Short communication

HMG-CoA reductase gene families that differentially accumulate transcripts in potato tubers are developmentally expressed in floral tissues

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Abstract

We isolated two full-length cDNA clones encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) from potato (*Solanum tuberosum*) L. tubers. The clones, designated *hmg*2.2 and *hmg*3.3, are members of previously described gene subfamilies. In addition to being induced by arachidonic acid in tubers, *hmg*2.2 transcript accumulates developmentally in young flowers, and in mature sepals and ovaries, whereas transcript for *hmg*3.3 accumulates in mature petals and anthers. Our data suggest that members of specific HMGR-encoding gene subfamilies might be involved in both defense responses and flower development. Accumulation of different HMGR transcripts could provide some control of isoprenoid biosynthesis by producing isoforms specific for classes of end-products produced in particular tissues.

Cellular control of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) has received a great deal of interest because of the probable importance of this enzyme in the regulation of isoprenoid biosynthesis. Isoprenoids in plants make up a huge array of compounds including many utilized in growth and development such as sterols, plant growth regulators, and carotenoids [6, 17]. In addition, commercially important isoprenoids like natural rubber, fragrant oils, and some pharmaceutical compounds are isolated from plants [12].

Antimicrobial phytoalexins from several plant families may also be isoprenoids; these include the sesquiterpene phytoalexins induced in potato tubers (*Solanum tuberosum* L.) upon infection with *Phytophthora infestans* (reviewed in [15]). A marked increase in HMGR activity accompanies accumulation of these sesquiterpenes in potato tubers after pathogen infection or treatment of tubers with arachidonic acid, an elicitor

from *P. infestans* [18, 23], suggesting an important role for this enzyme in defense against pathogens.

Enzymatic activity of HMGR in animals is known to be regulated by numerous means, both transcriptional and post-transcriptional [14]. It is believed that some of these same regulatory mechanisms are also at work in the control of the plant HMGR [22]. Genes encoding HMGR have been isolated from a large number of plant sources (e.g. [1, 5, 10, 13, 16, 19]), and transcriptional regulation of HMGR has been extensively studied.

Potato contains a small gene-family encoding HMGR and it is clear that accumulation of specific *hmg* transcripts can be differentially affected by numerous stimuli. Transcript levels of members of the potato *hmg* gene family can be either up- or down-regulated in tubers by wounding, bacterial and fungal pathogens, arachidonic acid, or methyl jasmonate [7, 8, 24, 25]. Choi *et al.* [8] isolated one full-length cDNA *hmg*1 and two partial cDNA (*hmg*2 and *hmg*3) clones to develop gene-specific probes and demonstrate this differential control. These genes were categorized by sequences in their 5′-terminal region

(*hmg*1) of 3′-untranslated regions (*hmg*2 and *hmg*3). Hybridization patterns indicate that each gene-specific probe will anneal with multiple fragments of restriction enzyme-digested potato genomic DNA [8, 22], suggesting the presence of gene subfamilies, each made up of several members. Transcript accumulation of *hmg*1 in potato tubers increases in response to wounding but this induction is repressed by arachidonic acid. The *hmg*2 and *hmg*3 transcripts are induced to a lower degree by wounding, but their levels are greatly enhanced by addition of arachidonic acid. These findings, along with a correlative increase of sesquiterpenoid phytoalexins with *hmg*2 transcripts [7] indicate that the *hmg*2 and *hmg*3 gene subfamilies might play a direct role in the production of defense-related molecules.

Previous work on transcriptional regulation of the potato HMGR gene families raised several questions. Choi *et al.* reported that a high level of transcript in potato anthers hybridized to a probe conserved among plant HMGRs, and that this signal was much greater than the cumulative signals hybridizing to the *hmg*1, *hmg*2, and *hmg*3 gene-specific probes. This suggested that one or more additional HMGR gene subfamilies are present in potato and expressed in anthers [8]. Based on those findings, it might be supposed that different HMGR-encoding genes are expressed during plant organogenesis and in response to external stimuli. Work from this laboratory however, showed that *hmg*3-hybridizing HMGR cDNAs were by far the most abundant type isolated from mature potato anthers and/or pollen [3], demonstrating the possibility that the same *hmg* gene subfamily could be filling dual roles, in both plant defense and development.

The work presented here was initiated to isolate full-length clones of HMGR genes involved in defense against pathogens. The previously isolated partial HMGR clones lacked sequence predicted to encode the N-terminal regions of the respective proteins. In addition, we hoped to gain a clearer understanding of how transcript accumulation of specific HMGR genes is regulated during plant organogenesis, especially during flower development. To that end, we produced a cDNA library (in the λZAPII vector, Stratagene) from seed-certified potato (cv. Kennebec) tubers that had been incubated with arachidonic acid for 20 h [23]. Using previously described *hmg* gene-specific probes [8] for screening, we isolated full-length cDNA clones of arachidonic acid-inducible genes. Blots of total tuber RNA show that 2.4 kb transcripts hybridizing to these probes accumulate to high levels upon elicitation (Fig. 1).
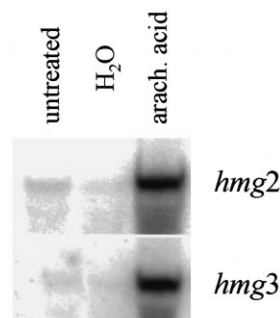

Figure 1. Analyses of total RNA from control and elicitor-treated potato tubers. Tuber disks were either fresh-cut, or treated with water or arachidonic acid and tissue was collected after 20 h as described [23]. In this and subsequent figures, 15 μg of total RNA was electrophoresed on 1% agarose/formaldehyde gels and blotted onto nylon membrane (GeneScreen Plus, NEN Research Products). Gene-specific probes for *hmg*2 and *hmg*3 were generated by polymerase chain reaction (PCR) using primers described [8]. Standard procedures were used for all steps [21].

The sequences of these new cDNAs bear great similarity to the partial clones described by Choi *et al.* In keeping with the established nomenclature, we designed the clones having significant similarity to *hmg*2 and *hmg*3 as *hmg*2.2 and *hmg*3.3, respectively. The insert size of *hmg*2.2 is 2019 bp and that of *hmg*3.3 is 2214 bp. However, the high similarity between *hmg*2, described by Choi *et al.*, and *hmg*2.2, reported here, ends at nucleotide (nt) 2000 of *hmg*2.2 (nt 845 of *hmg*2). Up to that point, the overlapping sequences are 99.8% identical. The poly(A) tail of *hmg*2.2 begins at nt 2007 whereas *hmg*2 has an additional 368 nt and lacks a poly(A) tail (outlined in Fig. 2). These results indicate that either there are two or more transcribed members of the *hmg*2 subfamily or different sites of polyadenylation can be utilized by the *hmg*2 gene. The region from nt 946 to nt 1095 of *hmg*2 has 82% identity to a portion of the sequence of the *Nicotiana sylvestris* HMGR gene [13], indicating that this is an authentic HMGR sequence. The point at which the similarity between *hmg*2.2 and *hmg*2 ends is 114 bases to the 3′ side of the end of the open reading frame, so that the predicted peptides encoded by the two sequences are the same in the overlapping regions. These differences could also be explained by the fact that the clones were derived from different potato cultivars, the clones of Choi *et al.* were isolated from cv. Russet Burbank [8], and our clones originated in the cv. Kennebec.

The similarity of *hmg*3.3 with the *hmg*3 clone of Choi *et al.* ends at nt 2164 of *hmg*3.3 (nt 1898 of *hmg*3). The sequence of *hmg*3 from nt 1898 to its 3′
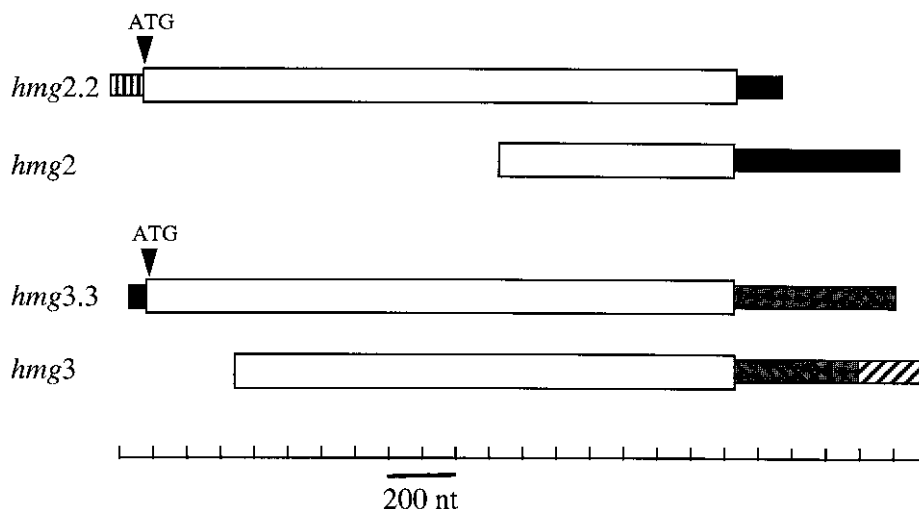
Figure 2. Schematic representation comparing the sequences of *hmg*2.2 and *hmg*3.3 with *hmg*2 and *hmg*3 [8], respectively. The large, open boxes represent the open reading frames beginning at the first ATG triplet, as indicated. The smaller boxes represent the flanking regions. Like boxes represent sequences with high levels of identity in the overlapping regions.

end is identical to a portion of the genome of potato virus X, indicating that this is probably a chimeric clone. This sequence does not hybridize to genomic DNA of potato cv. Kennebec (data not shown). Again, the discrepancies in sequence occur to the 3′ side of the open reading frame. The gene-specific probes that were used to isolate the cDNAs described here spanned the divergent regions in *hmg*2 and *hmg*3 but were nonetheless effective in hybridizing to elicitor-inducible transcripts.

The predicted open reading frames of the new, full-length potato HMGR clones are shown in Fig. 3 along with that predicted from *hmg*1 [8]. As with most plant HMGR peptides, the majority of the sequence variability is in the amino-terminal one-third of the protein, whereas the carbonyl two-thirds of the protein, containing the catalytic domain, show a high degree of conversation [2].

The predicted proteins contain other sequences common to plant HMGRs, such as two putative membrane-spanning domains and potential PEST sites, found in proteins with rapid turnover rates [20]. Phosphorylation has been proposed as a means of enzymatic control of HMGR, and inactivation of a plant HMGR through phosphorylation by a serine kinase from *Brassica oleracea* was recently demonstrated [11]. All of the HMGR clones thus far isolated from potato predict the presence of the conserved serine residue thought to be phosphorylated by this kinase (Fig. 3). In addition, *hmg*2.2 contains a consensus sequence for phosphorylation by a tyrosine kinase at residue 12. The tyrosine phosphorylation consensus site, present in *hmg*2.2 but not in the other *hmg* genes, could represent yet another means of differential control of the isoforms encoded by this gene family.

In an attempt to answer some of the aforementioned questions concerning HMGR transcript accumulation in potato, we assessed the expression of all of the characterized HMGR gene families in whole flowers. Figure 4 shows that the *hmg*1 subfamily is strongly expressed in the earliest stages of flower development but the transcript levels decrease with flower maturity. The *hmg*2 probe reacts most strongly with transcripts at the earliest and latest stages of flower development. Transcripts hybridizing to *hmg*3 are virtually absent until flowers are fully opened. The hybridization pattern seen with probe spanning a well conserved region of the *hmg* genes, *hmg*-C, shows that transcripts encoding all HMGRs are most abundant at stages 1 and 5 of flower development (Fig. 4B).

Figure 5 illustrates the abundance of the various transcripts in organs isolated from mature, stage five, flowers. Transcripts for *hmg*1 accumulate to high levels in ovaries but to relatively low levels in the other tissues examined. Transcripts hybridizing to *hmg*2 are abundant in all tissues examined but have highest levels in sepals and ovaries, whereas *hmg*3 transcripts are abundant only in petals and anthers. We have previously isolated HMGR cDNA clones from mature potato anthers [3]. Nearly all of the *hmg* clones isolated

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                                                                1              ↓
hmg1      KWLNTQYTLSLSLISLFFFSSFQNSGVPTGKSTKFTMDVRRRPVKPLYTSKDASAGE-PL
hmg2.2                                   KMDVRRRSEKPVYPSKVFGADEKPL
hmg3.3    ------------------IPPF--SSVNSGDRKSFTMDVRRRPVKPLYPSEHISSGE-PL
                                          ******. **.*.*.   ...* **


hmg1      K-QQEVSS-------PKASDALPLPLYLTNGLFFTMFFSVMYFLLVRWREKIRNSIPLHV
hmg2.2    KPHNNQQQEDNNTLLIDASDALPLPLYLTNGLFFTMFFSVMYFLLSRWREKIRNSTPLHV
hmg3.3    KPHNQDSS-------VKASDALPLPLYLTNGLFFTMFFSVMYFLLHRWREKIRNGIPLHV
          *  ...            .****************************** ******* ..****


hmg1      VTLSELLAMVSLIASVIYLLGFFGIGFVQSFVSRSNSDSWDIEDENAEQLIIEEDSRRGP
hmg2.2    VTLSELGAIVSLIASVIYLLGFFGIGFVQTFVSRGNNDSWD---ENDEEFLLKEDSR---
hmg3.3    LNFSELVAMVSLIASVIYLLGFFGIGFVQSFVSKGNNDSWDVEDESPEQFIDRT------
          ...*** *.****************.***..*.****    *. *...  .


hmg1      CAAATTLGCVVPPPPVRKIAPMVPQQPAKVALSQTEKPSPIIMPALSEDDEEIIQSVVQG
hmg2.2    CGPATTLGCAIPAPPARQISPMAPPQP---AMSMVEKPSPLITPASSEEDEEIINSVVQG
hmg3.3    ----------VTPPPVRRNIPMKS-------VPVAEKTAQIITPFSSEDDEVVIKSVVEG
                    ...**.*.   ** .         .. .**....* *   **.** .*.***.*


hmg1      KTPSYSLESKLGDCMRAASIRKEALQRITGKSLEGLPLEGFDYSSILGQCCEMPVGYVQI
hmg2.2    KFPSYSLVIQLGDVSAAASLRKEVMQRITGKSLEGLPLEGFTYESILGQCCEMPIGYVQI
hmg3.3    RIPSYSLESKLGDCKRAAFIRKEALQRSSGKSLEGLPLDGFDYESILGQCCEMPIGYIQI
          . *****  .***    **.***..**  .*********.**.*.**********.**.**


hmg1      PVGIAGPLLLDGREYSVPMATTEGCLVASTNRGCKAIFVSGGADSVLLRDGMTRAPVVRF
hmg2.2    PVGIAGPLLLNGKEFSVPMATTEGCLVASTNRGCKAIYASGGATCIVLRDGMTRAPCVRF
hmg3.3    PVGIAGPLLLNGKEFSVPMATTEGCLVASTNRGCKAIYVSGGATSVLFRDAMTRAPVVRF
          **********.*.*.********************..****.....**.***** ***


hmg1      TTAKRAAELKFFVEDPLNFETLSLMFNKSSRFARLQGIQCAIAGKNLYITFSCSTGDAMG
hmg2.2    GTAKRAAELKFFVEDPIKFETLANVFNQSSRFGRLQRIQCAIAGKNLYMRFVCSTGDAMG
hmg3.3    GSAKRAAELKFFVEDPMNFETLSVVFNKSSRFARLQNIQCAIAGKNLYMRFSCSTGDAMG
          ..**************..****. .**.****.*** ***********. * _____


hmg1      MNMVSKGVQNVLDYLQSEYPDMDVIGISGNFCSDKKPAAVNWIEGRGKSVVCEAIIKEEV
hmg2.2    MNMVSKGVQNVLDYLQNEYPDMDVIGISGNFCSDKKPAAVNWIEGRGKSVVCEAIITEEV
hmg3.3    MNMVSKGVQNVLDYLQNEYPDMDIIGISGNYCSDKKPAAVNWIEGRGKSVVCEAIIKEDV
          ****************.******.******.***********************.*.*


hmg1      VKKVLKTEVAALVELNMLKNLTGSAMAGALGGFNAHASNIVSAVYLATGQDPAQNVESSH
hmg2.2    VKKVLKTEVAALVELNMLKNLTGSAMAGALGGFNAHASNIVSAVFIATGQDPAQNIESSH
hmg3.3    VKKVLKTEVATLVELNMLKNLTGSAMAGALGGFNAHASNIVSAVYLATGQDPAQNIESSH
          **********.***************************************..*********.****


hmg1      CITMMEAVNDGKDLHVSVTMPSIEVGTVGGGTQLASQSACLNLLGVKGANRDAPGSNARL
hmg2.2    CITMMEAVNDGKDLHISVTMPSIEVGTVGGGTQLASQSACLNLLGVKGANREAPGSNARL
hmg3.3    CITMMEAVNDGKDLHISVTMPSIEVGTVGGGTQLASQSACLNLLGVKGANREAPGSNARL
          **************.*****************************************.*******
                                          ◆
hmg1      LATIVAGSVLAGELSLMSAISAGQLVKSHMKYNRSIKDISK
hmg2.2    LATVVAGSVLAGELSLMSAISAGQLVNSHMKYNRSTKA-SS
hmg3.3    LATIVAGSVLAGELSLMSAISAGQLVKSHMKYNRSCKDVTK
          ***.*******************.********* *.  ..
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Figure 3. The longest predicted open reading frames of the *hmg*2.2 and *hmg*3.3 clones aligned with that of *hmg*1 as reported in [8]. (*) indicates a match across all sequences, (•) indicates a conservative substitution. A **1** indicates the first Met residue of each sequence, ↓ indicates the potential tyrosine phosphorylation site at residue 12 of *hmg*2.2, and (◆) indicates the conserved Ser residue thought to be phosphorylated. The region spanned by a conserved probe, *hmg*-C, is underlined. The alignment was performed with the Clustal V program on the Wisconsin GCG package. Sequencing was performed on an Applied Biosystems model 372A automated sequencer.
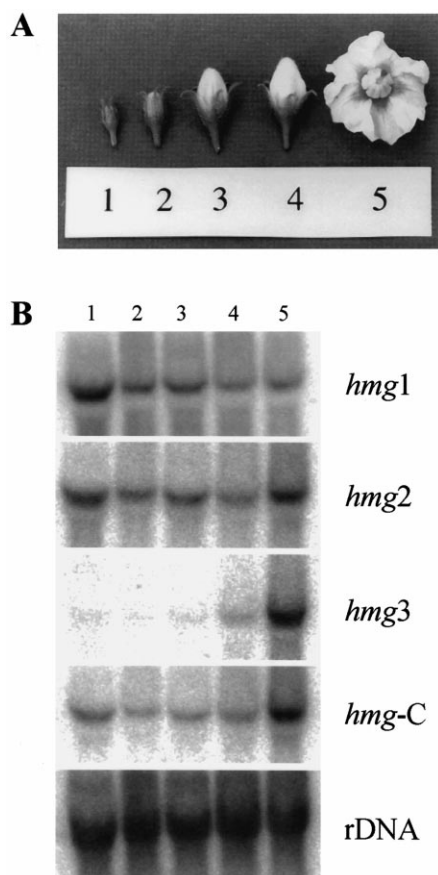
Figure 4. Levels of HMGR transcripts in potato flowers. **A**. Flowers were categorized according to maturity as stages 1 through 5. Whole flowers were detached and immediately frozen in liquid N_2 for subsequent RNA analysis. **B**. Blots of total RNA isolated from flowers as described [9]. Lane numbers correspond to flower stage numbers. The hmg1 and hmg2 probes were the same as designed by Choi et al. [8]. The hmg3 probe was PCR-generated using 5'-gcaacaacacagctaattc-3' and 5'-gatacaacactctgtgcaga-3' as primers and the hmg3.3 clone as templete. hmg-C is a 652 nt probe that spans a highly conserved region of the hmg genes; it was generated by PCR using 5'-agcaccggtctcgagatgggaatgaacatggtg-3' and 5'-tttcatgtggctaagcggtagttgcccagccgagat-3' as primers and the hmg2.2 clone as template. These primers were designed to create XhoI and EspI sites near the termini of the hmg-C fragment. The gene-specific probes and hmg-C were all of approximately the same specific activity. The rDNA probe hybridizes to the 18S ribosomal RNA and was included to demonstrate approximate equal loading in each lane.

from this tissue hybridized to the hmg3 gene-specific probe. A full-length cDNA clone from anther tissue was sequenced and found to be identical to the corresponding region of hmg3.3. That clone was designated hmg3.2 and the sequence at its 5' terminus has been reported [3]. The sequences of hmg3.2 and hmg3.3 are identical over the nearly 2200 nt where they over-
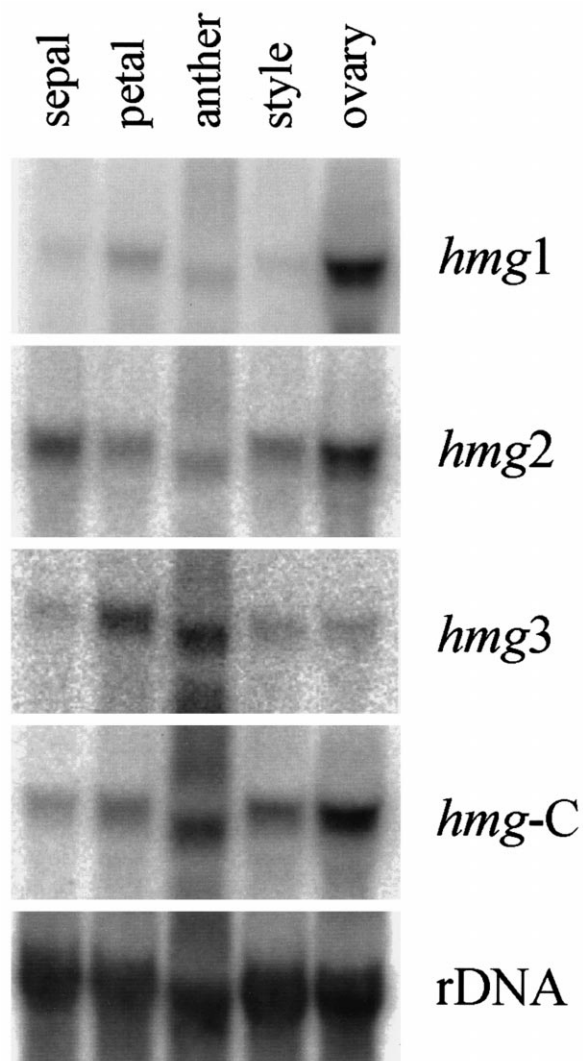

Figure 5. RNA blot analyses of HMGR transcript levels in potato floral organs. Blots were probed with the same gene-specific sequences as described for Fig. 4. Total RNA was isolated as described [9] from sepal, petal, anther, style/stigma, or ovary tissue frozen in liquid N_2 immediately after dissection from stage 5 flowers.

lap, including 450 nt in the 3'-flanking region of the genes, where the highest degree of variability occurs in the potato HMGR cDNAs examined thus far. Results from RNA blot analyses (Fig. 5) confirm that hmg3-hybridizing transcripts are the most abundant type in anthers.

The results obtained with an hmg probe spanning a conserved region demonstrate that the overall level of HMGR-encoding transcripts is highest in the anthers and ovaries (Fig. 5). Based on these results, most of the

hmg transcript in anthers, and the other tissues, could be accounted for by these three gene subfamilies. This contradicts the findings of Choi *et al.*, who reported that a large amount of HMGR transcript in anthers did not hybridize to any of the gene-specific probes [8]. In addition, we show an abundance of *hmg*3-hybridizing mRNA in petals (Fig. 5) whereas Choi *et al.* showed very little signal in that tissue. Both studies of transcript accumulation were carried out on potato cv. Kennebec. The discrepancies may have their origins in the different probes that were used. About 20% of the original *hmg*3 probe consisted of sequences probably derived from potato virus X, thus lowering its effectiveness. Because we designed a new gene-specific probe based on the 3′-flanking sequences of *hmg*3.3 (described in the legend of Fig. 4), the probes may be sufficiently different to explain some of the apparent contradictions. It is also possible that growing conditions, environmental factors, or the specific floral stages sampled, influenced observed levels of these transcripts.

The results in Figs. 4 and 5 might also clarify some of our earlier findings. Previously, we used 5′-flanking regions of an *hmg*1 genomic sequence to drive expression of the β-glucuronidase (GUS) enzyme in transgenic tobacco and potato plants [3]. Strong activity of the GUS enzyme was observed in mature pollen grains. One therefore might expect to find high levels of *hmg*1 transcript in mature pollen. However, by far the most abundant class of cDNAs isolated from a mature-anther library were members of the *hmg*3 subfamily. In addition, probing RNA blots with *hmg*1 and *hmg*3 now indicates that, while there is some level of *hmg*1 transcript present, there is much more *hmg*3 transcript in mature anthers (Fig. 5). The previous results might be explained by the fact that GUS is a fairly stable enzyme. Transcription controlled by *hmg*1.2 might have occurred early in flower development, when *hmg*1 transcripts are abundant (Fig. 4), and the enzyme accumulated to higher levels over time. The *hmg*1 subfamily is the largest encoding HMGR in potato [22] and the particular clone we tested, *hmg*1.2, only drove expression of GUS in pollen among flower tissues. It is possible that the *hmg*1.2 promoter is only active during the late stages of pollen development, and that accumulation of high levels of *hmg*1 transcripts in other tissues is driven by other promoters within the *hmg*1 gene subfamily. However, based on RNA analyses, the level of *hmg*1.2-produced transcripts in anthers is still much lower than the level of *hmg*3 transcripts.

Low levels of HMGR transcripts accumulate in roots, stolons, stems, and mature leaves, whereas higher levels of transcripts hybridizing to *hmg*1 and *hmg*2 accumulate in expanding leaves around the leaf apical bud (data not shown). This corresponds to a high level of HMGR enzyme activity often observed in young, rapidly growing leaf tissue [4; K.L. Korth and B.A. Stermer, unpublished results].

In addition to the already characterized control of *hmg* mRNA accumulation by external stimuli, we have shown that members of the *hmg* family in potato are developmentally expressed in different issues. Transcripts for gene subfamily types induced in tubers by methyl jasmonate (*hmg*1), or by pathogen infection (*hmg*2 and *hmg*3), also accumulate in different flower parts. The probes used in this study each hybridize to several bands on genomic DNA blots [22], indicating that there are potentially several loci producing transcripts that would react with these probes on RNA blots. It is possible that expression of different members within a subfamily could be controlled by different promoters, but that each would hybridize to the gene-specific probes. An alternative explanation is that the same gene encoding HMGR could be expressed in order to drive biosynthesis of isoprenoids needed in response to pathogens or for plant development.

Inducible transcript accumulation of *hmg*1 has been correlated with an increase in steroid glycoalkaloids in potato tubers, whereas inducible *hmg*2 accumulation correlates with an increase in sesquiterpene phytoalexins [7]. This strongly suggests that the isoforms encoded by these genes are active in separate pathways giving rise to discrete end-products. It has been suggested that isoprenoid biosynthesis in plant cells could be divided into metabolic channels, each specific for certain classes of end-products [6]. This is an attractive model that could, at least to some degree, explain the metabolic control of this very complex pathway. It is quite possible that developmental expression of specific HMGR genes at the transcriptional level might also correlate with certain metabolites needed in specific plant tissues.

The work presented here helps to clarify previous studies in the area of transcriptional regulation of potato HMGR genes. It also describes the differential accumulation of specific HMGR gene-subfamily transcripts during floral development, and further highlights the complexity of the HMGR gene family in potato.

References

1. Aoyagi K, Beyou A, Moon K, Fang L, Ulrich T: Isolation and characterization of cDNAs encoding wheat 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Physiol 102: 623–628 (1993).
2. Bach TJ, Wettstein A, Boronat A, Ferrer A, Enjuto M, Gruissem W, Narita JO: Properties and molecular cloning of plant HMG-CoA reductase. In: Patterson GW, Nes WD (eds) Physiology and Biochemistry of Sterols, pp. 29–49. American Oil Chemists' Society, Champaign, IL (1991).
3. Bhattacharyya MK, Paiva NL, Dixon RA, Korth KL, Stermer BA: Features of the *hmg*1 subfamily of genes encoding HMG-CoA reductase in potato Plant Mol Biol 28: 1–15 (1995).
4. Brooker JD, Russell DW: Properties of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase from *Pisum sativum* seedlings. Arch Biochem Biophys 167: 723–729 (1975).
5. Caelles C, Ferrer A, Balcells L, Hegardt FG, Boronat A: Isolation and structural characterization of a cDNA encoding *Arabidopsis thaliana* 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Mol Biol 13: 627–638 (1989).
6. Chappell J: The biochemistry and molecular biology of isoprenoid metabolism. Plant Physiol 107: 1–6 (1995).
7. Choi D, Bostock RM, Avdiushko S, Hildebrand DF: Lipid-derived signals that discriminate wound- and pathogen-responsive isoprenoid pathways in plants: methyl jasmonate and the fungal elicitor arachidonic acid induce different 3-hydroxy-3-methylglutaryl coenzyme A reductase genes and antimicrobial isoprenoids in *Solanum tuberosum* L. Proc Natl Acad Sci USA 91: 2329–2333 (1994).
8. Choi D, Ward BL, Bostock RM: Differential induction and suppression of potato 3-hydroxy-3-methylglutaryl coenzyme A reductase genes in response to *Phytophthora infestans* and to its elicitor arachidonic acid. Plant Cell 4: 1333–1344 (1992).
9. Chomczynski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159 (1987).
10. Chye M-L, Tan C-T, Chua N-H: Three genes encode 3-hydroxy-3-methylglutaryl-coenzyme A reductase in *Hevea brasiliensis: hmg*1 and *hmg*3 are differentially expressed. Plant Mol Biol 19: 473–484 (1992).
11. Dale S, Arró M, Becerra B, Morrice NG, Boronat A, Hardie DG, Ferrer A: 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 (1995).
12. Fraga BM: Sesquiterpenoids. In: Charlwood BV, Banthorpe DV (eds) Methods in Plant Biochemistry, pp. 145–185. Academic Press, London (1991).
13. Genschik P, Criqui M-C, Parmentier Y, Marbach J, Durr A, Fleck J, Jamet E: Isolation and chaterization of a cDNA encoding a 3-hydroxy-3-methylglutaryl coenzyme A reductase from *Nicotiana sylvestris*. Plant Mol Biol 20: 337–341 (1992).
14. Goldstein JL, Brown MS: Regulation of the mevalonate pathway. Nature 343: 425–430 (1990).
15. Kuc J: Phytoalexins from the solanaceae. In: Bailey JA, Mansfield JW (eds) Phytoalexins, pp. 81–105. John Wiley, New York (1982).
16. Maldonado-Mendoza IE, Burnett RJ, Nessler CL: Nucleotide sequence of a cDNA encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase from *Catharanthus roseus*. Plant Physiol 100: 1613–1614 (1992).
17. McGarvey DJ, Croteau R: Terpenoid metabolism. Plant Cell 7: 1015-1026 (1995).
18. Oba K, Kondo K, Doke N, Uritani I: 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 (1985).
19. Park H, Denbow CJ, Cramer CL: Structure and nucleotide sequence of tomato *HMG2* encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase. Plant Mol Biol 20: 327–331 (1992).
20. Rogers S, Wells R, Rechsteiner M: Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science 234: 364–368 (1986).
21. Sambrook J, Fritsch EF, Maniatis T: Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989).
22. Stermer BA, Bianchini GM, Korth KL: Regulation of HMG-CoA reductase activity in plants. J Lipid Res 35: 1133–1140 (1994).
23. Stermer BA, Bostock RM: Involvement of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the regulation of sesquiterpenoid phytoalexin synthesis in potato. Plant Physiol 84: 404–408 (1987).
24. Stermer BA, Edwards LA, Edington BV, Dixon RA: Analysis of elicitor-inducible transcripts encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase in potato. Physiol Mol Plant Path 39: 135–145 (1991).
25. Yang Z, Park H, Lacy GH, Cramer CL: Differential activation of potato 3-hydroxy-3-methylglutaryl coenzyme A reductase genes by wounding and pathogen challenge. Plant Cell 3: 397–405 (1991).