Environmental Medicine, 47, 2003 (page 1 ~ 11) **Review Article**

# The Involvement of 3β-hydroxysterol-$\Delta^{24}$-reductase, A Post-squalene Enzyme in Cholesterol Biosynthesis, in Desmosterolosis

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**Abstract:** As a structural component of mammalian cell membranes, cholesterol is essential for increasing the rigidity of the lipid bilayer and thus decreasing membrane fluidity and permeability. Cholesterol or its precursors are also essential for the synthesis of a wide variety of molecules that affect diverse physiological functions including nutrient absorption, vitamin balance, stress responses, reproductive function and osmoregulation. Hence, an elaborate regulatory system involving more than 100 genes has evolved to maintain the concentration of cholesterol within narrow limits. During the past 10 years, a number of defects in the late stages of the biosynthetic pathway have been associated with diseases. These diseases are linked with the accumulation of a variety of intermediate precursors which will in many cases at least partially compensate for the associated hypocholesterolemia. One such condition is desmosterolosis, an autosomal recessive disorder which results from mutations in the 3β-hydroxysterol-$\Delta^{24}$-reductase gene. The associated pathology underscores the essential roles of cholesterol not only in the maintenance of normal cell functioning but also during ontogeny. The discovery of this and other post-squalene genetic disorders provides a new tool to better understand the essential roles of cholesterol in homeostasis.

**Key words:** 3β-hydroxysterol-$\Delta^{24}$-reductase (DHCR24), desmosterolosis, cholesterol metabolism

## Introduction

It has been more than 70 years since the structure of cholesterol was first defined and considerable progress on understanding and elucidating its complex biosynthetic pathway (see Liscum, 2002 for review) has been made. However, scientific interest in cholesterol has not waned and a search on PubMed using "cholesterol" as a keyword yields almost 125 thousand citations while a search using "cholesterol and review" still produces more than 10 thousand references. The voluminous literature underscores the many critical roles that cholesterol plays in mammalian pre- and postnatal life. As a structural component of cell membranes, it is vital for altering gel-sol transitions and hence membrane fluidity and permeability as well as organizing microdomain structures. Furthermore, cholesterol, or its precursors, are substrates for the formation of steroid hormones, bile acids, oxysterols, meiosis activating sterols (MAS), vitamin D and a variety of non-sterol compounds. In addition, it is also required during ontogenesis for the maturation of the Hedgehog family of morphogens. Of medical interest, cholesterol is correlated with a number of disease states such as atherosclerosis, Alzeimer's and hypercholestenemia.

Since cholesterol biosynthesis is essential for normal development and maintenance of tissues, it is not surprising that relatively few mutations associated with loss of function have been identified in this pathway. Classically, the synthetic pathway has been divided into 2 parts according to the steps for sterol synthesis. The pre-sqalene pathway encompasses the synthesis of acetate to lanosterol (the first sterol produced), whereas, the post-sqalene pathway encompasses the path from lanosterol to cholesterol. The first and only recognized defect in the pre-squalene part of the pathway was identified in 1986 and is associated with mutations in the mevalonate kinase enzyme (see Waterham, 2002 for review) causing mevalonic aciduria. More recently, heritable human disorders associated with defects in various enzymes in the post-squalene pathway have been identified (Table 1: for reviews see Kelly and Herman, 2001; Waterham, 2002; Herman, 2003). These include Smith-Lemli-Opitz (SLO) syndrome (3β-hydroxysterol-$\Delta^{7}$-reductase), desmosterolosis (3β-hydroxysterol-$\Delta^{24}$-reductase), hydrop-ectopic calcification-moth-eaten (HEM) skeletal dysplasia (3β-hydroxysterol-$\Delta^{14}$-reductase), congenital hemidysplasia with ichtyosiform erythroderma and limb defects (CHILD) syndrome (3β-hydroxysteroid dehydrogenase), X-linked dominant chondrodysplasia (CDPX2: 3β-hydroxysterol-$\Delta^{8}$-$\Delta^{7}$-isomerase) and Antley-Bixler syndrome (lanosterol 14α-demethylase). In general, all of these conditions are associated with major malformations, dysmorphic

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Table 1 Inherited defects in the human cholesterol biosynthesis pathway.

| Syndrome | Enzyme defect | Gene (chromosome) | Accumulated product |
|---|---|---|---|
| Melvalonic aciduria | Mevalonate kinase | MVK (12q24) | Mevalonic acid |
| SLO syndrome | 3β-hydroxysterol Δ7 reductase | DHCR7 (11q13) | 7-dehydrocholesterol |
| Desmosterolosis | 3β-hydroxysterol Δ24 reductase | DHCR24 (1p31.1-p33) | Desmosterol |
| HEM skeletal dysplasia | 3β-hydroxysterol Δ14 reductase | LBR (1q42.1) | Cholesta-8,14-dien-3β and Cholesta-8,14,24-trien-3β-ol |
| CHILD (most cases) | 3β-hydroxysteroid dehydrogenase | NSDHL (Xq28) | 4-methyl sterols |
| CDPX2 | 3β-hydroxysterol-Δ8-Δ7-isomerase | EBP (Xp11.22-23) | Cholesta-8(9)-en-3β-ol and Cholesta-5,8(9)-dien-3β-ol |
| Antley-Bixler (some cases) | Lanosterol-14α-demethylase | CYP51 (7q21.2-21.3) | Lanosterol |

Nd: not determined

facial features and have the not unusual sequalae, of pre- or perinatal death. Indeed, murine models of SLO syndrome in which DHCR7 (Fitzky et al., 2001; Wassif et al., 2001) has been knocked out share many of the pathological symptoms expressed in the human. However, the pathology is attenuated due to higher levels of maternal transfer of cholesterol in murine species compared to the human (Woollett, 2001). To date, the pathogenesis underlying these polymalformation syndromes is not well understood. However, these newly discovered genetic lesions in sterol metabolism may provide valuable insights into the biochemical importance of cholesterol in normal cell function and in ontogeny.

This review will present an overview of one of these metabolic malformation syndromes caused by mutations in 3β-hydroxysterol-$\Delta^{24}$-reductase (DHCR24), namely desmosterolosis and examine some of the consequences of defects in DHCR24.

## Overview of the Cholesterol Synthetic Pathway

Cholesterol is synthesized by the isoprenoid pathway from acetate by a series of about 40 enyzmes which are compartmentalized in the cytoplasm, endoplasmic reticulum (ER) and/or peroxisome (Liscum, 2002). Lanosterol is the first sterol intermediate produced by condensation of squalene (C30). Subsequently, in a 19 step process in the post-squalene pathway, 9 enzymes catalyze 3 demethylations (C-14 and C-4 positions), 5 reductions, 1 desaturaturation and 1 isomerization reaction to produce cholesterol from lanosterol (Figure 1). Typically, the position of the C24 reduction by DHCR24 is placed as the last step in the pathway such that cholesta-7,24-dien-3β-ol is converted first to 7-dehydrodesmosterol then to desmosterol before the C24 reduction to form cholesterol. However, Bae and Paik (1997) have determined the Kcat of DHCR24 to be 3.3 for cholesta-7,24-dien-3β-ol, 1.1 for zymosterol, 1 for desmosterol and 0.2 for lanosterol. Hence, cholesta-7,24-dien-3β-ol is the preferred substrate and is more easily reduced than the other intermediates. As a consequence,

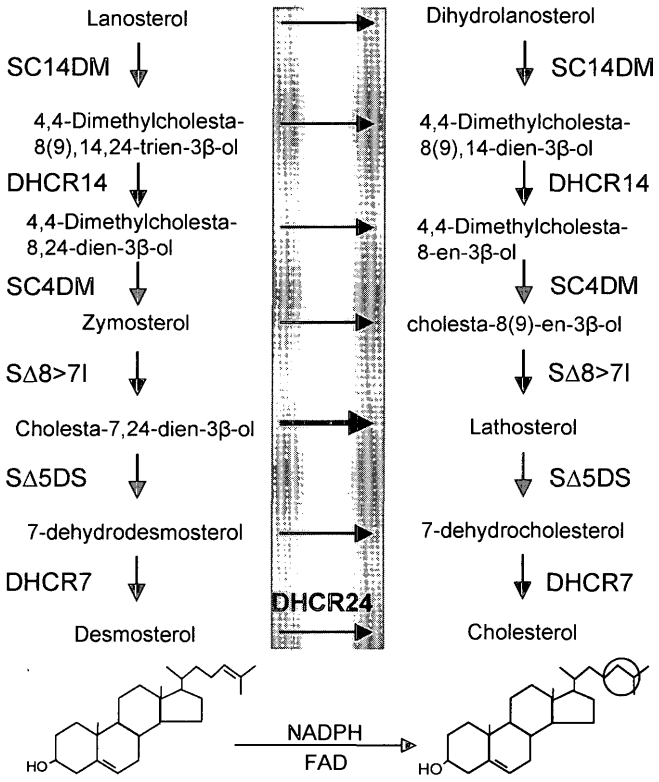


Fig. 1 Post-squalene biosynthesis of cholesterol. The structures of desmosterol and cholesterol are shown at the bottom of figure and represent 1 of the Δ24 reductions (shaded bar) catalyzed by DHCR24 (3β-hydroxysterol Δ24 reductase). The location of the reduced C24 is circled on cholesterol. The darker arrow indicates the preferred substrate for DHCR24. Other enzyme abbreviations are: SC14DM (3β-hydroxysterol C14 demethylase); DHCR14 (3β-hydroxysterol Δ14 reductase); SC4DM (3β-hydroxysterol C4 demethylase complex); SΔ8>7I (3β-hydroxysterol Δ8-Δ7 isomerase); SΔ5DS (3β-hydroxysterol Δ5 desaturase); and DHCR7 (3β-hydroxysterol Δ7 reductase).

under normal conditions the C24 reduction probably occurs using cholesta-7,24-dien-3β-ol as a substrate and thus, the reduction of the Δ7 C by DHCR7 is the last step to produce cholesterol. In support of this observation, inhibitors of DHCR7 (Gaoua et al., 2000) or the 2 knock out models of the DHCR7

gene (Fitzky et al., 2001; Wassif et al., 2001) result in the accumulation of 7-dehydrocholesterol. However, the accumulation of precursors at various steps in the pathway would also favour the reduction of C24 by DHCR24 despite having a lower substrate affinity. The Km and Kcat has not been assessed for all potential substrates in the pathway but it appears that DHCR24 has a relatively low specificity and functions at multiple steps within the pathway depending on substrate availability. Deficiency of DHCR24 will result in the accumulation of desmosterol and desmosterolosis (FitzPatrick et al., 1998; Waterham et al., 2001; Andersson et al., 2002).

DHCR24 was independently cloned in 3 separate studies: by Greeve et al. (2000) for its involvement in Alzheimer's Disease (AD); by us (Sarkar et al., 2001) for its involvement in adrenocortical adenomas; and by Waterham et al. (2001) for its involvement in desmosterolosis. The gene spanned 46,415 bp, contained 9 exons and was localized to chromosome 1p31.1-p33. The mRNA had an open reading frame (ORF) of 1,548 bp predicted to encode a protein of 60.1 kDa and which requires NADPH and FAD as cofactors for maximal activity.

## Regulation of DHCR24

Similar to many other enzymes in the cholesterol synthesis pathway, levels of DHCR24 are regulated by cholesterol concentration. The inclusion of the cholesterol lowering drugs, lovostatin and cholestyramine into rat diets led to a 40 fold induction of DHCR24 over the control diet (Bae and Paik, 1997). Furthermore, this induction was further enhanced by diurnal rhythm and a more than 120 fold induction was observed. This was the largest observed induction for any of the post-squalene enzymes and implies the presence of a sterol response element (SRE: discussed below and Figure 4) within the promoter region of the gene. Indeed, SREs can be localized to the promoter region of the DHCR24 gene in both humans and mice. However, the nucleotide sequences of SREs vary greatly (Edwards et al., 2000) and whether or not they are functional in DHCR24 awaits testing. However, DHCR7 is also inducible (5 fold) by cholesterol lowering drugs (Bae et al., 1999) and analysis of the promoter revealed multiple cis-acting elements including an active SRE (Kim et al., 2001). In view of the much greater induction (40 fold) of DHCR24 by sterol depletion, it is likely these elements are functional in the DHCR24 promoter. In addition, the studies of Sarkar et al. (2001) with forskolin and ACTH indicate that DHCR24 is activated by the PKA pathway.

DHCR24 is also regulated in a tissue specific fashion and during ontogeny. For example, during neural development, tissue levels of desmosterol as a percentage of total sterols are markedly elevated (Wassif et al., 2001) and this ratio decreases during maturation (Lutjohann et al., 2002). Similarly, desmosterol levels are concentrated in the membrane of spermatozoa and efflux of this desmosterol is required for final maturation (ie. capacitation) (Nimmo and Cross, 2003). Furthermore, a gradient of desmosterol concentration occurs within the epididymis (Lindenthal et al., 2002a). How the differential control of desmosterol levels is regulated within tissues and during ontogeny is not known.

## Desmosterolosis

Desmosterolosis was the second (SLO syndrome first) human malformation syndrome described affecting post-squalene sterol synthesis (FitzPatrick et al., 1998) and it was subsequently shown to arise from autosomal recessive mutations in the DHCR24 gene (Waterham et al., 2001). These mutations resulted in an accumulation of desmosterol in tissues. To date, only 2 patients with desmosterolosis have been reported, hence the phenotypic spectrum that may be presented remains yet to be defined. The first patient (46, XX) was delivered by Caesarean section due to fetal distress at 34 weeks and died within 1 h of delivery from respiratory complications (FitzPatrick et al., 1998). She had a profound increase in the desmosterol to cholesterol ratio, macrocephaly, craniofacial abnormalities including a hypoplastic nasal bridge, thickening of the alveolar ridges, cleft palate, hypoplastic lungs, renal hypoplasia, virilized genitalia, short limbs and generalized osteosclerosis. The second patient (46, XY) was born at 41 weeks and has survived to date (4 years). Post-natal analysis of plasma sterols indicated elevated levels of desmosterol but cholesterol was within the normal range (Andersson et al., 2002) Since dietary intake affects plasma sterols, an *in vitro* analysis was done. Culture of the boy's lymphoblasts in delipidated serum showed that levels of desmosterol were 260 fold higher than the control, whereas cholesterol levels were 0.7 fold of the control. The patient's pathological symptoms included microcephaly, agenesis of the corpus callosum, limb malformation, dysmorphic facial features and developmental delay. Waterham et al. (2001) determined that the expression of the 3 missense mutations in the first patient resulted in less than 1% of wild type DHCR24 activity in a yeast expression system. In agreement with the less severe phenotype in the boy, the mutated alleles expressed in yeast had 20% of the wild type activity. The parents of both cases had slightly elevated levels of desmosterol and each had mutated alleles consistent with the autosomal recessive mode of inheritance.

Since only 2 cases have been detected, desmosterolosis is a relatively rare disease compared to SLO syndrome (about 1 in 26,000 to 1 in 60,000). However, as reported by Nowaczyk et al. (2001) many mild cases of SLO are not detectable before the first birthday and prenatal losses due to developmental abnormalities may remain undiagnosed. As a result, Opitz (2001) has estimated that the homozygote frequency of SLO syndrome could be as high as 1 in 2500. In view of the more

recent discovery of desmosterolosis, the possible range of developmental defects and the lack of consanguinity amongst the 4 known heterozygotes, it is likely many more cases are undiagnosed. Furthermore, the observation that plasma desmosterol levels are highly variable among individuals with an estimated heritability of 28% (Berge et al., 2002) supports the likelihood that many more cases will be detectable by genetic or physiological testing.

## Teratogenesis Associated with Abnormal Cholesterol Metabolism

It was first noted in the 1960's that the administration of Triparanol (4-chloro-α-[4-[2-diethylamino] ethoxy] phenyl)-α-(4 methylphenyl) benzene ethanol), an inhibitor of DHCR24 into pregnant rats caused the accumulation of desmosterol, zymosterol, hypocholesterolemia and was highly teratogenic (Roux et al., 2000; Gofflot et al., 2003). The administration of Triparanol had no effect on embryonic mortality but induced frequent embryonic malformations which were not dissimilar to those observed in SLO syndrome and desmosterolosis. These included growth inhibition, limb abnormalities and holoproencephaly with facial dysmorphia, and microcephaly. Notably, the feeding of hypercholesterolemic diets reversed the effects of the induced teratogenicity, suggesting that either hypocholesterolemia or the accumulation of sterol intermediates was the causative factor (Gaoua et al., 2000). Since, cholesterol is known to modulate the activity of a family of cell signalling proteins, the Hedgehog (Hh) proteins which act to modulate growth, patterning and morphogenesis during embryonic ontogeny (Mann and Beachy, 2000; Rallu et al., 2002; Ruiz I Altaba et al., 2002a), it has been proposed that the teratogenic effects of cholesterol may be effected through Hh signalling pathways. In addition, various knock out mice have been produced in the Hh pathway which produce malformation phenotypes and a number of human disorders are linked with disruption of the Hh pathway (Bale, 2002).

The Hedgehog gene family is composed of Sonic (SHh), Indian (IHh) and Desert (DHh) which are expressed during development in a variety of tissues and play key roles in inductive interactions during differentiation. The signalling process involves establishment of a Hh morphoghen gradient from a localized Hh source and both short and longer range signalling (tens of cell diameters) to induce cell fates in a concentration dependent fashion. In general, SHh is expressed during embryogenesis in the head, posterior limb mesenchyme, epithelium of hair, lung, gut, bladder, urethra and vas deferens, whereas, IHh is expressed predominantly in the gut and chondrocytes of cartilage (Table 2). Desert Hh is associated with the development of the gonads, Schwann cells, vascular endothelium and endocardium. By virtue of its short range diffusibility, the Hhs appear to be critical for embryonic patterning. However, the expression of Hhs is not limited to embryogenesis. For example, diabetic neuropathy induced in maturing experimental rats is associated with a decrease in DHh and injection of a SHh fusion construct results in increases in peripheral nerve conduction velocity to control levels (Calcutt et al., 2003). In addition, Hh signalling has been implicated in tumour development and in stem cell differentiation (Ruiz I Altaba et al., 2002b).

The Hh proteins consist of a signal peptide that targets signal sequence cleavage in the secretory pathway. But more interestingly, the Hh precursor proteins undergo an autocatalytic internal cleavage due to the formation of a thioester link between glycine197 and cysteine 198 residues (Figure 2). This is followed by nucleophilic attack of the thioester bond by the C3 hydroxyl group of cholesterol. This autocleavage is facilitated by the C-terminus of the precursor and results in a 19 kDa N-terminal peptide covalently modified with cholesterol and a 26 kDa C-terminus peptide. Covalent modification of N-terminal peptide by the cholesterol moiety, limits its diffusion within the cell membrane and thus allows for local concentration in the Hh producing cell membranes. In addition, the N-terminus is further modified by palmitoylation in a process mediated by the Skinny Hh protein. It has been suggested that modified SHh forms multimers at lipid rafts with the hydrophobic lipid modifications projecting into the interior of the complex (Zeng et al., 2001). Such a structure may account for increased diffusibility and hence formation of a longer range morphogen gradient affecting patterning. In addition, since Hh becomes membrane anchored as a consequence of lipid modification, movement within the morphogen gradient is

Table 2 Human hedgehog (Hh) gene family and some of their functions.

| Family Member | Gene (chromosome) | Role |
|---|---|---|
| Sonic | SHH (7q36) | Cell proliferation (neural tissue, stem cells, gut, hair, muscle precursors) |
| | | Tissue patterning (neural tissue, somites, limbs,) |
| | | Organogenesis (lung, pituitary, pancreas, prostate, heart) |
| Indian | IHH (2q33-q35) | Cell proliferation (cartilage, gut, hematopoiesis) |
| | | Cell differentiation in endochondral skeleton |
| Desert | DHH (12q12q13.1) | Peripheral nerve sheath formation |
| | | Testes organogenesis |

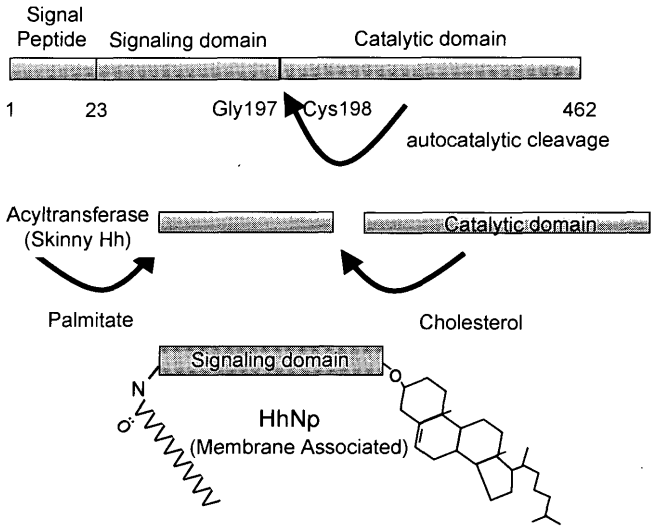


Fig. 2 Autocatalytic processing of human Sonic Hedgehog. The 462 amino acid precursor protein undergoes maturational steps to become bioactive. The signal peptide (1–23) is cleaved then the C terminal domain autocatalytically cleaves the precursor between Gly197 and Cys198 by trans-esterification which is coupled to addition of a cholesterol adduct. The N terminal domain is palmitoylated by Skinny hedgehog. Both lipids project from the same face of the signalling domain (HhNp) and interact with the cell membrane to restrict diffusion. Modification can also occur using desmosterol to attack the thioester bond but this will reduce the hydrophobicity of the signalling domain due to steric hinderence effects of the C24 double bond on van der Waals interactions within the lipid bilayer.

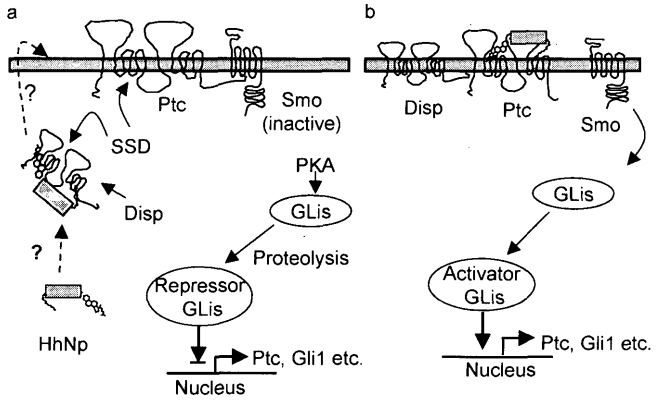


Fig. 3 Activation of cell signalling by hedgehog (Hh) producing cells.
a) Lipid modified Hh (HhNp) is translocated to the apical surface of the cell membrane. This movement from the endoplasmic reticulum to the cell membrane may involve the 12 transmembrane (tm) protein Dispatched (Disp) which is also required for the establishment of a long range signalling morphogen gradient. Both Disp and Patched (Ptc: the receptor for HhNp) contain sterol sensing domains (SSD) that may interact with cholesterol rich microdomains of the cell membrane (lipid rafts) to restrict their movement. In the absence of ligand binding the 12 tm receptor Ptc inhibits the 7 tm protein smoothened (Smo) and GLis are phoshorylated by protein kinase A (PKA). These are proteolytically cleaved to produce inhibitory Glis that act in the nucleus to suppress transcription of target genes.
b) Binding of HhNp to Ptc releases the inhibitory effect of Ptc on Smo and activator GLis are produced. Due to its lipid modification, diffusion of HhNp along the cell membranes from its site or origin is limited (autocrine/paracrine). To establish a longer range gradient over a distance of tens of cell diameters Disp is required but the mechanism is unknown. High levels of desmosterol in desmosterolosis may increase diffusional distance of Hh as well as destabilizing Disp/Ptc/Smo concentration in lipid rafts.

facilitated by the dispatched (Disp) protein (Kawakami et al., 2002). The molecular mechanism of movement is unresolved but notably Disp contains a sterol sensing domain (SSD) which will be discussed later.

Hedgehog signalling (Figure 3) is effected by binding to its receptor, Patched (Ptc). Patched is a 12 transmembrane domain that in the absence of Hh represses the 7 transmembrane protein Smoothened (Smo). Binding of Hh to Ptc removes the inhibition of Smo which in turn activates the Gli family of transcription factors (Ruiz I Altaba et al., 2002a). There are 3 Glis (Gli1, Gli2 and Gli3) and all are zinc finger containing transcription factors which in the absence of Hh signalling are cleaved by the proteosome and the C terminal fragment acts as dominant repressors. In the presence of Hh signalling, proteolysis is inhibited and full length activators of transcription are produced. These transcription factors interact in concert to affect cell function. For example, Hh signalling inhibits repressor formation by Gli3 but not by Gli2, whereas, Gli1 does not have a strong repressor function. In addition, neural patterning effects are affected differently by knock out of specific Gli. For example, in mice that lack Gli1 and Gli2, developmental effects are minimal, whereas, knock out of Gli3, results in abnormal brain development (Rallu et al., 2002).

A number of human disorders, including holoprosencephaly, Grieg cephalopolysyndactyly, Gorlin syndrome, sporadic basal-cell carcinoma and glioblastoma have been linked to disruption of the Hh pathway (Bale, 2002). Therefore, it is reasonable to propose that conditions that produce accumulation of aberrant sterols such as SLO syndrome and desmosterolosis may also be linked to disruption of the pathway. Indeed, other sterols including 7DHC and desmosterol could efficiently substitute for cholesterol as adducts in the autocatalytic processing of Shh in a cell line overexpressing SHh (Cooper et al., 1998). However, using a neural explant system and exogenous SHh, the authors determined that the repression of a dorsal plate marker, Pax7, by cholesterol modified SHh did not occur when distal inhibitors of cholesterol biosynthesis (AY9944 or Triparanol) were included in the culture medium. AY9944 and Triparanol induce accumulation of 7DHC and desmosterol, respectively. The latter sterols were presumably both active in SHh modification in cell culture but the modified SHh had no effect on the suppression of Pax7. This suggests that although a number of C27 sterol intermediates may act as adducts for SHh, the response of target tissues may be reduced. However, the direct effects of desmosterol or 7DHC modified SHh were not assessed.

It is notable however that the teratogenic effects of AY9944

in pregnant rats can be reversed by oral doses of cholesterol (Gaouna et al., 2000). Indeed, sterol depletion of CHO cells by compactin, sodium mevolonate (to inhibit sterol biosynthesis) and cyclodextrin (to complex hydrophobic compounds) resulted in decreased rates of autoprocessing and increased Shh precursor degradation (Guy, 2000). Cooper et al. (2003) reported that similar treatment of mouse embryonic fibroblasts (MEFs) lacking 7DHC reductase did not affect processing but did affect signalling. Furthermore, since total sterols (mainly 7DHC and cholesterol) inversely correlated with inhibition of signal responses, they proposed that 7DHC modified SHh may contribute to cell signalling. However, a competitive assay between cholesterol and 7DHC modified Hh on signalling remains to be done.

Associated with reduced function in DHCR24, there are dramatic changes in the sterol precursor to cholesterol ratio. In desmosterolosis, post-mortem analysis of the desmosterol to cholesterol ratio in the female patient was 0.309 in the liver compared to 0.0004–0.0029 in age-matched controls (FitzPatrick et al., 1998), whereas, in cultured lympoblasts from the surviving male patient the ratio was 0.7 compared to 0.002 in the controls (Andersson et al., 2002). Similarly, in rat fetuses on day 15 post-coitus and 6 days after triparanol treatment the ratio was 1.2 versus 0.09 in the controls (Gofflot et al., 2003). In the latter study, levels of zymosterol also increased from undetectable levels in the controls to a zymosterol to cholesterol ratio of 4.3 in the treatment group. It is open to speculation whether or not these precursor sterols would compete with cholesterol as adducts for Hh proteins. Clearly, cholesterol depletion results in diminished Hh signalling (Guy, 2000; Cooper et al., 1998, 2003; Gofflot et al., 2003) but whether or not there is some compensation via alternatively modified Hh is not clear. In this regard, the potency of SHh is enhanced by a wide variety of modifications that increase hydrophobicity at mainly the N- but also at the C-terminus (Taylor et al., 2001). Although the effects of a desmosterol adduct (C24 double bond) was not assessed, both lipid modified termini are known to project from the same face of the SHh molecule. Hence the decrease in hydrophobicity of a desmosterol adduct may subtly modify the anchoring of Hh to the membrane. In this regard, due to its C24 double bond increasing steric bulk (reduced van der Waals interaction with phospholipids tails) desmosterol is known to increase membrane fluidity and permeability which might in turn affect trafficking of the lipid modified SHh and downstream signalling.

How perturbations of lipid membrane physiology due to increased desmosterol content affecting membrane fluidity and permeability would affect morphogenic movements of Hh and downstream signalling is unknown. However, in triparanol treated rats, increased levels of sterol intermediates (mainly zymosterol and desmosterol) were associated with the loss of long range signalling of SHh (Gofflot et al., 2003) during embryonic limb development. Notably, the expression pattern of SHh in the zone of polarizing activity was much more diffuse than in the control embryos. This may suggest that increased levels of sterol intermediates may cause lipid perturbations to both SHh and the lipid bilayer which may affect movement of the ligand. In support of this, desmosterol is effluxed from cell membranes with about a 3 fold higher efficiency than cholesterol in the presence of HDL or other sterol acceptors (Phillips et al., 1998). Similarly, other intermediates such as zymosterol which rose to higher levels than desmosterol in the study of Gofflot et al. (2003) are also effluxed from cell membranes at a much greater rate than cholesterol (Lusa et al., 2003). Furthermore, it is notable that knockout of Dispatched (Disp) which seems to be required for both short and long range signal Hh signalling (Kawakami et al., 2002) produces a phenotype similar to Smo null mice (i.e. embryonic lethality at day 9.5 with cyclopia and holoprosencephaly). The authors also showed that Disp was not required for Hh synthesis or processing but was required for movement and establishment of a morphogen gradient from its site of synthesis in the notochord and along the floor plate of the neural tube. Both IHh and SHh had overlapping expression profiles with Disp. In addition, Disp and Ptc contain sterol sensing domains (SSDs) and are postulated to be associated with lipid rafts therefore, it is conceivable that changes in lipid bilayer dynamics may modulate their function.

## Sterol Sensing Domain Proteins

The best understood of the SSD containing proteins is SCAP (sterol regulatory element binding protein (SREBP) cleavage activating protein). SCAP is responsible for the proteolytic cleavage of SREBP which in turn regulates cellular cholesterol content. In cells deficient in SCAP, cells require exogenous cholesterol for growth (Rawson et al., 1999), whereas mutations in the SSD of SCAP renders the cell insensitive to the accumulation of cholesterol (Yabe et al., 2002a). Most data support the model proposed by Goldstein et al. (2002) where SCAP functions both as a membrane bound sterol sensor and as an escort protein for activation of SREBP (Figure 4). When cholesterol levels are adequate, SCAP complexes with-SREBP via interaction of their C-termini and the complex is retained in the ER by binding of the protein Insig-1 or Insig-2 to the region of the SSD of SCAP (Yabe et al., 2002b; Yabe et al., 2003). When cholesterol levels are low, there is a conformation change in this transmembrane region, Insig-1 is displaced and the SCAP-SREBP complex is translocated to the Golgi apparatus by common coat protein (COP)II coated vesicles (Espenshade et al., 2002). In the Golgi, cleavage of SCAP by site-1-protease (S1P) between transmembrane regions 6-7 produces the substrate for cleavage near the transmembrane region of SREBP by site-2-protease (S2P). These

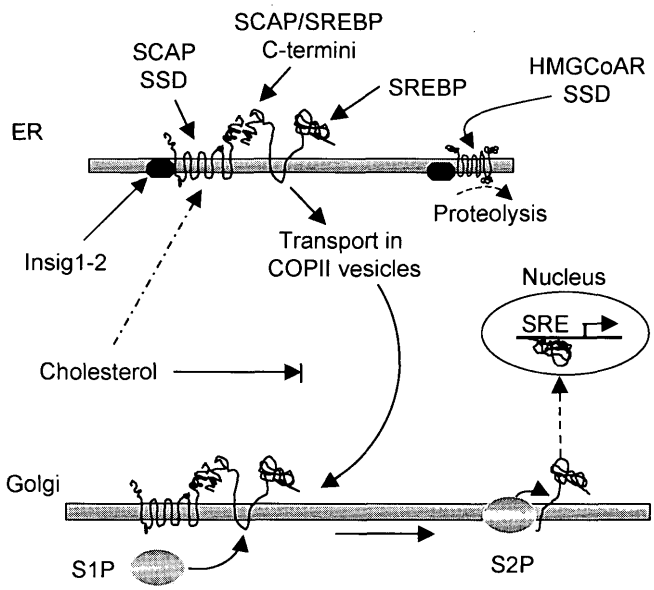


Fig. 4 Model of sterol sensing domain (SSD) containing proteins. In the endoplasmic reticulum (ER), the C-terminus of SCAP (sterol regulatory element binding protein (SREBP) cleavage activating protein) binds to the C-terminus of SREBP via 5 WD repeats. When cell cholesterol levels are adequate, the ER protein (either) Insig-1 or Insig-2 binds to SCAP in the region of the SSD and the complex is retained in the ER. When cholesterol levels decrease, the SSD responds by a conformational change within its 5 transmembrane (tm) region that results in dissociation of Insig from the complex. The SCAP/SREBP complex then moves from the ER to the Golgi by the classical secretory pathway in common coat protein (COP)II coated vesicles. In the Golgi, site-1 protease (S1P) cleaves SREBP in the intramembrane loop which provides the substrate for site-2 protease to cleave the N terminus 3 aa into the tm region. This releases the nuclear form of SREBP (helix-loop-helix transcription factor) which translocates to the nucleus and participates in the activation of target genes with SREs. The latter include many cholesterol biosynthetic enzymes including the rate limiting enzyme HMGCoAR (3-hydroxy-3-methylglutaryl coenzymeA reductase). Notably, HMGCoAR also contains a SSD. In the ER, it competes with SCAP for binding of Insig-1. However, whereas, high levels of cholesterol cause retention of SCAP/SREBP/Insig within the ER membrane, binding of Insig-1 to HMGCoAR causes rapid proteolysis of HMGCoAR within the ER.

cleavages release the transcriptionally active nuclear form of SREBP. In membrane preparations from sterol depleted cells overexpressing SCAP, 25 μM desmosterol was able to induce a similar conformational change as cholesterol (Brown et al., 2002). This suggests that the high levels of desmosterol associated with DHCR24 deficiency may compensate for cholesterol in inducing conformational changes in the SSD of SCAP, however the biological consequences remain unknown.

Other SSD containing proteins include the receptor for Hh signalling (Ptc), and movement (Disp), as well as, the rate limiting enzyme for cholesterol biosynthesis HMGCoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase. Understanding the role of the SSD in these proteins is not as advanced as SCAP and there are likely to be differences in the mechanism. For example, HMGCoA reductase is rapidly degraded when cells are replete with cholesterol. In the ER, both HMGCoA reductase and SCAP appear to compete for the same binding site of Insig-1 (Sever et al., 2003). However, binding of Insig-1 to HMGCoA reductase promotes its rapid proteolysis, whereas, binding to SCAP causes its retention within the ER. Why binding of Insig-1 to its 2 ligands causes such diverse consequences is not known.

Several genes related to cholesterol metabolism have been shown to contain SREs in their promoter and respond to cholesterol depletion. These include the rate limiting enzyme in cholesterol synthesis (HMG-CoA reductase) as well as HMG-CoA synthase, farnesyl-PP synthesase, squalene synthase and 7DHC (Kim et al., 2001). In addition, the rate limiting enzyme for steroiogenesis (StAR) and for receptor mediated uptake of LDL cholesterol are responsive to SREBP.

## DHCR24 and Apoptosis

DHCR24 was independently cloned in 2 studies for its association with aberrant cell functioning using differential mRNA display approaches. In the first, DHCR24 was selectively down regulated in the inferior temporal lobes compared with the sensorimotor cortex in Alzheimer diseased brains (Greeve et al., 2000). The authors named this cDNA of unknown function (at that time) seladin-1 (selective Alzheimer disease indicator 1). They found that over-expression of DHCR24 in cell lines conferred increased resistance to apoptosis induced by oxidative stress due to hydrogen peroxide or to β-amyloid peptides. Furthermore, since DHCR24 was shown to be a substrate for caspase 3 and 6, they proposed that it might be an important factor in protecting neurons from oxidative damage associated with neurodegeneration during the progression of Alzheimer's disease. Subsequent studies indicated that DHCR24 was down-regulated by about 30% in brain regions affected by Alzheimer's disease although there was no correlation with _-amlyoid accumulation (Iivonen et al., 2002). In addition, DHCR24 was up-regulated, albeit non-significantly, in a mouse neuroblastoma cell line induced to undergo apoptosis by okadaic acid. These data suggest that DHCR24 may contribute to the overall response to oxidative stress but the mechanism at this time requires further study. However, it is interesting that increased levels of DHCR24 are also associated with decreased apoptosis in adrenocortical adenomas (Sarkar et al., 2001) and the effects of experimentally induced neuropathy in rats is associated with a decrease in levels of DHh mRNA (Calcutt et al., 2003). Since treatment with SHh restored peripheral nerve conduction velocity and axonal caliber, this may suggest that the anti-apoptotic effects are also mediated through the cholesterol biosynthetic pathway and activation of Hh signalling.

## DHCR24 and the Adrenal

Using a differential display approach, Sarkar et al. (2001) identified DHCR24 in the adrenal of patients with Cushing's syndrome based on the subtraction between the adrenocortical adenoma and its adjacent atrophic tissue. The gene of unknown function at that time was named hDiminuto due to its similarity to the "diminuto-like protein" or dwarf-1, a cell elongation factor in *Arabidopsis thaliana*. DHCR24 was expressed about 3 fold higher in the adenoma compared to its adjacent non-tumourous tissue. The mRNA was detected in all layers of the cortex in normal adrenal tissue with most abundance in the zona fasiculata. This suggested a role in steroidogenesis and indeed, DHCR24 was inducible by forskolin in a time and concentration dependent fashion in H295 cells. Similarly, dexamethasone suppressed DHCR24 about 4 fold in rat adrenals and ACTH induced a time dependent increase to control levels suggesting that it might be involved in adrenal hyperplasia and/or steroidogenesis. Interestingly, in a microarray comparison of human fetal and adult adrenal glands, Rainey et al. (2001) identified DHCR24 as an EST that was expressed at 4 fold higher levels in the fetus than the adult and was one of the most highly expressed transcripts in the fetal adrenal gland (15–20 weeks). They further noted that the fetal adrenal has the greatest capacity to *de novo* synthesize cholesterol among all tissues and that 8 additional cholesterol synthetic genes were up-regulated. Since the fetal adrenal undergoes hyperplasia and the LDL receptor was also up-regulated, they proposed that cholesterol level may be rate limiting for fetal steroidogenesis. This may recapitulate the situation observed in Cushing's Syndrome by Sarkar et al. (2001).

Trophic hormones of pituitary origin are the main regulators of steroidogenesis and act in a biphasic manner. The acute phase which requires seconds to minutes involves increased mobilization of cholesterol to the mitochondria, whereas, the chronic phase, which requires hours to days involves increased transcription and translation of the enzymes involved in steroidogenesis. The rate limiting step appears to be substrate availability hence cholesterol transport across the mitochondrial membranes is critical. This process is mediated by steroidogenic acute regulatory protein (StAR). Two models have been proposed to explain how cholesterol may be transported from the outer (relatively cholesterol rich) to the inner (relatively cholesterol deficient) cell membrane of the mitochondrion (reviewed by Stauss et al., 2003). These involve either cholesterol desorption from the outer to the inner membrane or an intermembrane shuttle system. Since the transfer of substrate by StAR is rate limiting for steroidogenesis, it is rapidly upregulated in steroidogenic cells by trophic hormones acting through the cAMP signal transduction pathway. Twelve fold higher levels of heterogenous nuclear StAR RNA occur within 15 minutes of stimulation (Christenson et al., 2001b). In addition, the StAR promoter also contains an SRE that responds to low cholesterol levels. It is unclear at this time, whether or not the elevated levels of desmosterol during desmosterolosis would alter the kinetics of SCAP mediated SREBP activation. However, it is pertinent to note that the association of StAR with artificial membranes was enhanced by the inclusion of cholesterol in the lipid bilayer (Christensen et al., 2001a). They concluded that cholesterol enhanced membrane heterogeneity and that StAR undergoes a conformational change that favours binding to cholesterol rich membranes under the experimental conditions. Due to its effects on membrane permeability and more rapid exchange within lipid bilayers, desmosterol may change the way StAR binds to membranes and thus, change the rate at which sterols are translocated across the mitochondrial membranes. Such an effect would result in decreased steroidogenesis and possibly to the accumulation of lipids within steroidogenic cells.

The accumulation of lipids within steroid producing cells is observed in the autosomal recessive disease, lipoid congenital adrenal hyperplasia (CAH). CAH is caused by mutations in StAR which reduce cholesterol uptake into mitochondria resulting in impaired steroid hormone synthesis and a variable phenotype according to the severity of the loss of function mutation (Stocco, 2002). The condition is typified by adrenal glands that progressively accumulate sterols that eventually interfere with cell function. Without steroid replacement therapy, death occurs within days or weeks although less severe mutations have a less severe phenotype. A mouse model with knock out of StAR mimics this disease (Caron et al., 1997). Similar to humans, following birth, pups had feminized genitalia, low levels of corticosterone and aldosterone and elevated ACTH and CRH (due to lack of negative feedback). Lipid deposits accumulated in the adrenal and testes and most died shortly after birth unless rescued with exogenous glucocorticoids and saline. The gonads of females were less affected at birth but in rescued mice they progressively accumulated lipids and no follicles developed beyond the early antral stage (Ishii et al., 2002). As a result, both males and females are infertile.

Very limited measurement of steroids has been done in desmosterolosis patients. In the single surviving patient, serum aldosterone levels were low, whereas, plasma cortisol and testosterone were both within the normal range. However, these measurements were made at 3 years of age when the infant had normal levels of cholesterol (via dietary intake), thus no strong inferences can be drawn on whether or not defects in DHCR24 has an effect on steroidogenesis. The development of a mouse model for desmosterolosis would greatly facilitate the study the effects of desmosterol on StAR activity.

It is likely that desmosterol would also act as a substrate in steroidogenesis. Following transfer to inner cell membrane, the side chain of cholesterol is cleaved by cholesterol side-chain cleavage enzyme (P450scc) to produce pregnenolone

and isocaproaldehyde (4-methylpentanal). Since the latter is cytotoxic, it is catabolized to isocaproic acid and isocapryl alcohol by a member of the aldose-keto reductase superfamily, mouse vas deferens protein in human as well as murine adrenal cells (MVDP: Lefrancois-Martinez et al., 1999). The specificity of this enzyme for 4-methylpentanal with a double bond at C3 has not been tested but conceivably accumulation of this product of desmosterol side chain cleavage could be cytotoxic itself. Alternatively, due to its proximity to reactive oxygen species (ROS) associated with steroid biosynthesis, it may be autooxidized and either produce a more cytotoxic aldehyde or have an anti-apoptotic effect by protecting cells against ROS. In this context, desmosterol, itself, can undergo autooxidation in cell culture to produce a mixture of 24(R)- and 24(S)-25-epoxy-cholesterol (Saucier et al., 1990) which may have effects on cell physiology. Hence, both steroidogenic cells and fertility may be affected in desmosterolosis patients. Furthermore, it is notable that the human brain and especially the corpus callosum, expresses the entire steroidogenic pathway (Yu et al., 2002). Deficiency in neural steroidogenesis associated with desmosterolosis may further exacerbate any putative effects of Hh signalling on brain morphology.

Additional affects on development and fertility in desmosterolosis patients may involve meiosis activating sterols (MAS). These are a family of C29 4,4-dimethylsterols which, at least at pharmacological levels, can induce oocytes to resume meiosis (Byskov et al., 2002). The best studied MASs were isolated from human follicular fluid (FF) and named FF-MAS (4,4-dimethyl-5α-cholesta-8,14,24-triene-3β-ol) and the other was isolated from bull testes, T-MAS (4,4-dimethyl-5α-cholest-8,24-diene-3β-ol). Their synthesis appear to be regulated by physiological concentrations of progestins which act to inhibit DHCR24 resulting in the accumulation of desmosterol and FF- and T-MAS (Lindenthal et al., 2001b). A deficiency in DHCR24 would therefore be expected to result in the accumulation of cholesterol precursors some of which may result in the accumulation of MAS which in turn may inappropriately activate meiosis in gametes.

## Conclusion

Desmosterolosis is an autorecessive genetic disorder caused by defects in DHCR24 which results in the accumulation of desmosterol and deficiency in cholesterol levels. The pathology is associated with major malformations and dysmorphic facial features which suggest that disruption of the cholesterol biosynthetic pathway has teratogenic effects. The teratogenicity may arise as consequence of lack of cholesterol, accumulation of desmosterol and/or other sterol intermediates due to abnormal regulation and to abnormal Hedgehog signalling during development. Increased levels of desmosterol would affect the biophysical properties of cell membranes by increasing membrane fluidity and permeability and moreover affecting the mobility and signalling of sterol modified Hh morphogens which are critical for cell fate determination in many developmental processes. In addition, defects in DHCR24 may be associated with increased apoptosis in the brain and adrenal and also affect the synthesis of many other compounds for which cholesterol or its intermediates are required. To date, very few studies have focussed on the physiological effects of perturbations of DHCR24 on development or normal cell functioning. Therefore, the pathogenesis underlying desmosterolosis is not well understood. The development of a mouse model would greatly facilitate studies of function.

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Received July 16, 2003; accepted July 23, 2003