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Structural and Functional Conservation between Yeast and Human 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductases, the Rate-Limiting Enzyme of Sterol Biosynthesis

MICHAEL E. BASSON,[1] MARY THORSNESS,[1] JANET FINER-MOORE,[2] ROBERT M. STROUD,[2] AND JASPER RINE[1]*

Department of Biochemistry, University of California, Berkeley, California 94720,[1] and Department of Biochemistry and Biophysics, School of Medicine, University of California, San Francisco, California 94143[2]

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The pathway of sterol biosynthesis is highly conserved in all eucaryotic cells. We demonstrated structural and functional conservation of the rate-limiting enzyme of the mammalian pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), between the yeast *Saccharomyces cerevisiae* and humans. The amino acid sequence of the two yeast HMG-CoA reductase isozymes was deduced from DNA sequence analysis of the *HMG1* and *HMG2* genes. Extensive sequence similarity existed between the region of the mammalian enzyme encoding the active site and the corresponding region of the two yeast isozymes. Moreover, each of the yeast isozymes, like the mammalian enzyme, contained seven potential membrane-spanning domains in the NH_2-terminal region of the protein. Expression of cDNA clones encoding either hamster or human HMG-CoA reductase rescued the viability of *hmg1 hmg2* yeast cells lacking this enzyme. Thus, mammalian HMG-CoA reductase can provide sufficient catalytic function to replace both yeast isozymes in vivo. The availability of yeast cells whose growth depends on human HMG-CoA reductase may provide a microbial screen to identify new drugs that can modulate cholesterol biosynthesis.

Analysis of sterol biosynthesis in the yeast *Saccharomyces cerevisiae* and in mammalian cells (7) led to the biochemical elucidation of this pathway, which produces all isoprene-containing compounds, including sterol, ubiquinone, dolichol, and isopentenylated adenosine in tRNA. The enzymatic steps of this pathway are highly conserved between *S. cerevisiae* and larger eucaryotes. Although the regulation of this pathway in yeast cells remains virtually unexplored, it has been examined extensively in mammalian cells. These studies have revealed that 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is the rate-limiting enzyme of the mammalian pathway.

The rates of synthesis and degradation of mammalian HMG-CoA reductase are controlled by negative feedback regulation. Serum cholesterol in the form of low-density lipoprotein reduces the steady-state level of HMG-CoA reductase mRNA (14, 32) and also decreases the half-life of the protein (11, 18). An unidentified nonsterol product of the pathway provides additional negative feedback regulation (9, 44). The response of HMG-CoA reductase to the diurnal cycle and to several hormones may be mediated by reversible phosphorylation of the enzyme (6, 55) and by allosteric effectors (49).

Mammalian HMG-CoA reductase is an integral membrane glycoprotein of the endoplasmic reticulum (8, 34) and has also been identified in peroxisomes (29). A structural model for the endoplasmic reticulum form of hamster HMG-CoA reductase has been proposed which is based on secondary-structure predictions from the primary sequence and on proteolysis experiments (33). The NH_2-terminal one-third of the protein (amino acids 1 to 339) is membrane bound and contains seven potential membrane-spanning domains and a single N-linked glycosylation site. The COOH-terminal two-thirds of the protein (amino acids 340 to 887) extends into the cytoplasm and contains the active site. The association of HMG-CoA reductase with membrane is not required for its activity, since a soluble COOH-terminal fragment of the enzyme retains catalytic function in vivo (22). However, the membrane-bound domain of HMG-CoA reductase is required for regulation of the half-life of the protein by low-density lipoprotein (22) and for the proliferation of smooth endoplasmic reticulum membrane caused by HMG-CoA reductase overproduction (2, 12, 27).

The mammalian genome contains a single gene encoding HMG-CoA reductase (35, 46). In contrast, yeast cells contain two genes encoding HMG-CoA reductase, designated *HMG1* and *HMG2* (5). Cells containing a mutant allele of *HMG1* or *HMG2* have only a subtle growth defect (4). However, cells containing mutant alleles of both *HMG1* and *HMG2* are inviable (5). Assays of HMG-CoA reductase activity in extracts from *hmg1* and *hmg2* cells indicate that *HMG1* contributes most of the activity found in wild-type cells. The deduced amino acid sequences obtained from partial DNA sequence analysis of *HMG1* and *HMG2* reveal sequence similarity to a region in the COOH-terminal half of hamster HMG-CoA reductase. Analysis of the localization of the HMG1 isozyme by immunoelectron microscopy indicates that, like mammalian HMG-CoA reductase, the yeast enzyme is membrane bound (R. W. Wright, M. E. Basson, L. D'Ari, and J. Rine, J. Cell Biol., in press).

In order to assess the extent of structural similarity between the two yeast HMG-CoA reductase isozymes and between the yeast and mammalian enzymes, we have deduced the complete amino acid sequence of the yeast isozymes from DNA sequence analysis of *HMG1* and *HMG2*. Since the region of mammalian HMG-CoA reductase required for intracellular localization and for sterol-mediated control of enzyme half-life is completely separable from the region required for catalysis, comparison of the yeast and mammalian enzymes could indicate the degree of conserva-

* Corresponding author.

tion of these functions. Furthermore, conservation of catalytic function was examined directly by testing the ability of mammalian HMG-CoA reductase to function in *hmg1 hmg2* yeast cells lacking both yeast isozymes.

MATERIALS AND METHODS

Strains, media, and genetic methods. To construct JRY1597 (α *hmg1::LYS2 hmg2::HIS3 lys2-801 his3Δ200 ura3-52 ade2-101 met*), a rare mating between JRY1159 (**a** *hmg1::LYS2 HMG2 lys2-801 his3Δ200 ura3-52 ade2-101 met*) and JRY1160 (**a** *HMG1 hmg2::HIS3 lys2-801 his3Δ200 ura3-52 ade2-101 met*) was performed with selection for His^+ Lys^+ diploid cells. The resulting diploid was sporulated on sporulation medium containing mevalonolactone, and asci were dissected into tetrads on YM medium containing mevalonolactone. JRY1597 was obtained as a His^+ Lys^+ segregant and required mevalonolactone for growth. Plasmids were introduced into JRY1597 by transformation, with selection for uracil independence. A 333-mg/ml stock solution of DL-mevalonolactone (Sigma Chemical Co.) in water was prepared and used at a concentration of 5 mg/ml. Yeast minimal medium containing 2% glucose (YM) and sporulation medium were prepared as described previously (3). Amino acid supplements were added, when needed, at a concentration of 30 μg/ml. Solid medium contained 2% agar. Standard genetic manipulations were performed as described before (39). Yeast cells were transformed by the method of Hinnen et al. (25), except that spheroplasts were prepared with lyticase, a gift from the Schekman laboratory.

DNA sequencing. For sequence analysis, recombinant plasmids were propagated in *Escherichia coli* DIH101 (constructed by D. Ish-Horowicz; provided by S. Mount), and single-stranded DNA was prepared and sequenced by the dideoxy-terminator method (51). To sequence *HMG1*, two restriction fragments from pJR59 (5) were used. The leftward 2.48-kilobase-pair (kbp) *Eco*RI fragment was inserted into the *Eco*RI site of pEMBL18 (constructed by H. Roiha by replacing the polylinker of pEMBL8 [17] with that of pUC18 [41]) in both orientations, generating pJR301 and pJR302. The rightward 1.71-kbp *Pst*I-*Eco*RI fragment was treated with T4 DNA polymerase to make both ends blunt, and the fragment was then inserted into the *Sma*I site of pEMBL18 in both orientations, generating pJR307 and pJR362. pJR301, pJR302, pJR307, and pJR362 were digested with *Pst*I and *Bam*HI, and nested deletions in the insert fragment were created by exonuclease III and S1 nuclease treatment as described before (24). To determine the sequence between the central *Eco*RI site and the *Pst*I site, the rightward 1.73-kbp *Eco*RI fragment was inserted into the *Eco*RI site of pEMBL18, generating pJR327. Nucleotide sequence was also determined by using oligonucleotide primers complementary to known nucleotide sequences. To sequence *HMG2*, a 4.90-kbp *Eco*RI fragment from pJR322 (5) was inserted into the *Eco*RI site of pEMBL18 in both orientations, generating pJR417 and pJR418. pJR417 and pJR418 were digested with *Xba*I and *Pst*I, and nested deletions in the insert fragment were created as described for *HMG1*. Sequence from two *Hin*dIII sites and from a *Bam*HI site was determined by inserting appropriate restriction fragments into pEMBL18. Nucleotide sequence was also determined by using oligonucleotide primers complementary to known nucleotide sequences.

Plasmid constructions. pJR168 (alias pBM150), obtained from Mark Johnston, contains *ARS1*, *CEN4*, the *GAL1* promoter, and the *URA3* gene as a selectable marker (28). To place the expression of coding sequences under the control of the *GAL1* promoter, the sequences were inserted into the *Bam*HI site located downstream of the promoter. The *HMG1* open reading frame was placed under the control of the *GAL1* promoter as follows. A 1.77-kbp *Sph*I-*Hin*dIII fragment from pJR59, containing the *HMG1* open reading frame from the initiating ATG at the *Sph*I site to an internal *Hin*dIII site, was inserted into the polylinker of pEMBL19 (constructed by H. Roiha by replacing the polylinker of pEMBL9 [17] with that of pUC19 [41]) at the corresponding sites, generating pJR419. The 28-base-pair (bp) *Bam*HI-*Sph*I fragment of pJR419 was replaced with a double-stranded fragment formed by annealing two oligonucleotides, 5′-GATCCGTCGACAAGCATG-3′ and 5′-CTTGTCGACG-3′, generating pJR421. This fragment contains a 5′ overhang at one end complementary to a *Bam*HI site and a 3′ overhang at the other end complementary to an *Sph*I site. The 1.74-kbp *Bam*HI-*Sst*I fragment of pJR421, containing the 5′ half of the open reading frame, was ligated to the 9.55-kbp *Bam*HI-*Sst*I fragment of pJR59, containing the 3′ half of the open reading frame and all vector sequences, generating pJR429. The 0.60-kbp *Bam*HI-*Nru*I fragment of pJR168 was replaced with the 4.12-kbp *Bam*HI-*Nru*I fragment of pJR429 that contains the complete *HMG1* open reading frame, generating pJR435. This procedure places the initiating ATG of *HMG1* 16 bp from the *Bam*HI site downstream of the *GAL1* promoter. A hamster cDNA clone encoding HMG-CoA reductase was placed under the control of the *GAL1* promoter as follows. The 4.76-kbp *Bam*HI fragment of pRed-227 (10) (obtained from M. S. Brown and J. L. Goldstein) was inserted into the *Bam*HI site of pJR168 so that the ATG initiating the open reading frame of the cDNA clone was 24 bp from the *Bam*HI site downstream of the *GAL1* promoter. This plasmid was designated pJR441. A human cDNA clone encoding HMG-CoA reductase was placed under control of the *GAL1* promoter as follows. The 4.3-kbp *Bam*HI fragment of pHred-102 (36) (provided by K. Luskey) was inserted into the *Bam*HI site of pJR168 so that the ATG initiating the open reading frame of the cDNA clone was 24 bp from the *Bam*HI site downstream of the *GAL1* promoter. This plasmid was designated pJR511. All standard recombinant DNA methods were performed by procedures found in Maniatis et al. (37). *Escherichia coli* DH1 (23) was used for the propagation of plasmids unless otherwise noted.

RESULTS

DNA sequence of *HMG1* and *HMG2*. The strategy for determining the nucleotide sequence of *HMG1* and *HMG2* is shown in Fig. 1. A single continuous open reading frame of 1,054 amino acids was identified in the *HMG1* sequence, encoding a protein with a calculated molecular mass of 115,639 daltons. Likewise, a single continuous open reading frame of 1,045 amino acids was identified in the *HMG2* sequence, encoding a protein with a calculated molecular mass of 115,705 daltons. The nucleotide and deduced amino acid sequences of *HMG1* and *HMG2* are shown in Fig. 2. The NH_2-terminus of the HMG1 protein is not definitively assigned, since there are two in-frame methionine codons at positions 1 and 11 of the amino acid sequence Since transcription of *HMG1* initiates far upstream of the first methionine codon and since *lacZ* gene fusions in which the *HMG1* promoter is fused to *lacZ* at either methionine codon are expressed at similar levels (M. Thorsness and J. Rine, unpublished results), translation of *HMG1* probably begins

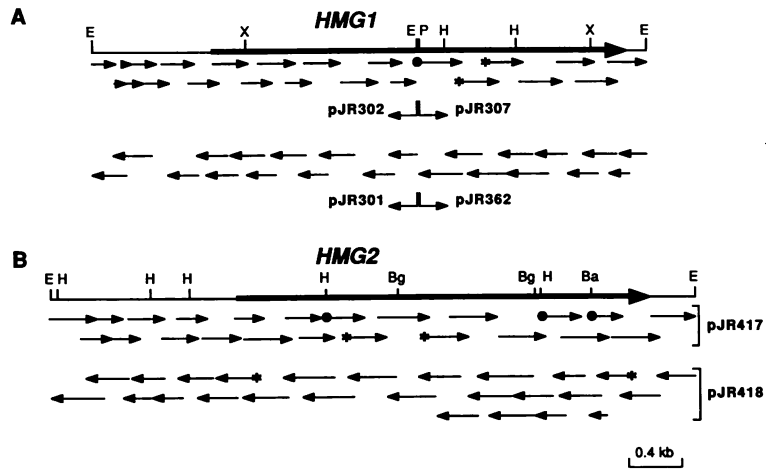


FIG. 1. Sequencing strategy for *HMG1* and *HMG2*. The position and direction of the open reading frames encoding *HMG1* and *HMG2* are indicated by a thick arrow drawn on the appropriate restriction map. (A) To sequence *HMG1*, nested deletions were generated as described in the Materials and Methods section. The extent of nucleotide sequence determined from each deletion analyzed is represented by an arrow. The extent of nucleotide sequence determined from pJR327 is represented by an arrow drawn with a solid circle. The extent of nucleotide sequence determined by using oligonucleotide primers complementary to known nucleotide sequence is represented by an arrow drawn with an asterisk. (B) To sequence *HMG2*, nested deletions in the insert fragment were created as described for *HMG1*. The extent of nucleotide sequence determined from each deletion analyzed is represented by an arrow. The extent of nucleotide sequence determined from two *Hin*dIII sites and from a *Bam*HI site is represented by arrows drawn with a solid circle. The extent of nucleotide sequence determined by using oligonucleotide primers complementary to known nucleotide sequence is represented by arrows drawn with an asterisk. Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; X, *Xba*I.

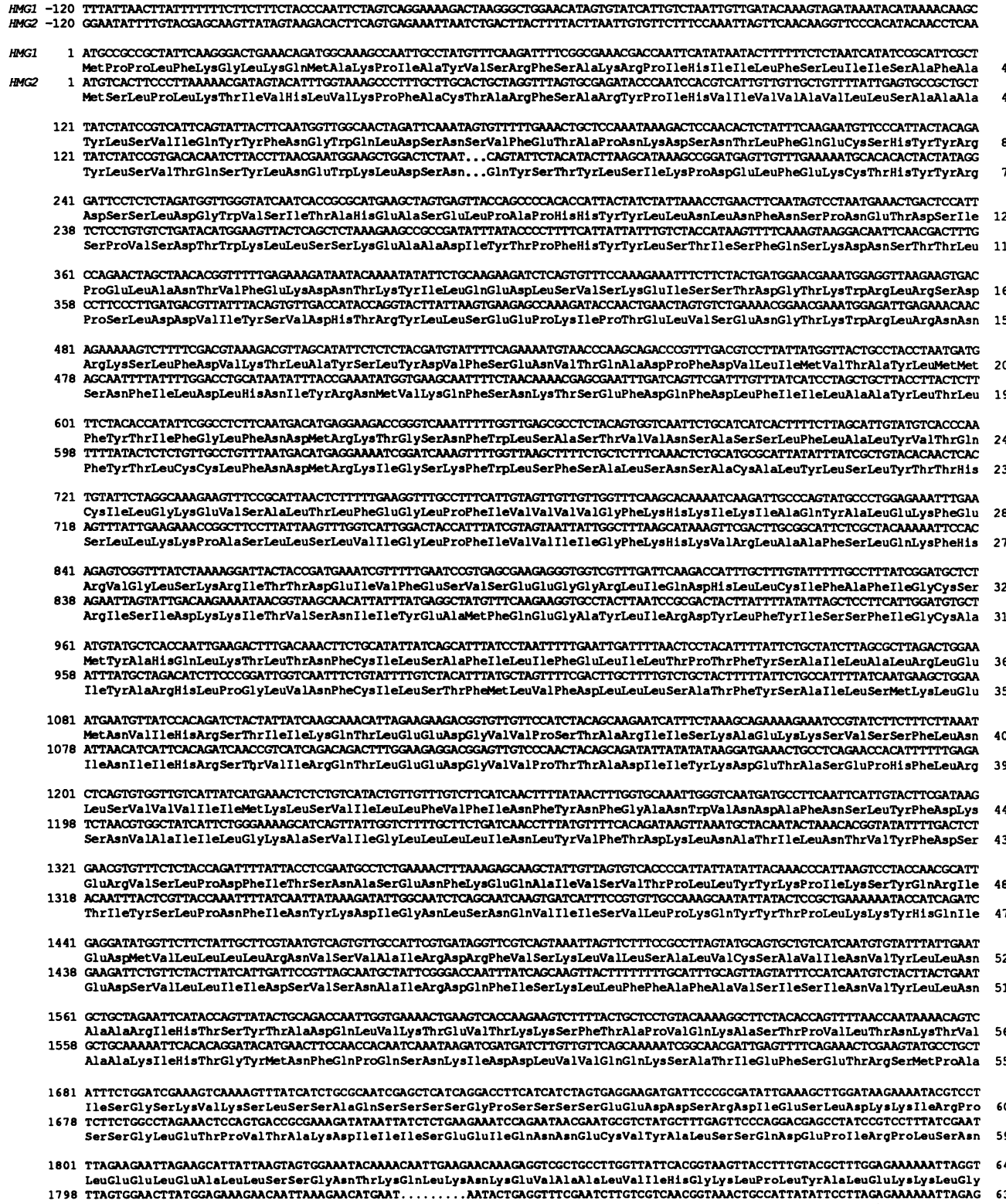
at the first methionine codon. A comparison of the amino acid sequences of the HMG1 and HMG2 proteins with each other and with the COOH-terminal region of human HMG-CoA reductase (36) is shown in Fig. 3. The sequences of the three proteins have been aligned to maximize their sequence similarity.

Model for the secondary structure of the HMG1 protein. To examine the structural similarities of the HMG1 protein with mammalian HMG-CoA reductase, we constructed a model for the secondary structure of the HMG1 protein. The methods and parameters used are outlined in the legend to Fig. 4. The secondary structures of the human and hamster proteins are predicted to be virtually identical, reflecting their high degree of sequence similarity: only 32 nonconservative amino acid replacements exist between the two proteins (10, 36). In contrast, there were many amino acid differences between the yeast and mammalian enzymes. The predicted structure of the NH_2-terminal half (amino acids 1 to 529) of the HMG1 protein is presented in Fig. 4A, and the predicted structure of the COOH-terminal half (amino acids 530 to 1054) is presented in Fig. 4B.

Comparison of hydrophobicity plots of the HMG1 and hamster HMG-CoA reductase proteins (Fig. 5A and B) showed that the NH_2-terminal region of both proteins was in general much more hydrophobic than the COOH-terminal region. Both NH_2-terminal regions exhibited the alternating hydrophobic and hydrophilic regions characteristic of membrane-bound domains containing several transmembrane helices. Moreover, both NH_2-terminal regions met the criteria for the prediction of membrane-bound domains suggested by Eisenberg et al. (19): both had at least one very hydrophobic stretch of 21 amino acids, which would highly favor a membrane environment, and both also contained several other moderately hydrophobic stretches whose hydrophilic groups would cluster on one side of an α-helix.

Eight potential membrane-spanning helices were identified in the hydrophobicity plot of the putative membrane-bound domain of the HMG1 isozyme. As shown in Table 1, these potential helices were evaluated in terms of overall hydrophobicity, amphipathicity, membrane propensity, and length (30, 45). We applied a predictive algorithm based on the length and membrane propensity of each of the putative membrane-spanning domains, as suggested by Rao and Argos (45). This algorithm has been shown to be fairly effective in distinguishing membrane-spanning helices from hydrophobic regions in soluble proteins (45), and it distinguished the seven long hydrophobic stretches in the membrane-bound domain from the predicted hydrophobic β-barrels of hamster HMG-CoA reductase. However, a very hydrophobic 20-residue stretch at the COOH-termini of both hamster HMG-CoA reductase and the HMG1 isozyme was strongly predicted to be a membrane-spanning region by this algorithm and all other criteria we considered, even though this stretch is part of a domain which appears to be soluble after proteolysis (33).

The six most hydrophobic of the eight potential membrane-spanning helices of the HMG1 isozyme, the regions labeled 1, 2, 4, 6, 7, and 8 in Fig. 4A, were strongly predicted to span the membrane. Regions 3 and 5, in contrast, had overall hydrophobicities at the lower end of the range observed for membrane-spanning helices, and both had large hydrophobic moments more characteristic of helices in globular proteins (Table 1) (19). Region three comprised the 29 amino acids between predicted membrane-spanning regions 2 and 4 and contained several moderately hydrophilic residues, such as serine, and two charged residues. This region was rather short for forming a membrane-spanning helix and two spacer regions and was therefore tentatively represented as a spacer region between helices 2 and 4. The COOH-terminal half of region 5 was very hydrophobic, but the NH_2-terminal half contained five charged residues which would lie on one side of the helix. It would therefore be rejected as a membrane-spanning helix by the rules of Rao and Argos (45). However, a clustering of charged residues on the cytoplasmic side of membrane-spanning helices has



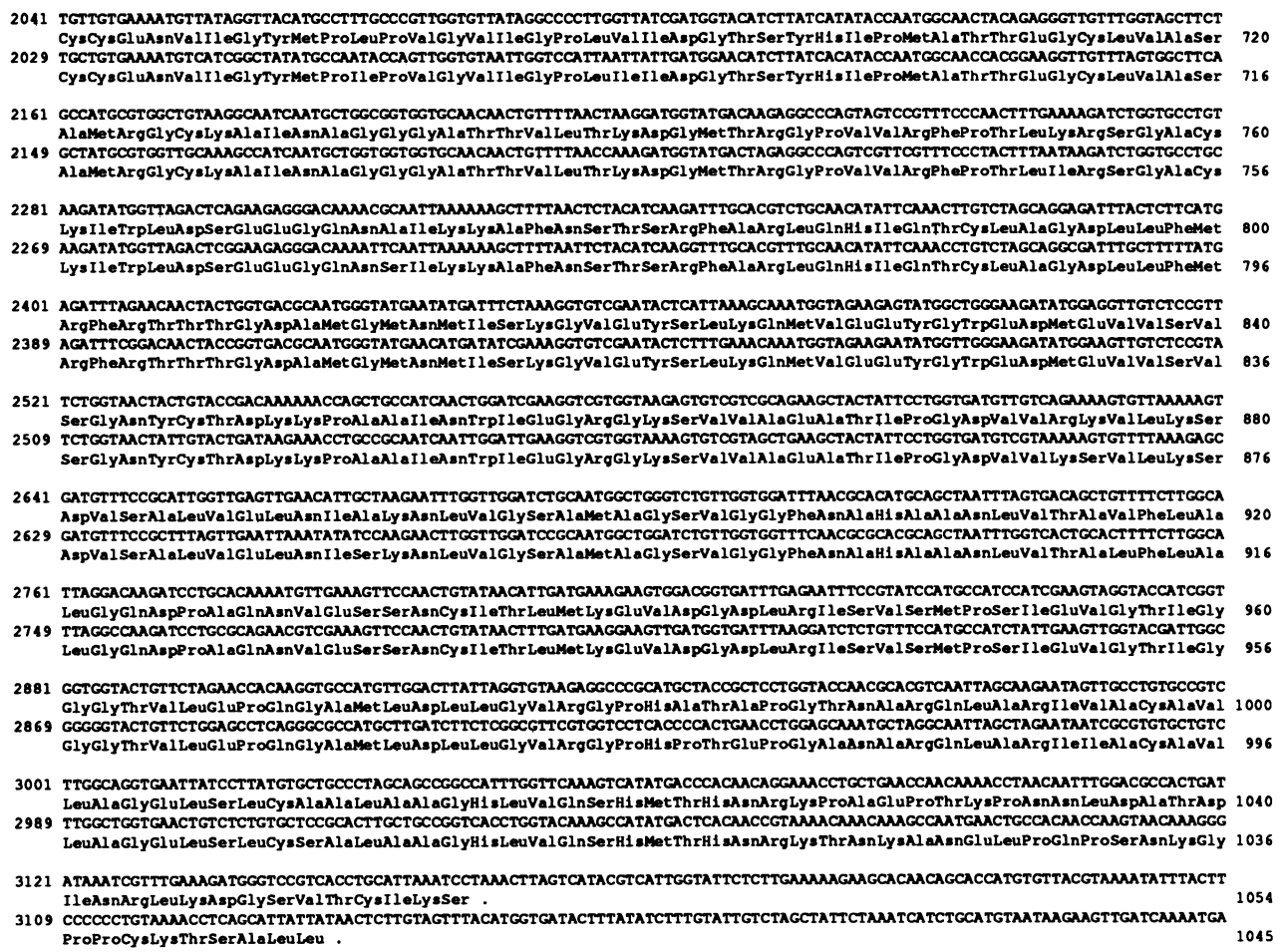


FIG. 2. Nucleotide and deduced amino acid sequence of *HMG1* and *HMG2*. The ATG that initiates each open reading frame is numbered +1. The two sequences have been aligned to maximize similarity.

been observed in the reaction center complex from the purple photosynthetic bacterium *Rhodopseudomonas viridis* (38). We have therefore tentatively represented region 5 as a membrane-spanning region.

The hydrophobicity plot of the HMG2 protein (Fig. 5C) was very similar to that of the HMG1 protein. Moreover, the predicted secondary structure of the NH_2-terminal half of the HMG2 protein contained seven membrane-spanning domains arranged in nearly identical positions as they are in the HMG1 protein and with similar characteristics (Table 1). Inspection of the sequence similarity between the NH_2-terminal halves of the HMG1 and HMG2 proteins revealed that the potential membrane-spanning domains were in general more conserved than the spacer regions between them (Fig. 6). However, individual spacer regions can differ markedly in the extent of their conservation. For example, the long spacer region between membrane-spanning domains 1 and 2 was weakly conserved, whereas the spacer region between membrane-spanning domains 5 and 6 was highly conserved (Table 2).

If it is assumed that the catalytic domain of yeast HMG-CoA reductase, like that of the hamster enzyme to which it is very similar, lies on the cytoplasmic side of the membrane, then the spacer region between the last two membrane-spanning domains can be reliably predicted to lie on the luminal side of the membrane. In this spacer region in both the HMG1 and HMG2 isozymes, two consensus sequences for N-linked glycosylation sites were found, although these sites were not conserved between the two proteins (amino acids 452 and 490 and amino acids 428 and 455 of the HMG1 and HMG2 proteins, respectively). Likewise, the single glycosylation site of the human and hamster enzymes also lay between the last two predicted membrane-spanning regions. Several other potential N-linked glycosylation sites were found in the membrane-bound domains of the HMG1 and HMG2 isozymes, as shown in Fig. 3.

Although the NH_2-termini of both the yeast and human proteins were predicted to contain seven membrane-spanning regions, no primary sequence similarity was observed in this region between either of the yeast proteins and the human protein. However, the predicted membrane-spanning domains of the yeast and human proteins were similar in that they contained amino acids that are usually charged. As noted previously (33), these charged residues might allow ion pairing within or between packed helices. Another similarity between the yeast and human NH_2-terminal regions was that, in both proteins, the spacer regions between the membrane-spanning domains were predicted to contain amphipathic helices which could associate with the lipid bilayer so that the hydrophobic face would be embedded in the interior of the bilayer and the hydrophilic face would

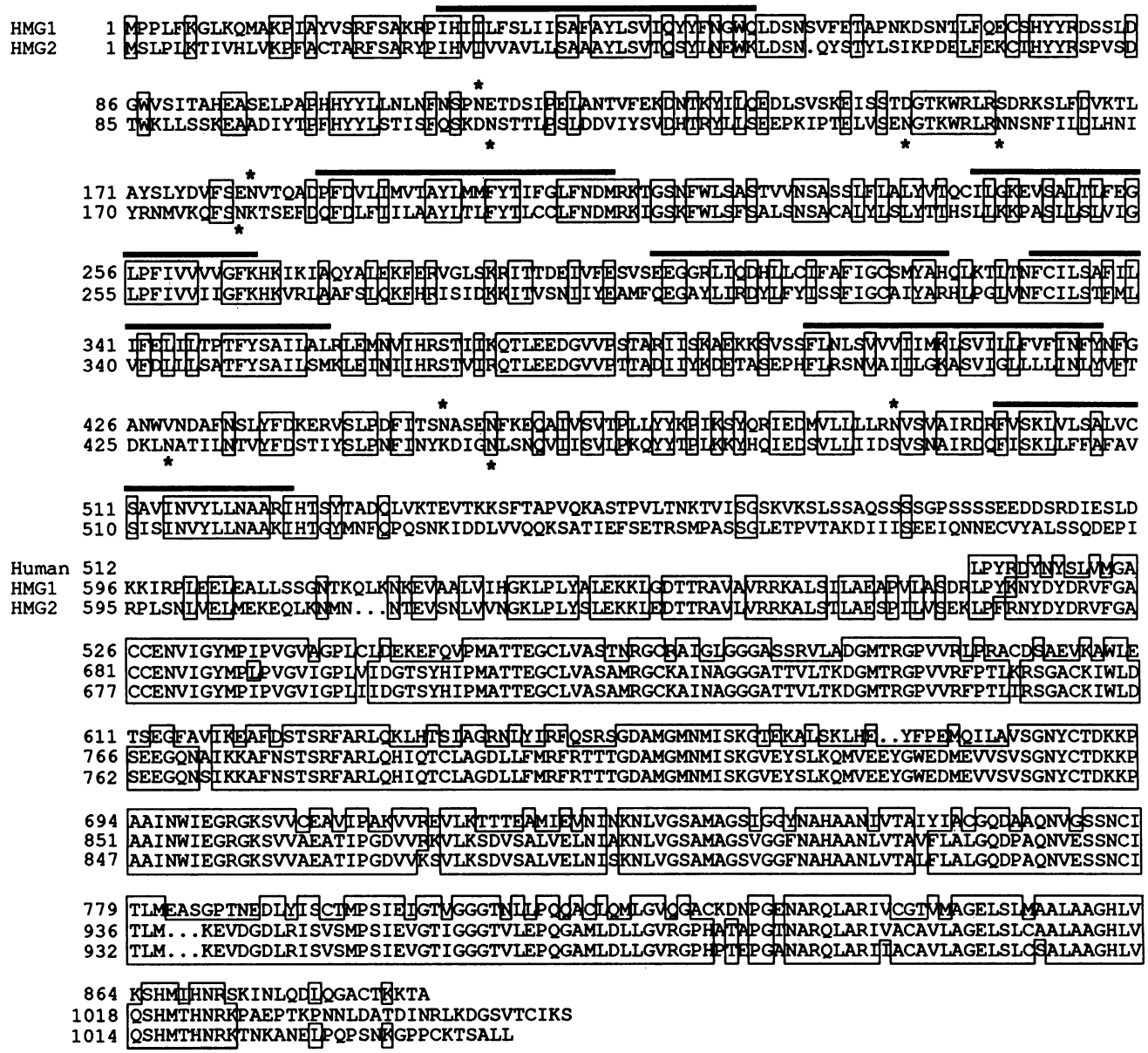


FIG. 3. Comparison of the HMG1, HMG2, and human HMG-CoA reductase proteins. No detectable sequence similarity between the human protein with either yeast protein was observed before amino acid 512 in the human protein. Identical residues are boxed. Bars above the sequence indicate predicted membrane-spanning domains. Asterisks indicate potential N-linked glycosylation sites in the membrane-bound region of the HMG1 and HMG2 proteins.

interact with the charged phospholipid head groups of the bilayer. No other structural similarity between the putative membrane-bound domains of the yeast and hamster HMG-CoA reductase proteins was revealed by our analysis. No correlation was observed, for example, between the relative hydrophobicities of the seven predicted membrane-spanning regions in the yeast and hamster proteins.

The region of the mammalian protein between the NH_2-terminal membrane-bound domain and the COOH-terminal cytoplasmic domain is termed the linker region. This region contained the greatest amount of sequence divergence between the hamster and human proteins, suggesting that this region of the protein is under the lowest selective pressure. The linker regions of the HMG1 and HMG2 proteins are defined as the regions between the last predicted membrane-spanning domain and the start of primary sequence identity between the two proteins (amino acids 525 to 617 and amino acids 524 to 613 in the HMG1 and HMG2 proteins, respectively). The linker regions of the two yeast proteins exhibited no significant sequence similarity to human HMG-CoA reductase. Except for the first few amino acids at the junction of the membrane-spanning and linker regions, there was also no detectable sequence similarity between the linker regions of the two yeast proteins. The linker region of the HMG1 protein was rich in serine residues (20 of 93 amino acids), and the linker regions of both the HMG1 and HMG2 proteins, like that of the mammalian protein, contained a large number of charged residues (25 of 93 amino acids in the HMG1 protein and 21 of 90 amino acids in the HMG2 protein). Many of these charged residues are predicted to lie

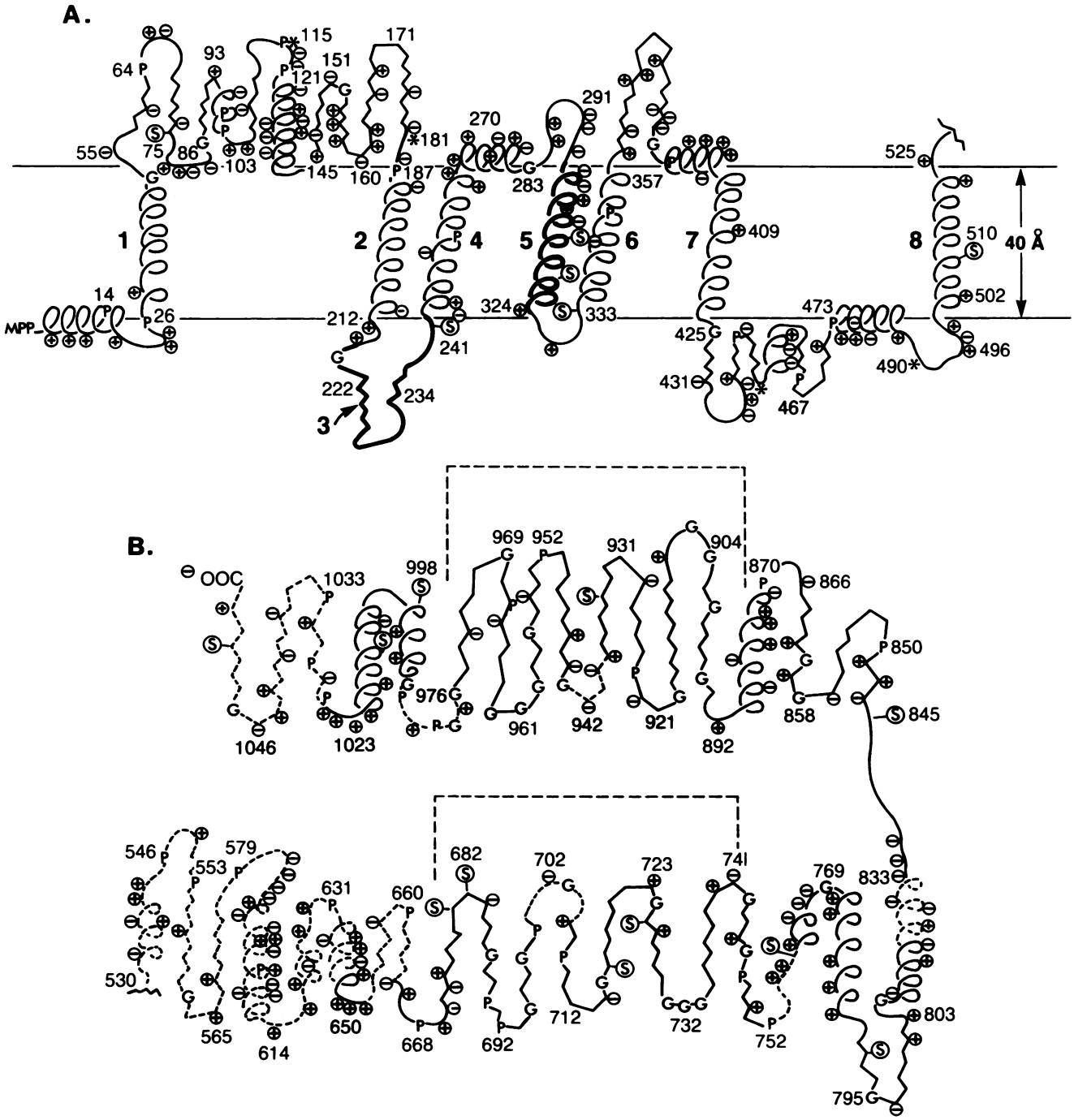


FIG. 4. Model of the predicted secondary structure of the HMG1 protein. Hydrophobic stretches of amino acids that could form membrane-spanning domains were located by the method of Kyte and Doolittle (31). A Fourier transform analysis designed to detect periodicities in hydrophobicity that suggest the presence of amphipathic α-helix or β-pleated sheet structure was used to identify regions of secondary structure (20). The algorithm of Garnier et al. (21), based on the frequency with which amino acids form different secondary structures in X-ray crystal structures, was also used. Charged amino acids are indicated by + or −. Cysteine residues are indicated by S, glycine residues by G, and proline residues by P. (A) Amino acids 1 to 529. The initial methionine is indicated by M. The boundaries of the lipid bilayer are drawn on either side of the predicted membrane-spanning domains. Regions 1, 2, 4, 6, 7, and 8 are strongly predicted to span the membrane. However, the secondary structures of regions 3 and 5 (indicated by bold lines) are less clearly predicted. Asterisks indicate potential N-linked glycosylation sites. (B) Amino acids 530 to 1054. Secondary structure drawn with dashed lines indicates regions with weak sequence similarity to hamster HMG-CoA reductase. As in the corresponding region of the hamster protein, amino acids 672 to 742 and 892 to 985, enclosed by dashed lines, each contain 8- to 11-amino-acid stretches of extended structure and are rich in cysteine, glycine, and proline residues.

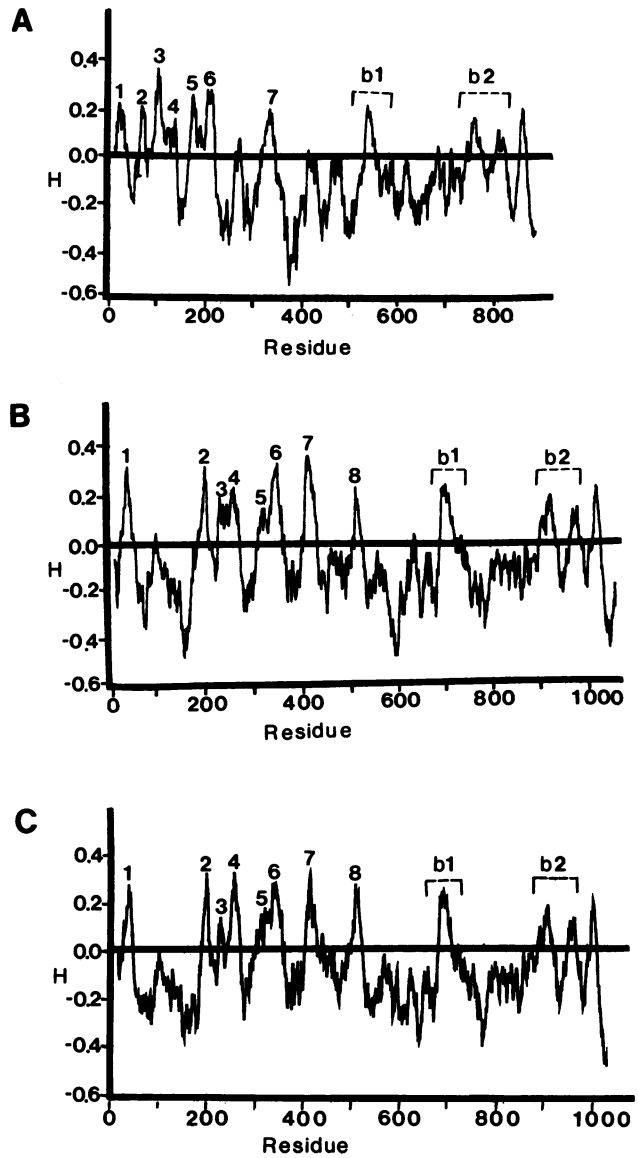


FIG. 5. Hydrophobicity plots of the amino acid sequences of hamster HMG-CoA reductase and the HMG1 and HMG2 proteins. The hydrophobicity of each residue, averaged over a window of 21 amino acids by the method of Kyte and Doolittle (31), is plotted as a function of residue number. (A) Hamster HMG-CoA reductase, redrawn from Liscum et al. (33). The seven predicted membrane-spanning domains are labeled 1 to 7. The regions labeled b1 and b2 are predicted to have β-structure and contain hydrophobic stretches. The corresponding regions of the HMG1 and HMG2 proteins predicted to have the same structure are similarly labeled (see Fig. 4). (B) HMG1 protein. Of the eight peaks of hydrophobicity (labeled 1 to 8), all except peak 3 are predicted to span the membrane (see Fig. 4). (C) HMG2 protein.

in amphipathic helices in the linker regions of both the yeast and mammalian proteins.

The COOH-terminal regions of the HMG1 and HMG2 proteins were highly conserved with respect to both mammalian HMG-CoA reductase and each other. Amino acids 667 to 1025 of the HMG1 protein were 65% identical and 25% conserved with amino acids 512 to 871 of human HMG-CoA reductase. Amino acids 618 to 1026 of the HMG1 protein were 93% identical and 3% conserved with amino acids 614 to 1022 of the HMG2 protein. The COOH-terminal region of these three proteins was the most conserved of any region within the protein. This strong sequence similarity suggests that the catalytic domain of the yeast isozymes, like that of the mammalian enzyme, is contained within this COOH-terminal region.

TABLE 1. Parameters for evaluation of putative membrane-spanning helices of the HMG1, HMG2, and hamster HMG-CoA reductase protein

Protein	Region[a]	H[b]	μ[c]	Peak	
				Height[d]	Length[e] (residues)
Yeast HMG1	1	0.73	0.34	1.33	18
	2	0.73	0.23	1.29	18
	3	0.53	0.41	1.24	23
	4	0.57	0.27	1.23	17
	5	0.44	0.53	1.32	13
	6	0.74	0.26	1.34	29
	7	0.79	0.30	1.33	25
	8	0.58	0.29	1.25	17
Yeast HMG2	1	0.60	0.20	1.32	16
	2	0.72	0.29	1.30	18
	3	0.45	0.30	1.17	19
	4	0.73	0.40	1.29	17
	5	0.47	0.47	1.22	13
	6	0.63	0.34	1.30	30
	7	0.72	0.13	1.28	20
	8	0.67	0.27	1.33	18
Hamster HMG-CoA reductase	1	0.57	0.28	1.26	17
	2	0.55	0.47	1.19	17
	3	0.79	0.37	1.31	22
	4	0.46	0.41	1.23	20
	5	0.62	0.27	1.21	25
	6	0.65	0.28	1.28	28
	7	0.54	0.30	1.30	13

[a] Regions for the HMG1 and HMG2 proteins are numbered as in Fig. 4A. The predicted membrane-spanning helices of hamster HMG-CoA reductase are described in Liscum et al. (33).

[b] Average hydrophobicity over most-hydrophobic 21-residue stretch calculated with the normalized consensus hydrophobicity scale of Eisenberg et al. (19).

[c] Hydrophobic moment over the 11-residue stretch with the most pronounced helical periodicity, calculated as described in Eisenberg et al. (19).

[d] Height of peak in buried-helix-parameter plot described by Rao and Argos (45).

[e] Width of peak in buried-helix-parameter plot at baseline of 1.05 (45).

The predicted secondary structure of the COOH-terminal region of the HMG1 protein (Fig. 4B) closely resembled that of the hamster protein. This region was predicted to consist largely of amphipathic helices flanking extended β-pleated sheet structure. As noted previously (33), it seems likely that residues from the putative β-domains form at least part of the active site. Regions of weak sequence similarity between the HMG1 and hamster proteins, indicated by dashed lines in Fig. 4B, fell in loops adjacent to β-strands or in amphipathic helices, as would be expected if the domains from the two proteins had similar tertiary structures. Thus, the predicted amphipathic helical structure is strongly conserved even when primary sequence is only weakly conserved.

The reaction by which HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate may involve the transient protonation of a histidine residue (47). Of the seven histidines conserved between the COOH-terminal regions of the two yeast proteins, four were also conserved with human HMG-CoA reductase at residues 909, 1015, 1020, and 1023 of the HMG1 protein.

In contrast to the general sequence similarity in the

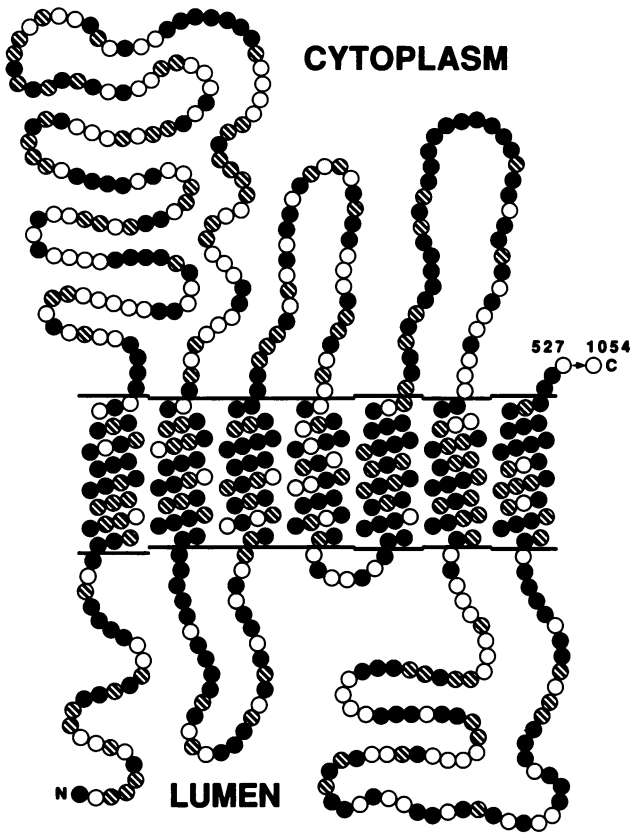


FIG. 6. Sequence conservation between the membrane-bound regions of the HMG1 and HMG2 proteins. Sequences are aligned as in Fig. 3. Identical amino acids are indicated by solid circles, conserved amino acids by hatched circles, and nonconserved amino acids by open circles. The criteria for conserved residues are given in Table 2, footnote *a*.

COOH-terminal regions of the two yeast isozymes to each other and to the human enzyme, no obvious sequence similarity existed between the final 28 residues of the HMG1 protein with either the HMG2 or human protein. Although the significance of this sequence divergence is unclear, we note that the sequences located at the COOH-terminus of both of the yeast enzymes as well as the hamster and human enzymes contained cysteine residues that could be used as sites for posttranslational fatty acid acylation (53), as is the case for several other integral membrane proteins, such as the G protein of vesicular stomatitis virus (50), the transferrin receptor (26), and rhodopsin (42).

Mammalian HMG-CoA reductase rescued yeast cells lacking this function. The similarities between yeast and mammalian HMG-CoA reductase strongly suggested functional conservation between these enzymes. We tested and confirmed this suggestion in vivo by rescuing otherwise inviable mutant yeast cells with the enzyme encoded by a mammalian gene.

Doubly mutant *hmg1 hmg2* yeast cells, lacking both HMG-CoA reductase isozymes, are inviable in standard minimal or rich yeast medium. However, as described in the Materials and Methods section, their growth can be rescued by supplementing either medium with a high concentration of mevalonate, the product of the reaction catalyzed by HMG-CoA reductase. As described in the Materials and Methods section, plasmids were constructed in which the inducible yeast *GAL1* promoter (28) was fused to the *HMG1* gene or to cDNA clones encoding either hamster or human HMG-CoA reductase. The *GAL1* promoter was inactive when cells were grown in glucose-containing medium, but active when cells were grown in galactose-containing medium. These plasmids were introduced separately into an *hmg1 hmg2* strain by transformation and tested for their ability to alleviate the mevalonate auxotrophy of this strain in galactose-containing medium.

As shown in Fig. 7, *hmg1 hmg2* cells harboring the *GAL1* promoter plasmid vector lacking HMG-CoA reductase coding sequences were inviable on galactose-containing solid medium lacking mevalonate. *hmg1 hmg2* cells harboring

TABLE 2. Conservation between the membrane-bound domains of the HMG1 and HMG2 proteins[a]

Amino acids[b]	Transmembrane domain[c]	No. of amino acids	% Identical	% Identical + % conserved
1–26		26	38	73
27–53	1	27	52	85
54–186		133	32	59
187–211	2	25	56	88
212–241		30	60	77
242–266	3	25	60	88
267–299		33	36	66
300–324	4	25	52	72
325–331		7	43	43
332–357	5	26	65	92
358–397		40	65	83
398–422	6	25	48	92
423–498		76	43	61
499–524	7	26	62	93

[a] Two amino acids are considered conserved if they are found together in one of the following classes: (i) alanine, valine, leucine, isoleucine, proline, phenylalanine, tryptophan, methionine, glycine, cysteine; (ii) serine, threonine, cysteine, tryosine, asparagine, glutamine; (iii) aspartic acid, glutamic acid; (iv) lysine, arginine, histidine; (v) phenylalanine, tyrosine, tryptophan; (vi) histidine, tryptophan (15, 16, 40).

[b] Amino acids are numbered as for the HMG1 protein. The alignment of the HMG1 and HMG2 proteins is shown in Fig. 3.

[c] Transmembrane domains are numbered sequentially. Note that transmembrane domains 3 through 7 correspond to regions 4 through 8 in Fig. 4 and 5.

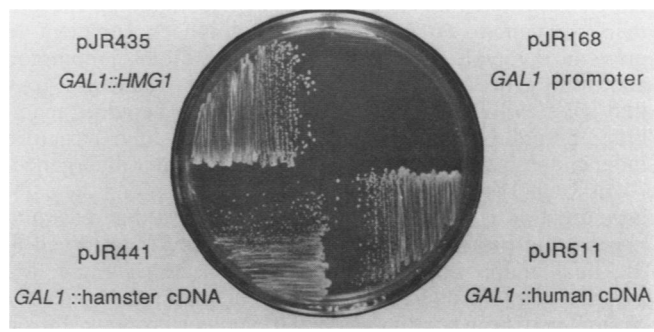


FIG. 7. Functional conservation between yeast and mammalian HMG-CoA reductase. JRY1597 (*hmg1 hmg2*) harboring pJR168 (containing the *GAL1* promoter without HMG-CoA reductase coding sequences), pJR435 (containing the *GAL1* promoter fused to the *HMG1* open reading frame), pJR441 (containing the *GAL1* promoter fused to a hamster HMG-CoA reductase cDNA clone), or pJR511 (containing the *GAL1* promoter fused to a human HMG-CoA reductase cDNA clone) was propagated on minimal glucose medium containing mevalonolactone and then streaked onto the plate shown containing minimal medium with 2% galactose and lacking mevalonolactone.

plasmids containing *GAL1* promoter fusions to either *HMG1*, the hamster HMG-CoA reductase cDNA, or the human HMG-CoA reductase cDNA were viable on this medium. The growth rate of cells containing mammalian HMG-CoA reductase was not readily distinguishable from that of cells containing the HMG1 protein. Therefore, hamster or human HMG-CoA reductase can function in yeast cells to meet their requirements for all essential products derived from mevalonate.

DISCUSSION

Evolution of HMG-CoA reductase. The mammalian genome apparently contains a single gene encoding HMG-CoA reductase (35, 46). No pseudogenes have been identified. The observation that yeast cells contain two genes encoding HMG-CoA reductase suggests that these two genes arose from the duplication of a single gene after the divergence of *S. cerevisiae* and mammals. We can make a minimum estimate of the time since this duplication event took place by considering the sequence divergence between the linker regions of the HMG1 and HMG2 proteins. As no similarity between these two regions was detected, we estimate that these regions have diverged by at least 90%. Assuming a divergence rate of 0.7 million years for each percent sequence divergence for sequences not under selection (54), then 90% sequence divergence would occur over 63 million years. However, it seems likely that the linker region is under some selective pressure. Comparison of the linker regions of human and hamster HMG-CoA reductase (36) indicates a divergence rate of 2.2 million years for each percent sequence divergence. This estimate of the rate of divergence of the linker regions would require that the duplication event generating *HMG1* and *HMG2* occurred at least 198 million years ago.

The ancient divergence of yeasts and mammals has allowed sequences not under stringent selective pressure to diverge completely. The only sequence similarity shared between yeast and mammalian HMG-CoA reductase occurs in the region of the protein expected to be under the most stringent selection, the catalytic domain. One explanation for the lack of sequence similarity observed between the membrane-bound domains of the yeast and mammalian enzymes is that the selective pressure maintaining the primary sequence of this domain has not been sufficient to prevent complete sequence divergence from a common ancestral gene. Alternatively, HMG-CoA reductase may first have evolved as a soluble enzyme that acquired membrane-bound NH_2-termini of roughly the same structure independently in yeasts and mammals after their divergence.

Although the COOH-terminal region, containing the catalytic domain, is generally conserved between the yeast and mammalian proteins, no sequence similarity is observed in the final amino acids of these proteins. Intriguingly, the position of an intron in the hamster gene marks the boundary of similarity between the yeast and hamster proteins. Intron 19 splits the codon for amino acid 870 of the hamster protein (46). This residue is the last to have sequence similarity with the yeast proteins, 29 amino acids from the end of the HMG1 protein and 24 amino acids from the end of the HMG2 protein. Thus the final exon of the hamster gene, encoding 16 amino acids, completely lacks sequence similarity with the yeast proteins. This observation suggests that the final amino acids of the yeast and mammalian proteins may have been added after the divergence of yeasts and mammals. In no other case was a correspondence between intron position in the hamster gene and sequence identity between the yeast and hamster proteins observed. The linker region of the HMG1 protein, like that of hamster HMG-CoA reductase, is rich in amino acids characteristic of PEST sequences. The linker region of the HMG2 protein is less highly enriched in these sequences. PEST sequences are present in many short-lived proteins (48). It has not yet been determined whether yeast HMG-CoA reductase has a short half-life, as does the mammalian enzyme.

Role of the membrane attachment of HMG-CoA reductase. The predicted membrane-bound domains of hamster and human HMG-CoA reductase are much more highly conserved than those of proteins with a single membrane-spanning domain (36). This high degree of conservation suggests that the complex architecture of the membrane-bound domain of mammalian HMG-CoA reductase not only serves as a membrane anchor but also has additional functions that are under selective pressure. Such functions may include regulation of the half-life of the protein (22) and a role in controlling the amount and type of internal membranes in the cell (27). The predicted structural similarities between the membrane-bound domains of yeast and mammalian HMG-CoA reductase suggest that the membrane-bound domains of the yeast proteins may also provide such functions. In support of this hypothesis, overexpression of the HMG1 protein leads to a proliferation of internal membranes that resembles the membrane proliferation observed at early times after overexpression of hamster HMG-CoA reductase (43, Wright et al., in press).

The membrane-bound domains of the HMG1 and HMG2 proteins differ from each other much more than do the membrane-bound domains of the human and hamster HMG-CoA reductase proteins. This increased sequence divergence may result simply from the much longer time during which the two yeast proteins are estimated to have had the opportunity to diverge compared with the human and hamster proteins. However, proteins serving duplicated functions in *S. cerevisiae* generally exhibit a very high degree of sequence similarity (1, 13, 52). Alternatively, the observed sequence divergence between the membrane-bound domains of the two yeast proteins may have resulted from selective pressure to allow their membrane attachment to serve specialized functions.

Functional conservation between yeast and mammalian HMG-CoA reductase. Expression of either human or hamster HMG-CoA reductase in yeast complemented the growth defect of an *hmg1 hmg2* double mutant. Since it is known that a soluble COOH-terminal fragment of the mammalian protein containing only the catalytic domain can function in mammalian cells, it is possible that the complementation observed in yeast cells is due to expression of not the intact mammalian protein, but rather the soluble catalytic domain. However, expression of the hamster or human genes from the strong *GAL1* promoter leads to a proliferation of internal membranes that appears identical to the membrane proliferation resulting from expression of the *HMG1* gene from the same promoter (R. Wright, personal communication). Since, in mammalian cells, the membrane-bound domain of mammalian HMG-CoA reductase is required for membrane proliferation, and since membrane proliferation is induced by expression of the mammalian gene in yeast cells, it seems likely that the intact mammalian protein is produced in yeast cells under these conditions.

The functional conservation between yeast and human HMG-CoA reductase may allow the use of yeast cells in identifying drugs with therapeutic value in the treatment of

hypercholesterolemia. *hmg1 hmg2* cells growing by virtue of expressing the human enzyme provide a simple microbial screen for specific inhibitors of human HMG-CoA reductase. Furthermore, we have recently found that the yeast enzyme is sensitive to feedback repression from products made later in the pathway (M. Thorsness and J. Rine, unpublished results). In light of the structural and functional conservation between the yeast and mammalian enzymes, the molecules that mediate feedback repression of yeast and mammalian HMG-CoA reductase may prove to be similar.

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LITERATURE CITED

1. **Abovich, N., and M. Roshbash.** 1984. Two genes for ribosomal protein 51 of *Saccharomyces cerevisiae* complement and contribute to the ribosomes. Mol. Cell. Biol. **4**:1871–1879.
2. **Anderson, R. G. W., L. Orci, M. S. Brown, L. M. Garcia-Segura, and J. L. Goldstein.** 1983. Ultrastructural analysis of crystalloid endoplasmic reticulum in UT-1 cells and its disappearance in response to cholesterol. J. Cell Sci. **63**:1–20.
3. **Barnes, G., W. J. Hansen, C. L. Holcomb, and J. Rine.** 1984. Asparagine-linked glycosylation in *Saccharomyces cerevisiae*: genetic analysis of an early step. Mol. Cell. Biol. **4**:2381–2388.
4. **Basson, M. E., R. L. Moore, J. O'Rear, and J. Rine.** 1987. Identifying mutations in duplicated functions in *Saccharomyces cerevisiae*: recessive mutations in HMG-CoA reductase genes. Genetics **117**:645–655.
5. **Basson, M. E., M. Thorsness, and J. Rine.** 1986. *Saccharomyces cerevisiae* contains two functional genes encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase. Proc. Natl. Acad. Sci. USA **83**:5563–5567.
6. **Beg, Z. H., J. A. Stonik, and H. B. Brewer, Jr.** 1987. Modulation of the enzymic activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase by multiple kinase systems involving reversible phosphorylation: a review. Metabolism **36**:900–917.
7. **Bloch, K.** 1965. The biological synthesis of cholesterol. Science **150**:19–28.
8. **Brown, D. A., and R. D. Simoni.** 1984. Biogenesis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, an integral glycoprotein of the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **81**:1674–1678.
9. **Brown, M. S., and J. L. Goldstein.** 1980. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J. Lipid Res. **21**: 505–517.
10. **Chin, D. J., G. Gil, D. W. Russell, L. Liscum, K. L. Luskey, S. K. Basu, H. Okayama, P. Berg, J. L. Goldstein, and M. S. Brown.** 1984. Nucleotide sequence of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a glycoprotein of endoplasmic reticulum. Nature (London) **308**:613–617.
11. **Chin, D. J., G. Gil, J. R. Faust, J. L. Goldstein, M. S. Brown, and K. L. Luskey.** 1985. Sterols accelerate degradation of hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase encoded by a constitutively expressed cDNA. Mol. Cell. Biol. **5**: 634–641.
12. **Chin, D. J., K. L. Luskey, R. G. W. Anderson, J. R. Faust, J. L. Goldstein, and M. S. Brown.** 1982. Appearance of crystalloid endoplasmic reticulum in compactin-resistant Chinese hamster cells with a 500-fold increase in 3-hydroxy-3-methylglutaryl coenzyme A reductase. Proc. Natl. Acad. Sci. USA **79**:1185–1189.
13. **Choe, J., D. Kolodrubetz, and M. Grunstein.** 1982. The two yeast histone H2A genes encode similar protein subtypes. Proc. Natl. Acad. Sci. USA **79**:1484–1488.
14. **Clarke, C. F., A. M. Fogelman, and P. A. Edwards.** 1985. Transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene in rat liver. J. Biol. Chem. **260**:14363–14367.
15. **Dayhoff, M. O.** 1972. Atlas of protein sequence and structure, vol. 5. National Biomedical Research Foundation. Washington, D.C.
16. **Dayhoff, M. O., W. C. Barker, and L. T. Hunt.** 1983. Establishing homologies in protein sequences. Methods Enzymol. **91**: 524–545.
17. **Dente, L. G., G. Cesareni, and R. Cortese.** 1983. pEMBL: a new family of single-stranded plasmids. Nucleic Acids Res. **11**:1645–1655.
18. **Edwards, P. A., S.-F. Lan, and A. M. Fogelman.** 1983. Alterations in the rates of synthesis and degradation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase produced by cholestyramine and mevinolin. J. Biol. Chem. **258**:10219–10222.
19. **Eisenberg, D., E. Schwarz, M. Komaromy, and R. Wall.** 1984. Analysis of membrane and surface protein sequences with the hydrophobic moment plot. J. Mol. Biol. **179**:125–142.
20. **Finer-Moore, J., and R. M. Stroud.** 1984. Amphipathic analysis and possible formation of the ion channel in an acetylcholine receptor. Proc. Natl. Acad. Sci. USA **81**:155–159.
21. **Garnier, J., D. J. Osguthorpe, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120**: 97–120.
22. **Gil, G., J. R. Faust, D. J. Chin, J. L. Goldstein, and M. S. Brown.** 1985. Membrane-bound domain of HMG-CoA reductase is required for sterol-enhanced degradation of the enzyme. Cell **41**:249–258.
23. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
24. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
25. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Yeast transformation. Proc. Natl. Acad. Sci. USA **75**:1929–1933.
26. **Jing, S., and I. S. Trowbridge.** 1987. Identification of the intermolecular disulfide bonds of the human transferrin receptor and its lipid-attachment site. EMBO J. **6**:327–331.
27. **Jingami, H., M. S. Brown, J. L. Goldstein, R. G. W. Anderson, and K. L. Luskey.** 1987. Partial deletion of membrane-bound domain of 3-hydroxy-3-methylglutaryl coenzyme A reductase eliminates sterol-enhanced degradation and prevents formation of crystalloid endoplasmic reticulum. J. Cell Biol. **104**:1693–1704.
28. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:1440–1448.
29. **Keller, G. A., M. Pazirandeh, and S. Krisans.** 1986. 3-Hydroxy-3-methylglutaryl coenzyme A reductase localization in rat liver peroxisomes and microsomes of control and cholestyramine-treated animals: quantitative biochemical and immunoelectron microscopical analyses. J. Cell Biol. **103**:875–886.
30. **Kuhn, L. A., and J. S. Leigh, Jr.** 1985. A statistical technique for predicting membrane protein structure. Biochim. Biophys. Acta **828**:351–361.
31. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
32. **Liscum, L., R. D. Cummings, R. G. W. Anderson, G. N. Demartino, J. L. Goldstein, and K. L. Luskey.** 1983. 3-Hydroxy-3-methylglutaryl coenzyme A reductase: a transmembrane glycoprotein of the endoplasmic reticulum with N-linked "high mannose" oligosaccharides. Proc. Natl. Acad. Sci. USA **80**: 7165–7169.
33. **Liscum, L., J. Finer-Moore, R. M. Stroud, K. L. Luskey, M. S. Brown, and J. L. Goldstein.** 1985. Domain structure of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a glycopro-

tein of the endoplasmic reticulum. J. Biol. Chem. **260**:522–530.
34. **Liscum, L., K. L. Luskey, D. J. Chin, Y. K. Ho, J. L. Goldstein, and M. S. Brown.** 1983. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and its mRNA in rat liver as studied with a monoclonal antibody and a cDNA probe. J. Biol. Chem. **258**:8450–8455.
35. **Luskey, K. L.** 1987. Conservation of promoter sequence but not complex intron splicing pattern in human and hamster genes for 3-hydroxy-3-methylglutaryl coenzyme A reductase. Mol. Cell. Biol. **7**:1881–1893.
36. **Luskey, K. L., and B. Stevens.** 1985. Human 3-hydroxy-3-methylglutaryl coenzyme A reductase. Conserved domains responsible for catalytic activity and sterol-regulated degradation. J. Biol. Chem. **260**:10271–10277.
37. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Michel, H., K. A. Weyer, H. Grunenberg, I. Dunger, D. Oesterhelt, and F. Lottspeich.** 1986. The "light" and "medium" subunits of the photosynthetic reaction centre from *Rhodopseudomonas viridis*: isolation of the genes, nucleotide and amino acid sequence. EMBO J. **5**:1149–1158.
39. **Mortimer, R. K., and D. C. Hawthorne.** 1969. Yeast genetics, p. 385–460. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 1. Academic Press, New York.
40. **Nathans, J., D. Thomas, and D. S. Hogness.** 1986. Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. Science **232**:193–202.
41. **Norrander, J., T. Kempe, and J. Messing.** 1983. Construction of improved M13 vectors using oligodeoxynucleotide-directed mutagenesis. Gene **26**:101–106.
42. **O'Brien, P. J., and M. Zatz.** 1984. Acylation of bovine rhodopsin by [^{3}H]palmitic acid. J. Biol. Chem. **259**:5054–5057.
43. **Pathak, R. K., K. L. Luskey, and R. G. W. Anderson.** 1986. Biogenesis of the crystalloid endoplasmic reticulum in UT-1 cells: evidence that newly formed endoplasmic reticulum emerges from the nuclear envelope. J. Cell Biol. **102**:2158–2168.
44. **Peffley, D., and M. Sinensky.** 1985. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase synthesis by a non-sterol mevalonate-derived product in Mev-1 cells. J. Biol. Chem. **260**: 9949–9952.
45. **Rao, J. K. M., and P. Argos.** 1986. A conformation preference parameter to predict helices in integral membrane proteins. Biochim. Biophys. Acta **869**:197–214.
46. **Reynolds, G. A., S. K. Basu, T. F. Osborne, D. J. Chin, G. Gil, M. S. Brown, J. L. Goldstein, and K. L. Luskey.** 1984. HMG-CoA reductase: a negatively regulated gene with unusual promoter and 5′ untranslated regions. Cell **38**:275–285.
47. **Rogers, D. H., S. R. Panini, and H. Rudney.** 1983. Properties of HMG CoA reductase and its mechanism of action, p. 58–75. *In* J. R. Sabine (ed.), 3-Hydroxy-3-methylglutaryl coenzyme A reductase. CRC Press, Inc., Boca Raton, Fla.
48. **Rogers, S., R. Wells, and M. Rechsteiner.** 1986. Amino acid sequences common to rapidly degraded proteins: the PEST hypothesis. Science **234**:364–368.
49. **Roitelman, J., and I. Shechter.** 1984. Regulation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Biol. Chem. **259**:870–877.
50. **Rose, J. K., G. A. Adams, and C. J. Gallone.** 1984. The presence of cysteine in the cytoplasmic domain of the vesicular stomatis virus glycoprotein is required for palmitate addition. Proc. Natl. Acad. Sci. USA **81**:2050–2054.
51. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
52. **Schatz, P. J., L. Pillus, P. Grisafi, F. Solomon, and D. Botstein.** 1986. Two functional α-tubulin genes of the yeast *Saccharomyces cerevisiae* encode divergent proteins. Mol. Cell. Biol. **6**:3711–3721.
53. **Sefton, B. M., and J. E. Buss.** 1987. The covalent modification of eukaryotic proteins with lipid. J. Cell Biol. **104**:1449–1453.
54. **Weisman, L. S., B. M. Krummel, and A. C. Wilson.** 1986. Evolutionary shift in the site of cleavage of prelysozyme. J. Biol. Chem. **261**:2309–2313.
55. **Zammit, V. A., and R. A. Easom.** 1987. Regulation of hepatic HMG-CoA reductase *in vivo* by reversible phosphorylation. Biochim. Biophys. Acta **927**:223–228.