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
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 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).
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 crystallizes as a trimer of dimers (which are composed of identical subunits), but HMGR crystallizes as a tetramer (of identical units). HMGR uses NADH as a coenzyme, whereas HMGR uses NADPH, but mutation to alanine of the aspartyl residue of HMGR that normally blocks binding of NADPH can allow NADPH to serve - albeit poorly - as the coenzyme for HMGR. A 180° difference in the orientation of the nicotinamide ring of the coenzyme suggests that

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**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.
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   | NY292877       |
| Blattella 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        |
| Nicotiana tabacum (tobacco) | Eukaryote   | AF004232        |
| Cucumis melo (muskmelon) | Eukaryote   | AB021862        |
| Hevea brasiliensis (rubber tree) | Eukaryote   | X54659          |
| Psidium satum (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   | AY034940        |
| Capsicum annuum (pepper) | Eukaryote   | AFI110383       |
| Camptotheca acuminata | Eukaryote   | U72145          |
| Saccharomyces cerevisiae (baker’s yeast) | Eukaryote   | CAB57937        |
| Schizosaccharomyces pombe (fission yeast) | Eukaryote   | AB012603        |
| Candido utilis | Eukaryote   | L7891           |
| Trachomonas cruzi (trypanosome) | Eukaryote   | M27294          |
| Schizosaccharomyces mansonii | Eukaryote   | M27294          |
| Leishmania major (trypanosome) | Eukaryote   | AF155593        |
| Dictyostelium discoideum | Eukaryote   | L19349          |
| Caenorhabditis elegans | Eukaryote   | NM_066225       |
| Strongylocentrus purpuratus (sea urchin) | Eukaryote   | NM_214559       |
| Dicentrarchus labrax (European sea bass) | Eukaryote   | AY424801        |
| Penicillium citrinum | Eukaryote   | AB072893        |
| Ustilago maydis | Eukaryote   | XM_400629       |
| Eremothecium rolfsii | Eukaryote   | NM_210364       |
| Gibberellae zeae | Eukaryote   | XM_389373       |
| Gibberella fujikuroi | Eukaryote   | X94307          |
| Sphaceloma manihotica | Eukaryote   | X94308          |
| Aspergillus nidulans | Eukaryote   | EAA60025        |
| Neurospora crassa | Eukaryote   | XM_324891       |
| Phycomyces blakesleeanus | Eukaryote   | X58371          |
| Archaeoglobus fulgidus | Archeae     | NC_000917       |
| Sulfolobus sulfurichorsis | Archeae     | U95360          |
| Oceanobacillus iheyensis | Eubacteria   | NC_004193       |
| Thermoplasmata volcanium | Eubacteria   | BAB60335        |
| Halobacterium sp | Eubacteria   | AAG20075        |
| Methanocarcina mazei | Eubacteria   | AAM30031        |
| Haloarcula hispanica | Eubacteria   | AF123438        |
| Thermoplasmata acidophilum | Eubacteria   | CAC11548        |
| Picrophilus torridus | Eubacteria   | AE017261        |
| Archaeoglobus veneficus | Eubacteria   | AJ299204        |

*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].

Table 1 (continued)

| Organism name* | Kingdom   | Accession number |
|----------------|-----------|------------------|
| Ferruglobus placidus | Archaeae | AJ299206        |
| Archaeoglobus profundus | Archaeae | AJ299205        |
| Archaeoglobus lithotrophicus | Archaeae | AJ299203        |
| Halofex axanarchii | Archaeae | M83531          |
| Pyrococcus furiosus | Archaeae | AAL81972        |
| Pyrococcus abyssi | Archaeae | AJ248284        |
| Methanococcus maripaludis | Archaeae | CAF29643        |
| Methanocaldococcus jannaschii | Archaeae | AA89699        |
| Methanosarcina acetivorans | Archaeae | AAM6446        |
| Methanolobus kandleri | Archaeae | AAM01570        |
| Sulfuromonas tokodai | Archaeae | AP009866        |
| Aeropyrum pernix | Archaeae | AP000622        |
| Methanobacterium thermoautotrophicus | Archaeae | AAB85063 |
| Pyrobaculum aerophilum | Archaeae | AAL64009 |
| Baldovibrio bacteriovorus | Eubacteria | BXY84260 |
| Lactobacillus plantarum | Eubacteria | AL93525 |
| Streptococcus agalactiae | Eubacteria | CAD47046 |
| Lactococcus lactis | Eubacteria | AE06387 |
| Vibrio cholerae | Eubacteria | AAF96622 |
| Vibrio vulniﬁcus | Eubacteria | AAO07090 |
| Vibrio parahaemoleticus | Eubacteria | BAC62311 |
| Enterococcus faecalis | Eubacteria | AAO81155 |
| Lactobacillus johnsonii | Eubacteria | AE017204 |
| Chloroflexus aurantius | Eubacteria | AJ299322 |
| Enterococcus faecium | Eubacteria | AF290094 |
| Listeria monocytogenes | Eubacteria | AE017324 |
| Listeria innocua | Eubacteria | CAC6503 |
| Streptococcus pneumoniae | Eubacteria | AF290098 |
| Staphylococcus epidermidis | Eubacteria | AF290090 |
| Staphylococcus haemoleticus | Eubacteria | AF290088 |
| Staphylococcus aureus | Eubacteria | AF290086 |
| Streptomyces griseolopes | Eubacteria | AB037907 |
| Streptomyces sp | Eubacteria | AB015627 |
| Streptococcus pyogenes | Eubacteria | AF290096 |
| Streptomyces mutans | Eubacteria | AANS8647 |
| Paracoccus zeaxanthinifaciens | Eubacteria | AF431706 |
| Pseudomonas aeruginosa | Eubacteria | MB2015 |
| Borelia burgdorferi | Eubacteria | AE001169 |
| Actinoplanes sp | Eubacteria | AB113568 |

that the stereospecificity of the HMGRH hydrogen transfer is opposite to that of HMGRP.

Comparisons between the HMGRP and HMGRH 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 104-fold higher Ki values for inhibition of HMGRP by statin relative to the inhibition of HMGRH (Ki 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
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 HMGR 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, neither of the two ER-anchored HMGR isozymes can provide the mevalonate needed for growth.

Enzyme mechanism

The reaction catalyzed by HMGR is:

\[(S)-HMG-CoA + 2 \text{NADPH} + 2 H^+ \rightarrow (R)-\text{mevalonate} + 2 \text{NADP}^+ + \text{CoA-SH}.\]

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<sub>p</sub> and of HMGR<sub>H</sub>, and inspection of the crystal structures of HMGR<sub>p</sub> and HMGR<sub>H</sub>, 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<sub>H</sub> 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<sub>H</sub> 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.

Degradation of HMGR<sub>H</sub> involves its transmembrane regions [14]: removal of two or more transmembrane regions abolishes the acceleration of HMGR<sub>H</sub> 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<sub>H</sub> [16]. Insig-1 also functions in the degradation of HMGR<sub>H</sub> [17]: when cholesterol levels are high, SCAP and HMGR<sub>H</sub> compete for binding to Insig-1. If SCAP binds Insig-1, the SCAP-Insig-1 complex is retained in the Golgi, whereas if HMGR<sub>H</sub> binds Insig-1, HMGR<sub>H</sub> is ubiquinated on lysine 248 and is rapidly degraded through a ubiquitin-proteasome mechanism [18].
The catalytic activity of the HMGRs of higher eukaryotes is attenuated by phosphorylation of a single serine, which in the case of HMGRH 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 activity therefore responds to hormonal control through AMP levels and PP2A activity. Phosphorylation of serine 577 of Arabidopsis 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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25. National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/) 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.