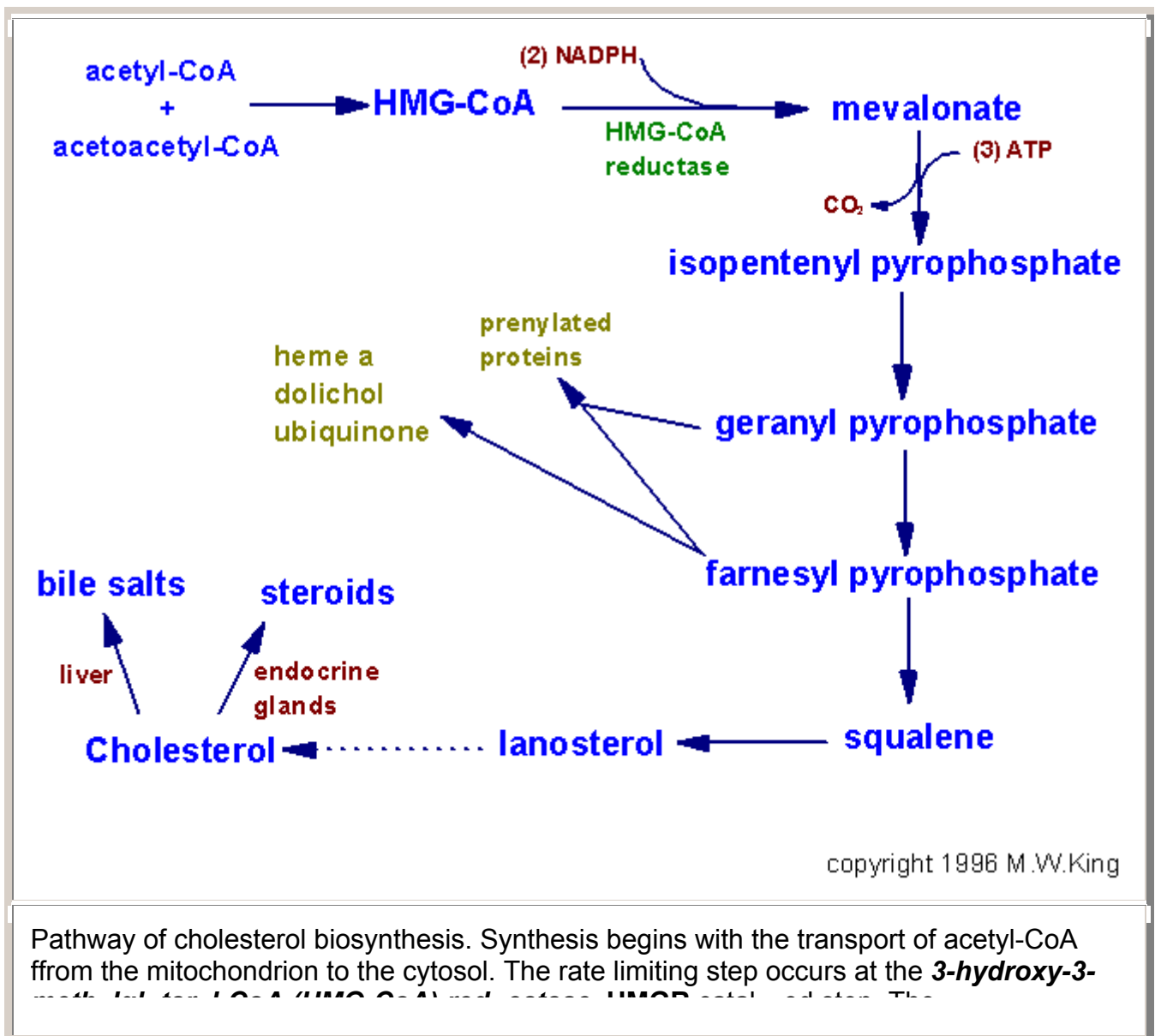


Biosynthesis of Cholesterol

Slightly less than half of the cholesterol in the body derives from biosynthesis *de novo*. Biosynthesis in the liver accounts for approximately 10%, and in the intestines approximately 15%, of the amount produced each day. Cholesterol synthesis occurs in the cytoplasm and microsomes from the two-carbon acetate group of acetyl-CoA.

The process has five major steps:

- 1. Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)
- 2. HMG-CoA is converted to mevalonate
- 3. Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP), with the concomitant loss of CO₂
- 4. IPP is converted to squalene
- 5. Squalene is converted to cholesterol.



phosphorylation reactions are required to solubilize the isoprenoid intermediates in the pathway. Intermediates in the pathway are used for the synthesis of prenylated proteins, dolichol, coenzyme Q and the side chain of heme *a*.

The acetyl-CoA utilized for cholesterol biosynthesis is derived from an oxidation reaction (eg, fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm by the same process as that [described for fatty acid synthesis](#). Acetyl-CoA can also be derived from cytoplasmic oxidation of ethanol by **acetyl-CoA synthetase**. All the reduction reactions of cholesterol biosynthesis use NADPH as a cofactor. The isoprenoid intermediates of cholesterol biosynthesis can be diverted to other synthesis reactions, such as those for dolichol (used in the synthesis of [N-linked glycoproteins](#), coenzyme Q (of the [oxidative phosphorylation](#)) pathway or the side chain of heme *a*. Additionally, these intermediates are used in the [lipid modification of some proteins](#).

Acetyl-CoA units are converted to mevalonate by a series of reactions that begins with the formation of **HMG-CoA**. Unlike the HMG-CoA formed during ketone body synthesis in the mitochondria, this form is synthesized in the cytoplasm. However, the pathway and the necessary enzymes are the same as those in the mitochondria. Two moles of acetyl-CoA are condensed in a reversal of the **thiolase** reaction, forming acetoacetyl-CoA. Acetoacetyl-CoA and a third mole of acetyl-CoA are converted to HMG-CoA by the action of **HMG-CoA synthase**. HMG-CoA is converted to **mevalonate** by **HMG-CoA reductase**, **HMGR** (this enzyme is bound in the endoplasmic reticulum, ER). **HMGR** absolutely requires NADPH as a cofactor and two moles of NADPH are consumed during the conversion of HMG-CoA to mevalonate.

The reaction catalyzed by **HMGR** is the rate limiting step of cholesterol biosynthesis, and this enzyme is subject to complex regulatory controls. Mevalonate is then activated by three successive phosphorylations, yielding **5-pyrophosphomevalonate**. In addition to activating mevalonate, the phosphorylations maintain its solubility, since otherwise it is insoluble in water. After phosphorylation, an ATP-dependent decarboxylation yields **isopentenyl pyrophosphate**, IPP, an activated isoprenoid molecule. Isopentenyl pyrophosphate is in equilibrium with its isomer, **dimethylallyl pyrophosphate**, **DMPP**. One molecule of IPP condenses with one molecule of DMPP to generate **geranyl pyrophosphate**, **GPP**. GPP further condenses with another IPP molecule to yield **farnesyl pyrophosphate**, **FPP**. Finally, the NADPH-requiring enzyme, **squalene synthase** catalyzes the head-to-tail condensation of two molecules of FPP, yielding squalene (squalene synthase also is tightly associated with the endoplasmic reticulum). Squalene undergoes a two step cyclization to yield **lanosterol**. The first reaction is catalyzed by **squalene monooxygenase**. This enzyme uses NADPH as a cofactor to introduce molecular oxygen as an epoxide at the 2,3 position of squalene. Through a series of 19 additional reactions, lanosterol is converted to cholesterol.

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Regulating Cholesterol Synthesis

Normal healthy adults synthesize cholesterol at a rate of approximately 1g/day and consume approximately 0.3g/day. A relatively constant level of cholesterol

in the body (150 - 200 mg/dL) is maintained primarily by controlling the level of *de novo* synthesis. The level of cholesterol synthesis is regulated in part by the dietary intake of cholesterol. Cholesterol from both diet and synthesis is utilized in the formation of membranes and in the synthesis of the steroid hormones and bile acids (see below). The greatest proportion of cholesterol is used in bile acid synthesis.

The cellular supply of cholesterol is maintained at a steady level by three distinct mechanisms:

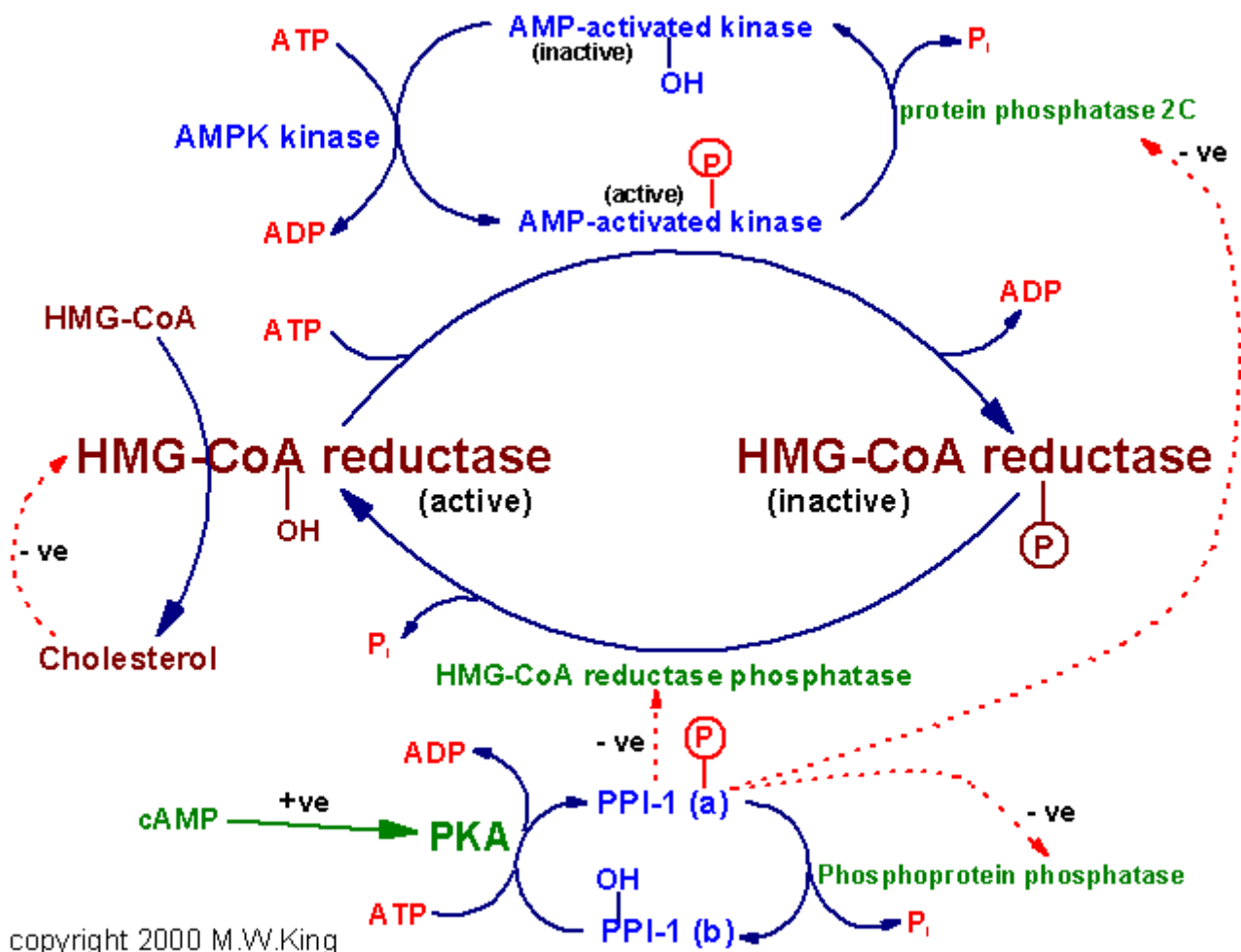
- 1. Regulation of **HMGR** activity and levels
- 2. Regulation of excess intracellular free cholesterol through the activity of **acyl-CoA:cholesterol acyltransferase, ACAT**
- 3. Regulation of plasma cholesterol levels via LDL receptor-mediated uptake and HDL-mediated reverse transport.

Regulation of **HMGR** activity is the primary means for controlling the level of cholesterol biosynthesis. The enzyme is controlled by four distinct mechanisms: feed-back inhibition, control of gene expression, rate of enzyme degradation and phosphorylation-dephosphorylation.

The first three control mechanisms are exerted by cholesterol itself.

Cholesterol acts as a feed-back inhibitor of pre-existing **HMGR** as well as inducing rapid degradation of the enzyme. The latter is the result of cholesterol-induced polyubiquitination of HMGR and its degradation in the proteosome (see [proteolytic degradation](#) below). This ability of cholesterol is a consequence of the **sterol sensing domain, SSD** of HMGR. In addition, when cholesterol is in excess the amount of mRNA for **HMGR** is reduced as a result of decreased expression of the gene. The mechanism by which cholesterol (and other sterols) affect the transcription of the HMGR gene is described below under [regulation of sterol content](#).

Regulation of **HMGR** through covalent modification occurs as a result of phosphorylation and dephosphorylation. The enzyme is most active in its unmodified form. Phosphorylation of the enzyme decreases its activity. **HMGR** is phosphorylated by **AMP-activated protein kinase, AMPK** (this is not the same as **cAMP-dependent protein kinase, PKA**). AMPK itself is activated via phosphorylation. The phosphorylation of **AMPK** is catalyzed by **AMPK kinase**.



Regulation of **HMGR** by covalent modification. **HMGR** is most active in the dephosphorylated state. Phosphorylation is catalyzed by **AMP-activated protein kinase**, AMPK, (used to be termed **HMGR kinase**), an enzyme whose activity is also regulated by phosphorylation. Phosphorylation of AMPK is catalyzed by **AMPK kinase**. Hormones such as glucagon and epinephrine negatively affect cholesterol biosynthesis by increasing the activity of the inhibitor of **phosphoprotein phosphatase inhibitor-1**, PPI-1. Conversely, insulin stimulates the removal of phosphates and, thereby, activates **HMGR** activity. Additional regulation of **HMGR** occurs through an inhibition of its' activity as well as of its' synthesis by elevation in intracellular cholesterol levels. This latter phenomenon involves the transcription factor SREBP described below.

The activity of **HMGR** is additionally controlled by the cAMP signaling pathway. Increases in cAMP lead to activation of **cAMP-dependent protein kinase**, PKA. In the context of **HMGR** regulation, PKA phosphorylates **phosphoprotein phosphatase inhibitor-1 (PPI-1)** leading to an increase in its' activity. PPI-1 can inhibit the activity of numerous **phosphatases** including **protein phosphatase 2C** and **HMG-CoA reductase phosphatase** which remove phosphates from AMPK and **HMGR**, respectively. This maintains AMPK in the phosphorylated and active state, and **HMGR** in the phosphorylated and inactive state. As the stimulus leading to increased cAMP production is removed, the level of phosphorylations decreases and that of

dephosphorylations increases. The net result is a return to a higher level of **HMGR** activity.

Since the intracellular level of cAMP is regulated by hormonal stimuli, regulation of cholesterol biosynthesis is hormonally controlled. Insulin leads to a decrease in cAMP, which in turn activates cholesterol synthesis.

Alternatively, glucagon and epinephrine, which increase the level of cAMP, inhibit cholesterol synthesis.

The ability of insulin to stimulate, and glucagon to inhibit, **HMGR** activity is consistent with the effects of these hormones on other metabolic pathways.

The basic function of these two hormones is to control the availability and delivery of energy to all cells of the body.

Long-term control of **HMGR** activity is exerted primarily through control over the synthesis and degradation of the enzyme. When levels of cholesterol are high, the level of expression of the **HMGR** gene is reduced. Conversely, reduced levels of cholesterol activate expression of the gene. Insulin also brings about long-term regulation of cholesterol metabolism by increasing the level of **HMGR** synthesis.

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Proteolytic Regulation of HMG-CoA Reductase

The stability of **HMGR** is regulated as the rate of flux through the mevalonate synthesis pathway changes. When the flux is high the rate of **HMGR** degradation is also high. When the flux is low, degradation of **HMGR** decreases. This phenomenon can easily be observed in the presence of the [statin drugs](#).

HMGR is localized to the ER and like SREBP (see below) contains a sterol-sensing domain, SSD. When sterol levels increase in cells there is an concomitant increase in the rate of **HMGR** degradation. The degradation of **HMGR** occurs within the proteasome, a multiprotein complex dedicated to protein degradation. The primary signal directing proteins to the proteasome is ubiquitination. Ubiquitin is a 7.6kDa protein that is covalently attached to proteins targeted for degradation by ubiquitin ligases. These enzymes attach multiple copies of ubiquitin allowing for recognition by the proteasome. **HMGR** has been shown to be ubiquitinated prior to its degradation. The primary sterol regulating **HMGR** degradation is cholesterol itself. As the levels of free cholesterol increase in cells, the rate of **HMGR** degradation increases.

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The Utilization of Cholesterol

Cholesterol is transported in the plasma predominantly as cholesteryl esters associated with [lipoproteins](#). Dietary cholesterol is transported from the small intestine to the liver within chylomicrons. Cholesterol synthesized by the liver, as well as any dietary cholesterol in the liver that exceeds hepatic needs, is transported in the serum within LDLs. The liver synthesizes VLDLs and these are converted to LDLs through the action of endothelial cell-associated lipoprotein lipase. Cholesterol found in plasma membranes can be extracted by HDLs and esterified by the HDL-associated enzyme LCAT. The cholesterol acquired from peripheral tissues by HDLs can then be transferred to VLDLs and LDLs via the action of cholesteryl ester transfer protein (apo-D) which is

associated with HDLs. **Reverse cholesterol transport** allows peripheral cholesterol to be returned to the liver in LDLs. Ultimately, cholesterol is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids in the liver.

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Regulation of Cellular Sterol Content

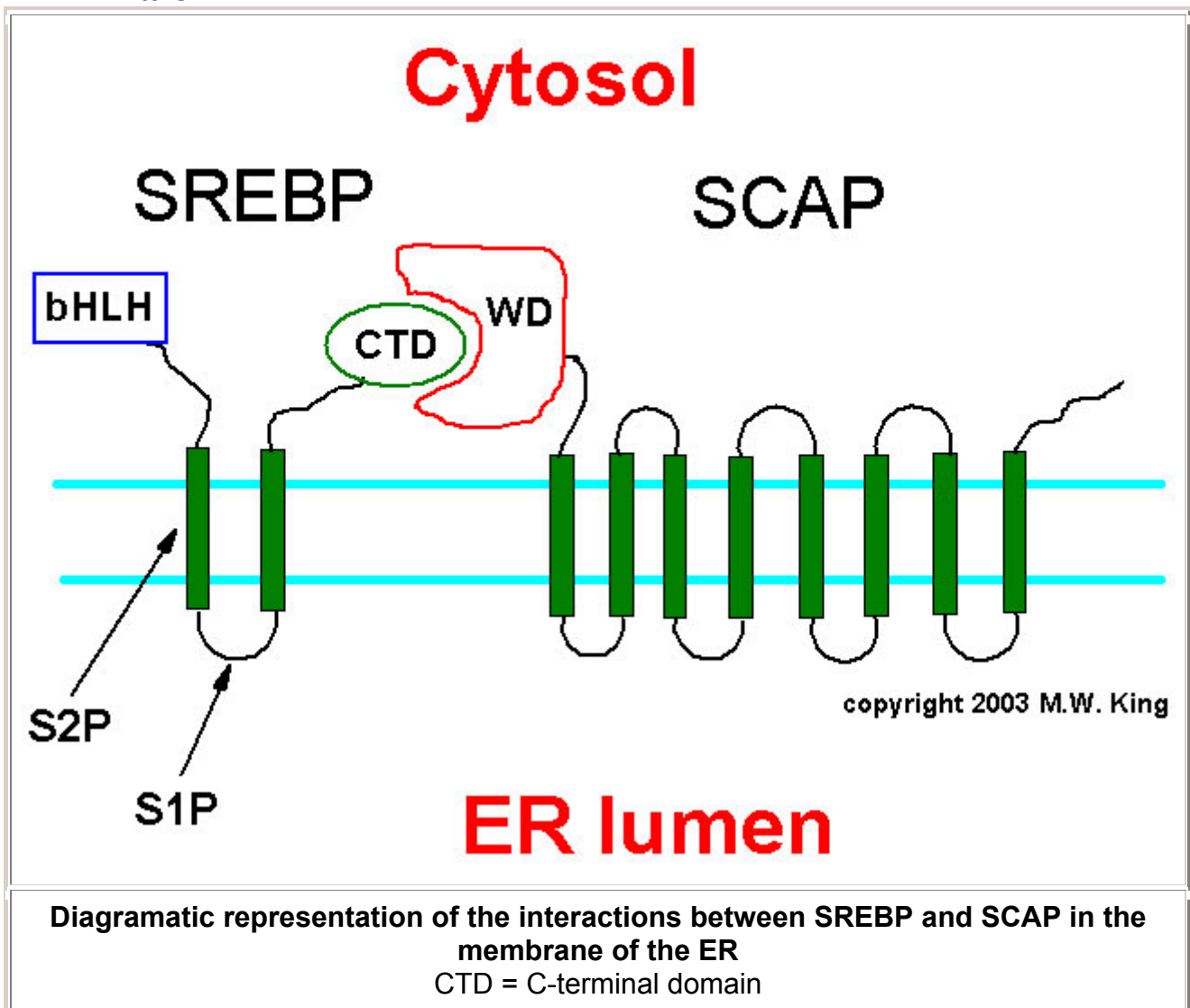
The continual alteration of the intracellular sterol content occurs through the regulation of key sterol synthetic enzymes as well as by altering the levels of cell-surface LDL receptors. As cells need more sterol they will induce their synthesis and uptake, conversely when the need declines synthesis and uptake are decreased. Regulation of these events is brought about primarily by sterol-regulated transcription of key rate limiting enzymes and by the regulated degradation of **HMGR**. Activation of transcriptional control occurs through the regulated cleavage of the membrane-bound transcription factor **sterol regulated element binding protein, SREBP**. As discussed above, degradation of **HMGR** is controlled by the ubiquitin-mediated pathway for proteolysis.

Sterol control of transcription affects more than 30 genes involved in the biosynthesis of cholesterol, triacylglycerols, phospholipids and fatty acids. Transcriptional control requires the presence of an octamer sequence in the gene termed the **sterol regulatory element, SRE-1**. It has been shown that SREBP is the transcription factor that binds to SRE-1 elements. It turns out that there are 2 distinct SREBP gene, SREBP-1 and SREBP-2. In addition, the SREBP-1 gene encodes 2 proteins, SREBP-1a and SREBP-1c as a consequence of alternative exon usage. All 3 proteins are proteolytically regulated by sterols. Full-length SREBPs have several domains and are embedded in the membrane of the endoplasmic reticulum (ER). The N-terminal domain contains a **transcription factor motif** of the basic helix-loop-helix (bHLH) type that is exposed to the cytoplasmic side of the ER. There are 2 transmembrane spanning domains followed by a large C-terminal domain also exposed to the cytosolic side. When sterols are scarce, cleavage of the full-length SREBP takes place with the result being that the N-terminal bHLH motif is released into the cytosol. The bHLH domain then migrates to the nucleus to direct transcription. Conversely, when sterols are abundant, cleavage of SREBP is inhibited. To control the level of SREBP-mediated transcription, the soluble bHLH domain is itself subject to rapid proteolysis. The cleavage of SREBP is carried out by 2 distinct enzymes, one of which is regulated by sterols. The regulated cleavage occurs in the luminal loop between the 2 transmembrane domains. This cleavage is catalyzed by **site-1 protease, S1P**. High sterol content blocks the activity of S1P. The second cleavage, catalyzed by **site-2 protease, S2P**, occurs in the first transmembrane span, leading to release of active SREBP. In order for S2P to act on SREBP, site-1 must already have been cleaved.

Additional studies on sterol-regulated gene expression demonstrated that cleavage of SREBP by S1P is controlled by the level and action of an additional protein termed, **SREBP cleavage-activating protein, SCAP**. SCAP is a large protein also found in the ER membrane and contains at least 8 transmembrane spans. The C-terminal portion, which extends into the cytosol, has been shown to interact with the C-terminal domain of SREBP. This C-

terminal region of SCAP contains 4 motifs called **WD40 repeats**. The WD40 repeats are required for interaction of SCAP with SREBP. Interestingly, the N-terminus of SCAP, including membrane spans 2-6, resembles **HMGR** which itself is subject to sterol-stimulated degradation (see above). This shared motif is called the **sterol sensing domain, SSD**. Several proteins whose functions involve sterols also contain the SSD. These include *patched*, an important development regulating receptor whose ligand, *hedgehog*, is modified by attachment of cholesterol and the **Neimann Pick C1 (NPC1)** protein which is involved in cholesterol transport in the secretory pathway. NPC1 is one of several genes whose activities, when disrupted, lead to severe neurological dysfunction.

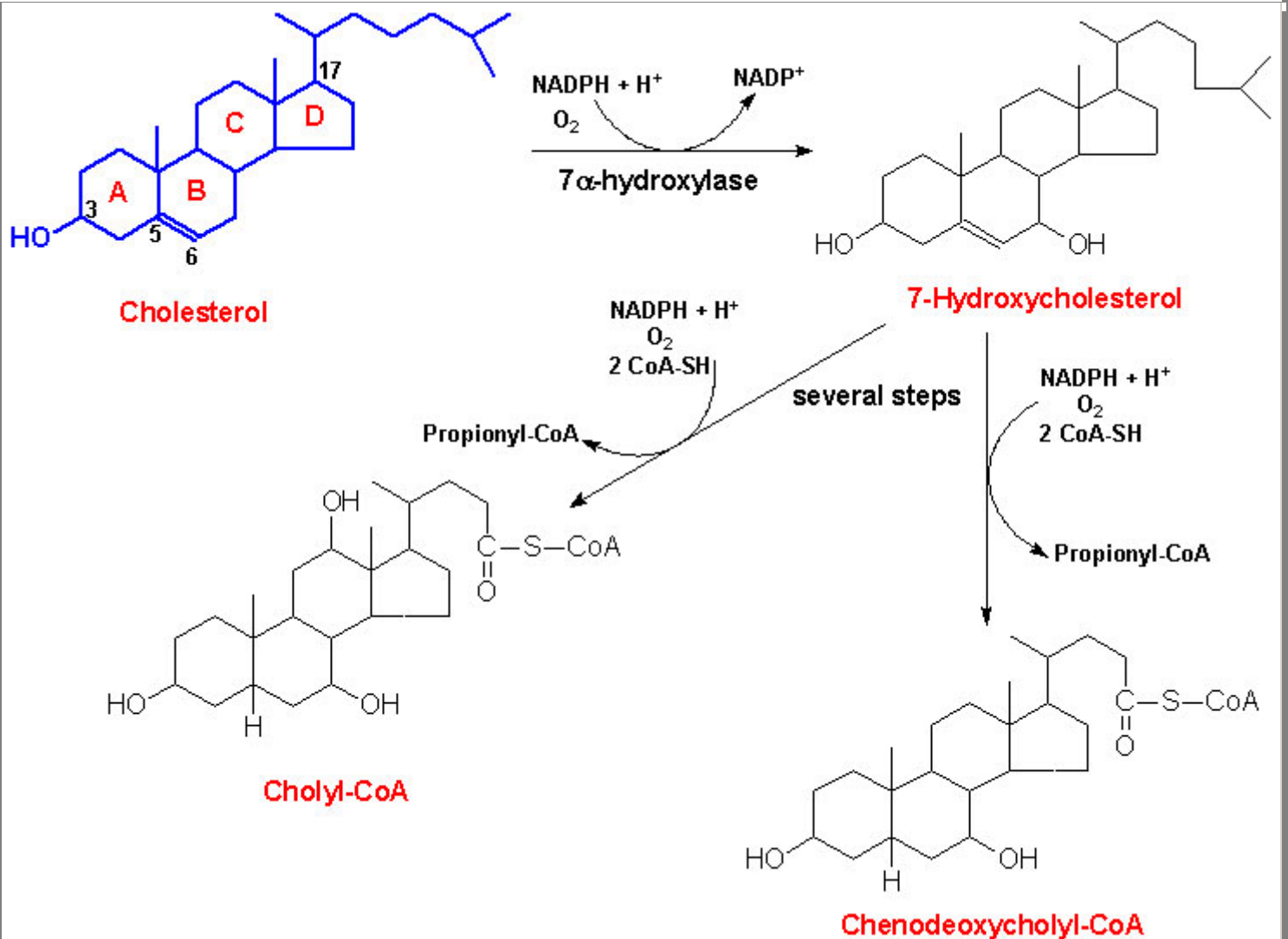
The function of SCAP is to positively stimulate S1P-mediated cleavage of SREBP. The function of sterols is to inhibit this positive action of SCAP. The activity of SCAP involves movement from the ER to the Golgi and back. Because the C-terminus of SCAP interacts with SREBP, movement of SCAP takes SREBP along for the ride. When sterols are low, SCAP and SREBP move to the Golgi. This transit is required for SREBP cleavage as S1P is Golgi-localized. When sterols are high, movement of SCAP is halted. Thus, the overall effect of sterols is to regulate the ability of SCAP to present SREBP to S1P.



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Bile Acids Synthesis and Utilization

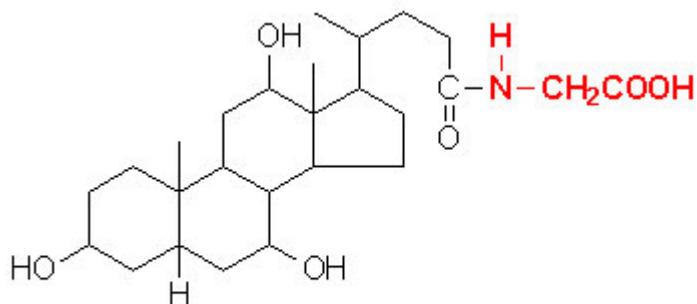
The end products of cholesterol utilization are the bile acids, synthesized in the liver. Synthesis of bile acids is one of the predominant mechanisms for the excretion of excess cholesterol. However, the excretion of cholesterol in the form of bile acids is insufficient to compensate for an excess dietary intake of cholesterol.



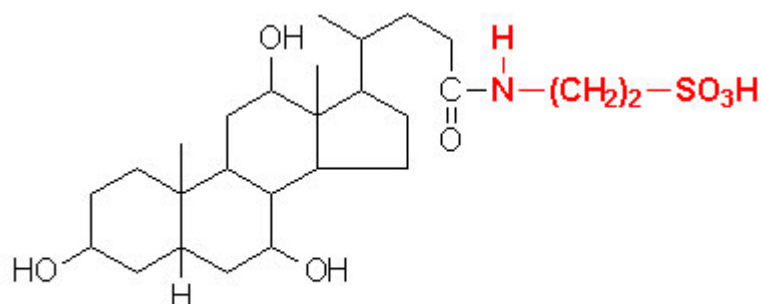
Synthesis of the 2 primary bile acids, cholic acid and chenodeoxycholic acid. The reaction catalyzed by the **7 α -hydroxylase** is the rate limiting step in bile acid synthesis. Conversion of 7 α -hydroxycholesterol to the bile acids requires several steps not shown in detail in this image. Only the relevant co-factors needed for the synthesis steps are shown.

The most abundant bile acids in human bile are **chenodeoxycholic acid** (45%) and **cholic acid** (31%). These are referred to as the **primary bile acids**. Within the intestines the primary bile acids are acted upon by bacteria and converted to the **secondary bile acids**, identified as deoxycholate (from

cholate) and lithocholate (from chenodeoxycholate). Both primary and secondary bile acids are reabsorbed by the intestines and delivered back to the liver via the portal circulation.



Glycocholic acid



Taurocholic acid

Structure of the conjugated cholic acids

Within the liver the carboxyl group of primary and secondary bile acids is conjugated via an amide bond to either glycine or taurine before their being resecreted into the bile canaliculi. These conjugation reactions yield **glycoconjugates** and **tauroconjugates**, respectively. The bile canaliculi join with the bile ductules, which then form the bile ducts. Bile acids are carried from the liver through these ducts to the gallbladder, where they are stored for future use. The ultimate fate of bile acids is secretion into the intestine, where they aid in the emulsification of dietary lipids. In the gut the glycine and taurine residues are removed and the bile acids are either excreted (only a small percentage) or reabsorbed by the gut and returned to the liver. This process of secretion from the liver to the gallbladder, to the intestines and finally reabsorption is termed the **enterohepatic circulation**.

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Clinical Significance of Bile Acid Synthesis

Bile acids perform four physiologically significant functions:

- 1. their synthesis and subsequent excretion in the feces represent the only significant mechanism for the elimination of excess cholesterol.
- 2. bile acids and phospholipids solubilize cholesterol in the bile, thereby preventing the precipitation of cholesterol in the gallbladder.
- 3. they facilitate the digestion of dietary triacylglycerols by acting as emulsifying agents that render fats accessible to pancreatic lipases.
- 4. they facilitate the intestinal absorption of fat-soluble vitamins.

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