Protein family review

The 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductases

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Summary

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase catalyzes the conversion of HMG-CoA to mevalonate, a four-electron oxidoreduction that is the rate-limiting step in the synthesis of cholesterol and other isoprenoids. The enzyme is found in eukaryotes and prokaryotes; and phylogenetic analysis has revealed two classes of HMG-CoA reductase, the Class I enzymes of eukaryotes and some archaea and the Class II enzymes of eubacteria and certain other archaea. Three-dimensional structures of the catalytic domain of HMG-CoA reductases from humans and from the bacterium *Pseudomonas mevalonii*, in conjunction with site-directed mutagenesis studies, have revealed details of the mechanism of catalysis. The reaction catalyzed by human HMG-CoA reductase is a target for anti-hypercholesterolemic drugs (statins), which are intended to lower cholesterol levels in serum. Eukaryotic forms of the enzyme are anchored to the endoplasmic reticulum, whereas the prokaryotic enzymes are soluble. Probably because of its critical role in cellular cholesterol homeostasis, mammalian HMG-CoA reductase is extensively regulated at the transcriptional, translational, and post-translational levels.

Gene organization and evolutionary history

The human *hmgr* gene that encodes the single human HMG-CoA reductase is located on chromosome 5, map location 5q13.3-5q14, and is over 24.8 kilobases (kb) long. The 20 exons of the 4,475-nucleotide transcript, which range in size from 27 to 1,813 base-pairs, encode the membrane-anchor domain (exons 2-10), a flexible linker region (exons 10 and 11), and the catalytic domain (exons 11-20) of the resulting 888-residue polypeptide (Figure 1).

Genome sequencing has identified *hmgr* genes in organisms from all three domains of life, and over 150 HMGR sequences are recorded in public databases. Higher animals, archaea, and eubacteria have only a single *hmgr* gene, although the lobster has both a soluble and a membrane-associated isozyme, both of which are encoded by a single gene). By contrast, plants, which use both HMGR-dependent and HMGR-independent pathways to synthesize isoprenoids, have multiple HMGR isozymes that appear to have arisen by gene duplication and subsequent sequence divergence [1]. Yeast has two HMGR isozymes derived from two different genes (*hmgr-1* and *hmgr-2*). Comparison of amino-acid sequences and phylogenetic analysis reveals two classes of HMGR, the Class I enzymes of eukaryotes and some archaea and the Class II enzymes of certain eubacteria and archaea, suggesting evolutionary divergence between the two classes (Figure 2, Table 1) [2,3]. The catalytic domain is highly conserved in eukaryotes, but the membrane-anchor domain (consisting of between two and eight membrane-spanning helices) is poorly conserved, and the HMGRs of archaea and of certain eubacteria lack a membrane-anchor domain.

Characteristic structural features

The HMGRs of different organisms are multimers of a species-specific number of identical monomers. High-resolution

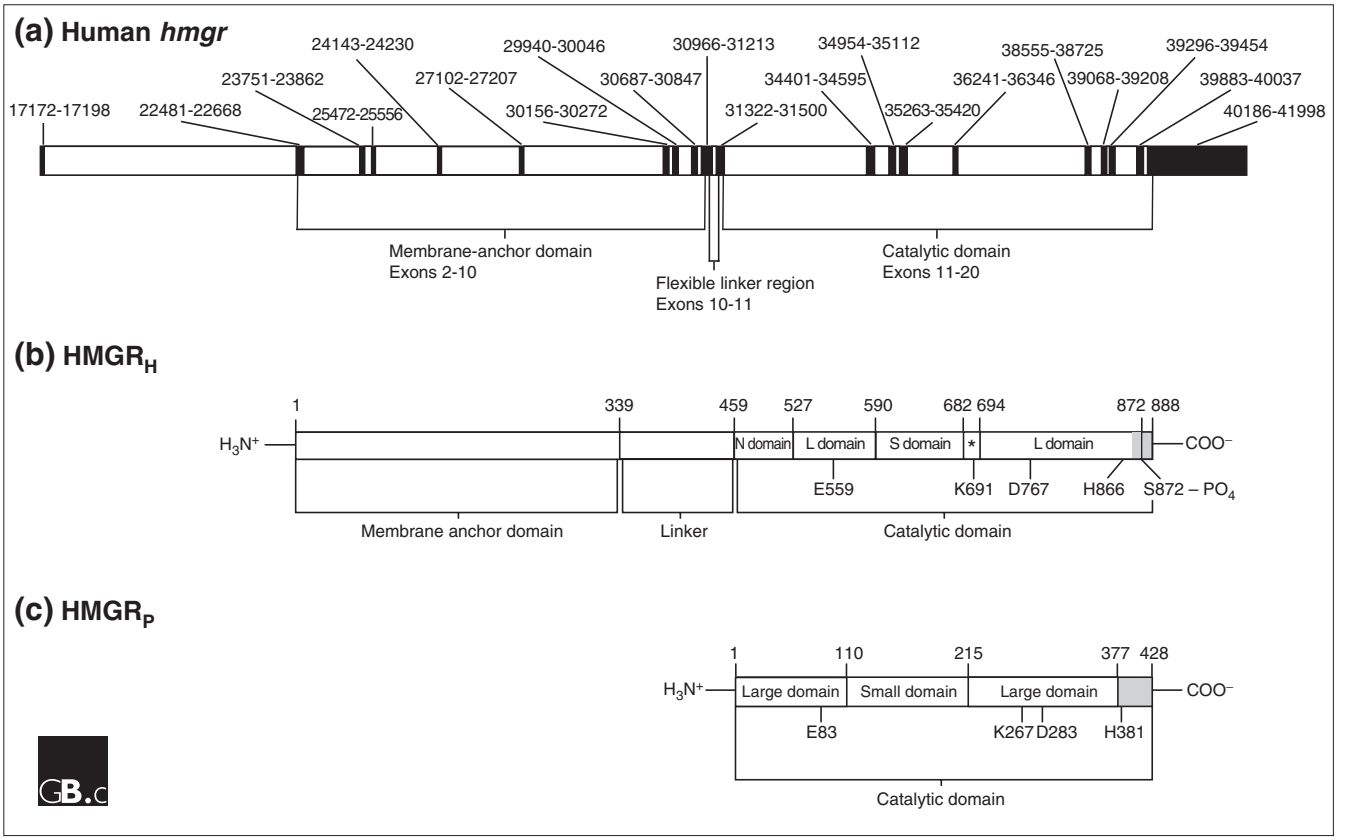

Figure 1

Schematic representation of the human *hmgr* gene and the human $HMGR_H$ and *P. mevalonii* $HMGR_P$ proteins. **(a)** The exon-intron structure of the human *hmgr* gene, which extends from position 74717172 to position 74741998 of the human genome; positions refer to the Ensembl Transcript ID for the human *hmgr* gene (ENST00000287936 [22]). The numbers indicate the start and end of each exon and intron and refer to the position in the human genome sequence, omitting the first three digits (747); exons are indicated as filled boxes. Exon 1 is an untranslated region (UTR), as are the last 1,758 nucleotides of exon 20. The exons encoding the membrane-anchor domain, a flexible linker region, and the catalytic domain are indicated below the gene structure. **(b)** Human HMGR protein ($HMGR_{H)}$ is comprised of three domains: the membrane-anchor domain, a linker domain, and a catalytic domain; within the catalytic domain subdomains have been defined. The N domain connects the L domain to the linker domain; the L domain contains an HMG-CoA binding region; and the S domain functions to bind NADP(H). The *cis*-loop (indicated by an asterisk), a region present only in $HMGR_H$ but not $HMGR_P$, connects the HMG-CoA-binding region with the NADPH-binding region. **(c)** The $HMGR_P$ protein does not contain the membrane-anchor domain or the linker domain but has a catalytic domain containing a large domain with an HMG-CoA binding region, and a small, NAD(H)-binding domain. The active site of HMG-CoA reductase is present at the homodimer interface between one monomer that binds the nicotinamide dinucleotide and a second monomer that binds HMG-CoA. The numbers underneath the diagrams in (b,c) denote amino acids (in the single-letter amino-acid code) that are implicated in catalysis; S872 of $HMGR_H$ is reversibly phosphorylated. At the extreme carboxyl terminus of each enzyme is a flap domain (approximately 50 amino acids in $HMGR_P$ and 25-30 amino acids in $HMGR_H$) that closes over the active site during catalysis; the flap domain is indicated by shading in (b,c).

crystal structures have been solved for the Class I human enzyme ($HMGR_H$) [4,5] and for the Class II enzyme of *Pseudomonas mevalonii* ($HMGR_P$) [6,7], including protein forms bound to either the HMG-CoA substrate or the coenzyme (NADH or NADPH) or both, or bound to statin drugs, which are potent competitive inhibitors of HMGR activity and thus lower cholesterol levels in the blood [8,9]. As reviewed in detail by Istvan [10], structural comparisons reveal both similarities and significant differences between the two classes of enzyme. The human HMGR has three major domains (catalytic, linker and anchor), whereas the *P. mevalonii* HMGR has only the catalytic domain (Figure 1). Both $HMGR_H$ and $HMGR_P$ have a dimeric active site with residues contributed by each monomer, and a non-Rossmann-type coenzyme-binding site (a three-dimensional structural fold that contains a nucleotide-binding motif and is found in many enzymes that use the dinucleotides NADH and NADPH for catalysis). The core regions containing the catalytic domains of the two enzymes have similar folds. Despite differences in amino-acid sequence and overall architecture, functionally similar residues participate in the binding of coenzyme A by the two enzymes, and the position and orientation of four key catalytic residues (glutamate, lysine, aspartate and histidine) is conserved in both classes of HMGR.

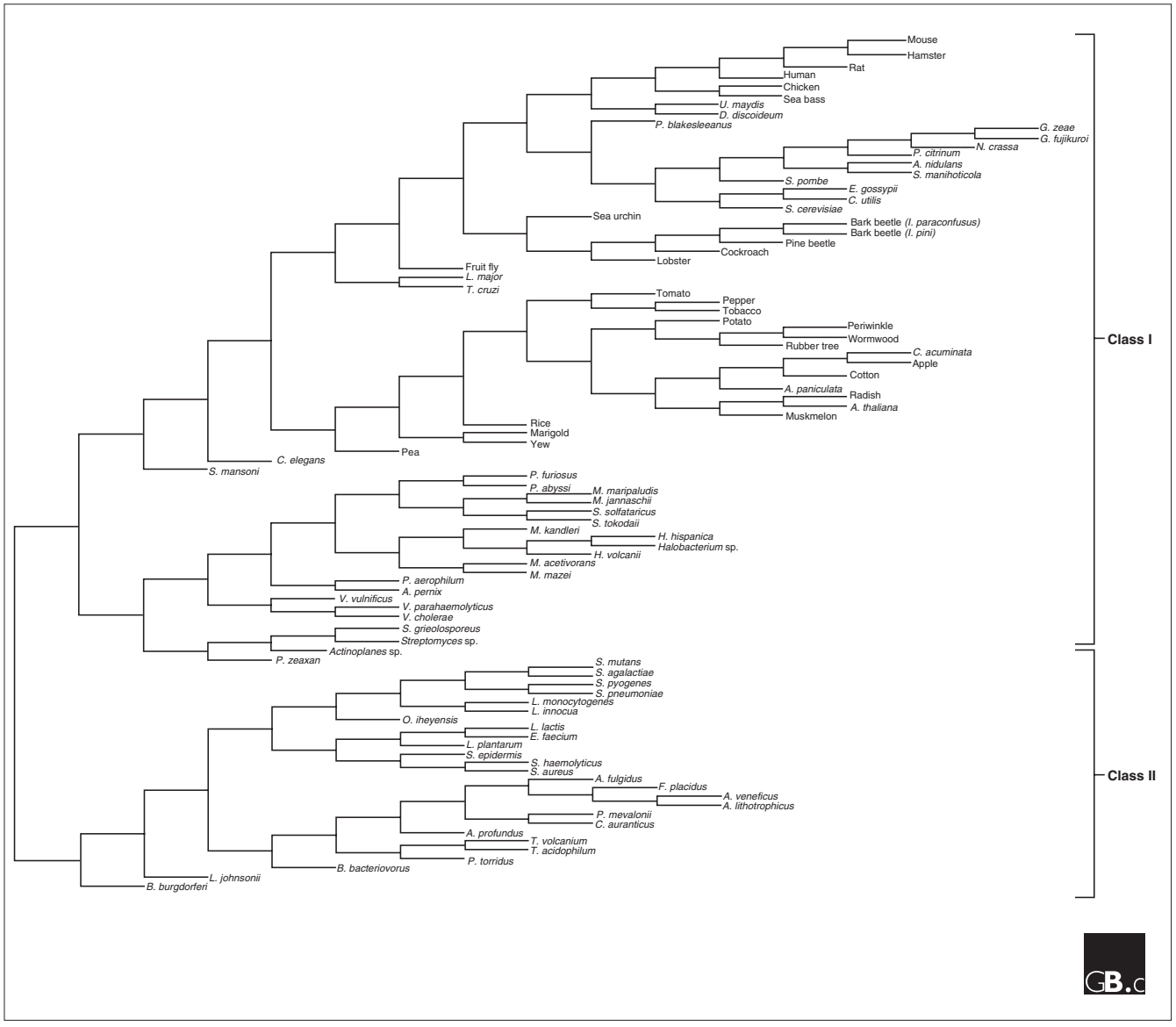

Figure 2
A phylogenetic tree of HMGRs. The tree includes 98 selected organisms that have *hmgr* genes; for plants, which have multiple isoforms, only isoform 1 of each species is included in the tree. Roman numerals indicate the division of the family into two classes [2,3]. Phylogenetic analysis was performed using aligned amino-acid sequences of HMGR catalytic domains; membrane anchor domains were excluded from analysis. Amino-acid sequence alignments were generated using ClustalW [23] and the phylogenetic tree constructed with TreeTop [24] using the cluster algorithm with PHYLIP tree-type output. Full species names and GenBank accession numbers of the sequences used are provided in Table 1.

Unlike the central cores, the amino- and carboxy-terminal regions of the catalytic domains show little similarity between the human and *P. mevalonii* HMGR structures. The active site of HMG-CoA reductase is at the interface of the homodimer between one monomer that binds the nicotinamide dinucleotide and a second monomer that binds the HMG-CoA. In human HMGR, the catalytic lysine is found on the monomer that binds the HMG-CoA and comes from the so-called *cis*-loop (a section that connects the HMG-CoA-binding region with the NADPH-binding region). In contrast, the *P. mevalonii* HMGR lacks the *cis*-loop and the catalytic lysine is contributed by the monomer that binds the nicotinamide dinucleotide. $HMGR_P$ crystallizes as a trimer of dimers (which are composed of identical subunits), but $HMGR_H$ crystallizes as a tetramer (of identical units). $HMGR_P$ uses NADH as a coenzyme, whereas $HMGR_H$ uses NADPH, but mutation to alanine of the aspartyl residue of $HMGR_P$ that normally blocks binding of NADPH can allow NADPH to serve - albeit poorly - as the coenzyme for $HMGR_P$. A 180° difference in the orientation of the nicotinamide ring of the coenzyme suggests that

Table 1

Details of the sequences used for the phylogenetic tree in Figure 2

Organism name*	Kingdom	Accession number
Mus musculus (mouse)	Eukaryote	XM_127496
Mesocricetus auratus (hamster)	Eukaryote	X00494
Rattus norvegicus (rat)	Eukaryote	BC064654
Homo sapiens (human)	Eukaryote	NM_000859
Gallus gallus (chicken)	Eukaryote	AB109635
Xenopus laevis (frog)	Eukaryote	M29258
Drosophila melanogaster (fruit fly)	Eukaryote	NM_206548
Homarus americanus (lobster)	Eukaryote	AY292877
Blatella germanica (cockroach)	Eukaryote	X70034
Dendroctonus jeffreyi (Jeffrey pine beetle)	Eukaryote	AF159136
Ips pini (bark beetle)	Eukaryote	AF304440
Ips paraconfusus (bark beetle)	Eukaryote	AF071750
Raphanus sativus (radish)	Eukaryote	X68651
Arabidopsis thaliana (thale-cress)	Eukaryote	NM_106299
Oryza sativa (rice)	Eukaryote	AF110382
Lycopersicon esculentum (tomato)	Eukaryote	AAL16927
Nicotinia tabacum (tobacco)	Eukaryote	AF004232
Cucumis melo (muskmelon)	Eukaryote	AB021862
Hevea brasiliensis (rubber tree)	Eukaryote	X54659
Pisum sativum (pea)	Eukaryote	AF303583
Solanum tuberosum (potato)	Eukaryote	L01400
Tagetes erecta (African marigold)	Eukaryote	AF034760
Catharanthus roseus (Madagascar periwinkle)	Eukaryote	M96068
Artemisia annua (wormwood)	Eukaryote	AF142473
Gossypium hirsutum (cotton)	Eukaryote	AF038046
Taxus x media (yew)	Eukaryote	AY277740
Andrographis paniculata (Indian herb)	Eukaryote	AY254389
Malus x domestica (apple)	Eukaryote	AY043490
Capsicum annuum (pepper)	Eukaryote	AF110383
Camptotheca acuminata	Eukaryote	U72145
Saccharomyces cerevisiae (baker's yeast)	Eukaryote	M22002
Schizosaccharomyces pombe (fission yeast)	Eukaryote	CAB57937
Candida utilis	Eukaryote	AB012603
Trypanosoma cruzi (trypanosome)	Eukaryote	L78791
Schistosoma mansoni	Eukaryote	M27294
Leishmania major (trypanosome)	Eukaryote	AF155593
Dictyostelium discoideum	Eukaryote	L19349
Caenorhabditis elegans	Eukaryote	NM_066225
Strongylocentrotus purpuratus (sea urchin)	Eukaryote	NM_214559
Dicentrarchus labrax (European sea bass)	Eukaryote	AY424801
Penicillium citrinum	Eukaryote	AB072893
Ustilago maydis	Eukaryote	XM_400629
Eremothecium gossypii	Eukaryote	NM_210364
Gibberella zeae	Eukaryote	XM_389373
Gibberella fujikuroi	Eukaryote	X94307
Sphaceloma manihoticola	Eukaryote	X94308
Aspergillus nidulans	Eukaryote	EAA60025
Neurospora crassa	Eukaryote	XM_324891
Phycomyces blakesleeanus	Eukaryote	X58371
Archaeoglobus fulgidus	Archaea	NC_000917
Sulfolobus solfataricus	Archaea	U95360
Oceanobacillus iheyensis	Archaea	NC_004193
Thermoplasma volcanium	Archaea	BAB60335
Halobacterium sp	Archaea	AAG20075
Methanosarcina mazei	Archaea	AAM30031
Haloarcula hispanica	Archaea	AF123438
Thermoplasma acidophilum	Archaea	CAC11548
Picrophilus torridus	Archaea	AE017261
Archaeoglobus veneficus	Archaea	AJ299204
Ferroglobus placidus	Archaea	AJ299206
Archaeoglobus profundus	Archaea	AJ299205
Archaeoglobus lithotrophicus	Archaea	AJ299203
Haloferax volcanii	Archaea	M83531
Pyrococcus furiosus	Archaea	AAL81972
Pyrococcus abyssi	Archaea	AJ248284
Methanococcus maripaludis	Archaea	CAF29643
Methanocaldococcus jannaschii	Archaea	AAB98699
Methanosarcina acetivorans	Archaea	AAM06446.
Methanopyrus kandleri	Archaea	AAM01570
Sulfolobus tokodaii	Archaea	AP000986
Aeropyrum pernix	Archaea	AP000062
Methanothermobacter thermautotrophicus	Archaea	AAB85068
Pyrobaculum aerophilum	Archaea	AAL64009
Bdellovibrio bacteriovorus	Eubacteria	BX842650
Lactobacillus plantarum	Eubacteria	AL935253
Streptococcus agalactiae	Eubacteria	CAD47046
Lactococcus lactis	Eubacteria	AE006387
Vibrio cholerae	Eubacteria	AAF96622
Vibrio vulnificus	Eubacteria	AAO07090.
Vibrio parahaemolyticus	Eubacteria	BAC62311
Enterococcus faecalis	Eubacteria	AAO81155
Lactobacillus johnsonii	Eubacteria	AE017204
Chloroflexus aurantiacus	Eubacteria	AJ299212
Enterococcus faecium	Eubacteria	AF290094
Listeria monocytogenes	Eubacteria	AE017324
Listeria innocua	Eubacteria	CAC96053
Streptococcus pneumoniae	Eubacteria	AF290098
Staphylococcus epidermidis	Eubacteria	AF290090
Staphylococcus haemolyticus	Eubacteria	AF290088
Staphylococcus aureus	Eubacteria	AF290086
Streptomyces griseolosporeus	Eubacteria	AB037907
Streptomyces sp.	Eubacteria	AB015627
Streptococcus pyogenes	Eubacteria	AF290096
Streptococcus mutans	Eubacteria	AAN58647
Paracoccus zeaxanthinifaciens	Eubacteria	AJ431696
Pseudomonas mevalonii	Eubacteria	M24015
Borrelia burgdorferi	Eubacteria	AE001169.
Actinoplanes sp.	Eubacteria	AB113568

*Common names are indicated in parentheses Accession numbers for each sequence are available from sequence databases accessible through the National Center for Biotechnology Information [25].

that the stereospecificity of the $HMGR_H$ hydrogen transfer is opposite to that of $HMGR_P$.

Comparisons between the $HMGR_P$ and $HMGR_H$ structures reveal an overall similarity in how they bind statins, which inhibit activity by blocking access of HMG-CoA to the active site. There is a considerable difference in specific interactions with inhibitor between the two enzymes, however [8,9], accounting for the almost 10^4-fold higher K_i values for inhibition of $HMGR_P$ by statin relative to the inhibition of $HMGR_H$ (K_i is the equilibrium constant for an inhibitor binding to an enzyme). There are significant differences in the regions of the two proteins that bind statins. In both enzymes the portion of the statin that resembles HMG (see

Figure 3) occupies the HMG portion of the HMG-CoA-binding pocket, and the non-polar region partially occupies a portion of the coenzyme-A-binding site. For $HMGR_P$, this impairs closure over the active site of the 'tail' domain that contains the catalytic histidine.

Localization and function

HMGRs of eukaryotes are localized to the endoplasmic reticulum (ER), and are directed there by a short portion of the amino-terminal domain (prokaryotic HMGRs are soluble and cytoplasmic). In humans, the reaction catalyzed by HMGR is the rate-limiting step in the synthesis of cholesterol, which maintains membrane fluidity and serves as a precursor for steroid hormones. In plants, a cytosolic HMG-CoA reductase participates in the synthesis of sterols, which are involved in plant development, certain sesquiterpenes, which are important in plant defense mechanisms against herbivores, and ubiquinone, which is critical for cellular protein turnover. In plastids, however, these compounds are synthesized via a pathway that does not involve mevalonate or HMGR [1]. Various plant HMGR isozymes function in fruit ripening and in the response to environmental challenges such as attack by pathogens. In yeast, either of the two ER-anchored HMGR isozymes can provide the mevalonate needed for growth.

Enzyme mechanism

The reaction catalyzed by HMGR is:

(*S*)-HMG-CoA + 2 NADPH + 2 H^+ → (*R*)-mevalonate + 2 $NADP^+$ + CoA-SH.

with the (*S*)-HMG-CoA and (*R*)-mevalonate designations referring to the stereochemistry of the substrate and product (enzymatic reactions are stereospecific and the (*R*)-HMG-CoA isomer is not a substrate for HMGR). This three-stage reaction involves two reductive stages and the formation of enzyme-bound mevaldyl-CoA and mevaldehyde as probable intermediates:

Stage 1: HMG-CoA + NADPH + H^+ → [Mevaldyl-CoA] + $NADP^+$

Stage 2: [Mevaldyl-CoA] → [Mevaldehyde] + CoA-SH

Stage 3: [Mevaldehyde] + NADPH + H^+ → Mevalonate + $NADP^+$

Kinetic analysis of point mutants of $HMGR_P$ and of $HMGR_H$, and inspection of the crystal structures of $HMGR_P$ and $HMGR_H$, has identified an aspartate, a glutamate, a histidine, and a lysine that are likely to be important and have suggested their probable roles in catalysis (Figure 4) [11].

Regulation

A highly regulated enzyme, $HMGR_H$ is subject to transcriptional, translational, and post-translational control [12] that can result in changes of over 200-fold in intracellular levels of the enzyme. The transcription factor sterol regulatory element-binding protein 2 (SREBP-2) participates in regulating levels of $HMGR_H$ mRNA in response to the level of sterols [13]; the regulatory process is as follows. At the ER membrane or the nuclear envelope, SREBP-2 binds to SREBP cleavage activating protein (SCAP) to form a SCAP-SREBP complex that functions as a sterol sensor. The proteins Insig-1 and Insig-2 bind to SCAP when cellular cholesterol levels are high and prevent movement of the SCAP-SREBP complex from the ER to the Golgi. In cells depleted of cholesterol, Insig-1 and Insig-2 allow activation of the SCAP-SREBP complex and its translocation to the Golgi, where SREBP is cleaved at two sites. Cleavage releases the amino-terminal basic helix-loop-helix (bHLH) domain, which enters the nucleus, where it functions as a transcription factor that recognizes non-palindromic decanucleotide sequences of DNA called sterol-regulatory elements (SREs). Binding of the bHLH domain of SREBP to an SRE promotes transcription of the *hmgr* gene.

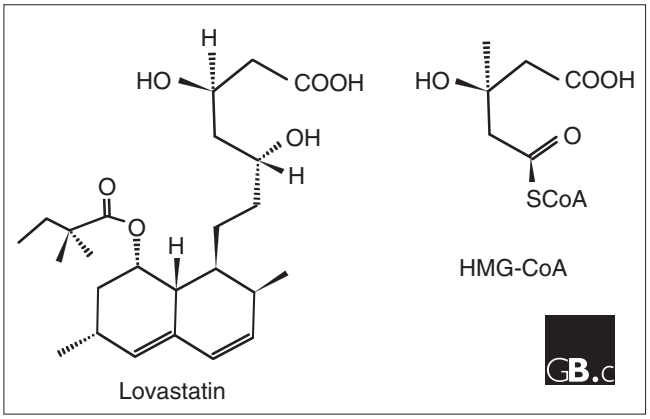

Figure 3
Structures of lovastatin, a statin drug that competitively inhibits HMGR, and of HMG-CoA. It can be seen that the portion of the drug shown here at the top resembles the HMG portion of HMG-CoA.

Degradation of $HMGR_H$ involves its transmembrane regions [14]: removal of two or more transmembrane regions abolishes the acceleration of $HMGR_H$ degradation that occurs under certain conditions [12,15]: degradation is induced by a non-sterol, mevalonate-derived metabolite alone or by a sterol plus a mevalonate-derived non-sterol metabolite, possibly farnesyl pyrophosphate or farnesol. Four conserved phenylalanines in the sixth membrane span of the transmembrane region are essential for degradation of $HMGR_H$ [16]. Insig-1 also functions in the degradation of $HMGR_H$ [17]: when cholesterol levels are high, SCAP and $HMGR_H$ compete for binding to Insig-1. If SCAP binds Insig-1, the SCAP-Insig-1 complex is retained in the Golgi, whereas if $HMGR_H$ binds Insig-1, $HMGR_H$ is ubiquinated on lysine 248 and is rapidly degraded through a ubiquitin-proteasome mechanism [18].

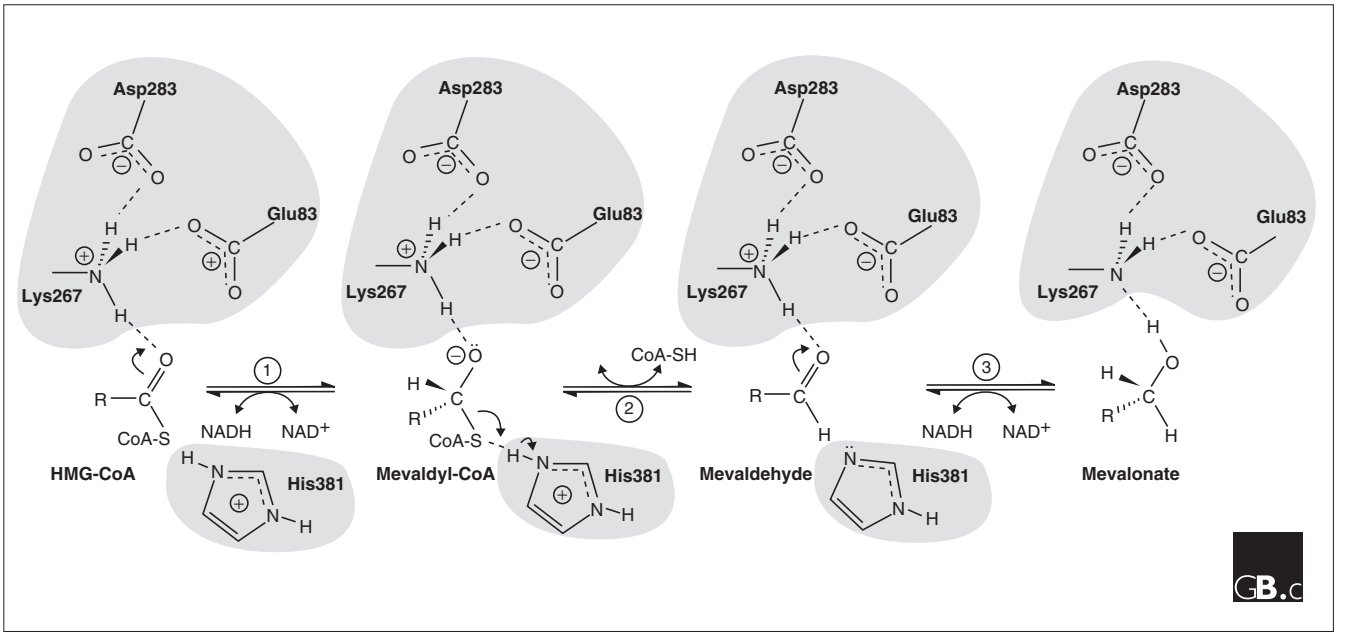

Figure 4
Proposed reaction mechanism for $HMGR_P$ [7,18]. The side groups of the key catalytic residues, Lys267, Asp283, Glu83, and His381, are shown, and the substrate and products are shown with R representing the HMG portion. The reaction follows three stages (see text for details). A basically similar mechanism has been proposed for $HMGR_H$ [4].

The catalytic activity of the HMGRs of higher eukaryotes is attenuated by phosphorylation of a single serine, which in the case of $HMGR_H$ is at position 872 [19]. The location of this serine - six residues from the catalytic histidine, a spacing conserved in all higher eukaryote HMGRs - suggests that the phosphoserine may interfere with the ability of this histidine to protonate the inhibitory $CoAS^-$ thioanion that is released in stage 2 of the reaction mechanism. Alternatively, it may interfere with closure of the flap domain, a carboxy-terminal region that is thought to close over the active site to facilitate catalysis, a step thought to be essential for formation of the active site [7]. Subsequent dephosphorylation restores full catalytic activity. HMGR kinase (also called AMP kinase) phosphorylates HMGR; the primary phosphatase *in vivo* is thought to be protein phosphatase 2A (PP2A), but both phosphatases 2A and 2B can catalyze dephosphorylation of vertebrate HMGR *in vitro* [20]. $HMGR_H$ activity therefore responds to hormonal control through AMP levels and PP2A activity. Phosphorylation of serine 577 of *A. thaliana* HMGR isozyme 1 by a plant HMGR kinase that does not require 5'-AMP attenuates activity, and restoration of HMGR activity follows from dephosphorylation [21]. As many plant genes encode a putative target serine surrounded by an apparent AMP kinase recognition motif, it is probable that most plant HMGRs are regulated by phosphorylation. Yeast HMGR activity is, however, unaffected by AMP kinase. The phosphorylation state of HMGR does not affect the rate at which the protein is degraded.

Frontiers

Several basic unresolved questions concern how phosphorylation controls the catalytic activity of HMGRs; solution of the structures of phosphorylated HMGRs should reveal more of the precise mechanism. The protein kinases, phosphatases, and signal-transduction pathways that participate in short-term regulation of HMGR activity are yet to be elucidated. Finally, the physiological roles served by the multiple ways in which HMGR is regulated require clarification. On the medical side, continuing intense competition between drug companies for a share of the lucrative worldwide market for hypercholesterolemic agents should result in new statin drugs with modified pharmacodynamic and metabolic properties that not only lower plasma cholesterol levels more effectively but more importantly minimize undesirable side effects.

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The NCBI contains a vast amount of sequence information, including protein and nucleic acid sequences for HMG-CoA reductases and information on the sequencing of genomes of organisms containing HMG-CoA reductase isoforms.