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Isoprenoid Biosynthesis in Plants: Carbon Partitioning Within the Cytoplasmic Pathway

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CONTENTS

I. INTRODUCTION

Plants produce a greater array of structurally diverse isoprenoids than other organisms, and these are conveniently divided into classes of primary and secondary metabolites (Figure 1). Isoprenoids that are primary metabolites include sterols, carotenoids, growth regulators (hormones), and the polyprenol substituents of dolichols, quinones, and proteins. These compounds function in plants much as they do in other organisms and are essential for membrane integrity, photoprotection, orchestration of developmental programs and anchoring of essential biochemical functions to specific membrane systems, respectively. Isoprenoids classified as secondary metabolites include monoterpenes, sesquiterpenes, and diterpenes, and it is this class of isoprenoids that are more unique to plants. Although compounds within this latter category are considered secondary because they are not essential for viability, they are important mediators of plant-plant,[10] plant-insect,[11] and plant-pathogen[3,12] interactions.

The topic of plant isoprenoids encompasses a rich and broad subject area, both of which are derived from a fascination in the diversity of isoprenoids found in nature and intense investigations into their biosynthesis. As such, many excellent reviews for particular classes of isoprenoids[4,6,8,13,14] and various aspects of their biosynthesis[2,9,15] exist. However, only the review of Gray in 1987[16] attempted to integrate the chemistry and biochemistry of all isoprenoids produced in plants as a means of identifying the many mechanisms possibly regulating isoprenoid biosynthesis in plants. At that time, Gray noted that, although activities for many enzymes constituting the central isoprenoid biosynthetic pathway had been measured, activities of the branch pathway enzymes had not. Gray also provided a critical assessment of how various branch pathways of the isoprenoid biosynthetic pathway may be localized to intracellular compartments, and how branch pathways may be integrated through metabolite transport mechanisms and mechanisms of feedback regulation. Finally, Gray listed criteria necessary for determining rate-limiting steps in the biosynthesis of any isoprenoid, and implied that, although modulations in the level of various biosynthetic enzymes had been documented, such documentation did not constitute proof of a rate-limiting step in and of itself. This chapter will revisit a very select and limited sampling of these issues.

Two important distinctions of isoprenoid biosynthesis in plants not depicted in Figure 1 are the regulation and intracellular compartmentation of this pathway. Both of these aspects are important in determining the synthetic capacity of any particular cell and, as will be discussed in this chapter, recent results from several laboratories have stimulated alternative views as to how isoprenoid metabolism in plants might be controlled. Figure 2 therefore depicts two models which attempt to address the issues of intracellular localization and regulation, and will be used throughout this chapter as a means of contrasting and comparing experimental observations. This chapter will also only address the synthetic capacities of plant cells for sterols and sesquiterpenes, metabolites known to be synthesized exclusively

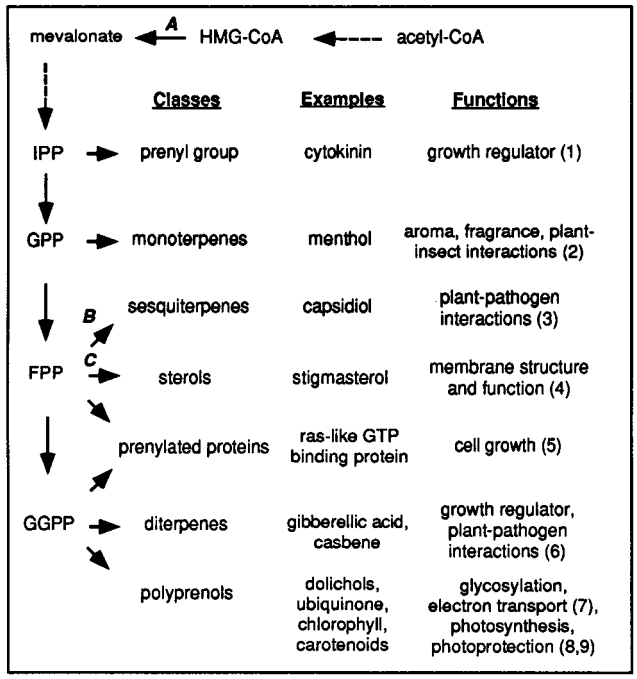

Figure 1 A depiction of the isoprenoid biosynthetic pathway in plants with respect to the types of end products and their physiological significance. Broken arrows indicate multiple steps or reactions. The rate-limiting step for sterol metabolism in mammals resides at the early step in the pathway catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR)[43](A). In plants the control of isoprenoid metabolism remains controversial. Sites other than HMGR that might regulate carbon flow include branch point enzymes such as sesquiterpene cyclases (B) for sesquiterpene biosynthesis and squalene synthase (C) for sterol biosynthesis. The regulation of these three enzymes as a means of controlling isoprenoid biosynthesis in plants is the subject of this chapter.

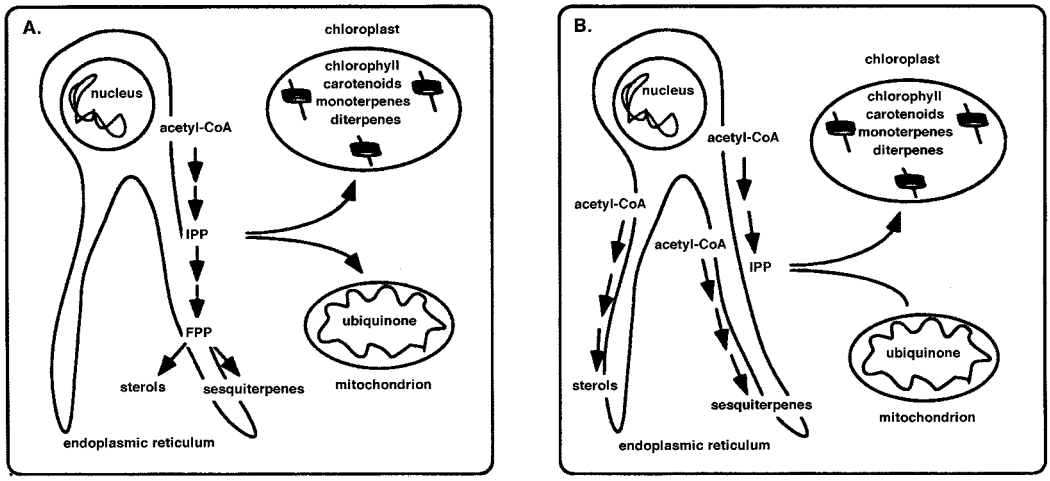

Figure 2 Hypothetical models to suggest that isoprenoid biosynthesis might be regulated by control of branch point enzymes and metabolite transport (A), or the coordinated expression of entire metabolic channels dedicated to the biosynthesis of specific classes of isoprenoids (B).

in the cytoplasm. This will purposefully narrow the subject matter to two contrasting views: the synthesis of sterols and sesquiterpenes can obviously be controlled by regulating the branch point enzymes that commit carbon to sterol or sesquiterpene biosynthesis (Figure 2A); or regulation might be more complicated, entailing the coordinated activity of entire separate metabolic units (Figure 2B).

II. BRANCH POINT REGULATION

The use of fruits for studies of sterol and carotenoid biosynthesis,[8,17] and the use of tuber tissues and plant cell cultures for studies of sterol accumulation and elicitation of sesquiterpene phytoalexins[18–26] have proven very useful for studying regulation of the biosynthesis of these metabolites. For example, early work[18,24] demonstrated a total absence of extracellular sesquiterpenes, but a steady accumulation of sterols corresponding to the rapid growth phase of the tobacco cell suspension cultures. Within 6 hours of elicitor addition to the cell cultures, sterol accumulation was suppressed to approximately 10% of the control cells, while sesquiterpene accumulation was induced. In pulse-labeling experiments, incorporation of radioactive acetate and mevalonate into sterol was suppressed upon elicitor-treatment of the cell cultures, and incorporation of label into extracellular sesquiterpenes was induced. These results suggested that regulation of sterol and sesquiterpene biosynthesis was downstream of mevalonate (and 3-hydroxy-3-methylglutaryl coenzyme A reductase [HMGR] activity). Additional work correlated the decline in sterol biosynthesis with a suppression of squalene synthase enzyme activity, and the induction of sesquiterpene biosynthesis with an induction of a sesquiterpene cyclase enzyme activity. Because these two enzymes are positioned at a putative branch point in the pathway, the induction of one enzyme and the suppression of the other were interpreted as an important mechanism controlling carbon flow and hence, end product formation (Figure 3).[18,24]

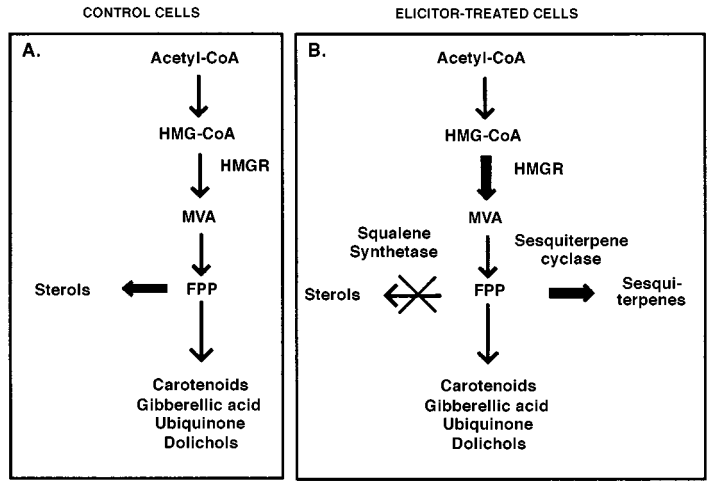

Figure 3 A summary of some of the biochemical changes that occur in elicitor-treated tobacco cell suspension cultures. Following elicitor addition, squalene synthetase is suppressed, sesquiterpene cyclase and HMGR are induced. These changes in enzyme activities result in the accumulation of extracellular sesquiterpene phytoalexins and suppression of sterol accumulation.

Zook and Kuć[26] described similar results in pathogen or elicitor-challenged potato tuber disks and extended the previous work by demonstrating that increased sterol production in response to wounding of potato tubers was correlated with an increase in squalene synthase activity. Furthermore, experiments with U18666A, an inhibitor of 2,3-oxidosqualene: cycloartenol cyclase, indicated that wounding stimulates carbon flow into the sterol biosynthetic pathway, while elicitor treatment decreases the flow of intermediates into the pathway.[27] Combined with the pulse labeling experiments and enzyme activity measurements, these studies suggest that squalene synthase is a major control point for the regulation of sterol biosynthesis in plants.

Investigators of ubiquinone and dolichol synthesis in mammalian systems came to a similar conclusion. That is, ubiquinone and dolichol biosynthesis are regulated independently of sterol biosynthesis.

In these studies the relevant branch point enzymes are squalene synthase for sterol biosynthesis and prenyltransferases that either by cis or trans addition of isopentenyl diphosphate (IPP) to FPP generate the prenyl lipid polymers of dolichol and ubiquinone, respectively. When mammalian cells or tissues were incubated in the presence of exogenous modulators of sterol biosynthesis (cholesterol, 25-hydroxycholesterol, and compactin) and the incorporation rate of ^{3}H-mevalonate into sterols and dolichols[28] or ubiquinone[29] determined, the incorporation of radiolabel into dolichols or ubiquinone was affected to a lesser extent than for sterols. These results implied some sort of independent regulation of the dolichol and ubiquinone branch pathways, and the investigators speculated about how this regulation might be imposed. One possibility assumed that the prenyl transferases responsible for shunting FPP into the respective branch pathways might have much lower K_ms for FPP than squalene synthase. This possibility recognized that as the level of HMGR activity was reduced in response to the various treatment of the cells, the amount of mevalonate and FPP dedicated to isoprenoid biosynthesis would also decrease. The more efficient prenyl transferases would then effectively compete for the scarce FPP, and the effect of decreased substrate concentrations on ubiquinone and dolichol synthesis would be moderated. An alternative explanation offered was that complete pathways for sterol, ubiquinone, and dolichols (consisting of isozymes) could be independently regulated.

Faust et al.[30] were to further demonstrate that squalene synthetase activity was indeed modulated in fibroblast cells responding to the availability of exogenous sterols. This study also demonstrated that the incorporation rate of ^{3}H-mevalonate into ubiquinones could be saturated at low external concentrations of mevalonate, but the incorporation rate into sterols was not saturated even at very high external concentrations of mevalonate. The relative insensitivity of the ^{3}H-mevalonate incorporation rate into ubiquinones by cells incubated in the presence of exogenous sterols was additionally shown to be saturated at relatively low concentrations of FPP, supporting the notion that the prenyltransferases responsible for ubiquinone synthesis have lower affinity constraints (K_m) for FPP than squalene synthetase. Although not directly demonstrated with respect to dolichol synthesis, the results of Dr. Kandutsch's laboratory are consistent with the suppression of FPP utilization for sterol synthesis by a decrease in squalene synthetase activity and the maintenance of a constant synthesis rate of dolichols by a high affinity of the appropriate prenyltransferases for FPP.

III. MOLECULAR BIOLOGY OF TERPENE CYCLASES AND SQUALENE SYNTHASE

The development of systems suitable for detailed biochemical studies have also provided means for cloning a number of branch point enzymes. For example, sequences for a monoterpene cyclase,[31] a sesquiterpene cyclase[32] and a diterpene cyclase[33] have recently been reported. These genes were cloned using conventional cloning strategies, such as preparing cDNA libraries with mRNA isolated from tissue or cells enriched for the cyclase mRNA, then screening the library with oligonucleotide probes designed from the amino acid sequence of the purified protein,[31] or using antibodies to the purified cyclase for immuno-screens.[34] Comparison of the deduced amino acid sequence of these three terpene cyclases has revealed a surprising degree of similarity between the proteins. The overall similarity and identity between the sesquiterpene and monoterpene cyclases are 53% and 33%, and between the sesquiterpene and diterpene cyclases are 64% and 42%, respectively.[33,35] This homology has been useful in conjunction with chemical modification experiments[36,37] in identifying domains and amino acid residues of putative importance to the enzymological reactions catalyzed by these enzymes. Testing of these domains and residues for their contribution to the enzymology is currently being assessed using domain-swapping types of experiments and expression of the chimeric genes in bacterial expression systems.[37a–d]

Clones for the terpene cyclase genes have allowed investigations into mechanisms controlling the level of the respective enzyme activities. For example, both the sesquiterpene cyclase and the diterpene cyclase mRNA levels are rapidly and transiently induced in elicitor-treated tissues. These changes in mRNA levels appear to arise from changes in the transcription rate of the terpene cyclase genes. Lois and West[34] used nuclear run-off experiments to correlate the accumulation of mRNA and cyclase enzyme activity with changes in the transcription rate of the gene, while Vögeli and Chappell[38] used the *in vivo* thiouridine method to demonstrate that the tobacco sesquiterpene cyclase gene was under stringent transcriptional control. Additional dissection of the promoter elements flanking these genes should provide additional insights into the regulatory mechanisms controlling their expression.

Our understanding of how squalene synthase enzyme activity and gene expression are regulated in plants is much more limited relative to that for the terpene cyclases. This is, in part, attributable to difficulties encountered in purifying this low-abundance, intrinsic membrane protein. However, recent

success in the isolation of the yeast squalene synthase gene using a complementation of the *erg9* mutant[39] and the isolation of mammalian squalene synthase clones using degenerate oligonucleotide probes to empirically determined amino acid sequence[40] have provided alternative means for obtaining the plant squalene synthase cDNA. In comparing the deduced amino acid sequences of the yeast and mammalian squalene synthase proteins, Robinson et al.[40] identified several conserved domains convenient for the design of polymerase chain reaction (PCR) primers and suggested using these primers for PCR cloning strategies. We also recognized this possibility in comparison of the yeast[39] and rat[40a] sequences. The feasibility of this approach was tested recently using tobacco cell mRNA to isolate and sequence a partial squalene synthase clone (unpublished data). The comparison of the deduced amino acid sequence of this clone to other squalene synthase sequences indicates the conservation of several sequence motifs (domains III, IV and V in the nomenclature of Robinson et al.) and amino acid residues previously implicated in the enzyme reaction mechanism by tagging experiments using chemical modifying reagents.

Subsequent reports by Jiang et al.[41] and Keller et al.[42] have presented convincing evidence that the mammalian squalene synthase genes are regulated by their transcription rate in response to exogenous sterols and inhibitors of sterol biosynthesis. In contrast, preliminary experiments in our laboratory using the tobacco-specific PCR clone suggest that the squalene synthase mRNA levels are not altered in elicitor-treated tobacco cells. This type of result implies some sort of posttranslational control of the squalene synthase enzyme activity. Several posttranslational control mechanisms for enzymes of isoprenoid biosynthesis are well established in mammalian systems (turnover of the enzyme protein, phosphorylation[43]), but remain to be defined in plants.[44]

IV. REGULATION AT HMGR

Although both the tobacco and potato studies mentioned above might be consistent with a simple model of regulation, earlier studies by Ôba et al.,[22,23] Stermer and Bostock,[45] Green and West,[46] and Vögeli and Chappell[18] provided evidence that other steps in the central isoprenoid pathway, and especially HMGR, were also regulated. HMGR has been a focal point for many of these studies because it catalyzes the irreversible conversion of HMG-CoA to mevalonate and is considered the key regulatory step controlling isoprenoid metabolism in animals,[43] fungi,[47] and perhaps insects.[48] Evidence for HMGR in such a role in plant isoprenoid metabolism is yet controversial. For example, a number of investigations, including those by ourselves[18] have reported a correlation between the induction of sesquiterpene phytoalexin biosynthesis and HMGR activity. However, this correlation has been considered somewhat tenuous, since the induction pattern of HMGR activity was rapid and transient while the accumulation time course for sesquiterpenes was much more protracted. Other studies using mevinolin and pathogen-derived suppressors of the inducible HMGR activity supported the notion that the inducible HMGR activity was necessary for sesquiterpene accumulation.[45,49] Nonetheless, these two observations, the necessity of induction of HMGR activity but a lack of correlation with sesquiterpene accumulation, have been difficult to reconcile using the model in Figure 2A.

Other investigations in other systems have also pointed to HMGR enzyme activity as a key step in the synthesis of sterols, but not all the evidence has added up to it being the rate-limiting or regulatory step. The Benveniste laboratory has used an alternative approach to screen for mutants in the isoprenoid biosynthetic pathway. These investigators selected UV-mutagenized, haploid protoplasts for growth in the presence of sterol biosynthetic inhibitors.[50,51] Relatively late steps in the pathway were targets of the inhibitors, a triazole compound which inhibits the demethylation of obtusifoliol and an *N*-alkyl morpholine which inhibits the isomerization of cycloeucalenol to obtusifoliol. The sterol composition of the selected tobacco mutants were dramatically altered and exhibited very significant increases in the proportion of cyclopropylsterols (intermediates in the sterol biosynthetic pathway). Interestingly, the extra sterol accumulating in the LAB1-4 mutant accumulates predominately in sterol esters associated with novel cytoplasmic lipid droplets.[52] Further studies of the LAB1-4 mutant have demonstrated that the phenotype of the mutation does not require continuous selection with the demethylase inhibitor, but that the altered sterol composition is attenuated in regenerated plants and dampened over generations.[53] Genetic studies also indicated that the alteration in sterol composition segregated as a single semidominant mutation,[53] and biochemical analysis suggested that the enzyme activities up to HMG-CoA synthase may not be enhanced, while the HMGR activity was increased by approximately threefold.[54] Whether the LAB1-4 mutation directly or indirectly affects the reductase or other downstream biosynthetic enzyme activities remains to be determined.

V. MOLECULAR BIOLOGY OF HMGR

The isolation of plant HMGR genes has been greatly facilitated by the high degree of homology observed in the catalytic domain of all HMGR proteins. This homology allowed the use of heterologous probes for library screening, and the design of degenerate oligonucleotides for PCR amplification of the conserved domain of HMGR from many different plants. A compilation of genes and cDNAs encoding HMGR from various plants are listed in Table 1.[55–66]

All of the plant HMGR genes identified to date have a similar genomic organization, with three introns inserted into identical positions within the coding sequence (Figure 4). The large C-terminal catalytic domain (Figure 4) is highly conserved among plants (72%–95% identical; 87%–98% similar) and shows significant homology to HMGRs from archaebacteria,[67] protozoa,[68] fungi,[69] and animals[70] and slight homology to a eubacteria HMGR.[71] Hydropathy plots indicate that a moderately conserved region of the plant HMGR proteins is comprised of two membrane-spanning domains connected by a short linker sequence. In contrast, the animal and yeast proteins contain eight membrane-spanning domains.[72,73] The presence of two transmembrane domains positions a variable N-terminal domain on the cytoplasmic face of the endoplasmic reticulum membrane with the catalytic domain. While the N-terminal domain of most HMGRs are quite different (rice lacks this domain entirely), certain genes such as the tomato HMG2 and the tobacco HMGR do show similarity in this region. Between the transmembrane and catalytic domains is a linker region that is variable in sequence, but similar in amino acid composition. This region is generally rich (>40%) in proline, aspartate, serine, threonine, and glutamate (P,E,S,T,D), and may be involved in regulating protein stability and turnover.[74]

One of the more intriguing findings arising from the molecular biology of the isoprenoid biosynthetic genes in plants has been the observation of gene families, especially for HMGR (Table 1). For example, *Arabidopsis* contains 2 or more HMGR genes,[55–57] *Hevea* contains at least 3 HMGR genes,[60,61] and *Solanum* has 3 or more genes.[19] Recent experiments have suggested unique roles for the multiple HMGR genes. Narita and Gruissem[17] found that the increase in fruit size of developing tomatoes was sensitive to mevinolin, a very potent competitive inhibitor of HMGR enzyme activity, but that carotenoid accumulation was not. Supplementing the mevinolin-treated fruits with exogenous mevalonate restored normal fruit development. In interpreting these results, the investigators note that fruit enlargement is correlated with cell division and enlargement, developmental phases requiring a significant level of sterol biosynthesis. As to why fruit enlargement but not carotenoid accumulation was sensitive to the mevinolin treatment, Narita and Gruissem[17] speculated that carotenoid biosynthesis must be regulated independently of sterol biosynthesis, and that HMGR activity must be limiting only for sterol biosynthesis under these conditions.

Choi et al.[19] took this concept one step further. These investigators have used potato tuber slices to measure a metabolic change-over similar to that described above for tobacco cell cultures. In potato, steroid accumulation is induced upon wounding or slicing the tuber tissue, but arrested if wounded tissue is treated with a fungal elicitor. Instead, sesquiterpene phytoalexins accumulate in the elicitor-treated tuber slices.[25] Using gene-specific probes for 3 potato HMGR genes (HMG1, 2, and 3), Choi et al.[19] correlated wound-inducible steroid accumulation with the induction of HMG1 gene expression, and the elicitor-inducible accumulation of sesquiterpene phytoalexins with HMG2 and HMG3 gene expression. HMG1 gene expression was not induced in the elicitor-treated tuber slices.

VI. METABOLIC CHANNELS

The results of these two studies are not consistent with the traditional view of isoprenoid metabolism occurring in a homogeneous environment with intermediates mixing freely and accessible to successive or competing enzymes. Instead, the results are more consistent with arrays of isozymes dedicated to the production of specific classes of isoprenoids, with each array regulated independently from one another (Figure 2). Regulation of end product formation would thus depend on the regulation of each metabolic unit by the accumulation of select isozymes, the insertion of those isozymes into the correct metabolic unit, and various posttranslational modifications modulating isozyme activity levels.

Consistent with this metabolic channel concept are the results of Chappell et al.[75] These investigators transformed into tobacco a truncated form of the hamster HMGR gene, encoding a soluble HMGR activity. As a means to measure how limiting the HMGR was for isoprenoid biosynthesis, the levels of sterols and elicitor-inducible sesquiterpenes (synthesized in the cytosol), and carotenoid and phytol (synthesized in the chloroplast) were measured and compared to nontransgenic plants. Surprisingly, only

Table 1 Compilation of Plant HMGR Sequences

Organism Gene	Accession Nos.		Comments[a]	Ref.
Arabidopsis thaliana				
HMG1	gene	L19261	Constitutive, higher levels in meristematic tissue	55–57
	cDNA	X15032		
	cDNA	J04537		
Arabidopsis thaliana				
HMG2	gene	L19262	Low levels in young seedlings, roots, inflorescences (meristematic tissue)	57
Camptotheca acuminata				
HMG1	gene	L10390	Expressed in seedlings but not older tissues, wound-induction suppressed by high [MeJas]	58
Catharanthus roseus (periwinkle)				
pHMC3	cDNA	M96068	Abundant transcripts in all tissues	59
Hevea brasilensis (rubber tree)				
HMG1	gene	X54657	Expression correlates with rubber production; ethylene-inducible, expressed in laticifers	60, 61
	cDNA	X54659		
Hevea brasilensis				
HMG2	cDNA	X54658		60
Hevea brasilensis				
HMG3	gene	M74798	Constitutive, housekeeping gene	61
	cDNA	M74800		
Lithospermum erythrorhizon	cDNA	X74783		
Lycopersicon esculentum (tomato)				
HMG1			Induced in young, developing fruit	17
Lycopersicon esculentum				
HMG2	gene	M63642	Elicitor inducible	62
Nicotiana sylvestris (tobacco)				
HMGR(2)	cDNA	X63649	Most similar to potato, tomato HMG2; induced by stresses such as protoplasting, $HgCl_2$, viral infection	63
Oryza sativa (rice)				
HMGR1	gene	L28995	Low level in vegetative, floral organs, elicitor-inducible	64
Raphanus sativus (radish)				
HMG1	protein	NCBI 322670		65
Raphanus sativus				
HMG2	cDNA	X68651		65
Solanum tuberosum (potato)				
HMG1	cDNA	L01400	3′-untranslated similar to *Arabidopsis hmg1*, tomato *hmg1*; expressed in flower primordia, anthers, petals, pistils, induced by wounding, low [MeJas], suppressed by AA, high [MeJas]	19, 20
Solanum tuberosum				
HMG2	cDNA	L01401	Expressed in anthers, induced in tubers by AA, high [MeJas], pathogen infection	19, 20
Solanum tuberosum				
HMG3	cDNA	L01402	Expressed in anthers, induced in tubers by AA, pathogen infection	19
Triticum aestivum (wheat)				
HMGR-10	protein	NCBI 545471	Most similar to rice *hmg1*	66
Triticum aestivum				
HMGR-18	protein	NCBI 478533		66
Triticum aestivum				
HMGR-23	protein	NCBI 545473		66

[a] abbreviations: AA, arachidonic acid MeJas, methyl jasmonate.

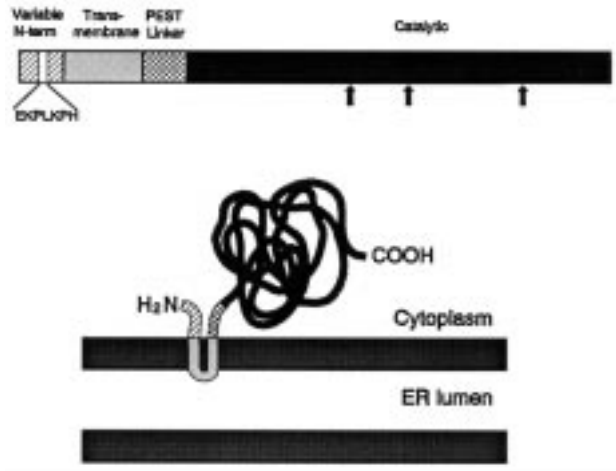

Figure 4 Schematic representations of the plant HMGR protein to highlight structure-function relationships (A) and likely orientation within the endoplasmic reticulum (B). Arrows indicate intron-exon junctions, and the N-terminal amino acid sequence shown is suspected of serving as a targeting signal for a sesquiterpene metabolic channel localized to a specific domain of the endoplasmic reticulum.

the level of sterols was significantly increased in the transgenic plants. Equally interesting was the observation that the predominant sterol in the transgenic plants was cycloartenol, an intermediate in the sterol biosynthetic pathway. These results suggest that HMGR activity is not limiting for the synthesis of campesterol, stigmasterol, and sitosterol, the major end-product sterol found in plants, but is limiting for cycloartenol synthesis. Also, because the carotenoid and phytol levels were unaltered in the transgenic plants, their synthesis is likely to occur independent of sterol biosynthesis. As depicted in Figure 2B, this may entail the coordination between a cytosolic and plastidic pathway, as suggested by Kleinig,[9] or the plastids could be entirely independent for their own isoprenoid biosynthetic needs. The recent report by Rohmer et al.[76] lends some support to this possibility.

Some predictions of such metabolic channels or metabolons[77] are obvious. Domains of the endoplasmic reticulum or some other cytoskeletal structure must be dedicated to the assembly of unique metabolons. Enzymes destined for metabolons must also have specific targeting information within their amino acid sequence or their secondary/tertiary structures. A putative signal sequence for the sesquiterpene metabolon has been deduced from a sequence comparison of the HMGR isozymes associated with sesquiterpene biosynthesis (potato, tomato, tobacco) and sesquiterpene cyclase enzymes from tobacco and *Hyoscyamus muticus*. A7-amino acid motif [EK(P/V)LKPH] is conserved among these proteins but is not present in HMGR proteins not associated with sesquiterpene synthesis, nor in other terpene cyclases sequenced to date. This sequence is present within the 30 extreme N-terminal amino acids of the HMGR proteins and within the 20 extreme C-terminal amino acids of sesquiterpene cyclase.

Genes coding for enzymes making up a specific metabolon must have similar transcriptional networks to coordinate expression of the metabolic unit. The recently cloned genes discussed above provide ample opportunities for testing some of these predictions. For example, if metabolic units are coordinately regulated at the transcriptional level, then one would expect to find conserved regulatory sequences or cis-sequence motifs within the promoters of elicitor-inducible HMGR and sesquiterpene cyclase genes. Also, if particular HMGR isozymes are limiting for each metabolic channel, then engineering high-level expression of each HMGR isoform should alter only one class of isoprenoids. These types of experiments are currently being pursued in a number of laboratories.

ACKNOWLEDGMENTS

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NOTES ADDED IN PROOF

With regard to the high degree of amino acid similarity observed between terpene cyclases, a recent article by K. Back and J. Chappell (Identifying functional domains within terpene cyclases using a domain-swapping strategy, *Proc. Natl. Acad. Sci. U.S.A.* 93, 6841, 1996) has provided experimental evidence that these enzymes do consist of domains, and that these domains do contribute directly to the enzymological properties of these enzymes.

Several other articles have recently appeared which address the rate-limiting nature of HMGR for isoprenoid biosynthesis in plants:

Re, E. D., Jones, D., and Learned, R. M., Co-expression of native and introduced genes reveals cryptic regulation of HMG CoA reductase expression in *Arabidopsis*, *Plant J.*, 7, 771, 1995.

Schaller, H., Grausem, B., Benveniste, P., Chye, M-L., Tan, C-T., Song, Y-H., and Chua, N-H., Expression of the *Hevea brasiliensis* (H.B.K.) Müll. Arg. 3-hydroxy-3-methylglutaryl-Coenzyme A reductase 1 in tobacco results in sterol overproduction, *Plant Physiol.*, 109, 761, 1995.

Chappell, J., Wolf, F., Proulx, J., and Saunders, C., 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, 1995.

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