l dry weight of platycosides and 1.8 mg/l dry weight of α-

Table 1
Manipulation of the MVA pathway and isoprenoid-related genes in plants.

Gene (s)	Origin	Promoter	Host	Products increase/yield	Reference/s
HMGR	Hamster	CaMV*35S*	Tobacco	Total phytosterol (2 fold); cycloartenol (>100 fold)	Chappell et al. (1995)
HMGR	*Hevea brasiliensis*	CaMV*35S*	Tobacco	Total phytosterol (6 fold)	Schaller et al. (1995)
t-HMGR	*H. brasiliensis*	CaMV*35S*	Tobacco	Total phytosterol (2.2 fold)	Holmberg et al. (2003)
t-HMGR	*H. brasiliensis*	Seed-specific	Tobacco	Total phytosterol (1.3 fold)	Holmberg et al. (2003)
t-HMGR	*H. brasiliensis*	Seed-specific	Tobacco	Total phytosterol (3.2 fold)	Harker et al. (2003)
t-HMGR	*H. brasiliensis*	CaMV*35S*	Tobacco	Total phytosterol (2.4 fold)	Harker et al. (2003)
t-HMGR1	*Arabidopsis thaliana*	CaMV*35S*	*L. latifolia*	Sitosterol and stigmasterol (~1.8 fold); essential oil constituents (~2.1 fold)	Muñoz-Bertomeu et al. (2007)
HMGR1	*Arabidopsis thaliana*	CaMV*35S*	Tomato	Total phytosterol (2.4 fold)	Enfissi et al. (2005)
HMGR	*Panax ginseng*	CaMV*35S*	*Platycodon grandiflorum*	Total phytosterol (1.1–1.6 fold); platycosides (1.6 mg/l-dry weight); α-spinasterol (1.8 mg/l-dry weight)	Kim et al. (2013)
HMGR2	*Withania somnifers*	CaMV*35S*	Tobacco	Total phytosterol (2.6 fold)	Singh et al. (2014)
HMGS1	*Brassica juncea*	CaMV*35S*	*Arabidopsis*	Total seedlings and leaf sterol (11.3% and 13.6%)	Wang et al. (2012)
S359A	*B. juncea*	CaMV*35S*	*Arabidopsis*	Total seedlings and leaf sterol (26.8% and 22.3%)	Wang et al. (2012)
HMGS1	*B. juncea*	CaMV*35S*	Tobacco	Total seedlings and leaf sterol (4.6% and 12.1%)	Liao et al. (2014a)
S359A	*B. juncea*	CaMV*35S*	Tobacco	Total seedlings and leaf sterol (22.9% and 18.7%)	Liao et al. (2014a)
HMGR	*Catharanthus roseus*	CaMV*35S*	*A. annua*	Artemisinin (38.9%)	Nafis et al. (2011)
HMGR + *ADS*	*C. roseus* and *Artemisia annua*	*Ubiquitin* + CaMV*35S*	*A. annua*	Artemisinin (7.7 fold)	Alam and Abdin (2011)
t-HMGR + *FPPS* + *CYP71AV1*	*Arabidopsis thaliana*	CaMV*35S*	*N. benthamiana*	Artemisinic acid-12-β-diglucoside (39.5 mg/kg)	van Herpen et al. (2010)
HMGR	*Salvia miltiorrhiza*	CaMV*35S*	*S. miltiorrhiza* hairy root	Total tanshinone (0.8–1.5 or 1.6 vs 0.5 or 0.6 mg/g)	Kai et al. (2011), Shi et al. (2014)
HMGR + *DXR*	*S. miltiorrhiza*	CaMV*35S*	*S. miltiorrhiza* hairy root	Total tanshinone (3.3 mg/g)	Shi et al. (2014)
HMGR1	*P. ginseng*	CaMV*35S*	*Arabidopsis* and ginseng	β-Sitosterol (1.6–2 fold), campersterol (1.6–1.8 fold), cycloartenol (1.8–2.6 fold), β-amyrin (1.6–2.5 fold), α-amyrin (2–3.7 fold); ginsenoside (1.5–2 fold)	Kim et al. (2014)
MVA pathway	*Saccharomyces cerevisiae*	16S-ribosomal rRNA	Tobacco plastids	Mevalonate (49 μmol/g), squalene (10 fold), sterols (2 fold), triacylglycerol (1.2 fold), carotenoids (1.4 fold)	Kumar et al. (2012)
MVA pathway	*S. cerevisiae*	16S-ribosomal rRNA	Tobacco	Artemisinic acid (0.1 mg/g-fresh weight)	Saxena et al. (2014)

Notes: *ADS*, gene encoding amorpha-4,11-diene synthase; *DXR*, gene encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase; *FPPS*, gene encoding farnesyl diphosphate synthase; *HMGS*, gene encoding 3-hydroxy-3-methylglutaryl-CoA synthase; MVA, mevalonate; *S359A*, gene encoding mutant HMGS S359A; *t-HMGR*, gene encoding truncated 3-hydroxy-3-methylglutaryl-CoA reductase.

spinasterol. According to Singh et al. (2014) the overexpression of *Withania somnifers* HMGR2 in tobacco elevated total phytosterol content by 2.6-fold (Table 1). This study further showed that the MVA pathway was the main source of intermediates for the biosynthesis of withanlides via steroid oxidation (Singh et al., 2014). Nonetheless, the degree of sterol overproduction remained relatively low in the studies mentioned above, which also suggests that downstream reactions in the pathway become limiting, and that membranes have a limited capacity to integrate a surplus of sterols and might even mask positive effects of a slight increase, see below.

HMGS has also been engineered to increase sterol content in *Arabidopsis* and tobacco (Table 1) (Liao et al., 2014a; Wang et al., 2012). The overexpression of wt BjHMGS1 (OE-wtBjHMGS1) in *Arabidopsis* and tobacco resulted in 11.3–13.6% and 4.6–12.1% elevations in total seedling and leaf sterol content, respectively (Liao et al., 2014a; Wang et al., 2012). *Arabidopsis* and tobacco OE-S359A achieved a better effect (26.8% and 22.3% enhancements for *Arabidopsis* and 22.9% and 18.7% for tobacco in total seedling and leaf sterol content) than OE-wtBjHMGS1 (Liao et al., 2014a; Wang et al., 2012). Despite lower sterol elevation in HMGS-OEs in comparison to HMGR-OEs (Chappell et al., 1995; Harker et al., 2003; Holmberg et al., 2003; Lange et al., 2015; Liao et al., 2014a; Schaller et al., 1995; Wang et al., 2012), HMGS-OEs showed unexpected enhancement in growth and seed yield, implying that the overexpression of mutant HMGS such as S359A represents an effective strategy in metabolic engineering. It remains to be determined whether other non-sterol (hormonal) effects on plant growth and seed yield had occurred in *Arabidopsis* and tobacco HMGS-OEs. All of the above observations demonstrate a positive role for plant HMGS and HMGR in sterol biosynthesis.

Recently, each gene from either the MVA pathway or the MEP pathway was individually overexpressed in *Arabidopsis*, and representative isoprenoids from both pathways (sterols from the MVA pathway, and chlorophylls and carotenoids from the MEP pathway) were analysed (Lange et al., 2015). Gene expression-to-metabolite correlation analysis was conducted to identify those genes that play the most important role in isoprenoid accumulation in *Arabidopsis* (Lange et al., 2015). For example, *AtHMGR1* was identified to be most important in influencing sterol biosynthesis followed by *AtHMGS* (Lange et al., 2015). Both *AtDXR* and *AtMDS* were proven to be important in chlorophyll and carotenoid biosynthesis in *Arabidopsis* (Lange et al., 2015). Furthermore, flux-bottlenecks were predicted to occur at reactions catalysed by STEROL METHYL OXIDASE1 (SMO1) and delta-24 sterol reductase (DWF1) (Lange et al., 2015). Experimental verification by the co-overexpression of HMGR1 with SMO1 or DWF1, culminated in higher sterol accumulation and lower intermediate retention (Lange et al., 2015). This study provided a new strategy in the pre-evaluation of a pathway for the selection of key genes in metabolic engineering of specific value-added isoprenoids in plants.

Besides sterols, the biosynthesis of other plant isoprenoid compounds such as artemisinin, pachoulol, and ginsenoside, can be regulated through metabolic engineering of the MVA pathway (Kai et al., 2011; Kim et al., 2014; Kumar et al., 2012; Nafis et al., 2011; Saxena et al., 2014; Shi et al., 2014; Tang et al., 2014; Wu et al., 2006; Zhao et al., 2014). Artemisinin is widely used for the treatment of malaria (Tang et al., 2014). When *Catharanthus roseus* L. HMGR (CrHMGR) was overexpressed in *A. annua* L., artemisinin content increased by 38.9% (Table 1) (Nafis et al., 2011). Co-overexpression of CrHMGR and *A. annua* L. amorpha-4,11-diene synthase (ADS) produced a 7.7-fold elevation in artemisinin content (1.7 mg/g DW) over the wt (Table 1) (Alam and Abdin, 2011), confirming that HMGR can be targeted to overproduce artemisinin. Furthermore, overexpression of both truncated *Arabidopsis* HMGR and FPPS in agroinfiltrated *Nicotiana*

benthamiana produced the artemisinin precursor amorphadiene, and when amorphadiene oxidase *35S::CYP71AV1* was subsequently introduced in the HMGR/FPPS co-overexpressing line, artemisinic acid-12-β-diglucoside accumulated up to 39.5 mg/kg fresh weight (Table 1) (van Herpen et al., 2010). It should also be noted that the co-overexpression of plastidial FPPS and ADS in tobacco increased amorphadiene content up to 25 mg/kg fresh weight (Table 1) (Wu et al., 2006). In addition, the sesquiterpene patchoulol content was enhanced more than 1000-fold by engineering soluble truncated HMGR (t-HMGR) together with an avian FPPS in conjunction with a terpene synthase such as pahcoulol synthase (Table 1) (Wu et al., 2006). The overexpression of *P. ginseng* 'Meyer' *HMGR1* in *Arabidopsis* elevated β-sitosterol, campesterol, cycloartenol, triterpene α-amyrin and β-amyrin by 1.6–3.7-fold, and the overexpression of *PgHMGR1* in ginseng increased triterpene ginsenoside production (Table 1). Also, overexpression of either *PgPPMD* or *PgFPPS* in *P. ginseng* hairy roots produced higher phytosterol and ginsenoside content (Kim et al., 2014) (Table 1). These results further confirmed a role for the MVA pathway and downstream genes/enzymes including FPPS in sterol biosynthesis and triterpene ginsenoside biosynthesis, although ginsenoside is apparently derived from both MVA and the MEP pathways (Zhao et al., 2014).

Plastid/chloroplast transformation of the whole MVA pathway was applied to overproduce mevalonate, squalene, sterols, triacylglycerol, carotenoids and artemisinic acid. Kumar et al. (2012) co-overexpressed all genes in the MVA pathway of *S. cerevisiae* in tobacco plastids, and enhanced mevalonate accumulation (49 μmol/g in comparison to undetectable mevalonate in the wt leaves or empty vector control). Also, squalene (10-fold), sterols (2-fold), triacylglycerol (1.2-fold) and carotenoids (1.4-fold) content increased (Table 1). This study suggested a positive role of the plastid-transformed MVA pathway in elevating the biosynthesis of squalene, triacylglycerol and carotenoid and feasibility in using multigene expression in metabolic engineering (i.e. simultaneous overexpression of all genes of the MVA pathway) in plants. Saxena et al. (2014) incorporated the *S. cerevisiae* MVA pathway with the artemisinic acid pathway in tobacco chloroplasts through multigene engineering and successfully produced artemisinic acid, the precursor of artemisinin (Table 1). Hence, it is feasible to engineer the whole MVA pathway in tobacco (*N. benthamiana* and *N. tabacum* cv. Petit Havana SR1) plastids/chloroplasts for generating artemisinin precursors including amorpha-4,11-diene and artemisinic acid, representing a low cost alternative to *A. annua* (Saxena et al., 2014). Overexpression of the complete MVA pathway in plastids/chloroplasts increased the metabolic pool, which may be partially due to a higher level of protein accumulation arising from chloroplast transformation.

Alternatively, cell and hairy root cultures have been utilised to enhance the production of specific valuable, pharmaceutical compounds normally harvested from roots (Buitelaar et al., 1991; Chen et al., 1997; Kamada et al., 1986; Liu et al., 1997; Palazón et al., 2003; Rijhwani and Shanks, 1998). HMGR was reported to support the biosynthesis of mainly-MEP derived tanshinone in hairy roots cultures. Kai et al. (2011) and Shi et al. (2014) overexpressed *S. miltiorrhiza* HMGR to enhance the biosynthesis of diterpene tanshinone in hairy roots, the transgenic lines contained moderately increased quantities of MEP-derived tanshinone (0.8–1.5 and 1.6 mg/g DW, respectively) in comparison to the control (0.5 and 0.6 mg/g DW, respectively) (Table 1). Interestingly, higher elevation in total tanshinone (2.7 mg/g DW and 3.3 mg/g DW) was achieved by co-overexpression of SmHMGR and SmGGPPS/SmDXR, respectively (Kai et al., 2011; Shi et al., 2014) (Table 1). These experiments indicated that cooperation between the MVA and MEP pathways enhanced tanshinone production (Kai et al., 2011; Shi et al., 2014). Furthermore, combined gene overexpression followed by elicitor treatment can stimulate metabolite production, possibly presenting a better strategy to further increase target compounds. The total tanshinone content in a transgenic hairy root line co-overexpressing SmHMGR and SmDXR (HR42) was enhanced from 3.3 to 6.7 mg/g DW by the treatment of Ag^{+} (Shi et al., 2014). These results suggested that the MVA pathway can regulate the biosynthesis of the mainly MEP-derived diterpene, tanshinone, to some extent, which might resemble the effects seen with MIA production in *C. roseus* that depends on the farnesylation of a regulatory protein, or the other way round, sesquiterpenoid capsidiol production in tobacco depending on protein geranylgeranylation, thus with a regulatory link to the MEP pathway.

There are many advantages in using plants for mass production of target compounds. Plants require only water, fertiliser, sunshine and soil for growth and can directly use carbon dioxide as a carbon substrate (not needing additional carbon sources such as glucose that are necessary for microbe growth) (Kempinski et al., 2015). However, their limitations include a longer cultivation time in comparison to microbes, and production and accumulation of target compounds which may occur only at specific developmental stages (Sawai and Saito, 2011). Also, it may be challenging to purify the specific compounds of interest because plants contain many other metabolites in addition to the desirable entities, the accumulation of which are often low (Wu et al., 2006). This poses severe difficulties such as establishing reliable and reproducible separation methods that can eventually be upgraded to industrial scale, to meet commercialization (Kempinski et al., 2015; Wu et al., 2006). Plants, as complex organisms with multicellular compartments may be difficult to engineer (Kempinski et al., 2015). It is also challenging to understand the mechanisms and regulatory role of each gene in the MVA pathway and the relationship between the MVA and MEP pathways. Furthermore, other metabolic pathways which utilise the same central precursors acetyl-CoA or pyruvate and glyceraldehyde 3-phosphate (GAP), need to be considered (Hemmerlin et al., 2012; Kempinski et al., 2015). Hence, improvement on plant isoprenoid production certainly requires a better understanding of the MVA or MEP pathways in relation to other primary/secondary metabolic routes.

3.2. Biotechnology of the MVA pathway for isoprenoid production in *E. coli*

E. coli is one of the most commonly used microbes for the heterologous production of isoprenoids (Chemler and Koffas, 2008). Naturally, *E. coli* has only the MEP pathway (Martin et al., 2003). Many studies report the metabolic engineering of the MVA pathway for heterologous isoprenoid production in *E. coli*. A summary is listed in Table 2. Tabata and Hashimoto (2004) manipulated the first three genes in the MVA pathway to produce MVA in *E. coli*. The maximal production of MVA in *E. coli* NM522 after 50 h was 47 g/l by fed-batch fermentation (Tabata and Hashimoto, 2004), exceeding *Saccharomycopsis fibuligera* (19 g/l MVA after 12 d) and *Streptomyces lividans* (0.7 g/l MVA after 8 d) (Kuzuyama et al., 2004; Tabata and Hashimoto, 2004). Hence, *E. coli* represents a good host with the highest efficiency in heterologous MVA production.

Martin et al. (2003) co-expressed the *S. cerevisiae* MVA pathway and ADS in *E. coli* to produce 24 mg/l of the sesquiterpene olefin precursor of artemisinin, amorphadiene. However, the flux rate in the engineered *E. coli* strain (Martin et al., 2003) was limited by HMG-CoA, and cell growth of this strain was inhibited by the high expression of the MVA pathway enzymes (Pitera et al., 2007). The bottleneck was eliminated by increasing the copy number of *t-HMGR1*, which led to an increase in MVA production (Pitera et al., 2007). Anthony et al. (2009) identified MK and ADS as the two rate-limiting enzymes for amorphadiene production in *E. coli*. By optimising the expression of these two proteins, a 7-fold increase of amorphadiene (293 mg/l) was produced (Martin et al., 2003). Redding-Johanson et al. (2011) further identified MK and PMK as potential bottlenecks through targeted proteomics and optimised the MVA pathway by elevating the expression of MK and PMK through codon-optimization as well as by using a more efficient promoter, to yield a 3-fold increase (>500 mg/l) of amorphadiene. Furthermore, Ma et al. (2011) enhanced the heterologous MVA pathway by using HMGR variants including HMGR from *Delftia acidovorans* and utilising NADH as cosubstrate, which was the best

Table 2
Manipulation of the MVA pathway and isoprenoid-related genes in *E. coli*.

Gene/s	Origin	Strategies	Products increase/yield	Reference/s
First three genes in the MVA pathway	*Enterococcus faecalis*	Overexpression	MVA ($C_6H_{12}O_4$) (47 g/l)	Tabata and Hashimoto (2004)
MVA pathway and *ADS*	*Saccharomyces cerevisiae*	Overexpression	Amorphadiene ($C_{15}H_{24}$) (24 mg/l)	Martin et al. (2003)
MVA pathway (especially *MK*) and *ADS*	*S. cerevisiae MK*, *Artemisia annua ADS*	Optimising the expression of *MK* and *ADS*	Amorphadiene ($C_{15}H_{24}$) (293 mg/l, 7 fold)	Anthony et al. (2009)
MK and *PMK*	*S. cerevisiae*	Optimising the expression of *MK* and *PMK* by codon optimisation and an efficient promoter	Amorphadiene ($C_{15}H_{24}$) (>500 mg/l)	Redding-Johanson et al. (2011)
HMGR	*S. cerevisiae*	Use of different *HMGR* variants	Amorphadiene ($C_{15}H_{24}$) (700 mg/l)	Ma et al. (2011)
HMGR	*Staphylococcus aureus*	Replace *SeHMGR* with *SaHMGR*, and regulate carbon and nitrogen	Amorphadiene ($C_{15}H_{24}$) (27.4 g/l)	Tsuruta et al. (2009)
MVA pathway, *ADS* and ATP-recycling enzyme	*MK*, *PMK*, *PPMD* from *S. cerevisiae*, *IDI*, *ispA*, and ATP-recycling enzymes from *Escherichia coli*, ADS from *A. annua*	Optimise the enzymatic ratio of MVA pathway, use of *ADS* and ATP-recycling enzymes to overcome *FPPS* caused inhibition, enzyme immobilisation strategy	Amorphadiene ($C_{15}H_{24}$) (2.2 g/l)	Chen (2014)
MVA pathway and lycopene biosynthetic genes	MVA pathway enzymes from *Streptomyces* sp. Strain CL190, lycopene biosynthetic enzymes from *Pantoea ananatis*	Overexpression	Lycopene ($C_{40}H_{56}$) (12.5 mg/g dry weight, 11.8 fold)	Harada and Misawa (2012)
MK, *PMK*, *PPMD*, *IDI*, *crtE*, *crtB*, *crtI* and *ipiHP1*	*Streptococcus pneumonia MK*, *PMK*, *PPMD*, *Erwinia herbicola crtE*, *crtB* and *crtI*, *Haematococcus pluvialis ipiHP1* and *E. coli IDI*	Overexpression	Lycopene ($C_{40}H_{56}$) (102 mg/l (22 mg/g dry cell weight), 5 fold)	Yoon et al. (2006)
MVA pathway, *IDI*, *crtE*, *crtB* and *crtI*	*atoB*, *IDI* and *ispA* from *E. coli*, *HMGS*, *t-HMGR1*, *MK*, *PMK* and *mvd1* from *S. cerevisiae*, *crtE*, *crtB* and *crtI* from *Pantoea ananatis*	Target metabolic engineering	Lycopene ($C_{40}H_{56}$) (1.2 g/l (34.3 mg/g dry cell weight))	Zhu et al. (2015)
MK, *PMK*, *PPMD*, *IDI*, *crtE*, *crtB*, *crtI*, *ipiHP1* and *crtY*	*S. pneumonia MK*, *PMK*, *PPMD*, *E. herbicola crtE*, *crtB* and *crtI*, *H. pluvialis ipiHP1*, *E. coli IDI* and *P. ananatis crtY*	Overexpression	β-Carotene ($C_{40}H_{56}$) (135 mg/l, 4 fold)	Yoon et al. (2007)
Whole MVA pathway and β-carotene biosynthetic genes	*mvaE* and *mvaS* from *E. faecalis*, *mvaK1*, *mvaK2*, and *mvaD* from *S. pneumoniae*, and *IDI* from *E. coli*	Overexpression	β-Carotene ($C_{40}H_{56}$) (465 mg/l)	Yoon et al. (2009)
Whole MVA pathway and β-carotene biosynthetic genes	*mvaE* and *mvaS* from *E. faecalis*, *mvaK1*, *mvaK2*, and *mvaD* from *S. pneumonia*, *IDI* from *E. coli*, *crtE*, *crtB*, and *crtI* from *Pantoea agglomerans*, *ipiHP1* from *H. plauvialis*, *crtY* from *P. ananatis*	Overexpression and cultured in batch or fed-batch	β-Carotene ($C_{40}H_{56}$) (663 mg/l)	Kim et al. (2009)
MVA pathway	*S. cerevisiae*	Overexpression	Abietadiene (100 mg/l, >1000 fold)	Morrone et al. (2010)
MVA pathway and gene encoding isoprene synthase	*S. cerevisiae*	Overexpression in flask	Isoprene (C_5H_8) (2.5 mg/l, 3 fold)	Yang et al. (2012a)
MVA pathway and gene encoding isoprene synthase	*S. cerevisiae*	Overexpression in fed-batch fermentation	Isoprene (C_5H_8) (532 mg/l, 5 fold)	Yang et al. (2012a)
HMGS, *AACT*, *HMGR*, *mvaS* and gene encoding isoprene synthase	*E. faecalis*	Replace *S. cerevisiae HMGS*, *AACT* and *HMGR* with *E. faecalis HMGS*, *AACT* and *HMGR* and site-mutated *mvaS* (A100G), overexpress isoprene synthase	Isoprene (C_5H_8) (6.3 g/l, 12 fold)	Yang et al. (2012b)
MVA pathway	*E. faecalis HMGS*, *HMGR*, *S. cerevisiae HMGR*, *MK*, *MDC*, *Sulfolobus sofataricus PMK* and *E. coli IDI*	Overexpress the MVA pathway and use of ATP and carbon from glycolysis of phosphoenolpyruvate	Efficiency 89.2% to 99% from acetyl-CoA, pyruvate and phosphoenolpyruvate to isoprene	Korman et al. (2014)
MVA pathway and ADP-ribose pyrophosphatase	*Bacillus subtilis* or *E. coli* ADP-ribose pyrophosphatase	Overexpress the MVA pathway and inhibiting IPP conversion to DMAPP by overexpressing ADP-ribose pyrophosphatase	Isoprenol (1.3 g/l single isoprenol), prenol (0.2 g/l prenol)	Zheng et al. (2013)
MVA pathway, especially *HMGS* and *MK*	*HMGS* from *Staphylococcus aureus*, *MK* from *S. cerevisiae*	Correlation analysing metabolites and proteins identify *HMGS* and *MK* as the major steps and adjusting the expression of *HMGS* and *MK*	Isopentenol ($C_5H_{10}O$) (1.5 g/l, 46% of theoretical yield, 3 or 5 fold higher than controls)	George et al. (2014)
MVA pathway, a mutated *GPPS* and a gene encoding truncated geraniol synthase	*E. coli FPPS* (S80P), *Ocimum basilicum*	Combinational overexpression	Geraniol ($C_{10}H_{18}O$) (182.5 mg/l, 2 or 13 fold higher than controls)	Zhou et al. (2014)
GPPS	*E. coli* FPPS (S80P)	Optimization of GPPS expression	Geraniol ($C_{10}H_{18}O$) (1119 mg/l, 6 fold)	Zhou et al. (2015)
MVA pathway, *ispA* and a gene encoding α-farnesene synthase (*AFS*)	*atoB*, *IDI* and *ispA* from *E. coli*, *HMGS*, *t-HMGR1*, *MK*, *PMK* and *mvd1* from *S. cerevisiae*, *AFS* from apple	Combinational overexpression	Farnesene ($C_{15}H_{24}$) (1.1 g/l, ~2000 fold)	Zhu et al. (2014)
MVA pathway and a gene encoding bisabolene synthase	*atoB*, *IDI*, *ispA* from *E. coli*, *t-HMGR*, *MK*, *PMK* and *PPMD* from *S. cerevisiae*, gene encoding bisabolene synthase from *Abies grandis*	Combinational overexpression and codon-optimization of bisabilene synthase	Bisabolene ($C_{15}H_{24}$) (1 g/l)	Peralta-Yahya et al. (2011)
MVA pathway, *IDI1*, *IDI*, *BTS1*, *ERG20*, *FPPS*	*HMGS*, *HMGR1*, *ERG8* (*PMK*), *IDI1*, *BTS1* (*GGPPS*) and *ERG20* from *S. cerevisiae*, *IDI* and *FPPS* from *E. coli*	Combinational overexpression	Farnesol ($C_{15}H_{26}O$) (135.5 mg/l, 350 fold)	Ohto et al. (2009)

Notes: *AACT*, gene encoding acetoacetyl-CoA thiolase; *atoB*, bacterial gene encoding acetoacetyl-CoA thiolase; *AFS*, gene encoding α-farnesene synthase; *BTS1*, yeast gene encoding geranylgeranyl diphosphate synthase; *crtB*, bacterial gene encoding phytoene synthase; *crtE*, bacterial gene encoding geranylgeranyl diphosphate synthase; *crtI*, bacterial gene encoding phytoene desaturase; *crtY*, bacterial gene encoding lycopene cyclase; *ERG20*, yeast gene encoding farnesyl diphosphate synthase; *IDI*, gene encoding isopentenyl diphosphate isomerase; *ipiHP1*, gene encoding isopentenyl pyrophosphate:dimethylallyl pyrophosphate isomerase; *ispA*, bacterial gene encoding farnesyl diphosphate synthase; *MDC*, gene encoding mevalonate pyrophosphate decarboxylase; *MK*, gene encoding mevalonate kinase; *mvaD*, bacterial gene encoding mevalonate 5-diphosphate decarboxylase; *mvaE*, the bifunctional bacterial gene encoding AACT and HMGR; *mvaS*, bacterial gene encoding HMG-CoA synthase; *MK*, gene encoding mevalonate kinase; *PMK*, gene encoding phosphomevalonate kinase; *PPMD*, gene encoding diphospho-mevalonate decarboxylase.

choice, amorphadiene production reached up to 700 mg/l after 48-h fermentation. The engineered *E. coli* strains reported by Newman et al. (2006) were enhanced by replacing *S. cerevisiae* HMGR with a more efficient soluble *Staphylococcus aureus* class II HMGR (cf., Hedl et al., 2004) plus regulating carbon and nitrogen in the fermentation process. A 50-fold elevation in amorphadiene (27.4 g/l) attained (Tsuruta et al., 2009), in comparison to the previous report (Newman et al., 2006). Garcia (2013) examined the kinetic properties of *S. cerevisiae* MK and PMK in *E. coli* and observed feedback inhibition of MK and non-feedback inhibition of PMK, thus providing more clues to further rationally optimise the heterologous-expressed MVA pathway. Proteomics and kinetic-based strategies were further applied to potentially optimise this pathway in *E. coli* (Weaver et al., 2015). Kinetic-related strategies combined with omics technology and computational prediction pose an alternative to metabolic engineering of an enzyme or pathway in microbes. Recently, Chen (2014) reported on combinational approaches to accumulate amorphadiene in *E. coli*, but the highest amorphadiene production was only 2.2 g/l, lower than in a previous study (Tsuruta et al., 2009). Chen (2014) optimised the enzymatic ratio of the whole MVA pathway by a so-called statistical experimental design; inhibition caused by FPP and ATP was overcome by introducing ADS and an ATP-recycling enzyme. Finally, the enzyme immobilisation strategy was applied to achieve the highest amorphadiene production (Chen, 2014).

To enhance the production of lycopene in *E. coli*, the MVA pathway was co-expressed with lycopene biosynthetic genes (*crtE*, *crtB*, *crtI* and *ipiHP1*) (Harada and Misawa, 2012). The engineered *E. coli* presented an 11.8-fold enhancement of lycopene (12.5 mg/g DW), over the control *E. coli* transformed with only the lycopene-producing plasmid (Harada and Misawa, 2012). When the genes from the lower MVA pathway encoding MK, PMK, PPMD and IDI were co-expressed with the lycopene biosynthetic genes, in *E. coli*, a 5-fold elevation of lycopene (102 mg/l, 22 mg/g DW) was produced, in comparison to a strain expressing only the lycopene biosynthetic genes (Yoon et al., 2006). By targeted metabolic engineering of both MVA and lycopene biosynthesis pathways, lycopene production reached 1.2 g/l (34.3 mg/g DW) when a scale-up was performed using 100-l cultures in 150-l fermenter by fed-batch fermentation (Zhu et al., 2015). When an *E. coli* strain harbouring the lycopene biosynthetic genes was further co-transformed with *crtY* and the genes encoding MK, PMK, PPMD and IDI, a 4-fold elevation in β-carotene (135 mg/l) was observed over the strain co-expressing the lycopene biosynthetic genes and *crtY* only (Yoon et al., 2007). Furthermore, the whole MVA pathway was engineered with β-carotene biosynthetic genes to produce β-carotene in *E. coli* (Nguyen et al., 2012; Yoon et al., 2009). These studies showed that an artificially added MVA pathway efficiently redirected acetyl-CoA into IPP, and improved β-carotene production (Nguyen et al., 2012; Yoon et al., 2009). The engineered *E. coli* carrying the biosynthetic genes encoding the whole MVA pathway and β-carotene biosynthetic pathway produced 465 mg/l β-carotene (Yoon et al., 2009). When the recombinant *E. coli* was further cultured in batch or fed-batch, the maximum β-carotene yield reached 663 mg/l (Kim et al., 2009). These investigations indicate that the exogenous MVA pathway is able to efficiently synthesise IPP for the production of lycopene or carotenoids in *E. coli*.

Furthermore, production of diterpene abietadiene in *E. coli* bioreactor-grown cultures by introduction of the heterologous MVA pathway was greater than achieved with the endogenous MEP pathway genes alone (Morrone et al., 2010), because abietadiene reached >1000-fold increase (100 mg/l) (Morrone et al., 2010) in comparison to a previous study (Cyr et al., 2007), thus yielding a more promising approach than in plants.

The production of isoprene in *E. coli* was accomplished when the exogenous MVA pathway from *S. cerevisiae* was co-expressed with a gene encoding isoprene synthase from *Populus alba* (Yang et al., 2012a). Production of isoprene enhanced 3-fold over control in 100-ml shake-flasks or 5-fold over control in 5-l fermenters by fed-batch fermentation (Yang et al., 2012a). To further enhance production, Yang et al. (2012b) optimised the MVA pathway by replacing *S. cerevisiae HMGS*, *AACT* and *HMGR* with *Enterococcus faecalis* equivalents as well as site-directed mutants of mvaS (A110G). The final *E. coli* strain contained a gene encoding isoprene synthase from *P. alba* and production of isoprene elevated (12-fold) than previously achieved (Yang et al., 2012a, b). Recently, isoprene was generated in *E. coli* using a synthetic biochemical pathway, which utilises ATP and carbon synthesised from the glycolysis intermediate phosphoenolpyruvate besides expressing the MVA pathway (Korman et al., 2014). The conversion efficiency from acetyl-CoA, pyruvate and phosphoenolpyruvate to isoprene in the engineered system was 89.2–92.5%, and 96.1–99%, respectively (Korman et al., 2014). Taken together, these studies demonstrated feasibility in engineering the MVA pathway for isoprene production in *E. coli*.

Given the better combustion efficiencies of the five-carbon alcohols derived from IPP or DMAPP including isoprenol (3-methyl-3-butenol), 3-methyl-2-butenol and 3-methylbutanol over ethanol, they are considered promising next-generation biofuels (Chou and Keasling, 2012). To this end, the production of isoprenol or 3-methyl-2-butenol in *E. coli* was enhanced up to 12% by inhibiting the conversion of IPP to DMAPP and by co-expression of a *Bacillus subtilis* or *E. coli* ADP-ribose pyrophosphatase, which uses IPP as a substrate to accumulate isoprenol or 3-methyl-2-butenol (Zheng et al., 2013). Two proteins (HMGS and MK) in the heterologous-applied MVA pathway were identified as major determinants for efficient production of isopentenol in *E. coli* by correlation analysis of target metabolites and proteins (George et al., 2014). After adjusting the expression of HMGS and MK, production of isopentenol (1.5 g/l, 46% of theoretical yield) was enhanced 5-fold (George et al., 2014) over using the original pathway (Chou and Keasling, 2012) and 3-fold over optimised isopentenol-producing strains (Zheng et al., 2013). Herein, HMGS was shown to be the first committed step for the heterologous production of isopentenol in *E. coli* (George et al., 2014).

The monoterpene alcohol, geraniol, which is promising in the fragrance industry, pharmacy, agrochemistry and is considered as an alternative gasoline over ethanol, was heterologously produced in *E. coli* by combinational engineering involving the whole MVA pathway, a mutated GPPS from *E. coli* FPPS (S80P) for GPP production and a truncated geraniol synthase from *Ocimum basilicum* (Zhou et al., 2014). The maximum production of geraniol (182.5 mg/l) in the final engineered strain *E. coli* was 2-fold higher than the wt control (96.5 mg/l) and about 13-fold higher than the *E. coli* strain that co-expressed MK, PMK, PPMD, IDI and GPPS (Zhou et al., 2014). Subsequently, a 6-fold elevation in geraniol (1119 mg/l) was obtained by optimising the GPPS expression in the MVA pathway engineered *E. coli* (Zhou et al., 2015).

Also, the MVA pathway was engineered in *E. coli* with FPPS and an α-farnesene synthase (AFS) to optimally generate farnesene, the precursor of next-generation jet fuel (Wang et al., 2011; Zhu et al., 2014). The production of α-farnesene increased 317-fold (from 1.2 mg/l to 380 mg/l) by overexpressing an exogenous MVA pathway and a fused FPPS synthase and AFS (Wang et al., 2011). The final optimised *E. coli* strain harbouring FPPS, AFS and the MVA pathway yielded an ~2000-fold increase in farnesene (1.1 g/l), in comparison to the control which contains only heterologous AFS (Zhu et al., 2014). Co-expression in *E. coli* of the heterologous MVA pathway with a codon-optimised bisabolene synthase from *Abies grandis* produced sesquiterpene bisabolene (1 g/l), which represents an alternative advanced biofuel and serves as an intermediate for the biosynthesis of many natural compounds, including the natural sweetener hernandulcin (Peralta-Yahya et al., 2011). Wang et al. (2010) reported a 350-fold enhancement of farnesol (135.5 mg/l) in comparison to previous results (Ohto et al., 2009) by co-expression of the entire MVA pathway and a FPP synthase from *E. coli*.

Overall, such obvious advances including the convenience of *E. coli* in genetic transformation for isoprenoid overproduction imply that

prokaryotic cell systems are more adaptable than plants. Perhaps the regulation in plants is too tightly regulated and complex, and pools needed for secondary metabolites appear to be shared besides being sources of primary metabolites. For example, the increase of amorphadiene is higher in *E. coli* than in plants (w/v vs w/w) (Tsuruta et al., 2009; van Herpen et al., 2010), and the culture time for *E. coli* is shorter (several to 16 hours). In contrast, plants are less efficient, needing several months/years (Sawai and Saito, 2011). Furthermore, it is easier to purify the compound from *E. coli* than from plants. Nonetheless, plants remain the preferred host when the desired compound can only be overproduced in a relatively higher amount in plants, when plants themselves are targeted for protection against insects or various adverse stresses, or when the compound is targeted for overproduction in specific organs, cellular compartments, crop or edible plant.

3.3. Biotechnology of the MVA pathway for isoprenoid production in *S. cerevisiae*

Besides *E. coli*, *S. cerevisiae* is another model and excellent organism for isoprenoid heterologous production (Nevoigt, 2008). *S. cerevisiae* is a eukaryote that has the native MVA pathway (Chambon et al., 1991; Tsay and Robinson, 1991) in contrast to *E. coli* which lacks this pathway (Martin et al., 2003). Therefore *S. cerevisiae* may tolerate the potentially toxic effects in *E. coli* caused by MVA pathway intermediates such as MVA, IPP (Fischer et al., 2008; Gruchattka et al., 2013; Martin et al., 2003). Similar to *E. coli*, attempts have been made for the heterologous production of different isoprenoids including amorphadiene, artemisinic acid, carotenoids, taxadiene, isoprene, monoterpenoid and tanshinone by metabolic engineering of *S. cerevisiae*. A summary is listed in Table 3.

Donald et al. (1997) overexpressed the catalytic domain of HMGR in *S. cerevisiae*, resulting in a 10-fold increase in squalene. Later, the MVA pathway was engineered in *S. cerevisiae* for the production of sesquiterpene cubebol and other sesquiterpenes including farnesol (18.4 mg/l), and the total sesquiterpene yield in the engineered strains was 52 mg/l (Asadollahi et al., 2010).

When t-HMGR was co-overexpressed with an *A. annua* epi-cedrol synthase in *S. cerevisiae*, 370 μg/l epi-cedrol (sesquiterpene alcohol) was produced (Jackson et al., 2003). Ro et al. (2006) co-overexpressed *S. cerevisiae* t-HMGR1, SQS (ERG9), FPPS (ERG20), *A. annua* ADS (for the production of amorphadiene) and/or CYP71AV1, which catalyses a three-step oxidative reaction of amorphadiene to artemisinic acid in *S. cerevisiae*. The maximum production of amorphadiene and artemisinic acid was 153 mg/l and greater than 100 mg/l, respectively (Ro et al., 2006). Later, by optimising the culture components and selection markers, the production of artemisinic acid was increased to 250 mg/l using shake-flask cultures and 1 g/l in bioreactors (Ro et al., 2008). Furthermore, an improved fed-batch fermentation process using galactose as the only carbon source for the engineered *S. cerevisiae* strain (Ro et al., 2006) increased the yield of artemisinic acid to 2.5 g/l (Lenihan et al., 2008). Recently, the whole MVA pathway and FPPS were co-overexpressed in *S. cerevisiae* and the production of amorphadiene reached more than 40 g/l following a developed fermentation process (Westfall et al., 2012). A year later, the whole artemisinin biosynthetic pathway could be completed and the production of artemisinic acid reached 25 g/l by engineering all the five *A. annua* enzymes participating in the oxidation of amorphadiene to artemisinic acid in *S. cerevisiae* (Paddon et al., 2013). Furthermore, by using chemical-derived singlet oxygen, artemisinic acid was efficiently converted into artemisinin (Paddon et al., 2013). This synthetic biotechnology obtained from the Keasling Laboratory (UC Berkeley) with collaborators Amyris Inc. and PATH Drug Solutions, has been licenced by Sanofi Aventis (Corsello and Garg, 2015; Paddon and Keasling, 2014). Most importantly, large-scale of semi-synthetic artemisinin (35 tons) with an overall yield of 55% was successfully produced from microbial derived artemisinic acid in 2013 by Sanofi and PATH (Corsello and Garg, 2015 and references cited therein). This represents a typical, successful combination of metabolic engineering, synthetic biology and synthetic chemistry for the production of a pharmaceutical agent, and such strategies are also applicable for the production of other useful compounds or pharmaceutical agents.

The MVA pathway was also engineered by co-overexpressing *Candida utilis* tHMGR1, GGPPS and β-carotene biosynthetic pathway genes including *crtE*, *crtI* and *crtYB* to produce β-carotene in *S. cerevisiae* and the production of β-carotene reached 5.9 mg/g DW (Verwaal et al., 2007). By combinational overexpression of HMGR and the addition of ergosterol inhibitors, the production of β-carotene rose to 6.3 mg/g DW (Yan et al., 2012). Recently, the MVA pathway was further optimised in *S. cerevisiae* and produced a 3-fold increase of β-carotene (18 mg/g DW) over the parent strain (Reyes et al., 2014). Xie et al. (2015a) further elevated the production of β-carotene to 20.8 mg/g DW in *S. cerevisiae* by inducer/repressor-free sequential control of downstream, upstream and competitive pathways of FPP following 120 h of fermentation. Furthermore, metabolic engineering of *S. cerevisiae* tHMGR1, *Xanthophyllomyces dendrorhous* CrtI, variants of *X. dendrorhous* CrtYB and CrtE in *S. cerevisiae* led to the production of 24.4 mg/g DW of lycopene after fed-batch fermentation (Xie et al., 2015b).

S. cerevisiae has been demonstrated a suitable host for the production of isoprene (Lv et al., 2014). By using a push–pull–restrain strategy for the native acetyl-CoA and MVA pathways of *S. cerevisiae*, isoprene accumulation elevated 782-fold (from 0.047 mg/l to 37 mg/l) (Lv et al., 2014).

Farnesene was also heterologously generated in *S. cerevisiae*. Co-overexpression of the native MVA pathway and a farnesene synthase resulted in up to 50% of the theoretical yield from glucose (23.8 g farnesene/100 g glucose) (Chandran et al., 2011). The C15 terpene alcohol (farnesol) as well as nerolidol and geranylgeraniol have also been produced by engineering the MVA pathway in *S. cerevisiae* (Ohto et al., 2009). HMGR was identified as the most efficient enzyme for the production of prenyl alcohols among genes tested in the *S. cerevisiae* MVA and prenyl diphosphate pathways (Ohto et al., 2009). Overexpression of HMGR in *S. cerevisiae* ATCC 200589 produced 145.7 mg/l farnesol, 98.8 mg/l nerolidol and 2.5 mg/l geranylgeraniol (Ohto et al., 2009).

HMGR was shown to be an effective target for the production of the key precursor of diterpene tanshinone in *S. cerevisiae* (Zhou et al., 2012). Co-overexpression of HMGR1 or tHMGR1 with three tanshinone biosynthesis downstream genes that encoding FPPS, GGPPS, copalyl diphosphate synthase (CPS) and kaurene synthase-like (KSL) in *S. cerevisiae* led to a 38% or a 2.6-fold enhancement of miltiradiene (2.7 mg/l) (Zhou et al., 2012). When tHMGR1 was co-expressed with combined GGPPS-FPPS and combined CPS-KSL in *S. cerevisiae*, the production of miltiradiene reached a 4-fold increase (12.5 mg/l), in comparison to the parent strain (Zhou et al., 2012). When the engineered *S. cerevisiae* strain was cultured in a 15-l bioreactor, 365 mg/l miltiradiene was produced (Zhou et al., 2012). Further enhancement of miltiradiene (488 mg/l) (Dai et al., 2012) was obtained by co-overexpression of a semi-dominant mutant allele which enhanced activities of the sterol uptake control protein 2 (UPC2) and a *Sulfolobus acidocaldarius* GGPPS in the above engineered *S. cerevisiae* strain (Zhou et al., 2012). This study indicated that similar to observations with hairy roots, HMGR also plays a significant role in the production of the precursor of diterpene tanshinone in *S. cerevisiae*.

Besides the isoprenoids mentioned above, other products such as patchoulol, β-amyrin (precursor of oleanane-type triterpenoids), protopanaxadiol, fecosterol, cineole and n-butanol were also generated in *S. cerevisiae* through manipulating HMGR or the MVA pathway (Hu and Lu, 2015 and references cited therein).

Overall, both *E. coli* and *S. cerevisiae* represent excellent model organisms for the production of valuable chemicals, drugs, and bio-fuels. The MVA pathway (especially HMGR) could be effectively engineered

Table 3
Manipulation of the MVA pathway and isoprenoid-related genes in *S. cerevisiae*.

Gene/s	Origin	Strategies	Products increase/yield	Reference/s
Catalytic domain of HMGR	*S. cerevisiae*	Overexpression	Squalene (10 fold)	Donald et al. (1997)
t-HMGR1 and *ERG9*	*S. cerevisiae*	Overexpression *t-HMGR1* and downregulation of *ERG9*	Sesquiterpene (52 mg/l), farnesol (18.4 mg/l)	Asadollahi et al. (2010)
t-HMGR and gene encoding epi-cedrol synthase	*S. cerevisiae t-HMGR* and gene encoding *Artemisia annua* epi-cedrol synthase	Overexpression	Sesquiterpene epi-cedrol ($C_{15}H_{26}O$) (370 μg/l)	Jackson et al. (2003)
t-HMGR, *ERG9*, *ERG20*, *ADS* and/or *CYP71AV1*	*S. cerevisiae t-HMGR*, *ERG9* and *ERG 20*, *A. annua ADS* and *CYP71AV1*	Combinational overexpression of *t-HMGR*, *ERG 20*, *ADS* and *CYP71AV1* or downregulation of *ERG9*	Amorphadiene ($C_{15}H_{24}$) (153 mg/l, ~500 fold), artemisinic acid (>100 mg/l)	Ro et al. (2006)
t-HMGR, *ERG9*, *ERG20*, *ADS* and/or *CYP71AV1*	*S. cerevisiae t-HMGR*, *ERG9* and *ERG20*, *A. annua ADS* and *CYP71AV1*	Optimization of the selection markers, culture components and conditions	Artemisinic acid (1 g/l)	Ro et al. (2008)
t-HMGR, *ERG9*, *ERG20*	*S. cerevisiae*	Optimization of the fed-batch fermentation process	Artemisinic acid (2.5 g/l)	Lenihan et al. (2008)
t-HMGR, *ERG8*, *ERG9*, *ERG10*, *ERG12*, *ERG13*, and *ERG20*, *IDI*, *ADE1*, *ADS*	*S. cerevisiae t-HMGR*, *ERG8*, *ERG9*, *ERG10*, *ERG12*, *ERG13*, and *ERG20*, *IDI* and *A. annua ADS*	Combinational overexpression and improve the fermentation process	Amorphadiene ($C_{15}H_{24}$) (40 g/l)	Westfall et al. (2012)
CYP71AV1, *CPR1*, *CYB5*, *ADH1* and *ALDH1* based on previously engineered strain (Westfall et al., 2012)	*A. annua CYP71AV1*, *CPR1*, *CYB5*, *ADH1* and *ALDH1*	Combinational overexpression and optimization of fermentation process	Artemisinic acid (25 g/l)	Paddon et al. (2013)
CYP71AV1, *CPR1*, *CYB5*, *ADH1* and *ALDH1* based on previously engineered strain (Westfall et al., 2012)	*A. annua CYP71AV1*, *CPR1*, *CYB5*, *ADH1* and *ALDH1*	Metabolic engineering, synthetic biology and synthetic chemistry	Artemisinin (55% overall yield)	(Paddon and Keasling, 2014; Corsello and Garg (2015) and references cited therein)
t-HMGR1, *crtE*, *crtI* and *crtYB*	*t-HMGR1* from *S. cerevisiae*, *crtE*, *crtI* and *crtYB* from *Xanthophyllomyces dendrorhous*	Combinational overexpression	β-Carotene ($C_{40}H_{56}$) (5.9 mg/g dry cell weight)	Verwaal et al. (2007)
t-HMGR1, *crtE*, *crtI* and *crtYB*	*t-HMGR1* from *S.* cerevisiae, *crtE*, *crtI* and *crtYB* from *Xanthophyllomyces dendrorhous*	Combinational overexpression, and addition of ergosterol inhibitors	β-Carotene ($C_{40}H_{56}$) (6.3 mg/g dry cell weight)	Yan et al. (2012)
t-HMGR1, *crtE*, *crtI* and *crtYB*	*t-HMGR1* from *S. cerevisiae*, *crtE*, *crtI* and *crtYB* from *Xanthophyllomyces dendrorhous*	Further optimization through adaptive laboratory evolution	β-Carotene ($C_{40}H_{56}$) (18 mg/g, 3 fold)	Reyes et al. (2014)
t-HMGR1, *ERG9*, *crtE*, *crtI* and *crtYB*	*t-HMGR1* and *ERG9* from *S. cerevisiae*, *crtE*, *crtI* and *crtYB* from *X. dendrorhous*	Inducer/repressor-free sequential control the downstream, upstream and competitive pathways of FPP	β-Carotene ($C_{40}H_{56}$) (20.8 mg/g dry cell weight)	Xie et al. (2015a)
t-HMGR1, *crtE*, *crtI* and *crtYB*	*t-HMGR1* from *S. cerevisiae*, *crtE*, *crtI* and *crtYB* from *X. dendrorhous*	Combinational overexpression, site-mutagenesis of crtE and crtYB	Lycopene ($C_{40}H_{56}$) (24.4 mg/g dry cell weight)	Xie et al. (2015b)
t-HMGR1, *ispS*, *IDI1*, *ACS2* and *ERG10* and *ERG20*	*t-HMGR1*, *IDI1*, *ACS2* and *ERG10* and *ERG20* from *S. cerevisiae*, *ispS* from *Populus alba*	Push–pull–restrain strategy	Isoprene (C_5H_8) (37 mg/l, 782 fold)	Lv et al. (2014)
MVA pathway and a gene encoding farnesene synthase	MVA pathway genes from *S. cerevisiae*, gene encoding farnesene synthase from *A. annua*	Combinational overexpression	Farnesene ($C_{15}H_{24}$) [50% of the theoretical yield (23.8 g/100 g glucose)]	Chandran et al. (2011)
HMGR	*S. cerevisiae*	Overexpression	Farnesol (145.7 mg/l), nerolidol (98.8 mg/l), geranylgeraniol (2.5 mg/l)	Ohto et al. (2009)
HMGR1/*t-HMGR1*, *FPPS*, *GGPPS*, *CPS* and *KSL*	*HMGR*, *ERG20* and *BTS1* from *S. cerevisiae*, *CPS* and *KSL* from *S. miltiorrhiza*	Combinational overexpression	Miltiradiene (2.7 mg/l, 38% or 2.6 fold)	Zhou et al. (2012)
t-HMGR1, combined *GGPPS-FPPS* and combined *CPS-KSL*	*HMGR*, *ERG20* and *BTS1* from *S. cerevisiae*, *CPS* and *KSL* from *S. miltiorrhiza*	Combinational overexpression	Miltiradiene (12.5 mg/l, 4 fold)	Zhou et al. (2012)
t-HMGR1, combined *GGPPS-FPPS* and combined *CPS-KSL*	*HMGR*, *ERG20* and *BTS1* from *S. cerevisiae*, *CPS* and *KSL* from *S. miltiorrhiza*	Cultured in a 15-l bioreactor	Miltiradiene (365 mg/l)	Zhou et al. (2012)
t-HMGR1, combined *GGPPS-FPPS*, combined *CPS-KSL*, a semi-dominant mutant allele enhancing sterol uptake control protein 2 activity and a *GGPPS*	*HMGR* from *S. cerevisiae*, *CPS* and *KSL* from *S. miltiorrhiza*, *GGPPS* from *Sulfolobus acidocaldarius*	Combinational overexpression and fed-batch fermentation	Miltiradiene (488 mg/l)	Dai et al. (2012)

Notes: *ACS2*,*ACETYL-COA SYNTHASE2*; *ADH1*, *ALCOHOL DEHYDROGENASE1*; *ALDH1*, *ALDEHYDE DEHYDROGENASE1*; *ADS*, gene encoding amorpha-4,11-diene synthase; *CPS*, gene encoding copalyl diphosphate; *crtE*, bacterial gene encoding geranylgeranyl diphosphate synthase; *crtI*, bacterial gene encoding phytoene desaturase; *crtYB*, bacterial gene encodes a bifunctional phytoene synthase and lycopene cyclase; *CPR1*, gene encoding NADPH-cytochrome P450 reductase; *CYB5*, gene encoding cytochrome b_5; *CYP71AV1*, gene encoding amorphadiene oxidase (cytochrome P450 enzyme); *ERG8*, yeast gene encoding phosphomevalonate kinase; *ERG9*, yeast gene encoding squalene synthase; *ERG10*, yeast gene encoding acetoacetyl-CoA thiolase; *ERG12*, yeast gene encoding mevalonate kinase; *ERG13*, yeast gene encoding 3-hydroxy-3-methylglutaryl-CoA synthase; *ERG20*, yeast gene encoding farnesyl diphosphate synthase; *FPPS*, gene encoding farnesyl diphosphate synthase; *IDI*, gene encoding isopentenyl disphosphate isomerase; *ispS*, gene encoding isoprene synthase; *KSL*, gene encoding kaurene synthase-like; *t-HMGR*, gene encoding truncated 3-hydroxy-3-methylglutaryl-CoA reductase.

to produce different isoprenoids in *E. coli* or *S. cerevisiae*. Although significant progress for heterologous expression of isoprenoids has been made the past several decades, the yield of any isoprenoid produced in *E. coli* or *S. cerevisiae* has not been high enough for commercialisation (Jensen and Keasling, 2015) except recently for the production of artemisinin by combined application of synthetic biology and synthetic chemistry (Corsello and Garg, 2015 and references cited therein). Problems such as the toxicity of the engineered intermediates to the *E. coli* host cell, and the effect of co-factors and feedback regulation by the accumulation of products in the engineered cell culture need to be addressed (Dai et al., 2015). As *S. cerevisiae* is more tolerant to some isoprenoids such as farnesene than *E. coli* (Chandran et al., 2011), the risk of contamination by other byproducts is lower. Nonetheless, using *S. cerevisiae* also poses difficulties (Dai et al., 2015). Squalene accumulation adversely affected growth and it takes longer to grow *S. cerevisiae* than *E. coli*, so the mechanism of each engineered enzyme/protein or pathway in *S. cerevisiae* needs to be further explored (Dai et al., 2015).

3.4. Summary of the methods and techniques used in engineering the MVA pathway in plants, E. coli and S. cerevisiae

Many strategies have been used to engineer the MVA pathway for isoprenoid production in plants, *E. coli* and *S. cerevisiae*. Common strategies include the overexpression of one or more genes in native or non-native plants utilising different promoters, a combinational expression of genes from the MVA and MEP pathways, and the use of truncated HMGR and the expression of site-mutated variants that show higher enzyme activity. In plants, two other techniques include plastid/chloroplast transformation of the whole MVA pathway and the use of plant cell and hairy root cultures as an alternative host (Kempinski et al., 2015; Yuan and Grotewold, 2015). In *E. coli*, two other methods include the regulation of carbon, nitrogen, or ATP and optimisation of enzymatic ratio or culture conditions (batch or fed-batch in flask or in fermenter). In *S. cerevisiae*, other strategies are the regulation of culture components, culture conditions, fermentation processes, or combinational use of metabolic engineering, synthetic biology and synthetic chemistry. It should be noted that different strategies are not independent and are usually applied together or consecutively especially in *E. coli* and *S. cerevisiae* which grow much faster than plants. Also, regulatory mechanisms for isoprenoid production in *E. coli* and *S. cerevisiae* are relatively clearer and simpler than in plants. Therefore, more complicated strategies including simultaneous overexpression of more than 10 genes and regulation of copy numbers of overexpressing gene(s) have been used in *E. coli* and *S. cerevisiae* than in plants. Synthetic biology combined with metabolic engineering and synthetic chemistry in *E. coli* and *S. cerevisiae* seem promising. With the development of new techniques such as system biology and image processing in phenotyping/genotyping, more information will be available for better understanding of the complex regulatory mechanisms for isoprenoid production in plants, *E. coli* and *S. cerevisiae*. By then, more precise strategies could be designed and applied for more efficient and higher production of target compounds. It is expected that the combination of synthetic biology with metabolic engineering, system biology and synthetic chemistry will take the research area (MVA pathway in isoprenoid production) to a new level in the near future.

4. Conclusions and perspectives

In summary, the updated role of the MVA pathway in seed production and miRNA-related function are summarised. The MVA pathway is necessary in the biosynthesis of isoprenoids such as sterols, rubber, sesquiterpene artemisinin, diterpene tanshinone, and triterpene ginsenoside. Advances in biotechnology/metabolic engineering of the MVA pathway and/or downstream genes in plants, *E. coli* and *S. cerevisiae* are deemed necessary in our perspectives provided herein. With such a strategy different kinds of isoprenoids including MVA, sesquiterpene amorphadiene, artemisinic acid or artemisinin, tetraterpene carotenoids, diterpene abietadiene, miltiradiene (precursor of tanshinone), isoprene (precursors of rubber), and next-generation fuels (isopentenol, geraniol, farnesene, bisabolene and farnesol) have been successfully produced in *E. coli* and in *S. cerevisiae* through metabolic engineering of the MVA pathway alone or in combination with other downstream genes of related target compounds. Notably, semi-synthetic artemisinin production has entered commercialization using a combination of metabolic engineering, synthetic biology and synthetic chemistry (Paddon and Keasling, 2014). New technology such as *cis*-genic vectors which only use host gene sequences in the transformation construct or marker-free vectors can be applied for overexpression of a gene or a pathway to reduce possible adverse effects posed from the transformation construct and will improve the acceptance of the products (Tester and Langridge, 2010). The newly developed genome editing techniques such as clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) regulatory systems provide alternative efficient ways to investigate the roles of single or multiple genes (Piatek et al., 2015; Schiml et al., 2014; Yan et al., 2015). Furthermore, the mechanism and regulatory role of the MVA pathway and its relationship with the MEP pathway and other metabolic pathways such as fatty acid metabolism, glycolysis, and amino acid metabolism, which also use acetyl-CoA or pyruvate as the central precursor, need more comprehensive investigations. Only then can the metabolic engineering of the MVA pathway for isoprenoid production be further improved in tandem with applications in system biology methods such as transcriptomics, proteomics, metabolomics and synthetic biology (Corsello and Garg, 2015; Jensen and Keasling, 2015; Keasling, 2012; Kizer et al., 2008; Liu et al., 2015) in the near future.

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