Biosynthesis of Isoprenoid Precursors in Arabidopsis

Manuel Rodríguez-Concepción, Narciso Campos,Albert Ferrer,and Albert Boronat

Abstract

Isoprenoids are the most functionally and structurally diverse group of plant metabolites reported to date. Many of them participate in essential processes, like respiration (ubiquinone), photosynthesis (carotenoids, chlorophylls, plastoquinone), and regulation of growth and development (cytokinins, brassinosteroids, gibberellins, abscisic acid). However, most plant isoprenoids are considered as secondary metabolites and play key roles in mediating the interactions of plants with their environment. Some plant isoprenoids also have biotechnological interest. Despite their structural and functional diversity, all isoprenoids derive from the same five-carbon precursors, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). Plants contain two pathways for the synthesis of IPP and DMAPP: the mevalonate pathway (located in the cytosol/endoplasmic reticulum) and the methylerythritol 4-phosphate pathway (located in the plastids). A limited exchange of IPP and/or prenyl diphosphates is known to take place between these compartments in plant cells. In recent years, the use of the model plant *Arabidopsis thaliana* in genetic and biochemical approaches has allowed the identification of all the genes and enzymes from both pathways and has accelerated the flow of information on their

regulation. A major challenge now is to understand how the production of common isoprenoid precursors in different subcellular locations conveys the information required to coordinate the fluxes of the two pathways. Here we review the current knowledge on these matters deduced mainly from work carried out in Arabidopsis.

Keywords
Arabidopsis • Dimethylallyl diphosphate • Isopentenyl diphosphate • Isoprenoid • Methylerythritol 4-phosphate • Mevalonate • Terpenoid

1 Introduction

Isoprenoids, also known as terpenoids, are the most functionally and structurally diverse group of plant metabolites reported to date. They are produced in all living organisms, but their abundance and variety in plants are unparalleled. From the tens of thousands of plant isoprenoid compounds, only a few can be considered as "primary" metabolites, i.e., those that are essential for plant function and are therefore common to all plant species. These include compounds that participate in respiration (ubiquinone), photosynthesis (carotenoids, chlorophylls, plastoquinone), and regulation of growth and development (cytokinins, brassinosteroids, gibberellins, abscisic acid). The rest are "secondary" metabolites, nonessential compounds whose biosynthesis is usually restricted to specific plant families or even to particular plant species. They typically function in protecting plants against herbivores and pathogens, in attracting pollinators and seed-dispersing animals, and as allelochemicals that influence competition among plant species (cf. Croteau et al. 2000; Bouvier et al. 2005). A large number of secondary isoprenoid metabolites have a commercial value as flavors, pigments, polymers, or drugs.

Despite the structural and functional diversity among isoprenoids, they all derive from the same five-carbon (C_5) precursors, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), also called isoprene units (Fig. 30.1). Addition of IPP units to DMAPP generates prenyl diphosphate molecules of increasing size such as geranyl diphosphate (GPP, C_{10}), farnesyl diphosphate (FPP, C_{15}), and geranylgeranyl diphosphate (GGPP, C_{20}). These are the starting points for the production of the huge variety of isoprenoids found in plants. Consistent with the compartmentalization of most plant metabolic pathways in plants (Lunn 2007), different steps of plant isoprenoid biosynthesis can take place in different plant tissues and subcellular compartments. Most notably, all plants use two different pathways for the production of the same universal isoprenoid precursors (Fig. 30.1). The mevalonic acid (MVA) pathway synthesizes cytosolic IPP for the production of sterols, brassinosteroids, sesquiterpenes, polyprenols, dolichols, and prenyl moieties used for protein modification. MVA-derived IPP is also transported to mitochondria for the biosynthesis of ubiquinone (Disch et al. 1998). Plastidial IPP and DMAPP precursors for the production of carotenoids, abscisic acid, gibberellins, monoterpenes, isoprene, and the side chain of chlorophylls, tocopherols, phylloquinones, and plastoquinone are synthesized by the methylerythritol 4-phosphate (MEP) pathway. A limited exchange of IPP and/or prenyl diphosphates is known to take place between these compartments in plant cells (Rodríguez-Concepción and Boronat 2002; Bouvier et al. 2005). In recent years, the use of the model plant *Arabidopsis thaliana* in genetic and biochemical approaches has allowed the identification of all the genes and

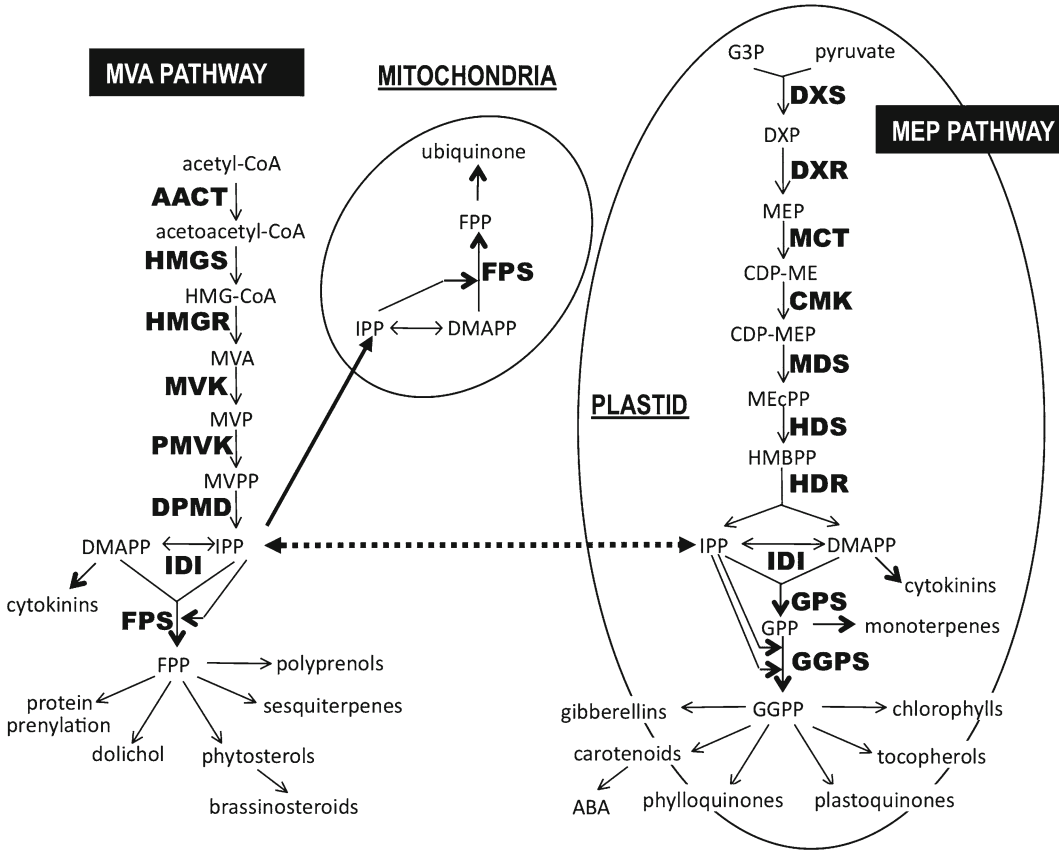

Fig. 1 *Organization and compartmentalization of isoprenoid biosynthesis in plants*. *HMG-CoA* 3-hydroxy-3-metylglutaryl coenzyme A, *MVA* mevalonate, *MVP* 5-phosphomevalonate, *MVPP* 5-diphosphomevalonate, *IPP* isopentenyl diphosphate, *DMAPP* dimethylallyl diphosphate, *FPP* farnesyl diphosphate, *G3P* D-glyceraldehyde 3-phosphate, *DXP* 1-deoxy-D-xylulose 5-phosphate, *MEP* 2-*C*-methyl-D-erythritol 4-phosphate, *CDP-ME* 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol, *CDP-MEP* 4-(cytidine 5′-diphospho)-2-*C*-methyl-D-erythritol phosphate, *MEcPP* 2C-methyl-D-erythritol 2,4-cyclodiphosphate, *HMBPP* 4-hydroxy-3-methylbut-2-enyl diphosphate, *GPP* geranyl diphosphate, *GGPP* geranylgeranyl diphosphate. Enzymes are *AACT* acetoacetyl-CoA thiolase, *HMGS* HMG-CoA synthase, *HMGR* HMG-CoA reductase, *MVK* MVA kinase, *PMVK* MVP kinase, *DPMD* MVPP decarboxylase, *IDI* IPP isomerase, *DXS* DXP synthase, *DXR* DXP reductoisomerase, *MCT* CDP-ME synthase, *CMK* CDP-ME kinase, *MDS* MEcPP synthase, *HDS* HMBPP synthase, *HDR* HMBPP reductase

enzymes from both pathways and has accelerated the flow of information on their regulation. A major challenge now is to understand how the production of common isoprenoid precursors in different subcellular locations conveys information between compartments in order to coordinate fluxes through both pathways. Here we review the current knowledge on these matters deduced mainly from work carried out in *Arabidopsis*.

2 Historical Perspective

Until relatively recent times, it was believed that IPP was synthesized from acetyl-CoA via MVA and then isomerized to DMAPP in all living organisms (Chappell 1995; McGarvey and Croteau 1995). The MVA pathway, discovered in the 1950s, was soon found to function in plant cells (Goldstein and Brown 1990). However, inconsistent data suggesting the existence of a

MVA-independent pathway in bacteria and plant plastids grew stronger until the early 1990s, when compelling new evidence for the existence of a completely novel pathway for the production of IPP and DMAPP was shown (reviewed in Lichtenthaler et al. 1997; Eisenreich et al. 1998; Lichtenthaler 1999; Rohmer 1999, 2008). It was soon established that the new pathway, currently known as the MEP pathway after what it is believed to be its first committed precursor in bacteria (following the same rule used to name the MVA pathway), was the only one present in most eubacteria and apicomplexan protozoa such as the malaria parasite. Following the first biochemical approaches, genetics and genomics (database searches) demonstrated the existence of the pathway in several plants, including *Arabidopsis*. By 2002, all the genes encoding MEP pathway enzymes in *Arabidopsis* had been identified (cf. Rodríguez-Concepción and Boronat 2002). Genomic tools also led to identify all the *Arabidopsis* genes potentially encoding MVA pathway enzymes, as well as those putatively involved in other isoprenoid biosynthetic pathways (Lange and Ghassemian 2003).

3 The MVA Pathway in *Arabidopsis*

The MVA pathway (Fig. 30.1) starts with the sequential condensation of three molecules of acetyl-CoA to yield 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) catalyzed by the enzymes acetoacetyl-CoA thiolase (AACT) and HMG-CoA synthase (HMGS). HMG-CoA is then con-verted to MVA in a functionally irreversible reaction catalyzed by HMG-CoA reductase (HMGR). MVA is sequentially phosphorylated and decarboxylated to generate IPP by the enzymes mevalonate kinase (MVK), 5-phospho-mevalonate kinase (PMVK), and 5-diphosphom-evalonate decarboxylase (DPMD). IPP along with DMAPP, formed by the action of isopente-nyl diphosphate isomerase (IDI), represent the building blocks for the synthesis of isoprenoid end products and derivatives in the cytosol, endoplasmic reticulum (ER), and mitochondria. With the only exception of PVMK, the rest of the MVA pathway enzymes have been characterized at the biochemical and genetic level in *Arabidopsis*.

AACT catalyzes the reversible Claisen-type condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. *Arabidopsis*contains two genes encoding AACT, *ACT1* (At5g47720) and *ACT2* (At5g48230). These genes encode two closely related AACT isoforms, designated as AACT1 and AACT2 respectively, located in dif-ferent cell compartments (Ahumada et al. 2008). While AACT1 is found in peroxisomes, AACT2 is localized in the cytosol and the nucleus. The peroxisomal localization of AACT1 depends on the presence of a C-terminal peroxisomal tar-geting sequence 1 (PTS1) motif (Ser-Ala-Leu) not previously found in other organisms (Ahumada et al. 2008). However, targeting of AACT1 to peroxisomes has also been associated to an N-terminal extension (PTS2) present in a splicing variant of the protein (Carrie et al. 2007). *ACT1* and *ACT2* genes are differentially expressed. Whereas *ACT2* expression is relatively high in all parts of the plant, the expression of *ACT1* is much lower and restricted to roots and in fl orescences (Ahumada et al. 2008). The expression of *ACT2*is induced by light during seedling development (Ahumada and Boronat, unpublished results).

Although the metabolic role(s) of AACT1 and AACT2 has not yet been unequivocally assigned, the characterization of *Arabidopsis* knockout mutants defective in the *ACT1* or *ACT2* genes suggests that only AACT2 is actually involved in isoprenoid biosynthesis. T-DNA insertion mutants affected in the expression of the *ACT2* gene are embryo-lethal, whereas null alleles of *ACT1* are viable and have no apparent growth phenotypes (Jin and Nikolau 2007). The meta-bolic function of AACT1 is not clear at present, although its particular peroxisomal localization might exclude a role in isoprenoid biosynthesis, and rather argues for its implication in β-oxida-tion of fatty acids, catalyzing the last step.

HMGS catalyzes the condensation of acetyl-CoA with acetoacetyl-CoA to produce HMG-CoA. A cDNA encoding HMGS was cloned by Montamat et al. (1995) and corresponds to the

only gene coding for this enzyme in *Arabidopsis* (*HMS*, At4g11820). Although HMGS behaves as a cytosolic enzyme, immunocytochemical analysis has shown that the enzyme is mainly found associated with the ER, the Golgi apparatus, and unknown globular structures (Díez and Boronat, unpublished results). *HMS* transcripts have been detected in all plant tissues, although they are particularly abundant in the hypocotyl and the root elongation zone of young seedlings and in the roots and the inflorescences of adult plants (Díez and Boronat, unpublished results). In contrast to other MVA pathway genes, the expression of *HMS* is not regulated by light (Díez and Boronat, unpublished results). A more detailed kinetic analysis has been done with HMGS1 from the closely related plant *Brassica juncea* (Indian mustard, Nagegowda et al. 2004). In this plant, four stress-inducible genes exist, encoding closely related isozymes (Alex et al. 2000), which are differentially expressed in the plant (Nagegowda et al. 2005).

HMGR has a well-recognized regulatory role in isoprenoid biosynthesis and is the best characterized enzyme of the MVA pathway. In *Arabidopsis*, the genes *HMG1* (At1g76490) and *HMG2 (*At2g17370) encode three HMGR isoforms (HMGR1S, HMGR1L, and HMGR2) (Caelles et al. 1989; Enjuto et al. 1994; Lumbreras et al. 1995). HMGR1S and HMGR1L isoforms only differ in their N-terminal region and derive from two mRNAs transcribed from two alternative promoters in the *HMG1* gene. The presence of an in-phase upstream AUG start codon in the HMGR1L mRNA results in the synthesis of the HMGR1L isoform that has 50 additional amino acid residues at its N-terminal end with respect to the HMGR1S isoform (Lumbreras et al. 1995). The three HMGR isoforms are primarily targeted to the ER and have the same topology in the membrane (Campos and Boronat 1995; Lumbreras et al. 1995). Like all known plant HMGRs, four regions have been defined in HMGR1S, HMGR1L, and HMGR2: the N-terminal region (highly divergent), two conserved membrane-spanning targeting sequences, the linker region (also highly divergent), and the catalytic domain, which is highly conserved among eukaryotic HMGRs. The N-terminal region and the catalytic domain are located in the cytosol, whereas only a short stretch of amino acids of the membrane-spanning domain are facing the ER lumen. In *Arabidopsis* epidermal cells, HMGR was immunolocalized in the ER, as expected, but also in spherical structures of the endomembrane system, 0.2–0.6 μm in diameter, that may represent a specific subcompartment for isoprenoid biosynthesis (Leivar et al. 2005). In a more recent study, using tobacco BY-2 cells expressing GFP proteins to which the membrane domains of two major HMGR isoforms were N-terminally fused suggested an association with the cytoskeleton of one form, which could be interconverted into the other one forming aggregates by just one amino acid exchange in a putative phosphorylation motif (Merret et al. 2007). The HMGR1S transcript is found in all tissues, but at fairly higher levels during the first stages of development and in the inflorescences (Enjuto et al. 1994). HMGR1L and HMGR2 transcripts are detected only in seedlings, roots, and inflorescences, and their abundance is much lower than that of the HMGR1S mRNA (Enjuto et al. 1995; Lumbreras et al. 1995). In transgenic tobacco plants harboring a *HMG2*:*GUS* quimeric gene, GUS expression was restricted to meristematic and floral tissues (Enjuto et al. 1995). Promoter analysis has revealed that sequence elements of the 5′ transcribed-untranslated region (5′-UTR) are important for expression in both *HMG1* and *HMG2* (Enjuto et al. 1995; Lumbreras et al. 1995). These observations suggest a housekeeping role for HMGR1S and a more specialised function for HMGR1L and HMGR2, which might be required in particular cell types or at specific developmental stages. In agreement with this, the analysis of an *Arabidopsis* null mutant affecting *HMG1* (*hmg1-1*) confirms the essential role of this gene on growth and development (Suzuki et al. 2004). Mutant *hmg1-1* plants show dwarfism, early senescence, and male sterility. In contrast, the disruption of *HMG2* does not affect the phenotype or the fertility of the plant under normal growth conditions (Ohyama et al. 2007). The *hmg1-1* mutant has a severe reduction in sterol and triterpenoid content, which was proposed to be related with

the observed phenotype (Suzuki et al. 2004). Consistently, the phenotype of the *hmg1-1* mutant was rescued by the exogenous application of MVA or squalene (Suzuki et al. 2004).

MVK catalyzes the phosphorylation of MVA to yield 5-phosphomevalonate. A cDNA encoding *Arabidopsis* MVK was cloned by functional complementation of a yeast mutant defective in this enzyme activity (Riou et al. 1994). *Arabidopsis* contains a single gene encoding MVK that is widely expressed throughout plant development, although the highest level of expression is detected in roots, predominantly in the meristematic region, and in floral organs. The *MVK* gene (At5g27450) expresses three major mRNA populations, which most likely encode the same protein, but have different 5′ ends. The 5′-UTR of the MVK transcripts includes several ATG codons located upstream of the MVK translation start codon, which suggests that MVK protein levels might be regulated at both transcriptional and translational levels (Lluch et al. 2000).

PMVK, the enzyme that phosphorylates 5-phosphomevalonate to produce 5-diphosphomevalonate, has not yet been characterized in plants. A putative gene encoding PMVK (At1g31910) has been annotated in the *Arabidopsis* genome by similarity to known eukaryotic PMVK gene sequences. Expression data available at the eFP Browser database indicate that the putative *PMK* gene is constitutively expressed.

The formation of IPP from 5-diphosphomevalonate, catalyzed by DPMD, represents the last step of the MVA pathway. A cDNA coding for DPMD was cloned in *Arabidopsis* by complementation of a yeast mutant strain defective in the DPMD enzyme (Cordier et al. 1999). *Arabidopsis* contains two annotated genes encoding DPMD, *MVD1*(At2g38700) *and MVD2* (At3g54250). *MVD1* encodes the DPMD isoform cloned by Cordier et al. (1999). The *MVD2* gene has not yet been characterized. Data available at the eFP Browser database show a similar and almost overlapping pattern of expression for the *MVD1* and *MVD2* genes, which are constitutively expressed.

A recent report has shown that both *Catharanthus roseus* and *Arabidopsis* PMVK and DPMD fused to YFP are targeted to peroxisomes in transfected *C. roseus* cells (Simkin et al. 2011). This, together with the previous finding that *Arabidopsis* IDI can also be targeted to peroxisomes in tobacco protoplasts (Sapir-Mir et al. 2008), has led to propose that the final steps of the MVA pathway are localized in peroxisomes.

4 The Plastidial MEP Pathway in *Arabidopsis*

The MEP pathway enzymes are encoded by nuclear genes and targeted to plastids. The initial reaction of the MEP pathway, catalyzed by deoxyxylulose 5-phosphate (DXP) synthase (DXS), involves the condensation of (hydroxyethyl)thiamine derived from pyruvate with the C1 aldehyde group of glyceraldehyde 3-phosphate (GAP) to produce DXP (Sprenger et al. 1997; Lange et al. 1998; Lois et al. 1998), a compound that may also serve as a precursor for the biosynthesis of vitamin B_1 (thiamine) (Julliard and Douce 1991). In the second step, an intramolecular rearrangement and reduction of DXP by the enzyme DXP reductoisomerase (DXR) yields MEP (Takahashi et al. 1998; Lange and Croteau 1999; Schwender et al. 1999). MEP is converted into methylerythritol 2,4-cyclodiphosphate (MEcPP) in three enzymatic steps (Fig. 30.1), catalyzed by 4-diphosphocytidyl-methylerythritol (CDP-ME) synthase (MCT), CDP-ME kinase (CMK), and MEcPP synthase (MDS) enzymes. A reduction of MEcPP catalyzed by hydroxymethylbutenyl diphosphate (HMBPP) synthase (HDS) produces HMBPP, which the enzyme HMBPP reductase (HDR) finally converts to a 5:1 mixture of IPP and DMAPP (reviewed in (Rodríguez-Concepción and Boronat 2002; Eisenreich et al. 2004; Bouvier et al. 2005). Rules for the nomenclature of the *Arabidopsis* MEP pathway genes, enzymes, and mutants have been recently proposed (Phillips et al. 2008).

DXS is encoded by more than one gene in several plants, particularly in those with an active secondary metabolism (Walter et al. 2002; Paetzold et al. 2010; Walter et al. this volume). It has been proposed that class 1 DXS would have a housekeeping function, whereas class 2 enzymes

might participate in the biosynthesis of secondary isoprenoids. No genes encoding class 2 DXS enzymes have been found in *Arabidopsis*, whose genome contains three open reading frames encoding sequences with homology to class 1 DXS isoforms (Rodríguez-Concepción and Boronat 2002). From these three putative isoforms, tentatively named DXS1 (At4g15560), DXS2 (At3g21500), and DXS3 (At5g11380), evidence of a role in the MEP pathway is only available for DXS1. Overexpression of DXS1 in transgenic plants leads to a higher production of MEP-derived isoprenoids (Estévez et al. 2001; Botella-Pavía et al. 2004; Carretero-Paulet et al. 2006; Muñoz-Bertomeu et al. 2006) and complements the pigmentation and developmental defects of DXS1-defective mutants (Crowell et al. 2000). Growth of *cla1* plants can also be rescued by adding deoxyxylulose (DX, the dephosphorylated product of DXS activity) to their growth medium (Estévez et al. 2003). Similarly, the decreased accumulation of plastidic isoprenoid pigments and the pale variegated phenotype of mutants such as *chs5* (*dxs-3*) and *lvr111* (*dxs-4*), which harbor point mutations in the gene encoding DXS1, was partially rescued with DX supplementation or DXS1 overexpression (Araki et al. 2000; Crowell et al. 2003). In contrast to the relatively high expression level of the *DXS1* gene, only a few ESTs from the *DXS2* and *DXS3* genes are available (Rodríguez-Concepción and Boronat 2002), suggesting that their expression is low and most likely restricted to particular tissues or developmental stages. The differential expression pattern could explain why the mutations in the *DXS1* gene are not rescued by the other two putative DXS isoforms. Alternatively, it is possible that DXS2 and DXS3 do not have DXS activity, as deduced from the fact that the predicted mature proteins lack amino acid residues that are conserved in all the bacterial and plant DXS (Rodríguez-Concepción and Boronat 2002; Phillips et al. 2008).

The rest of the *Arabidopsis* MEP pathway enzymes appear to be encoded by a single gene (Rodríguez-Concepción and Boronat 2002). Experimental data have confirmed that all the MEP pathway enzymes harbor N-terminal sequences that target them to plastids (Araki et al. 2000; Carretero-Paulet et al. 2002; Querol et al. 2002; Hsieh and Goodman 2005; Hsieh et al. 2008). A screening for *Arabidopsis* seedling-lethal mutants from T-DNA collections identified albino mutants being disrupted in the genes encoding DXS1 (At4g15560), DXR (At5g62790), and MCT (At2g02500). These lines have been renamed *dxs-2*, *dxr-1*, and *mct-3*, respectively (Phillips et al. 2008). The same phenotype displayed by these and the DXS-defective *cla1* (*dxs-1*) mutant (Mandel et al. 1996; Estévez et al. 2000) was found in loss-of-function mutants of the genes encoding CMK (At2g26930), MDS (At1g63970), HDS (At5g60600), and HDR (At4g34350) in *Arabidopsis* (Gutiérrez-Nava et al. 2004; Guevara-García et al. 2005; Hsieh and Goodman 2005; Hsieh and Goodman 2006; Hsieh et al. 2008). These mutants typically display chloroplasts with virtually no development of thylakoids and almost undetectable levels of photosynthetic pigments (chlorophylls and carotenoids). As expected, the accumulation of these and other plastidic isoprenoids is reduced in partial loss-of-function mutants and antisense lines with reduced activity levels of DXS, CMS, or HDS (Araki et al. 2000; Okada et al. 2002; Crowell et al. 2003; Flores-Pérez et al. 2008b). Most interestingly, some of these lines have unveiled connections between the MEP pathway and other metabolic pathways and cell processes. For instance, the *csb3* (*hds-3*) mutant, which harbors a point mutation in the gene encoding HDS (Gil et al. 2005) and causing a decreased production of isoprenoid pigments (Flores-Pérez et al. 2008b), was unexpectedly found in a search for mutants resistant to biotrophic pathogens, suggesting the involvement of the MEP pathway in defense responses. Also unexpectedly, the decreased DXS activity in the pale *lvr111* (*dxs-4*) mutant correlates with an enhanced resistance to the block of the MVA pathway with lovastatin (also known as mevinolin), a specific inhibitor of HMGR (Crowell et al. 2003), suggesting a crosstalk between the two pathways leading to the production of isoprenoid precursors.

5 Crosstalk Between Pathways and Exchange of Common Pathway Products

Mutant *Arabidopsis* seedlings with a block in the MEP pathway have been shown to still accumulate low levels of plastid isoprenoids such as chlorophylls and carotenoids, suggesting an import of cytosolic MVA-derived isoprenoid precursors to the plastids (Araki et al. 2000; Estévez et al. 2000; Nagata et al. 2002). Labeling experiments have demonstrated that a bidirectional exchange of common isoprenoid precursors between cell compartments takes place in *Arabidopsis*. For example, gibberellins are formed from precursors derived mostly from the MEP pathway, but also from the MVA pathway (Kasahara et al. 2002). The exchange rate, however, is not high enough to rescue the production of all types of isoprenoids when one of the two pathways is pharmacologically or genetically blocked in *Arabidopsis*. The extent of the exchange can be increased by feeding of intermediates of the MVA or the MEP pathways and by metabolic, environmental, or developmental cues (Kasahara et al. 2002; Nagata et al. 2002; Schuhr et al. 2003; Rodríguez-Concepción et al. 2004). Together, these results suggest a coordination of the activities of the MVA and MEP pathways to ensure the availability of precursors for the plethora of isoprenoid end products synthesized in plant cells. The nature of the mechanisms involved, however, has remained largely unknown.

Factors responsible for the inter-pathway crosstalk are now being identified using molecular, genetic, and genomic approaches. A recent analysis of transcriptional profiles of genes of the MVA and MEP pathways under various experimental conditions could not detect a close connection between these pathways, suggesting that the crosstalk at the transcriptional level may be restricted to single genes in both pathways (Wille et al. 2004). In particular, the gene encoding HMGR1, the most widely distributed HMGR isoform in *Arabidopsis* (Enjuto et al. 1994), appeared as a good candidate for inter-pathway crosstalk since it had few connections to the MVA pathway and was found to be negatively correlated to the module of fully connected genes in the MEP pathway (those encoding DXR, MCT, CMK, MDS, and HDS), suggesting a mutually exclusive pattern of expression.

A second module of MEP pathway genes was formed by *DXS1* and *HDR*, which were only connected to each other.

A complementary approach to investigate the crosstalk between the MVA and MEP pathways is the isolation and characterization of mutants resistant to a block of these pathways. The specific block of the MVA pathway with mevinolin (MEV, an inhibitor of HMGR) or the MEP pathway with fosmidomycin (FSM) in *Arabidopsis* results in a developmental block at the seedling stage (Rodríguez-Concepción et al. 2004). A strategy based on the isolation of *Arabidopsis* mutants capable of overcoming the inhibition of the MVA pathway with MEV which could also survive in the presence of FSM resulted in the identification of *rim1*, which was shown to be defective in phytochrome B, the major light stable photoreceptor of red light in *Arabidopsis* seedlings. The MEV-resistant phenotype of *rim1* seedlings was shown to be caused by increased levels of HMGR enzyme activity due to an activation of gene expression, whereas the FSM-resistant phenotype of mutant seedlings was proposed to derive from an enhanced intake of MVA-derived isoprenoid precursors by the plastid (Rodríguez-Concepción et al. 2004). The analysis of FSM resistance of mutants being impaired in light perception and signal transduction indicated that the light-triggered signaling pathway eventually leading to downregulation of the cytosol-to-plastid transport of prenyl diphosphates is initiated by phytochromes, but did not require the participation of other factors required for HMGR upregulation and MEV resistance, thereby revealing the existence of distinct light perception and signaling pathways for the control of the MVA pathway and the exchange of common products of the MVA and MEP pathways. The identification and characterization of other mutants resistant to a block in the MVA or the

MEP pathways should provide further insights into the molecular networks coordinating inter-pathway crosstalk in plants.

6 Regulation of the Metabolic Flux Through the MVA Pathway

Several lines of evidence sustain that HMGR has a key role in the regulation of the MVA pathway. The first indications in this direction came from studies on the distribution of HMGR activity in pea and radish seedlings (Brooker and Russell 1975; Grumbach and Bach 1979; Bach et al. 1980; Bach and Lichtenthaler 1984) and HMGR transcripts in wheat and *Arabidopsis* (Aoyagi et al. 1993; Enjuto et al. 1994; Enjuto et al. 1995; Lumbreras et al. 1995). Both the enzymatic activity and transcript levels are high in rapidly growing parts of the plant, such as apical buds and roots, where very active sterol biosynthesis occurs, and low in more mature structures. In agreement, the levels of HMGR activity tightly correlate with the rate of sterol production in developing seeds of rape and tobacco (Harker et al. 2003a). HMGR activity and transcript levels are also high in actively dividing *Arabidopsis*, tomato and tobacco culture cells (Lumbreras et al. 1995; Jelesko et al. 1999; Hemmerlin and Bach 2000; Hemmerlin et al. 2004). On the other hand, it was found that the challenge of some solanaceous species with pathogens or elicitor treatment induces sharp increases in HMGR activity and transcript levels, that precede the accumulation of sesquiterpenoid phytoalexins (Suzuki et al. 1975; Ôba et al. 1985; Chappell and Nable 1987; Yang et al. 1991). Thus, the regulatory role of HMGR in the MVA pathway seems to be important not only for normal plant growth and development but also for the adaptation to challenging environmental conditions.

More conclusive evidence in favor of the limiting role of plant HMGR in the biosynthesis of MVA-derived isoprenoid products has been obtained by expression of different variants of this enzyme in homologous and heterologous systems. The level of total phytosterols was increased about eightfold in leaves of tobacco plants by constitutive expression of either the *Hevea HMG1* gene (Schaller et al. 1995) or the catalytic domain of hamster HMGR (Chappell et al. 1995). The overexpression of the *Arabidopsis* HMGR isoforms in transgenic *Arabidopsis* plants also led to higher accumulation of leaf phytosterols (González 2002; Manzano et al. 2004; Leivar et al. 2005). The increase was moderate with ectopic expression of HMGR1L and HMGR2 (1.3- and 1.5-fold, respectively) and more prominent with HMGR1S (2.5-fold). However, the highest success in the homologous *Arabidopsis* system was achieved by expression of the catalytic domain of either HMGR1 (11.5-fold phytosterol increase) or HMGR2 (fivefold). Higher phytosterol accumulation in tobacco leaves and seeds was also obtained when the catalytic domain of Hevea HMGR1 was expressed instead of the complete protein (Harker et al. 2003b). In all the above-mentioned systems, the HMGR activity correlates positively with the phytosterol production level. Therefore, the cumulative data indicate that plant HMGR activity is downregulated by its membrane domain. The elimination of this domain allows deregulation of HMGR and more active phytosterol biosynthesis. However, regulation of downstream enzymes in the phytosterol pathway still limits the production of end-of-chain phytosterols, and the excessively accumulated intermediates are largely esterified with fatty acids and accumulate as lipid droplets in the cytosol of HMGR overexpressing plant cells (Schaller et al. 1995).

Additional proof of the limiting role of HMGR in plant isoprenoid biosynthesis was obtained by the overexpression of cytosolic or mitochondrial FPP synthase in *Arabidopsis* plants (Masferrer et al. 2002; Manzano et al. 2004; Manzano et al. 2006). High levels of FPP synthase activity without a concomitant increase of MVA production led to a reduction of isoprenoid precursors for cytokinin biosynthesis, the formation of necrotic lesion, and premature senescence. A strict control of the level of isoprenoid intermediates is thus required to avoid deleterious effects on plant growth and development (Manzano et al. 2004). All these observations underline the

importance of plant HMGR in the control of the metabolic flux to cytosolic and mitochondrial isoprenoid products. In sharp contrast, no evidence has been provided to date in support of such a regulatory role for other enzymes of the MVA pathway.

In agreement with its fundamental role in the control of the MVA pathway, plant HMGR is modulated by a variety of developmental and environmental signals such as phytohormones, calcium, calmodulin, light, wounding, elicitor treatment, and pathogen attack (cf. Stermer et al. 1994). The response to these factors involves a complex regulatory network that operates at both transcriptional and post-translational level. It has been proposed that the major changes in HMGR activity would be determined at the transcriptional level, whereas the posttranscriptional control would allow a finer and faster adjustment (Chappell 1995). In *Arabidopsis*, development and light have major effects on HMGR transcript and activity levels. The expression of the *Arabidopsis HMG1* gene is triggered by germination, and the *HMG1* mRNA levels increase rapidly as the seedling develops (Learned and Connolly 1997). In rosette leaves, HMGR activity declines with age (Manzano et al. 2004), but *HMG1* and *HMG2* expression is induced in the inflorescences (Enjuto et al. 1994; Enjuto et al. 1995; Lumbreras et al. 1995). This alternating profile of HMGR levels, encompassing seedling, leaf, and inflorescence development, is not exclusive of *Arabidopsis*, but occurs in other plant systems as well (Brooker and Russell 1975; Aoyagi et al. 1993; Moore and Oishi 1993; Korth et al. 1997; Jain et al. 2000; Korth et al. 2000). Light is a negative regulator of *Arabidopsis HMG1* gene expression (Learned 1996). This control is exerted at the transcriptional level and depends on the fluence rate, time of illumination, and spectral quality. In agreement, genetic evidence was provided showing that phytochrome and cryptochrome are required for downregulation of *Arabidopsis HMG1* and *HMG2* transcript levels (Rodríguez-Concepción et al. 2004). The *HMG1* promoter was activated by light deprivation in immature *Arabidopsis* leaves, but not in roots, indicating differential responsiveness in different organs (Learned and Connolly 1997). Direct control by light and the light-dependent developmental program likely contribute to the higher HMGR activity and transcript levels observed in etiolated seedlings versus light-exposed seedlings of several plant species (Brooker and Russell 1975; Bach 1986; Ji et al. 1992; Enjuto et al. 1994).

Evidences showing posttranscriptional regulation of plant HMGR are still scarce. Pioneering work in pea indicated that phytochrome can repress HMGR activity at the posttranslational level (Brooker and Russell 1979; Wong et al. 1982). Downregulation of potato HMGR occurring along development and in response to light is most likely mediated by protein degradation (Korth et al. 2000). It has been shown that the increase or reduction of the flux through the sterol pathway in tobacco cell culture and *Arabidopsis* seedlings causes compensatory response in HMGR activity, which is regulated at the posttranslational level (Wentzinger et al. 2002; Nieto et al. 2009). The block of sphingolipid biosynthesis in *Arabidopsis* also led to post-translational repression of HMGR activity and concomitant reduction in the sterol content, indicating a regulatory crosstalk between the two lipid biosynthetic pathways (Nieto et al. 2009). In microsomal preparations from pea seedlings, addition of increasing nanomolar concentrations of calcium caused a proportional reversible decrease of HMGR activity (Russell et al. 1985). This effect was not mediated by direct action of Ca^{2+} on HMGR, Ca^{2+}-induced proteolysis or interaction with calmodulin. Reversible inhibition of HMGR activity by calcium was also observed in *Arabidopsis* extracts (Antolín and Campos, unpublished results), thus indicating that a still unknown mechanism for calcium-dependent regulation of enzyme activity might be conserved in plants. It has been shown that *Arabidopsis* HMGR1 can be phosphorylated at a conserved serine residue (Ser577 in the HMGR1S sequence) by SnF1-related kinase (SnRK1) of cauliflower and spinach cell-free extracts (Dale et al. 1995; Sugden et al. 1999). This modification completely inactivates the HMGR enzyme and could, therefore, determine the flux through the MVA pathway. In fact, phytosterol levels in

transgenic tobacco seeds are higher when a variant of *Arabidopsis* HMGR1S without the phosphorylation site is used instead of the unmodified enzyme (Hey et al. 2006). However, the gain in phytosterol content (19 % increase, with respect to the non-transformed control, and 10 % increase, with respect to tobacco expressing the original HMGR1S) was moderate in comparison with that obtained after constitutive expression of single copy HMGR catalytic domain (170 % increase) (Harker et al. 2003b). Thus, further work is required to reinforce the view that phosphorylation of plant HMGR catalytic domain has a major role in the posttranslational control of the MVA pathway.

Protein phosphatase 2A (PP2A) was recently identified as a multilevel regulator of HMGR in *Arabidopsis* (Leivar et al. 2011). Both HMGR1L and HMGR1S interact with PP2A through B″ regulatory subunits of PP2A, which are also Ca^{2+}-binding proteins (Leivar et al. 2011). Whereas the *Arabidopsis* B″β variant is involved in the posttranscriptional repression of HMGR in unchallenged seedlings, B″α modulates HMGR transcript, protein, and activity levels in response to salt challenge (Leivar et al. 2011). When *Arabidopsis* seedlings were transferred to salt-containing medium, B″α and PP2A mediated the decrease and subsequent increase of HMGR activity, which resulted from a steady rise of *HMG1* transcripts levels and an initial sharper reduction of HMGR protein level (Leivar et al. 2011). A similar biphasic profile of HMGR activity was previously observed when bright yellow-2 tobacco cells were subjected to a block with mevinolin (Hemmerlin et al. 2003) or when potato tubers were subjected to wounding (Yang et al. 1991), suggesting that these responses might be also mediated by PP2A through B″ regulatory subunits. Since PP2A is involved in auxin, abscisic acid, ethylene, and brassinosteroid signaling and the B″-PP2A subunit is a Ca^{2+}-binding protein, these observations uncover the potential of PP2A to integrate developmental and Ca^{2+}-mediated environmental signals in the control of plant HMGR (Antolín-Llovera et al. 2011).

7 Regulation of the Metabolic Flux Through the MEP Pathway

The first two and the last two enzymes of the MEP pathway (DXS, DXR, HDS, and HDR) were first proposed as potential control points of the metabolic flux to plastidial isoprenoids. Pioneer studies in tomato supported such a role for DXS and HDR, but not for DXR or HDS, mainly based on the correlation between the accumulation of transcripts and proteins from these genes and the production of MEP-derived products (carotenoids) during fruit ripening (Lois et al. 2000; Rodríguez-Concepción et al. 2001; Rodríguez-Concepción et al. 2003; Botella-Pavía et al. 2004). During light-induced de-etiolation of *Arabidopsis* seedlings, high IPP and DMAPP levels are also required to support an increased production of plastid isoprenoids such as carotenoids and chlorophylls. But the analysis of transcript accumulation in de-etiolating *Arabidopsis* seedlings has shown that all the genes encoding MEP pathway enzymes are upregulated after the light treatment (Mandel et al. 1996; Botella-Pavía et al. 2004; Rodríguez-Concepción 2006; Hsieh et al. 2008; Cordoba et al. 2009). More compelling evidence for the role of DXS, DXR, HDS, and HDR in controlling plastidial IPP and DMAPP production came from the analysis of transgenic *Arabidopsis* plants in which their levels had been altered (Estévez et al. 2001; Botella-Pavía et al. 2004; Carretero-Paulet et al. 2006; Flores-Pérez et al. 2008b). Transgene-mediated upregulation and/or downregulation of DXS, DXR, or HDR levels in *Arabidopsis* correlated with concomitant changes in the levels of MEP-derived isoprenoid end products such as chlorophylls and carotenoids (Estévez et al. 2001; Botella-Pavía et al. 2004; Carretero-Paulet et al. 2006). Similarly, transgenic tomato fruit overexpressing a bacterial DXS enzyme showed a significant increase in the production of carotenoids (Enfissi et al. 2005), whereas peppermint plants overexpressing DXR also accumulated enhanced levels of MEP-derived monoterpenes (Mahmoud and Croteau 2001).

By contrast, no effect on the accumulation of MEP-derived isoprenoids was observed in transgenic lines overexpressing HDS (Flores-Pérez et al. 2008b). These results demonstrate that not all the MEP pathway enzymes cause a significant change in the pathway flux when their concentration is altered, supporting the metabolic control analysis (MCA) conclusion that several enzymes can share control over the flux of the pathway, with different enzymes exhibiting different degrees of control (Thomas and Fell 1998; Libourel and Shachar-Hill 2008). Based on the overexpression studies described above, the control of flux through the MEP pathway should be shared by the rate-determining enzymes DXS, DXR and HDR, whereas the contribution of HDS to overall flux appears to be comparatively much lower. Nothing is known yet about the relative contribution of the rest of MEP pathway enzymes (MCT, CMK, and MDS) to flux control. A thorough examination of the relevant MCA parameters, including a detailed study of the effect of the overexpression of each enzyme of the MEP pathway on the activity of the rest, is now needed to identify what enzymes besides DXS, DXR, and HDR are "rate-determining" (a MCA term used when changes in the concentration of an enzyme cause a change in the pathway flux) and to assign which steps have greater influence in regulating flux to IPP and DMAPP. This analysis is particularly important for the rational design of metabolic engineering approaches aimed to overproduce MEP-derived isoprenoids in plant systems.

Changes in gene expression appear to act as a coarse control to regulate the MEP pathway enzyme levels, but they do not always lead to similar changes in protein levels and, most importantly, enzyme activities and metabolite production (Rodríguez-Concepción 2006). For instance, the partial block of the MVA and MEP pathways in *Arabidopsis* seedlings grown in the presence of sublethal concentrations of specific inhibitors causes a reduction in derived isoprenoid levels without changes in gene expression (Laule et al. 2003). The levels of DXS protein, but not transcripts, are upregulated in FSM-treated *Arabidopsis* seedlings, and a posttranscriptional increase of DXS and HDR protein levels has been recently shown to occur specifically in *Arabidopsis* null mutants of the MEP pathway (Guevara-García et al. 2005). Posttranscriptional events also appear to modulate the accumulation of MEP pathway proteins during seedling development, resulting in discrete changes on protein levels despite strong upregulation of gene expression (Guevara-García et al. 2005). These and other results have led to suggest that the accumulation of at least some MEP pathway enzymes (including flux-controlling DXS and HDR) in *Arabidopsis* might be regulated by changes in the metabolic flux through the pathway. Further experiments are, however, required to ascertain the importance of this type of potential regulation.

The identification of *Arabidopsis* mutants that are resistant to FSM (*rif* mutants) has recently unveiled a mechanism for the posttranscriptional accumulation of active MEP pathway enzymes in plastids (Sauret-Güeto et al. 2006; Flores-Pérez et al. 2008a). The characterization of the *rif1* and *rif10* mutants showed that their FSM resistance phenotype was the result of upregulated DXS and DXR levels without major changes in the expression of the corresponding (nuclear) genes. Both RIF1 (Flores-Pérez et al. 2008a) and RIF10 (Sauret-Güeto et al. 2006) proteins are required for the proper expression of the plastid genome (plastome), and their loss of function in *rif1* and *rif10* lines cause a reduction in the production of plastome-encoded proteins linked to the posttranscriptional accumulation of DXS, DXR, HDS, and HDR in mutant plastids. Mutant seedlings were shown to have altered levels of the plastome-encoded ClpP1 protein, one the catalytic subunits of the stromal Clp protease complex (Peltier et al. 2004), likely resulting in a decreased proteolytic activity of the entire complex as a consequence of impaired subunit stoichiometry (Sjögren et al. 2006). Consistent with a role of this protease in the regulation of MEP pathway enzyme levels, mutants with a decreased Clp protease activity also showed an increased accumulation of active DXS and DXR enzymes, leading to FSM resistance (Flores-Pérez et al. 2008a). The presence of similar Clp protease core

complexes in all plastid types, their abundance, and the essential character of the catalytic subunits suggests that it plays a central role in plastid homeostasis and biogenesis, similar to that of the ubiquitin-proteasome system for plant development (Sjögren et al. 2006). However, little is currently known concerning the specific function of this stromal protease or its protein substrates in higher plants. Although the participation of the Clp protease in the degradation of at least some of the MEP pathway enzymes within plastids is clear (Flores-Pérez et al. 2008a), current evidence does not allow to conclude whether these proteins, which are also localized in the stroma (Carretero-Paulet et al. 2002; Oudin et al. 2007), are direct targets (actual substrates) of this protease. Thus, it is possible that downregulation of Clp activity in plastids results in changes that are ultimately responsible for the observed accumulation of DXS, DXR and/or the other proposed substrates of this proteolytic complex. In summary, the metabolic flux through the MEP pathway appears to be controlled by several enzymes (with a major contribution of DXS and HDR) regulated at transcriptional and posttranscriptional levels in response to metabolic (feedback regulation), environmental (light), and developmental cues.

Acknowledgments Work from our groups was supported by grants from the Spanish Ministerio de Ciencia e Innovación (MICINN) (BIO2011-23680 to MRC, BFU2006-14655 to NC, BIO2009-06984 to AF, and BIO2009-09523 to AB, all including FEDER funds) and the Generalitat de Catalunya (GC) (grant 2009SGR-26) and carried out within the Centre CONSOLIDER on Agrigenomics (CSD2007-00036) funded by MICINN and Xarxa de Referencia en Biotecnologia funded by GC.

References

Ahumada I, Cairó A, Hemmerlin A, González V, Pateraki I, Bach TJ, Rodríguez-Concepción M, Campos N, Boronat A (2008) Characterization of the gene family encoding acetoacetyl-CoA thiolase in Arabidopsis. Funct Plant Biol 35:1100–1111

Alex D, Bach TJ, Chye ML (2000) Expression of *Brassica juncea* 3-hydroxy-3-methylglutaryl CoA synthase is developmentally regulated and stress-responsive. Plant J 22:415–426

Antolín-Llovera M, Leivar P, Arró M, Ferrer A, Boronat A, Campos N (2011) Modulation of plant HMG-CoA reductase by protein phosphatase 2A. Positive and negative control at a key node of metabolism. Plant Signal Behav 6:1127–1131

Aoyagi K, Beyou A, Moon K, Fang L, Ulrich T (1993) Isolation and characterization of cDNAs encoding wheat 3-hydroxy-3-methylglutaryl coenzyme-A reductase. Plant Physiol 102:623–628

Araki N, Kusumi K, Masamoto K, Niwa Y, Iba K (2000) Temperature-sensitive *Arabidopsis* mutant defective in 1-deoxy-D-xylulose 5-phosphate synthase within the plastid non-mevalonate pathway of isoprenoid biosynthesis. Physiol Plant 108:9–24

Bach TJ (1986) Hydroxymethylglutaryl-CoA reductase, a key enzyme in phytosterol synthesis? Lipids 21:82–88

Bach TJ, Lichtenthaler HK (1984) Application of modified Lineweaver-Burk plots to studies of kinetics and regulation of radish 3-hydroxy-3-methylglutaryl-CoA reductase. Biochim Biophys Acta 794:152–161

Bach TJ, Lichtenthaler HK, Rétey J (1980) Properties of membrane-bound 3-hydroxy-3-methylglutaryl coenzyme A reductase (EC. 1.1.1.34) from radish seedlings and some aspects of its regulation. In: Mazliak P, Benveniste P, Costes C, Douce R (eds) Biogenesis and function of plant lipids. Elsevier, Amsterdam, pp 355–362

Botella-Pavía P, Besumbes O, Phillips MA, Carretero-Paulet L, Boronat A, Rodríguez-Concepción M (2004) Regulation of carotenoid biosynthesis in plants: evidence for a key role of hydroxymethylbutenyl diphosphate reductase in controlling the supply of plastidial isoprenoid precursors. Plant J 40:188–199

Bouvier F, Rahier A, Camara B (2005) Biogenesis, molecular regulation and function of plant isoprenoids. Prog Lipid Res 44:357–429

Brooker JD, Russell DW (1975) Properties of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase from *Pisum sativum* seedlings. Arch Biochem Biophys 167:723–729

Brooker JD, Russell DW (1979) Regulation of Microsomal 3-hydroxy-3-methylglutaryl coenzyme-A reductase from pea seedlings – rapid posttranslational phytochrome-mediated decrease in activity and *in vivo* regulation by isoprenoid products. Arch Biochem Biophys 198:323–334

Caelles C, Ferrer A, Balcells L, Hegardt FG, Boronat A (1989) Isolation and structural characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl coenzyme-A reductase. Plant Mol Biol 13:627–638

Campos N, Boronat A (1995) Targeting and topology in the membrane of plant 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Cell 7:2163–2174

Carretero-Paulet L, Ahumada I, Cunillera N, Rodríguez-Concepción M, Ferrer A, Boronat A, Campos N (2002) Expression and molecular analysis of the *Arabidopsis* DXR gene encoding 1-deoxy-D-xylulose 5-phosphate reductoisomerase, the first committed enzyme of the

2-*C*-methyl-D-erythritol 4-phosphate pathway. Plant Physiol 129:1581–1591

Carretero-Paulet L, Cairo A, Botella-Pavia P, Besumbes O, Campos N, Boronat A, Rodríguez-Concepción M (2006) Enhanced flux through the methylerythritol 4-phosphate pathway in *Arabidopsis* plants overexpressing deoxyxylulose 5-phosphate reductoisomerase. Plant Mol Biol 62:683–695

Carrie C, Murcha MW, Millar AH, Smith SM, Whelan J (2007) Nine 3-ketoacyl-CoA thiolases (KATs) and acetoacetyl-CoA thiolases (ACATs) encoded by five genes in *Arabidopsis thaliana* are targeted either to peroxisomes or cytosol but not to mitochondria. Plant Mol Biol 63:97–108

Chappell J, Nable R (1987) Induction of sesquiterpenoid biosynthesis in tobacco cell-suspension cultures by fungal elicitor. Plant Physiol 85:469–473

Chappell J, Wolf F, Proulx J, Cuellar R, Saunders C (1995) Is the reaction catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase a rate-limiting step for isoprenoid Biosynthesis in Plants. Plant Physiol 109:1337–1343

Chappell J (1995) Biochemistry and molecular biology of the isoprenoid biosynthetic pathway in plants. Ann Rev Plant Physiol Plant Mol Biol 46:521–547

Cordoba E, Salmi M, León P (2009) Unravelling the regulatory mechanisms that modulate the MEP pathway in higher plants. J Exp Bot 60:2933–2943

Cordier H, Karst F, Bergès T (1999) Heterologous expression in *Saccharomyces cerevisiae* of an *Arabidopsis thaliana* cDNA encoding mevalonate diphosphate decarboxylase. Plant Mol Biol 39:953–967

Croteau R, Kutchan T, Lewis N (2000) Natural products (secondary metabolites). In: Buchanan B, Gruissem W, Jones R (eds) Biochemistry and molecular biology of plants. American Society of Plant Biologists, Rockville, pp 1250–1268

Crowell DN, Packard CE, Pierson CA, Giner JL, Downes BP, Chary SN (2003) Identification of an allele of *CLA1* associated with variegation in *Arabidopsis thaliana*. Physiol Plant 118:29–37

Dale S, Arró M, Becerra B, Morrice NG, Boronat A, Hardie DG, Ferrer A (1995) Bacterial expression of the catalytic domain of 3-hydroxy-3-methylglutaryl-CoA reductase (isoform Hmgr1) from *Arabidopsis thaliana*, and its inactivation by phosphorylation at Ser577 by *Brassica oleracea* 3-hydroxy-3-methylglutaryl-CoA reductase kinase. Eur J Biochem 233: 506–513

Disch A, Hemmerlin A, Bach TJ, Rohmer M (1998) Mevalonate-derived isopentenyl diphosphate is the biosynthetic precursor of ubiquinone prenyl side chain in tobacco BY-2 cells. Biochem J 331:615–621

Eisenreich W, Bacher A, Arigoni D, Rohdich F (2004) Biosynthesis of isoprenoids via the non-mevalonate pathway. Cell Mol Life Sci 61:1401–1426

Eisenreich W, Schwarz M, Cartayrade A, Arigoni D, Zenk MH, Bacher A (1998) The deoxyxylulose phosphate pathway of terpenoid biosynthesis in plants and microorganisms. Chem Biol 5:R221–R233

Enfissi EMA, Fraser PD, Lois LM, Boronat A, Schuch W, Bramley PM (2005) Metabolic engineering of the mevalonate and non-mevalonate isopentenyl diphosphate-forming pathways for the production of health-promoting isoprenoids in tomato. Plant Biotech J 3:17–27

Enjuto M, Balcells L, Campos N, Caelles C, Arró M, Boronat A (1994) *Arabidopsis thaliana* contains two differentially expressed 3-hydroxy-3-methylglutaryl-CoA reductase genes, which encode microsomal forms of the enzyme. Proc Natl Acad Sci USA 91:927–931

Enjuto M, Lumbreras V, Marin C, Boronat A (1995) Expression of the Arabidopsis *HMG2* gene, encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase, is restricted to meristematic and floral tissues. Plant Cell 7:517–527

Estévez JM, Cantero A, Reindl A, Reichler S, León P (2001) 1-Deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. J Biol Chem 276:22901–22909

Estévez JM, Cantero A, Romero C, Kawaide H, Jiménez LF, Kuzuyama T, Seto H, Kamiya Y, León P (2000) Analysis of the expression of *CLA1*, a gene that encodes the 1-deoxyxylulose 5-phosphate synthase of the 2-*C*-methyl-D-erythritol-4-phosphate pathway in *Arabidopsis*. Plant Physiol 124:95–103

Flores-Pérez U, Sauret-Güeto S, Gas E, Jarvis P, Rodríguez-Concepción M (2008a) A mutant impaired in the production of plastome-encoded proteins uncovers a mechanism for the homeostasis of isoprenoid biosynthetic enzymes in *Arabidopsis* plastids. Plant Cell 20:1303–1315

Flores-Pérez U, Pérez-Gil J, Rodríguez-Villalón A, Gil MJ, Vera P, Rodríguez-Concepción M (2008b) Contribution of hydroxymethylbutenyl diphosphate synthase to carotenoid biosynthesis in bacteria and plants. Biochem Biophys Res Commun 371:510–514

Gil MJ, Coego A, Mauch-Mani B, Jordà L, Vera P (2005) The *Arabidopsis csb3* mutant reveals a regulatory link between salicylic acid-mediated disease resistance and the methyl-erythritol 4-phosphate pathway. Plant J 44:155–166

Goldstein JL, Brown MS (1990) Regulation of the mevalonate pathway. Nature 343:425–430

González V (2002) Caracterización funcional de la 3-hydroxi-3-metilmetilglutaril CoA reductasa de *Arabidosis thaliana*. Ph.D. Thesis. Department of Biochemistry and Molecular Biology. University of Barcelona

Grumbach KH, Bach TJ (1979) The effect of PSII herbicides, amitrol and SAN 6706 on the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and the incorporation of (2-^{14}C) acetate and (2-^{3}H) mevalonate into chloroplast pigments of radish seedlings. Z Naturforsch 34c:941–943

Guevara-García A, San Román C, Arroyo A, Cortés ME, Gutiérrez-Nava ML, León P (2005) Characterization of the *Arabidopsis clb6* mutant illustrates the importance of posttranscriptional regulation of the methyl-D-erythritol 4-phosphate pathway. Plant Cell 17:628–643

Gutiérrez-Nava ML, Gillmor CS, Jiménez LF, Guevara-García A, León P (2004) *CHLOROPLAST BIOGENESIS* genes act cell and noncell autonomously in early chloroplast development. Plant Physiol 135:471–482

Harker M, Hellyer A, Clayton JC, Duvoix A, Lanot A, Safford R (2003a) Co-ordinate regulation of sterol biosynthesis enzyme activity during accumulation of sterols in developing rape and tobacco seed. Planta 216:707–715

Harker M, Holmberg N, Clayton JC, Gibbard CL, Wallace AD, Rawlins S, Hellyer SA, Lanot A, Safford R (2003b) Enhancement of seed phytosterol levels by expression of an N-terminal truncated *Hevea brasiliensis* (rubber tree) 3-hydroxy-3-methylglutaryl-CoA reductase. Plant Biotechnol J 1:113–121

Hemmerlin A, Bach TJ (2000) Farnesol-induced cell death and stimulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in tobacco cv bright yellow-2 cells. Plant Physiol 123:1257–1268

Hemmerlin A, Gerber E, Feldtrauer JF, Wentzinger L, Hartmann MA, Tritsch D, Hoeffler JF, Rohmer M, Bach TJ (2004) A review of tobacco BY-2 cells as an excellent system to study the synthesis and function of sterols and other isoprenoids. Lipids 39:723–735

Hemmerlin A, Hoeffler JF, Meyer O, Tritsch D, Kagan IA, Grosdemange-Billiard C, Rohmer M, Bach TJ (2003) Cross-talk between the cytosolic mevalonate and the plastidial methylerythritol phosphate pathways in tobacco bright yellow-2 cells. J Biol Chem 278:26666–26676

Hey SJ, Powers SJ, Beale MH, Hawkins ND, Ward JL, Halford NG (2006) Enhanced seed phytosterol accumulation through expression of a modified HMG-CoA reductase. Plant Biotechnol J 4:219–229

Hsieh MH, Goodman HM (2005) The *Arabidopsis* IspH homolog is involved in the plastid nonmevalonate pathway of isoprenoid biosynthesis. Plant Physiol 138:641–653

Hsieh MH, Goodman HM (2006) Functional evidence for the involvement of *Arabidopsis* IspF homolog in the nonmevalonate pathway of plastid isoprenoid biosynthesis. Planta 223:779–784

Hsieh MH, Chang CY, Hsu SJ, Chen JJ (2008) Chloroplast localization of methylerythritol 4-phosphate pathway enzymes and regulation of mitochondrial genes in *ispD* and *ispE* albino mutants in Arabidopsis. Plant Mol Biol 66:663–673

Jain AK, Vincent RM, Nessler CL (2000) Molecular characterization of a hydroxymethylglutaryl-CoA reductase gene from mulberry (*Morus alba* L.). Plant Mol Biol 42:559–569

Jelesko JG, Jenkins SM, Rodríguez-Concepción M, Gruissem W (1999) Regulation of tomato *HMG1* during cell proliferation and growth. Planta 208:310–318

Ji W, Hatzios KK, Cramer CL (1992) Expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in maize tissues. Physiol Plant 84:185–192

Jin H, Nikolau BJ (2007) Genetic, biochemical and physiological studies of acetyl-CoA metabolism via condensation. In: Benning C, Ohlrogge J (eds) Current advances in the biochemistry and cell biology of plant lipids. Aardvark Global Publishing Company, LCC, Sal Lake City, p 177

Julliard JH, Douce R (1991) Biosynthesis of the thiazole moiety of thiamin (vitamin B1) in higher-plant chloroplasts. Proc Natl Acad Sci USA 88:2042–2045

Kasahara H, Hanada A, Kuzuyama T, Takagi M, Kamiya Y, Yamaguchi S (2002) Contribution of the mevalonate and methylerythritol phosphate pathways to the biosynthesis of gibberellins in *Arabidopsis*. J Biol Chem 277:45188–45194

Korth KL, Stermer BA, Bhattacharyya MK, Dixon RA (1997) HMG-CoA reductase gene families that differentially accumulate transcripts in potato tubers are developmentally expressed in floral tissues. Plant Mol Biol 33:545–551

Korth KL, Jaggard DAW, Dixon RA (2000) Developmental and light-regulated post-translational control of 3-hydroxy-3-methylglutaryl-CoA reductase levels in potato. Plant J 23:507–516

Lange BM, Croteau R (1999) Isoprenoid biosynthesis via a mevalonate-independent pathway in plants: cloning and heterologous expression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase from peppermint. Arch Biochem Biophys 365:170–174

Lange BM, Ghassemian M (2003) Genome organization in *Arabidopsis thaliana*: a survey for genes involved in isoprenoid and chlorophyll metabolism. Plant Mol Biol 51:925–948

Lange BM, Wildung MR, McCaskill D, Croteau R (1998) A family of transketolases that directs isoprenoid biosynthesis via a mevalonate-independent pathway. Proc Natl Acad Sci USA 95:2100–2104

Laule O, Fürholz A, Chang HS, Zhu T, Wang X, Heifetz PB, Gruissem W, Lange M (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 100:6866–6871

Learned RM (1996) Light suppresses 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression in *Arabidopsis thaliana*. Plant Physiol 110: 645–655

Learned RM, Connolly EL (1997) Light modulates the spatial patterns of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression in *Arabidopsis thaliana*. Plant J 11:499–511

Leivar P, González VM, Castel S, Trelease RN, López-Iglesias C, Arró M, Boronat A, Campos N, Ferrer A, Fernàndez-Busquets X (2005) Subcellular localization of *Arabidopsis* 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Plant Physiol 137:57–69

Leivar P, Antolín-Llovera M, Ferrero S, Closa M, Arró M, Ferrer A, Boronat A, Campos N (2011) Multilevel control of *Arabidopsis* 3-hydroxy-3-methylglutaryl coenzyme A reductase by protein phosphatase 2A. Plant Cell 23:1494–1511

Libourel IGL, Shachar-Hill Y (2008) Metabolic flux analysis in plants: from intelligent design to rational engineering. Annu Rev Plant Biol 59:625–650

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 50:47–65

Lichtenthaler HK, Rohmer M, Schwender J (1997) Two independent biochemical pathways for isopentenyl diphosphate and isoprenoid biosynthesis in higher plants. Physiol Plant 101:643–652

Lluch MA, Masferrer A, Arró M, Boronat A, Ferrer A (2000) Molecular cloning and expression analysis of the mevalonate kinase gene from *Arabidopsis thaliana*. Plant Mol Biol 42:365–376

Lois LM, Rodríguez-Concepción M, Gallego F, Campos N, Boronat A (2000) Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. Plant J 2: 503–513

Lois LM, Campos N, Putra SR, Danielsen K, Rohmer M, Boronat A (1998) Cloning and characterization of a gene from *Escherichia coli* encoding a transketolase-like enzyme that catalyzes the synthesis of D-1-deoxyxylulose 5-phosphate, a common precursor for isoprenoid, thiamin, and pyridoxol biosynthesis. Proc Natl Acad Sci USA 95:2105–2110

Lumbreras V, Campos N, Boronat A (1995) The use of an alternative promoter in the *Arabidopsis thaliana HMG1* gene generates a mRNA that encodes a novel 3-hydroxy-3-methylglutaryl coenzyme A reductase isoform with and extended N-terminal region. Plant J 8:541–549

Lunn JE (2007) Compartmentation in plant metabolism. J Exp Bot 58:35–47

Mahmoud SS, Croteau RB (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. Proc Natl Acad Sci USA 98:8915–8920

Mandel MA, Feldmann KA, Herrera-Estrella L, Rocha-Sosa M, Leon P (1996) *CLA1*, a novel gene required for chloroplast development, is highly conserved in evolution. Plant J 9:649–658

Manzano D, Fernández-Busquets X, Schaller H, González V, Boronat A, Arró M, Ferrer A (2004) The metabolic imbalance underlying lesion formation in *Arabidopsis thaliana* overexpressing farnesyl diphosphate synthase (isoform 1S) leads to oxidative stress and is triggered by the developmental decline of endogenous HMGR activity. Planta 219:982–992

Manzano D, Busquets A, Closa M, Hoyerova K, Schaller H, Kaminek M, Arró M, Ferrer A (2006) Overexpression of farnesyl diphosphate synthase in *Arabidopsis* mitochondria triggers light-dependent lesion formation and alters cytokinin homeostasis. Plant Mol Biol 61:195–213

Masferrer A, Arró M, Manzano D, Schaller H, Fernández-Busquets X, Moncaleán P, Fernández B, Cunillera N, Boronat A, Ferrer A (2002) Overexpression of *Arabidopsis thaliana* farnesyl diphosphate synthase (FPS1S) in transgenic *Arabidopsis* induces a cell death/senescence-like response and reduced cytokinin levels. Plant J 30:123–132

McGarvey DJ, Croteau R (1995) Terpenoid metabolism. Plant Cell 7:1015–1026

Merret R, Cirioni J, Bach TJ, Hemmerlin A (2007) A serine involved in actin-dependent subcellular localization of a stress-induced tobacco BY-2 hydroxymethylglutaryl-CoA reductase isoform. FEBS Lett 581:5295–5299

Montamat F, Guilloton M, Karst F, Delrot S (1995) Isolation and characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl-coenzyme A synthase. Gene 167:197–201

Moore KB, Oishi KK (1993) Characterization of 3-hydroxy-3-methylglutaryl coenzyme-A reductase activity during maize seed development, germination, and seedling emergence. Plant Physiol 101:485–491

Muñoz-Bertomeu J, Arrillaga I, Ros R, Segura J (2006) Up-regulation of 1-deoxy-D-xylulose-5-phosphate synthase enhances production of essential oils in transgenic spike lavender. Plant Physiol 142:890–900

Nagata N, Suzuki M, Yoshida S, Muranaka T (2002) Mevalonic acid partially restores chloroplast and etioplast development in *Arabidopsis* lacking the non-mevalonate pathway. Planta 216:345–350

Nagegowda DA, Bach TJ, Chye ML (2004) *Brassica juncea* 3-hydrox-3-methylglutaryl (HMG)-CoA synthase 1: expression and characterization of recombinant wild-type and mutant enzymes. Biochem J 383: 517–527

Nagegowda DA, Ramalingam S, Hemmerlin A, Bach TJ, Chye ML (2005) *Brassica juncea* HMG-CoA synthase: localization of mRNA and protein. Planta 221:844–856

Nieto B, Forés O, Arró M, Ferrer A (2009) *Arabidopsis* 3-hydroxy-3-methylglutaryl-CoA reductase is regulated at the post-translational level in response to alterations of the sphingolipid and the sterol biosynthetic pathways. Phytochemistry 70:58–64

Ôba K, Kondo K, Doke N, Uritani I (1985) Induction of 3-hydroxy-3-methylglutaryl CoA reductase in potato tubers after slicing, fungal infection or chemical treatment, and some properties of the enzyme. Plant Cell Physiol 26:873–880

Ohyama K, Suzuki M, Masuda K, Yoshida S, Muranaka T (2007) Chemical phenotypes of the *hmg1* and *hmg2* mutants of *Arabidopsis* demonstrate the *in-planta* role of HMG-CoA reductase in triterpene biosynthesis. Chem Pharm Bull 55:1518–1521

Okada K, Kawaide H, Kuzuyama T, Seto H, Curtis IS, Kamiya Y (2002) Antisense and chemical suppression of the nonmevalonate pathway affects *ent*-kaurene biosynthesis in *Arabidopsis*. Planta 215:339–344

Oudin A, Mahroug S, Courdavault V, Hervouet N, Zelwer C, Rodríguez-Concepción M, St-Pierre B, Burlat V (2007) Spatial distribution and hormonal regulation of gene products from methyl erythritol phosphate and monoterpene-secoiridoid pathways in *Catharanthus roseus*. Plant Mol Biol 65:13–30

Paetzold H, Garms S, Bartram S, Wieczorek J, Urós-Gracia EM, Rodríguez-Concepción M, Boland W, Strack D, Hause B, Walter MH (2010) The isogene 1-deoxy-D-xylulose 5-phosphate synthase 2 controls

isoprenoid profiles, precursor pathway allocation, and density of tomato trichomes. Mol Plant 3:904–916

Peltier JB, Ripoll DR, Friso G, Rudella A, Cai Y, Ytterberg J, Giacomelli L, Pillardy J, van Wijk KJ (2004) Clp protease complexes from photosynthetic and non-photosynthetic plastids and mitochondria of plants, their predicted three-dimensional structures, and functional implications. J Biol Chem 279:4768–4781

Phillips MA, León P, Boronat A, Rodríguez-Concepción M (2008) The plastidial MEP pathway: unified nomenclature and resources. Trends Plant Sci 13:619–623

Querol J, Campos N, Imperial S, Boronat A, Rodríguez-Concepción M (2002) Functional analysis of the *Arabidopsis thaliana* GCPE protein involved in plastid isoprenoid biosynthesis. FEBS Lett 514:343–346

Riou C, Tourte Y, Lacroute F, Karst F (1994) Isolation and characterization of a cDNA encoding *Arabidopsis thaliana* mevalonate kinase by genetic complementation in yeast. Gene 148:293–297

Rodríguez-Concepción M, Ahumada I, Diez-Juez E, Sauret-Güeto S, Lois LM, Gallego F, Carretero-Paulet L, Campos N, Boronat A (2001) 1-Deoxy-D-xylulose 5-phosphate reductoisomerase and plastid isoprenoid biosynthesis during tomato fruit ripening. Plant J 27:213–222

Rodríguez-Concepción M, Boronat A (2002) Elucidation of the methylerythritol phosphate pathway for isoprenoid biosynthesis in bacteria and plastids. A metabolic milestone achieved through genomics. Plant Physiol 130:1079–1089

Rodríguez-Concepción M, Querol J, Lois LM, Imperial S, Boronat A (2003) Bioinformatic and molecular analysis of hydroxymethylbutenyl diphosphate synthase (*GCPE*) gene expression during carotenoid accumulation in ripening tomato fruit. Planta 217:476–482

Rodríguez-Concepción M, Forés O, Martínez-García JF, González V, Phillips MA, Ferrer A, Boronat A (2004) Distinct light-mediated pathways regulate the biosynthesis and exchange of isoprenoid precursors during *Arabidopsis* seedling development. Plant Cell 16:144–156

Rodríguez-Concepción M (2006) Early steps in isoprenoid biosynthesis: multilevel regulation of the supply of common precursors in plant cells. Phytochem Rev 5:1–15

Rohmer M (1999) The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. Nat Prod Rep 16:565–574

Rohmer M (2008) From molecular fossils of bacterial hopanoids to the formation of isoprene units: discovery and elucidation of the methylerythritol phosphate pathway. Lipids 43:1095–1107

Russell DW, Knight JS, Wilson TM (1985) Pea seedling HMG-CoA reductases: regulation of activity *in vitro* by phosphorylation and Ca^{2+}, and posttranslational control *in vivo* by phytochrome and isoprenoid hormones. In: Randall DD, Blevins DG, Larson RL, Kagawa T (eds) Current topics in plant biochemistry and physiology. University of Missouri-Columbia, Columbia, pp 191–206

Sapir-Mir M, Mett A, Belausov E, Tal-Meshulam S, Frydman A, Gidoni D, Eyal Y (2008) Peroxisomal localization of *Arabidopsis* isopentenyl diphosphate isomerases suggests that part of the plant isoprenoid mevalonic acid pathway is compartmentalized to peroxisomes. Plant Physiol 148:1219–1228

Sauret-Güeto S, Botella-Pavía P, Flores-Pérez U, Martínez-Garcia JF, San Román C, León P, Boronat A, Rodríguez-Concepción M (2006) Plastid cues posttranscriptionally regulate the accumulation of key enzymes of the methylerythritol phosphate pathway in *Arabidopsis*. Plant Physiol 141:75–84

Schaller H, Grausem B, Benveniste P, Chye ML, Tan CT, Song CT, Chua NH (1995) Expression of the *Hevea brasiliensis* (Hbk) Mull Arg 3-hydroxy-3-methylglutaryl-coenzyme A reductase-1 in tobacco results in sterol overproduction. Plant Physiol 109:761–770

Schuhr CA, Radykewicz T, Sagner S, Latzel C, Zenk MH, Arigoni D, Bacher A, Rohdich F, Eisenreich W (2003) Quantitative assessment of crosstalk between the two isoprenoid biosynthesis pathways in plants by NMR spectroscopy. Phytochem Rev 2:3–16

Schwender J, Müller C, Zeidler J, Lichtenthaler HK (1999) Cloning and heterologous expression of a cDNA encoding 1-deoxy-D-xylulose-5-phosphate reductoisomerase of *Arabidopsis thaliana*. FEBS Lett 455:140–144

Simkin AJ, Guirimand G, Papon N, Courdavault V, Thabet I, Ginis O, Bouzid S, Giglioli-Guivarc'h N, Clastre M (2011) Peroxisomal localisation of the final steps of the mevalonic acid pathway in planta. Planta 234:903–914

Sjögren LL, Stanne TM, Zheng B, Sutinen S, Clarke AK (2006) Structural and functional insights into the chloroplast ATP-dependent Clp protease in *Arabidopsis*. Plant Cell 18:2635–2649

Sprenger GA, Schorken U, Wiegert T, Grolle S, de Graaf AA, Taylor SV, Begley TP, Bringer-Meyer S, Sahm H (1997) Identification of a thiamin-dependent synthase in *Escherichia coli* required for the formation of the 1-deoxy-D-xylulose 5-phosphate precursor to isoprenoids, thiamin, and pyridoxol. Proc Natl Acad Sci USA 94:12857–12862

Stermer BA, Bianchini GM, Korth KL (1994) Regulation of HMG-CoA reductase activity in plants. J Lipid Res 35:1133–1140

Sugden C, Donaghy PG, Halford NG, Hardie DG (1999) Two SNF1-Related protein kinases from spinach leaf phosphorylate and inactivate 3-hydroxy-3-methylglutaryl-coenzyme A reductase, nitrate reductase, and sucrose phosphate synthase *in vitro*. Plant Physiol 120:257–274

Suzuki H, Ôba K, Uritani I (1975) Occurrence and some properties of 3-hydroxy-3-methylglutaryl coenzyme A reductase in sweet-potato roots infected by *Ceratocystis fimbriata*. Physiol Plant Pathol 7:265–276

Suzuki M, Kamide Y, Nagata N, Seki H, Ohyama K, Kato H, Masuda K, Sato S, Kato T, Tabata S, Yoshida S, Muranaka T (2004) Loss of function of *3-hydroxy-3-methylglutaryl coenzyme A reductase 1 (HMG1)* in *Arabidopsis* leads

to dwarfing, early senescence and male sterility, and reduced sterol levels. Plant J 37:750–761

Takahashi S, Kuzuyama T, Watanabe H, Seto H (1998) A 1-deoxy-D-xylulose 5-phosphate reductoisomerase catalyzing the formation of 2-*C*-methyl-D-erythritol 4-phosphate in an alternative non-mevalonate pathway for terpenoid biosynthesis. Proc Natl Acad Sci USA 95:9879–9884

Thomas S, Fell DA (1998) The role of multiple enzyme activation in metabolic flux control. Adv Enzyme Regul 38:65–85

Walter MH, Hans J, Strack D (2002) Two distantly related genes encoding 1-deoxy-D-xylulose 5-phosphate synthases: differential regulation in shoots and apocarotenoid-accumulating mycorrhizal roots. Plant J 31:243–254

Wentzinger L, Bach TJ, Hartmann M-A (2002) Inhibition of squalene synthase and squalene epoxidase in tobacco cells triggers an up-regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Physiol 130:334–346

Wille A, Zimmermann P, Vranová E, Fürholz A, Laule O, Bleuler S, Hennig L, Prelic A, von Rohr P, Thiele L, Zitzler E, Gruissem W, Bühlmann P (2004) Sparse graphical Gaussian modeling of the isoprenoid gene network in *Arabidopsis thaliana*. Genome Biol 5:R92. doi:10.1186/gb-2004-5-11-r92

Wong RJ, McCormack DK, Russell DW (1982) Plastid 3-hydroxy-3-methylglutaryl coenzyme A reductase has distinctive kinetic and regulatory features – properties of the enzyme and positive phytochrome control of activity in pea-seedlings. Arch Biochem Biophys 216:631–638

Yang ZB, Park HS, Lacy GH, Cramer CL (1991) Differential activation of potato 3-hydroxy-3-methylglutaryl coenzyme A reductase genes by wounding and pathogen challenge. Plant Cell 3:397–405