review

# Regulation of HMG-CoA reductase activity in plants

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**Abstract** This brief review summarizes the current literature on the regulation of HMG-CoA reductase (HMGR) in plants. The mevalonate pathway, which starts with the synthesis of mevalonate by HMGR, has more branch pathways in plants than in most other organisms, leading to a tremendous variety of isoprenoid products. Evidence suggests that HMGR is an important control point for the synthesis of many of these plant isoprenoids, including some that are vital for primary metabolism and pest resistance. Plant HMGR activity responds in vivo to a variety of developmental and environmental signals, such as cell division, light, and infection. Plants regulate HMGR activity at the level of mRNA by differential induction of HMGR gene family members, and posttranslationally by enzyme modification. Calcium, calmodulin, and proteolytic degradation may also have a role in regulation of plant HMGR.—**Stermer, B. A., G. M. Bianchini, and K. L. Korth.** Regulation of HMG-CoA reductase activity in plants. *J. Lipid Res.* 1994. **35:** 1133–1140.

**Supplementary key words** multigene family • cysteine protease • light regulation • disease resistance • mevalonate pathway

## INTRODUCTION

The mevalonate pathway, which starts with the synthesis of mevalonate by HMG-CoA reductase (HMGR), provides precursors for the diverse spectrum of isoprenoid compounds produced by a cell. Isoprenoids such as sterols, dolicol, ubiquinone, and isopentenylated tRNAs are widespread in eukaryotes. In addition to these ubiquitous compounds, plants produce an exceptional variety of isoprenoids. Many are known to play key roles in plant growth and development, photosynthesis, and resistance to pests; and some plant isoprenoids, like fragrant oils and natural rubber, have considerable commercial value. Evidence is accumulating that implicates HMGR as an important control point for the mevalonate pathway in plants (1–4), as has already been demonstrated for this enzyme in yeast and animals. HMGR is among the most highly regulated enzymes in animals (5), and the greater number of roles that isoprenoids play in plants compared to animals suggests that regulation of HMGR activity in plants may be even more complex. Despite the fact that HMGR is the most studied enzyme of the mevalonate pathway in plants, much remains to be learned about its regulation.

The ability to clone and analyze HMGR genes from plants has attracted more researchers to the field and has accelerated studies on this enzyme. At the same time, progress is being made in studies of the posttranslational regulation of plant HMGR. This article summarizes current information on the regulation of HMGR in plants and points out areas where further research is needed.

### Isoprenoids play many vital roles in plants

The growth and differentiation of plants is coordinated to a large degree by growth regulators that are isoprenoids (6). Of the five major groups of growth regulators, or plant hormones as they are sometimes called, three, abscisic acid, gibberellins, and cytokinins, are isoprenoid compounds (**Fig. 1**). A sixth group of compounds with growth-regulating activity in plants are the brassinolides, sterol-based structures active at concentrations of $10^{-8}$ M (7). Photosynthesis requires several isoprenoid compounds, some like chlorophyll or plastoquinone have prenyl side chains necessary for their function, and others such as the tetraterpene carotenoids are completely derived from the mevalonate pathway (8). Recent studies indicate that, as in other eukaryotes, isoprenylation is critical for the function of some proteins in plants (9). Many plant steroids have potent effects in animals. For instance, some plants accumulate steroids that appear to act in defense against insects, such as the steroid glycoalkaloids which are insect antifeedants (10). Other steroids that occur in plants, like cardiac glycosides, digitonin, and progesterone, which have poorly defined functions in plants, are pharmacologically active in animals (8). One highly derivatized diterpenoid from the Pacific yew tree, taxol, has attracted considerable interest because of its properties as an antitumor agent (11). Another important group of isoprenoids in plants are phytoalexins (12). These are antimicrobial compounds synthesized in response to pathogen attack and apparently play an important part in disease resistance. Although not all phytoalexins are derived from the mevalonate pathway,

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HMGR, HMG-CoA reductase.

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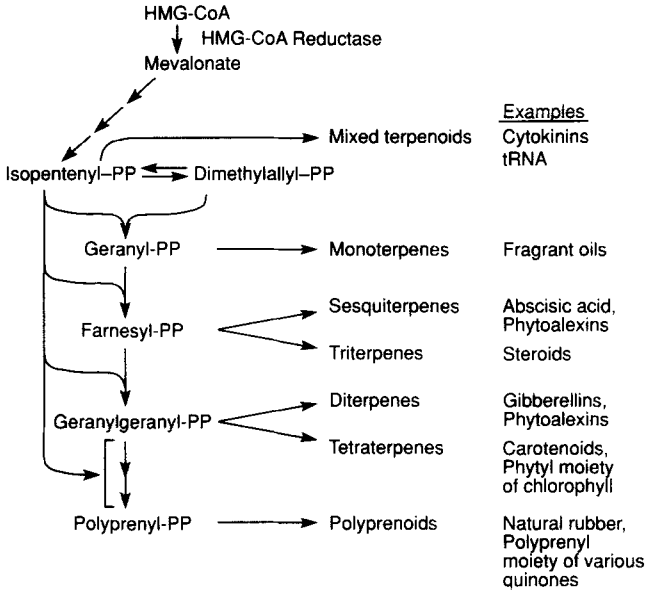


**Fig. 1.** Mevalonate pathway and typical end products in plants.

several important crop plants do have isoprenoid phytoalexins. Solanaceous plants such as potato, tomato, and pepper produce related sesquiterpenoid defense compounds. Cotton produces another class of sesquiterpenoid phytoalexins, and plants like rice and castor bean produce diterpenoid phytoalexins. Also, many of the flavonoid phytoalexins of legumes contain prenyl groups.

### Characterization of plant HMGR

Studies with HMGR activity from plants have left many unanswered questions. Purification of HMGR from several plants has been reported, but these studies have led to conflicting results regarding apparent molecular mass and the presence of multimer forms (13). Similar to animal and yeast HMGR, we know that plant HMGR activity utilizes NADPH and requires a reduced thiol group for optimal activity. However, the subcellular localization of HMGR in plants is still controversial (see Gray (14) for a detailed discussion). The localization of HMGR has important regulatory implications for the potential involvement of this enzyme in independent subcellular pathways. Plant HMGR, which appears to be an integral membrane protein as in other eukaryotes, has been localized to three subcellular sites by fractionation studies, plastids, mitochondria, and the cytoplasmic side of the endoplasmic reticulum. Supporting the presence of HMGR in several cell compartments are reports that the HMGR activity of distinct cell fractions have different kinetic parameters and exhibit differential regulation (13). As was the case with mammalian HMGR, many of the physical characteristics of HMGR from plants were uncertain until the cloning of genes encoding the enzyme.

Many genes encoding plant HMGR have been cloned in recent years (15–23). From analysis of the primary sequences it is evident that plant HMGRs have a strong similarity to other eukaryotic HMGRs, especially at the carboxy-terminal portion which contains the catalytic domain. The major difference in protein structure is in the membrane domains which consist of two spanning regions in plants compared to the eight found in animal HMGRs (24). The differences in the membrane domains may be significant in light of the requirement for this region in animals for feedback regulation by sterols (5).

The number of genes encoding HMGR in plants varies depending on the species. HMGR is encoded by at least two distinct genes in *Arabidopsis* (17), three in *Hevea* (18), at least three in tomato (25), and even larger multigene families in maize and pea (17) and potato (19). Clearly, differential expression of multiple HMGR genes in plants could play an important role in regulation of HMGR activity. The presence of multiple genes is consistent with the hypothesis that different isoforms of HMGR are involved in separate subcellular pathways for isoprenoid biosynthesis.

## PLANT HMGR ACTIVITY RESPONDS TO MANY SIGNALS

### Development

Because HMGR is a key enzyme in a pathway leading to compounds with diverse and important functions in plants, it is not surprising that HMGR activity in plants is controlled by a variety of developmental and environmental signals. Higher levels of HMGR activity are usually associated with the rapidly growing parts of the plant, such as apical buds and roots, with much reduced activity found in mature tissues (26, 27). For example, the relative activity of microsomal HMGR from the mature leaves of pea seedlings was only 7% of that observed in the apical buds (26). In tomato fruit, HMGR activity was highest in the early stages of fruit development when rapid cell division occurs, yet later in ripening tomato fruits there was a very low level of reductase even though large amounts of carotenoid pigment are synthesized during this period (25). In developing maize seeds, the highest activity was during stages of rapid mitotic divisions 10 to 12 days after pollination (28). Maize HMGR activity was associated exclusively with microsomal membranes. During later stages of seed maturation the maize endosperm HMGR activity decreased to one-fifth the maximal activity, and the embryo activity was relatively higher at one-half the maximal activity. The activity of both the endosperm and embryo decreased to a much lower level in the desiccated seed. In maize seeds germinated under white light, HMGR activity in roots was 2- to 4-fold higher than shoot activity.

### Light

Many metabolic changes in plants, including changes in the abundance of isoprenoid compounds, can be triggered by light. Red light has been reported to stimulate the accumulation of many isoprenoids including mono- and sesquiterpenes in maritime pine (29), gibberellins in etiolated wheat leaves (30), carotenoids in the etiolated seedlings of mustard (31), and monoterpenes, sterols, and carotenoids in the etiolated cotyledons of thyme (32, 33). Consistent with a key role for HMGR in isoprenoid biosynthesis, the activity of HMGR in plants is also modulated by light. When dark-grown pea seedlings received 5 min of irradiation with red light and were then returned to darkness, the HMGR activity contained in the plastid fraction doubled after 1.75 h (34). In contrast, the microsomal HMGR activity rapidly declined to a low level where it remained for about 24 h (35). Reversal of the action of red light by subsequent far red irradiation suggests that phytochrome is the receptor pigment involved in signal transduction. Similarly, HMGR activity of the microsomal and heavy membrane fractions of radish seedlings appeared to be independently regulated by phytochrome (36). Incubation of potato tuber tissue in white light during induction of HMGR activity by fungal elicitor markedly reduced the HMGR activity of the microsomal fraction compared to treatments carried out in darkness. Associated with this light-inhibition of activity was a similar reduction in accumulation of sesquiterpenoid phytoalexins (3). In maize seedlings germinated in the dark, both root and shoot HMGR activities were 1- to 5-fold higher relative to activities of seedlings in white light (28).

### Infection

In plants that synthesize isoprenoid phytoalexins as part of their defense against microbes, exposure to pathogens or pathogen-derived elicitor compounds can cause large increases in the HMGR activity of the plant. For example, sweet potato root tissue has very low HMGR activity, and the activity increased only slightly after the tissue is wounded by slicing. However, when infected by a fungal pathogen, HMGR activity increased rapidly to a maximum at 2 days post inoculation then decreased rapidly (37). Formation of the furanoterpene phytoalexin ipomearmarone followed the increase in HMGR activity. Similarly, wounding of potato tubers produced a temporary increase in HMGR activity of the microsomal and organelle fractions, and treatment of wounded tuber tissue with the sesquiterpenoid phytoalexin elicitor arachidonic acid further increased and prolonged the activity (3, 38). In tobacco cell cultures HMGR activity was transiently induced by elicitor treatment leading to accumulation of sesquiterpenoid phytoalexins (39). Mevinolin, a strong inhibitor of plant HMGR, inhibited elicitor-induced phytoalexin accumulation, thus supporting the involvement of elicitor-induced HMGR activity in the phytoalexin biosynthesis of these plants (3, 39).

### Isoprenoids

There have been some reports that HMGR activity is modulated in vivo by isoprenoid products. Certain sterols, such as stigmasterol and cholesterol, when sprayed on pea seedlings reduced the HMGR activity found in tissue extracts by 30 to 35% (40). Application of the isoprenoid growth regulator abscisic acid also inhibited HMGR activity in pea (40%), but zeatin and gibberellin, which are also isoprenoid growth regulators, increased the activity of HMGR (40). Auxin and 2,4-D, nonisoprenoid growth regulators, had no effect on pea HMGR. However, in carrot cell suspension culture, the presence of 2,4-D in the medium resulted in higher levels of HMGR activity (41). It is unknown whether the effect of the isoprenoid plant hormones is due to their isoprenoid nature (feedback regulation) or their action as growth regulators. Experiments with mevinolin indicate that HMGR activity may be feedback repressed by isoprenoid compounds. When the alga *Ochromonas malhamensis* was grown in 10 $\mu$M mevinolin there was a 10- to 15-fold increase in microsomal HMGR activity (after removal of the inhibitor) with little effect on cell growth (1). Mevinolin treatment of potato tuber tissue also resulted in an induction of microsomal HMGR activity (B. Stermer, unpublished results).

## MECHANISMS REGULATING PLANT HMGR

### Transcription

The activity of HMGR in plants is regulated in part by the level of its mRNA. Several studies have reported increases in the abundance of HMGR mRNA which are associated with increased HMGR activity (16–18, 42, 43). A key feature of this regulation is the differential induction of members of the plant's HMGR gene family. In *Hevea brasiliensis,* a plant used for production of natural rubber, HMGR is encoded by a small gene family comprised of three members, *hmg*1, *hmg*2, and *hmg*3. The expression of *Hevea hmg*1 and *hmg*3 has been examined by Northern, primer extension, and in situ hybridization analyses which revealed that *hmg*1 is inducible by ethylene while *hmg*3 is constitutively expressed (18). Furthermore, *hmg*1 is expressed predominantly in the laticifers, the cells specific to rubber biosynthesis, but *hmg*3 expression is not cell type specific. Three classes of HMGR genes have also been described for potato (19). Designated as *hmg*1, *hmg*2, and *hmg*3, consistent with the designation of the corresponding HMGR genes in tomato, each of these classes contains multiple genes bringing the total of HMGR genes in potato to 12 or more in all. Southern blot analysis

with probes specific for each class indicated that the potato *hmg*1 class contains seven or more distinct genes whereas *hmg*2 and *hmg*3 contain only one or two each (**Fig. 2**). These results expand on the previous work (19) that reported only one to three *hmg*1 class genes in potato. Sequence analysis of genomic clones supports the presence of at least seven different *hmg*1 genes in potato (M. Bhattacharyya, N. Paiva and B. Stermer, unpublished results). RNA blot analyses using class specific probes showed that potato *hmg*1 was strongly induced in tuber tissue by wounding but the wound induction was strongly suppressed by treatment of the tissue with the elicitor arachidonic acid or by inoculation with the potato pathogen *Phytophthora infestans* (19). The *hmg*2 and *hmg*3 mRNAs accumulated at lower levels in response to wounding, but in contrast to *hmg*1, these mRNAs were strongly enhanced by arachidonic acid or inoculation (19, 42).

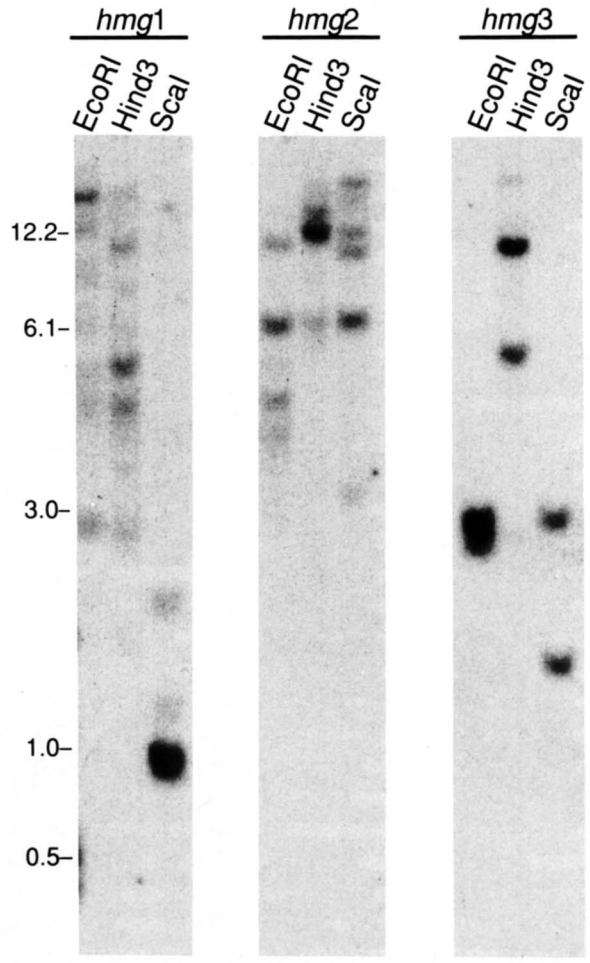


**Fig. 2.** Southern blot analysis of the HMGR gene family in potato. Genomic DNA from potato cv. Kennebec was digested with the indicated restriction enzymes; duplicate samples were run on an agarose gel, then blotted on to a membrane. Three identical blots were hybridized with the *hmg*1, *hmg*2, or *hmg*3 gene probes described by Choi et al. (19).

Preliminary studies with other plants indicate that differential regulation of HMGR expression at the mRNA level is a widespread phenomenon. *Arabidopsis* HMG1 mRNA accumulated in leaves and light grown seedlings, and transcripts from a second gene, HMG2, accumulated to lesser levels mainly in the roots (16, 44). A cDNA clone isolated from a tomato library derived from young fruit hybridized to a HMGR mRNA that had the highest levels during early states of fruit development when HMGR enzyme activity was also the highest (25). In addition, sequencing of cDNA clones from radish and pea has demonstrated that these plants also expressed more than one HMGR gene, although differential expression patterns are yet to be reported (16). Recent work with *Camptotheca acuminata,* a Chinese tree that produces the anti-cancer monoterpenoid camptothecin, describes a gene encoding HMGR (22). Northern blot analysis showed transcripts corresponding to *Camptotheca hmg*1 only in young seedlings and not in the vegetative organs of older plants. Expression of translational fusions of the *hmg*1 promoter with the $\beta$-glucuronidase (GUS) reporter gene in transgenic tobacco indicated promoter activity in the epidermis of young leaves and stems. However, roots also showed high levels of GUS expression suggesting that the transcript for *hmg*1 in *Camptotheca* is rapidly degraded in roots. The *Camptotheca hmg*1::GUS promoter fusion was also induced by wounding in tobacco and this induction was suppressed by treatment with methyl jasmonate. A potato *hmg*1 promoter-GUS fusion gave a similar pattern of expression in transgenic potato and tobacco, but the strongest expression was in pollen (M. Bhattacharyya, K. Korth, and B. Stermer, unpublished results). The potato *hmg*1 transcript also appears to be rapidly degraded.

### Reversible phosphorylation

HMGR activity is controlled not only at the mRNA level but also posttranslationally. Some studies have suggested that HMGR activity in plants is regulated by reversible phosphorylation, as it is in animals. The plant enzyme is inactivated by incubation with supernatant fractions in the presence of MgATP and can be activated by phosphatase (45, 46). Recent work isolating and characterizing a specific HMGR kinase from the supernatant fraction of both monocots and dicots has provided more direct evidence for phosphorylation of HMGR in plants (47). The plant kinase inactivated HMGR purified from plants or mammals. The only significant difference between the plant and animal protein kinases was the lack of plant HMGR kinase activation by AMP. The AMP-activated protein kinase of animals does not play a role in the end product feedback regulation of HMGR, but instead responds to low cellular ATP levels (48). The plant kinase does appear to be part of a protein kinase cascade conserved with the animal HMGR kinase, as the plant kinase is inactivated by mammalian protein phosphatases

and reactivated by mammalian kinase kinase (47). Further studies will be needed to determine whether the rapid inactivation of plant HMGR by red light or isoprenoids such as stigmasterol, cholesterol, or abscisic acid occurs via HMGR phosphorylation.

### Calcium

The regulation of HMGR activity may also involve calcium and calmodulin, but studies to date are difficult to interpret. Wititsuwannakul, Wititsuwannakul, and Dumkong (49) reported that purified calmodulin from *Hevea* latex was able to increase HMGR activity 2.5-fold. This activation required the presence of $Ca^{2+}$ and was inhibited by the calmodulin antagonist trifluoperazine. Interestingly, their results with sodium fluoride, a phosphatase inhibitor, suggested that calmodulin may indirectly activate *Hevea* HMGR activity via regulation of enzymes involved in reversible phosphorylation of the enzyme. In contrast, Russell, Knight, and Wilson (45) reported that nanomolar levels of free $Ca^{2+}$ caused marked inhibition of microsomal HMGR activity from pea. The action of a $Ca^{2+}$-dependent protease or a $Ca^{2+}$-dependent protein kinase activity seems to be excluded by the reversibility of the $Ca^{2+}$ response and the absence of other cofactor requirements, respectively. Furthermore, calmodulin antagonists had no effect on the $Ca^{2+}$ response. Consistent with this, the addition of $Ca^{2+}$ in the presence or absence of bovine brain calmodulin had no effect on HMGR kinase activity in crude preparations from cauliflower, carrot, pea, or rapeseed, or on the purified cauliflower enzyme (47). These varying results may be due to qualitative differences in enzyme preparations or variations found between plant species. More work will be needed to resolve the role of $Ca^{2+}$ and calmodulin in HMGR regulation.

### Proteolytic degradation

Proteolytic degradation may also serve to help regulate plant HMGR activity as it does in animal cells. A recent report suggests that a cysteine protease is involved in the mevalonate-accelerated and basal degradation of mammalian HMGR (50). In pea seedlings, a previous observation of a cofactor-independent HMGR-inactivation enzyme in the soluble protein fraction, which was active only at DTT concentrations above 20 to 25 mM (51), may be explained by the presence of a cysteine protease. Consistent with a role for the protease in plants, in potato extracts, inclusion of cysteine protease inhibitors increases recovered HMGR activity 10-fold, while other types of protease inhibitors had no effect (**Fig. 3**). Also, the linker region of the plant HMGR contains a PEST motif, similar to that of mammalian HMGR, which is found in proteins with rapid turnover rates (18, 44). However, the relationship of the PEST motif to proteolytic regulation of plant HMGR is unknown.

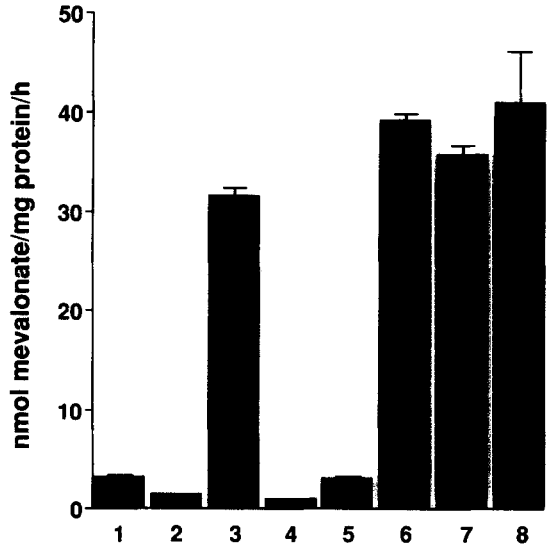


**Fig. 3.** Microsomal HMGR activity recovered from potato tubers in the presence of protease inhibitors. The HMGR activity was induced in tuber disks by application of arachidonic acid as described previously (3). The protease inhibitors were present in the extraction and assay buffers at the indicated concentrations: 1, none; 2, 1 mM PMSF; 3, 5 μg/ml leupeptin; 4, 5 μg/ml phosphoramidon; 5, 2 μg/ml trasylol; 6, 5 μg/ml E-64; 7, 5 μg/ml leupeptin + 5 μg/ml E-64; 8, 5 μg/ml leupeptin, 2 μg/ml trasylol + 5 μg/ml E-64.

### Subcellular compartmentation

Much of the isoprenoid biosynthesis in plants is localized in specific compartments within a cell which allows for the independent regulation of parallel pathways that produce different end products (14, 52). For example, carotenoids, ubiquinone and sterols are isoprenoids synthesized in the chloroplasts, mitochondria, and cytoplasm, respectively (8). The significance of subcellular compartmentation for HMGR regulation lies in the organelle membranes that affect the nature of the proteins present and the permeability of the compartment to substrates, intermediates, and products (53). Thus, not only could HMGR isoforms with distinct regulatory properties be present in separate compartments, but the activity of the enzyme could be affected by the chemical environment of the organelle. The production or import of NADPH and HMG-CoA as well as competing reactions for these substrates in subcellular compartments could have a major impact on the in vivo activity of HMGR. However, despite the potential importance of compartmentation, the subcellular localization of HMGR in plants is still unresolved.

The debate on the spatial organization of isoprenoid biosynthesis in plant cells has been presented in several recent articles (14, 52, 53). There are two major views concerning the localization of HMGR in plant cells. One hypothesis is that three cell compartments (plastids,

mitochondria, and cytoplasm) each contain a separate mevolonate pathway complete with HMGR. The alternative hypothesis is that all steps in the mevalonate pathway prior to isopentenyl diphosphate, which would include HMGR, are located solely in the cytoplasm and the isopentenyl diphosphate is transported into the subcellular compartments. Obviously, the presence of HMGR in multiple subcellular locations could bring greater complexity to the regulation of this enzyme.

## CONCLUDING REMARKS

This brief overview has highlighted the major features of our current understanding of HMGR regulation in plants. There are several facets to the regulation of HMGR in plants that are not found in animals or yeasts. One example is the influence of ambient light conditions on plant HMGR activity. Light has profound effects on numerous aspects of plant development and metabolism that often occur coordinate with changes in HMGR activity. Another attribute of plant HMGR is the induction of its expression by pathogen attack in plants that synthesize isoprenoid phytoalexins. Because many of the end products of the mevalonate pathway are unique to plants, HMGR activity in plants responds to stimuli not reported for other groups of organisms. Evidently, plants have signal transduction pathways linking the detection of pathogens or light to mechanisms that control expression of HMGR.

Studies have indicated that an important component of HMGR regulation in plants is at the level of its mRNA. This is also true for mammalian HMGR where the rate of transcription is repressed by sterols. However, in the case of plants, studies have focused on alterations in transcript levels and have not differentiated between changes in transcription and mRNA stability. The HMGR promoter-GUS reporter gene fusions would suggest that transcription does have an important part in controlling the activity of this enzyme. Further studies are needed to confirm regulation by transcription.

HMGR activity in plants is also regulated after transcription. In animal systems, at least three different control points have been described that serve to regulate HMGR activity posttranscriptionally: translation rate, enzyme degradation, and enzyme inactivation (5). Of these, only enzyme inactivation has been reported for plants. Like animals, plants contain HMGR kinase and apparently can regulate HMGR activity by reversible phosphorylation. The rapid effects of light on HMGR activity make phosphorylation a good candidate for the mechanism mediating light effects. Although some sterols and nonsterol isoprenoids have been reported to inhibit HMGR activity in plants, the level at which they regulate activity is not known.

Unlike its mammalian counterpart, different isoforms of HMGR are encoded by a family of genes. This makes possible the regulation of HMGR activity by differential expression of its genes. For example, in potato, *hmg*1 is activated during the biosynthesis of sterols while *hmg*2 and *hmg*3 are induced during biosynthesis of sesquiterpenoid phytoalexins (19). Multiple genes could encode HMGR enzymes with distinct kinetic properties for specific metabolic tasks and may provide the added flexibility of having HMGR transcription plugged into two different regulatory networks. Consistent with this, kinetically distinct isoforms of HMGR have been reported in studies of several plant species (13).

There are still many important questions about the regulation of HMGR in plants that remain to be answered. We need to learn more about the possibility of separate mevalonate pathways, targeting of HMGR, and the role of the multiple genes encoding this enzyme in plant cells. The subcellular localization of HMGR is essential to the understanding of its regulation. In addition, posttranslational regulation and signal transduction mechanisms require more attention. Because HMGR has a role in so many aspects of plant metabolism, an understanding of the regulation of HMGR will be needed as opportunities become available to alter plant metabolism for crop improvement or production of valuable products.

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