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An Overview of 3-Hydroxy-3-Methylglutaryl CoA Reductase (HMGR) in Plants

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Abstract – Isoprenoids biosynthesis in plants involves two separate pathways, mevalonate (MVA) pathway and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. A large group of isoprenoids are found to play crucial roles in common plant biochemical functions and have been produced on a large scale for commercial applications. 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is the key enzyme that catalyses the first committing step in the MVA pathway. In mammals and yeast, HMGR is a well-studied enzyme as many studies have been done on this enzyme due to its important function in the biosynthesis of cholesterol. In plants, many researches on HMGR have been done on different plant species, for example, *Arabidopsis thaliana*, tobacco, gingko, *Zea mays*, potato, rose, rubber tree, muskmelon, ginseng and others, in the past decades since it was discovered. Previous researches that worked on plant HMGR focused on the cloning and characterisation of its physiological functions. Little is known about the aspect of regulation and structural characteristics of plants' HMGR. This review is aimed at providing an overview of the characteristics and structure of HMGR, the transcriptional and post-translational events related to HMGR that have been reported in plants, and proposes areas on the regulation event of HMGR in plants that can be explored to further enhance understanding towards HMGR regulatory interactions.

Keywords: 2-C-methyl-D-erythritol 4-phosphate pathway, 3-hydroxy-3-methylglutaryl-coenzyme Areductase, isoprenoid biosynthesis, HMGR, mevalonate pathway

Introduction

Isoprenoids, also known as terpenoids, are among the largest variety group of natural products in plants derived from isopentenyldiphosphate (IPP) and dimethylallyldiphosphate (DMAPP). Isoprenoids are categorised into primary and secondary metabolites. Primary metabolites are essential in common plant functions where they are involved in many biochemical functions: acting in the electron transport chains (quinones), as membrane components (sterols), pigments (carotenoids) and phytohormones (gibberellins, brassinosteroids, abscisic acid, strigolactones and cytokinins) (reviewed by Pulido, Perello, & Rodriguez-Concepcion, 2012). For secondary metabolites, they are the non-essential compounds with specific functions involved in the interaction between plants and the environment. These secondary metabolites are normally produced specifically by plant families, species, and/or organs. Compounds that are produced as secondary metabolites include: monoterpenes, diterpenes, triterpenes, sesquiterpene and polyterpenes. Most of these terpenoids are applied in the industries as flavoring agents, pigments and drugs (reviewed by Rodríguez-Concepción & Boronat, 2015).

Isoprenoids biosynthesis involves two different pathways, the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Fig. 1). These two pathways are located in separate subcellular sections with the MVA pathway in the cytosol and the MEP pathway in the plastid (Nagegowda, 2010). Metabolites that are derived from the MVA pathway, for example, cytochrome a3 ,

phytosterols, prenylated proteins and ubiquinones are mostly involved in the cell's primary functions and are important in promoting cell growth (Hemmerlin et al., 2004; Opitz, Nes, & Gershenzon, 2014), whereas in the MEP pathway, the enzymes are encoded by nuclear genes and lead to the production of precursors for examples abscisic acid, carotenoids, gibberellins, monoterpenes and the side chain of chlorophylls, tocopherols, plastoquinone and phylloquinones that are crucial in plant growth and development (Hemmerlin, Harwood, & Bach, 2012; Rodríguez-Concepción, 2006; Ton, Flors, & Mauch-Mani, 2009; Van Schie et al., 2007). In addition, some of the compounds produced from MEP pathway are found to possess medicinal and nutritional values, and are important for biotechnological manipulation (Cordoba, Salmi, & Leon, 2009; Du, Yu, Xu, & Li, 2014; Yang & Guo, 2014).

The MVA pathway (Fig. 1) begins with the condensation of acetyl-CoA, catalysed by acetoacetyl-CoA thiolase (AACT) and HMG-CoA synthase (HMGS) to produce 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). Then, the HMG-CoA is irreversibly converted to MVA catalysed by HMG-CoA reductase (HMGR). This conversion of HMG-CoA to MVA is considered as the first committed step of MVA pathway and the enzyme involved in this step, HMGR is also thought as catalysing the bottleneck step in the biosynthesis of isoprenoids (Leivar, et al., 2011; Suwanmanee, Sirinupong, & Suvachittanont, 2013). In the MEP pathway (Fig. 1), the reaction starts when 1-deoxy-D-xylulose 5-phosphate (DXP) is formed, involving the condensation of glyceraldehyde 3-phosphate and pyruvate, catalysed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS). DXP acts as an intermediate for the biosynthesis of isopentenyldisphosphate (IPP) and dimethylallyldiphosphate (DMAPP). There has been a suggestion that the reaction catalysed by DXS could be the first committed step in MEP pathway for the isoprenoids biosynthesis (Gong, Liao, Guo, Sun, & Tang, 2006).

Though these two pathways work independently in their own subcellular compartments, metabolic cross-talk between them has been found (Bick & Lange, 2003; Mendoza-poudereux et al., 2015). However, in-depth understanding on how MVA and MEP pathways contribute to the biosynthesis of various plant isoprenoids especially the cross-talk mechanisms between these two pathways and their regulation is still lacking, except for the discovery that isoprenoid secondary metabolites tend to be produced from both MVA and MEP pathways (Opitz et al., 2014). Thus, new approaches are expected to be available in the near future to further understand the plant isoprenoid metabolism; consequently, more metabolic engineering approaches can be designed to improve the isoprenoid production that has industrial values.

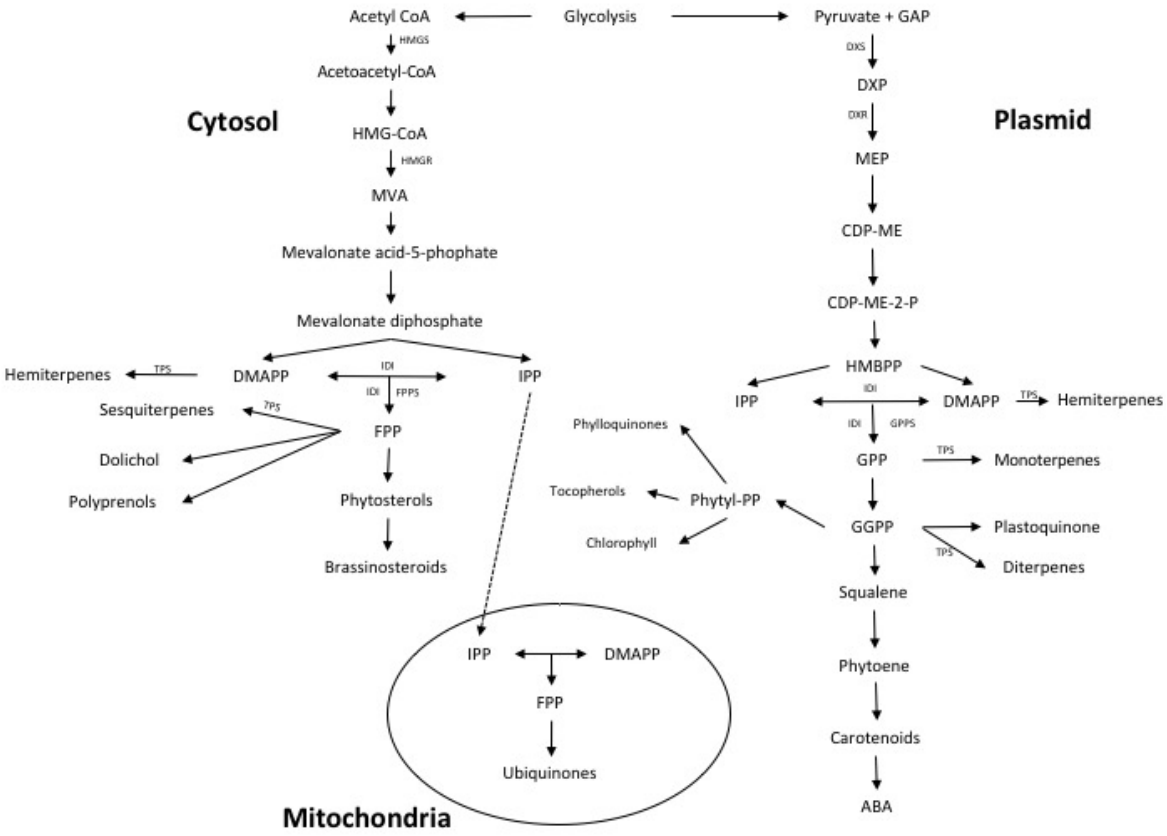

Figure 1: The enzymatic steps of melavonate (MVA) pathway and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway in plant. HMGS, 3-hydroxy-3-methylglutaryl synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; HMGR, 3-hydroxy-3-methylglutaryl CoA reductase; MVA, mevalonate; IPP, isopentenyldiphosphate; DMAPP, dimethylallyldiphosphate; IDI, isopentenyldiphosphateisomerase; FPP, famesyldiphosphate; FPPS, farnesyldiphosphate synthase; TPS, terpene synthase; GAP, glyceraldehyde-3-phosphate; DXP, 1-deoxy-D-xylulose; DXS, 1-deoxy-D-xylulose synthase; DXR, MEP, 2-C-methyl-D-erythritol 4-phosphate; CDP-ME, 4-(Cytidine 5'-diphospho)-2-C-Methyl-D-erythritol; CDP-ME-2-P, 4-(Cytidine 5'-diphospho)-2-C-Methyl-D-erythritol 2-phosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate; GPP, geranyldiphosphate; GPPS, geranyldiphosphate synthase. [Sources: Hemmerlin, Harwood, & Bach (2012); Kumari, Priya, Misra, & Yadav (2013); Rodríguez-Concepción (2006)]

Characteristics and Structure of 3-Hydroxy-3-Methylglutaryl CoA Reductase (HMGR)

The HMGR is the most studied enzyme in the MVA pathway and it is also known as a key enzyme as it catalyses the first committed step in the isoprenoid biosynthesis pathway (Li et al., 2014). The plants' cytosolic HMGR is involved in the synthesis of sterols (for plants' development), sesquiterpenes (defence against herbivores) and ubiquinone (turnover of cellular protein). However, in plastid, monoterpenes, diterpenes, carotenoids, abscisic acid, gibberellins, side chains of chlorophylls and prenylquinones are mainly synthesised in the MEP pathway (Rodríguez-Concepción & Boronat, 2015). Besides that, in the isoprenoid biosynthesis pathway, HMGR appeared to be the only membrane-bound enzyme in plants while the other enzymes are predicted to be soluble cytosolic proteins. However, the membrane domain is not necessary for catalytic activity (Kumari et al., 2013). All eukaryotic HMGRs are similar in their basic functional features, though they possess different structures in plant, human and yeast. The membrane and catalytic domains in human and yeast HMGR show correlated functions. The membrane domains of HMGR in human and yeast control catalytic activity by determining the targeting, turnover and ability to induce propagation of endoplasmic reticulum (ER); and oligomerisation state of the catalytic domain affects the tertiary structure and function of the membrane

domain (Ferrero et al., 2015). Therefore, it is suggested that this structural organisation in the enzyme's architecture in plant cells possess functional and regulatory purposes (Hemmerlin et al., 2012).This suggestion is further confirmed by Ferrero et al. (2015) who reported that the membrane domain of plant HMGR played a part in the proliferation of ER and the biogenesis of organised smooth endoplasmic reticulum (OSER) as overexpression of the membrane domain of Arabidopsis HMGR caused ER hypertrophy, and knockout of HMG1 gene that encodes major HMGR isoforms caused aggregation of ER.

There are three regions in the HMGR sequence which are: the N region with two transmembrane parts, a linker region, and a catalytic region that contains approximately 400 amino acids. In the catalytic region of plants' HMGR, there are three domains: the N domain with a small helical amino-terminal, a large central L domain with two motifs (TTEGCLVA and EMPVGYVQIP) that bind to the substrate HMG-CoA, and the motif (GTVGGGT) that binds to NADPH and a small helical S domain with motif (DAMGMNM) that binds to NADPH (Li et al., 2014). The variants of HMGR were previously known to target primarily the endoplasmic reticulum (ER) but it has been found that the primary reservoir of Arabidopsis HMGR in cotyledons is within the novel cytoplasmic and intravacuolar vesicular structures derived from the sub-compartments of ER instead of reticular ER (Leivar et al., 2005). The families of plant HMGR genes encoded no less than three isozymes, depending on the species of the plants, and each isozyme encoded was found to have a different function in plants (Hemmerlin, Harwood, & Bach, 2012). It was suggested by Kim, Lee, Oh, Jang, & Yang, (2014) that these isozymes of HMGR could possibly evolve differently based on the production of specific products instead of duplication. This suggestion was made because from their research the HMGR isozymes from ginseng have similar identities with HMGR isozymes of *Acanthopanax* and *Camptotheca acuminata*. Thus, the HMGR isozymes of ginseng may have originated from gene evolution event and evolved accordingly to synthesised triterpene ginsenoside. However, the molecular mechanism on how the HMGRs affect the downstream metabolites and how their respective functions are being distributed are yet to be clarified (Muranaka & Ohyama, 2013). Every HMGR may own a specific function in plant and the involvement of each HMGR isozyme in the mechanism of synthesising isoprenoids may vary based on their functions.

Regulation of HMGR

HMGR plays a crucial role in the regulation of the MVA pathway. The HMGR activity and its transcripts level is high in plant parts where sterols biosynthesis is actively occurring and with low structure maturity, such as in the apical buds and roots (Rodríguez-Concepción, Campos, Ferrer, & Boronat, 2013).

The plants' HMGRs respond to various external stimuli, such as light (Korth, Jaggard, & Dixon, 2000; Rodríguez-Concepción et al., 2004), elicitor treatment (Cao, Li, Miao, Zheng, & Jiang, 2011; Cools, Chope, Hammond, Thompson, & Terry, 2011; Diarra, He, Wang, & Li, 2013; Suh, Hyun, Kim, Lee, & Choi, 2013; Wang, Zheng, Zhao, Zhao, & Wang, 2014), attacks of pathogens and pests (Arimura et al., 2007; Tian et al., 2015), woundings (Akhtar, Gupta, Sangwan, Sangwan, & Trivedi, 2013), and exposure to heavy metals (Hojati et al., 2015; Rai, Pandey, & Rai, 2011). The responses of the HMGR family paralogs towards endogenous molecules like sterol metabolites, phytohormones and external stimuli are different depending on their differences in the patterns of developmental and tissue-specific expression (Tholl, 2015).

The HMGR is highly regulated at both the transcriptional and post-transcriptional levels. However, the correlations between protein quantities and/or mRNA levels and enzymes activities are yet to be shown in studies on the regulation of HMGR (Hemmerlin, Harwood, & Bach, 2012). The regulation of plant HMGR upon sensing and transducing the environmental light situation had been suggested to be a mechanism in controlling the metabolism of isoprenoids and the development of seedlings (Rodríguez-Concepción et al., 2004). Light was shown to have negative regulation on gene expression in *Arabidopsis thaliana* (Learned & Connolly, 1997; Rodríguez-Concepción et al., 2013), *L. erythrorhizon* (Markus Lange, Severin, Bechthold, & Heide, 1998) and mulberry (*Morus alba*) (Jain, Vincent, & Nessler, 2000). This metabolic control that occurred at the transcriptional level was found to be

influenced by illumination period and the quality of spectra (Rodríguez-Concepción, Campos, Ferrer, & Boronat, 2013). However, when ginseng (*Panax ginseng* Meyer) was exposed to darkness, HMGR activity was induced and this increased the production of ginsenosides because ginsenosides biosynthesis is dark-dependent. Thus, it is believed that HMGRs are involved in the regulation of triterpene ginsenoside biosynthesis (Kim, Lee, Oh, Jang, & Yang, 2014).

Post translational modification is important in regulating the metabolic pathways in plants where it revises the functionality of the proteins, interaction with other proteins, stability of proteins and their subcellular localisations. HMGRs have been studied using *in vitro* extracts method and the characterisation of this enzyme modification was only done *in vitro* or from purified proteins. Therefore, post-translational modification of enzymes in plant isoprenoid biosynthesis pathways with intelligible *in vivo* data is still finite (Hemmerlin et al., 2012; Iqbal et al., 2014; Leivar et al., 2011).

It has been proposed that the transcriptional modulation of HMGR is present in several plant systems; however, the information on post-translational control of HMGR is still limited (Rodríguez-Concepción, Campos, Ferrer, & Boronat, 2013). Currently, it is believed that plant enzymes in the beginning stages of the isoprenoid pathways are regulated by phosphorylation or by ubiquitin-dependent proteolytic degradation (Hemmerlin, Harwood, & Bach, 2012). Phosphorylation was the main protein modification identified in enzymes at the early stages of isoprenoid biosynthesis in plants. It was found that protein phosphatase 2A (PP2A) in *Arabidopsis thaliana* (*A. thaliana*) acted as a negative regulator of HMGR activity in post-translational regulation whereby it involved dephosphorylation and degradation of the HGMR (Antolín-Llovera et al., 2011; Leivar et al., 2011).This modulation of HMGR by PP2A could be the end point of the signal transduction network for the integration of phytohormone signals and environmental calcium-mediated stimuli. Therefore, it would be interesting to find out to what level PP2A is involved in the modulation of HMGR by phytohormones, and the exact roles of phytohormones and calcium signals in this matter. Under salt stress condition, B" regulatory subunit (B"α) and PP2A were found to mediate the decrease and subsequent increase of the HMGR activity that arose due to a consistent rise of HMGR1-encoding transcript levels and an early more pronounced decrease of HMGR protein level. However, based on the research, the reason behind the increase in HMGR protein levels which occurred along with a lower increase in HMGR activity is still unknown, although it has been suggested that it could be due to the simultaneous post-translational repression. The changes in transcript and protein levels of HMGR might happen at various rates, relying on the plant system and type of challenges (Leivar et al., 2011).

Proteolysis was identified as a regulatory mechanism to remove regulatory protein(s) when necessary, while at the same time transforming other proteins from dormant into biologically active states (Ehrmann & Clausen, 2004). Proteolysis degradation has been suggested in several researches to be involved in the control of HMGR in plants. It was shown that the degradation of HMGR helps to control its activity in potato leaf tissues as cysteine protease increases the HMGR protein recovery and its activity (Korth, Jaggard, & Dixon, 2000). On the other hand, there was a decline of HMGR activity in both potato tuber tissue and sweet potato root when measuring the HMGR protein level by immunotitration using specific polyclonal antibody and by radioimmunoassay using monoclonal antibody. This suggested that inactivation of HMGR was caused by its proteolytic degradation(Kondo, Uritani, & Oba, 2003). It was also suggested that proteolytic degradation possibly took place in the regulation of HMGR expression of melon (*Cucumis melo* L.). Western blot analysis demonstrated that there is a protein cross-reaction with the anti-HMGR antibody, thus, the protein band was suspected to contain a proteolytic fragment that lacked the transmembrane domains (Takahashi, Kobayashi, Sato-Nara, Tomita, & Ezura, 2002).

Conclusion

In conclusion, plant HMGR plays important roles in the biosynthesis of isoprenoids. Research on plant HMGR constantly showed that hormonal differences, environment signals, and metabolic needs can affect the activity of HMGR. The understanding of the role of plant HMGR in post-translational regulation event is still limited. Thus, to further study the post-translational regulation, pure regulatory

enzymes extracted from plants and/or recombinant proteins should be subjected to systemic kinetic properties analysis.

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