Tissue-specific transcriptional regulation of the cholesterol biosynthetic pathway leads to accumulation of testis meiosis-activating sterol (T-MAS)[1]

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Abstract Lanosterol 14α-demethylase (CYP51) produces follicular fluid meiosis-activating sterol (FF-MAS), which is converted further to testis meiosis-activating sterol (T-MAS). MAS are intermediates in the cholesterol biosynthetic pathway, with the ability to trigger resumption of oocyte meiosis in vitro. In contrast to the liver, where pre- and post-MAS genes are upregulated coordinately at the level of transcription by a cholesterol feedback mechanism through sterol regulatory element-binding proteins (SREBP), regulation differs in the testis. Genes encoding pre-MAS enzymes [HMG-CoA synthase (SYN), HMG-CoA reductase (RED), farnesyl diphosphate synthase (FPP), squalene synthase (SS), and CYP51] are upregulated during sexual development of the testis, although not all genes are turned on at the same time. Furthermore, two post-MAS genes, C-4 sterol methyl oxidase and sterol Δ^7-reductase, are expressed at low levels and are not upregulated either in rat or human. This transcriptional discrepancy seems to be SREBP independent. Besides cAMP/cAMP-responsive element modulator, other unknown transcription factors control expression of individual cholesterogenic genes during spermatogenesis. HPLC analysis shows an 8-fold increase in T-MAS during development of rat testis whereas MAS is barely detectable in livers of the same animals. We propose that the lack of a coordinate transcriptional control over the cholesterol biosynthetic pathway contributes importantly to overproduction of the signaling sterol T-MAS in testis.—Fon Tacer, K., T. B. Haugen, M. Baltsen, N. Debeljak, and D. Rozman. **Tissue-specific transcriptional regulation of the cholesterol biosynthetic pathway leads to accumulation of testis meiosis-activating sterol (T-MAS).** ***J. Lipid Res.*** **2002.** 43: **82–89.**

Supplementary key words cholesterol biosynthesis • cytochrome P450 • lanosterol 14α-demethylase • spermatogenesis and meiosis-activating sterol

Cholesterol is an essential constituent of cell membranes, with a crucial role in membrane function (1). It is also a precursor for bile acids and steroid hormones (2). Having indispensable roles on the one hand, while on the other hand being harmful in excess, the cholesterol level must be under precise control. Cholesterol homeostasis is maintained by a series of regulated pathways that control the acquisition of cholesterol from endogenous and exogenous sources, biosynthesis of cholesterol do novo, and elimination of cholesterol through conversion to bile acids and steroids (3). This homeostasis is regulated initially at the level of transcription by transcription factors of the sterol regulatory element-binding protein (SREBP) family (4). Mature SREBP is released from its membrane-bound precursor by a transport-dependent proteolytic cleavage (5). Cholesterol and oxysterols block this cleavage and regulate their own synthesis by a negative cholesterol feedback loop (6). In addition to cholesterol-dependent transcriptional regulation by SREBP, which is believed to be common to all involved genes, control at the posttranscriptional level also exists, at least in the case of HMG-CoA reductase (RED), which catalyzes the rate-limiting reaction of the mevalonate pathway (7). The quantity of RED is regulated at multiple levels (8), from transcription (9) to mRNA stabilization (10), translational efficiency (9, 11), reversible phosphorylation (12), and protein degradation (13).

In contrast to the liver, in haploid male germ cells

Abbreviations: CREM-τ, cAMP-responsive element modulator; EST, expressed sequence tag; FSH, follicle-stimulating hormone; FDS MAS, meiosis-activating sterol; FPP, farnesyl disphosphate synthase; RED, HMG-CoA reductase; SREBP, sterol regulatory element-binding protein; SS, squalene synthase; SYN, HMG-CoA sythase; T-MAS, testis meiosis-activating sterol.

[1] An article showing that the entire pathway of cholesterol synthesis is induced by SREBP in the liver has been published during the time that our manuscript was being revised (Sakakura, Y., H. Shimano, H. Sone, A. Takahashi, K. Inoue, N. Toyoshima, S. Suzuki, and N. Yamada. 2001. *Biochem. Biophys. Res. Commun.* **286:** 176–183).

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(spermatids) lanosterol 14α-demethylase (CYP51), one of the postlanosterol genes of cholesterol biosynthesis, seems to escape the SREBP-dependent regulation and is upregulated primarily by a cAMP/cAMP-responsive element modulator (CREM-τ) (14). Spermatids develop in male puberty after the second meiotic division in the process of sexual maturation (15). Elevated CYP51 mRNA expression is observed in step 2–3 round spermatids, remaining high until step 14 (16), leading to elevated levels of CYP51 protein (17) in the same stages as the mRNA is observed. This suggests no delay in translation of CYP51 mRNA. The translated CYP51 protein is functional, showing a higher CYP51 activity in germ cells of sexually mature animals compared with prepubertal animals (16). Elevated mRNA (16) and protein (17) levels have been observed during male germ cell development also for squalene synthase (SS), which is a prelanosterol enzyme of cholesterol biosynthesis. Translation of this protein might be time-delayed because a rise in mRNA was observed earlier (pachytene spermatocytes), as the protein was immunodetected (step 3 round spermatids). Even if expression of some early cholesterogenic genes and the corresponding enzymes seems to be elevated during male germ cell development, male germ cells are not efficient in synthesizing cholesterol. The rates of [^{14}C]acetate incorporation into cholesterol decreased in late pachynema remained low at all subsequent stages of meiosis, and were low in mature sperm (18). During capacitation, up to 40% of cholesterol is depleted from the plasma membrane of sperm cells in different mammalian species, because a relatively low cholesterol concentration in sperm cell membranes is prerequisite for successful fertilization (19). Thus, coupling the cholesterol efflux to activation of signaling events (20, 21) suggests low cholesterol synthesis in germ cells, whereas high expression of CYP51 and SS is observed (16, 22).

CYP51 is a member of the cytochrome P450 superfamily (http://drnelson.utmem.edu/CytochromeP450.html). It removes a C-32 methyl group at position 14α from lanosterol, forming follicular fluid meiosis-activating sterol (FF-MAS; 4,4-dimethyl-5α-cholesta-8,14,24-triene-3β-ol). FF-MAS is converted to testis meiosis-activating sterol (T-MAS; 4,4-di-methyl-5α-cholesta-8,24-diene-3β-ol) by sterol Δ^{14}-reductase (**Fig. 1A**). MAS were isolated from the follicular fluid and testis, and have roles not only in cholesterol biosynthesis but also in signaling, with the ability to trigger resumption of oocyte meiosis (23–25). It is well established that intermediates of the prelanosterol portion of the pathway are involved in biological processes other than cholesterol synthesis (production of heme A and dolichol, farnesylation of proteins, etc.). The postlanosterol portion has been considered to be devoted exclusively to cholesterol (7). The discovery of FF-MAS and T-MAS in gonads pointed to the existence of additional roles also for postlanosterol intermediates (23). However, the postlanosterol portion of cholesterol biosynthesis is not well understood. The precise sequence of reactions is difficult to determine because of the broad substrate specificity of most postlanosterol enzymes (Fig. 1A) (26, 27). Furthermore, sterol Δ^{14}-reductase, a crucial gene required for T-MAS production, has not yet been characterized, whereas Δ^{24}-reductase has been characterized (28).

We describe herein a detailed study of mRNA expression of pre- and postlanosterol cholesterogenic genes in the male gonad. We propose that differences in cholesterogenic gene expression contribute importantly to tissue-specific sterol profiles.

MATERIALS AND METHODS

HPLC assay of sterols

Sterols were separated by modification of a previously published method (29). Frozen testis was freeze-dried and lipids were extracted in 75% *n*-heptane-25% isopropanol (v/v). The organic phase was dried, reconstituted in mobile phase for HPLC straight-phase (SP) separation, and loaded onto a ChromSpher Si (5 μm, 250 × 4.6 mm; Varian, Palo Alto, CA) HPLC column running in (v/v) 99.65% *n*-heptane (BDH, Poole, UK)-0.35% isopropanol (J. T. Baker, Phillipsburg, NJ) at 1 ml/min. FF-MAS was read from SP chromatograms. The windows containing 4,4-dimethylsterols (window 2, 10 min–14 min), cholesterol and desmosterol (window 3, 23 min–26 min), and 5,7-ene sterols (window 4, 26 min 24 s–30 min) were collected automatically. Individual SP elution windows were dried and subjected to reversed-phase (RP) separation by reconstitution in acetonitrile and loading onto a LiChrospher RP (8.5 μm, 250 × 4.6 mm) HPLC column running in (v/v) 92.5% acetonitrile (Fisher Scientific, Pittsburgh, PA)-7.5% water at 1 ml/min, 40°C. All analytes were clearly identifiable on the basis of retention time (Fig. 1D, panel a) and UV absorption at 200–300 nm (Fig. 1D, panel b). Quantification was done by comparing the eluted peaks with runs of commercial standards lanosterol (L5768; Sigma, St. Louis, MO), 7-dehydrocholesterol (C3000; Steraloids, Newport, RI), desmosterol (C3150; Steraloids), and cholesterol (C6760; Steraloids) or laboratory standards [FF-MAS and T-MAS (29)]. Recovery was assessed by direct and transferred standards as explained previously (M. Baltsen, unpublished observations) and was between 70% and 130% for all analytes.

Preparation of probes

Mouse and human expressed sequence tag (EST) clones containing sequences related to late cholesterogenic genes were found after a BLAST search against the dbEST database (http://www.ncbi.nlm.nih.gov/blast). Clones containing potential mouse sterol Δ^7-reductase (I.M.A.G.E. ID 875323, AA475208); human Δ^7-reductase clone (I.M.A.G.E. ID 378803, AA683516), identical to human Δ^7-reductase cDNA (AF034544); mouse sigma-1 receptor (S1R; I.M.A.G.E. ID 570543, AA108525), similar to the fungal ERG2 sterol $\Delta^{8,7}$-isomerase cDNA (M74037); and mouse C-4 sterol methyl oxidase (4,4MO; I.M.A.G.E. ID 22082, AA106129) have been selected and obtained from the RZPD/Reference Library Database (30). Plasmid DNA was isolated by standard procedures (31) and inserts were excised by a double restriction with *Not*I and *Eco*RI or *Eco*RI and *Xho*I (4,4MO). All EST clones were sequenced by an automatic sequencer and ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Norwalk, CT). Plasmids containing partial mouse cDNA encoding RED (pmHMG-CoA Red/CRII) (32), HMG-CoA synthase (SYN) (pmHMG-CoA Syn/CRII) (32), and farnesyl diphosphate synthase (FPP) (pmFPP/CR2.1) (33) were received from the laboratory of J. L. Goldstein and M. S. Brown (Department of Molecular Genetics and Biochemistry, University of Texas South-

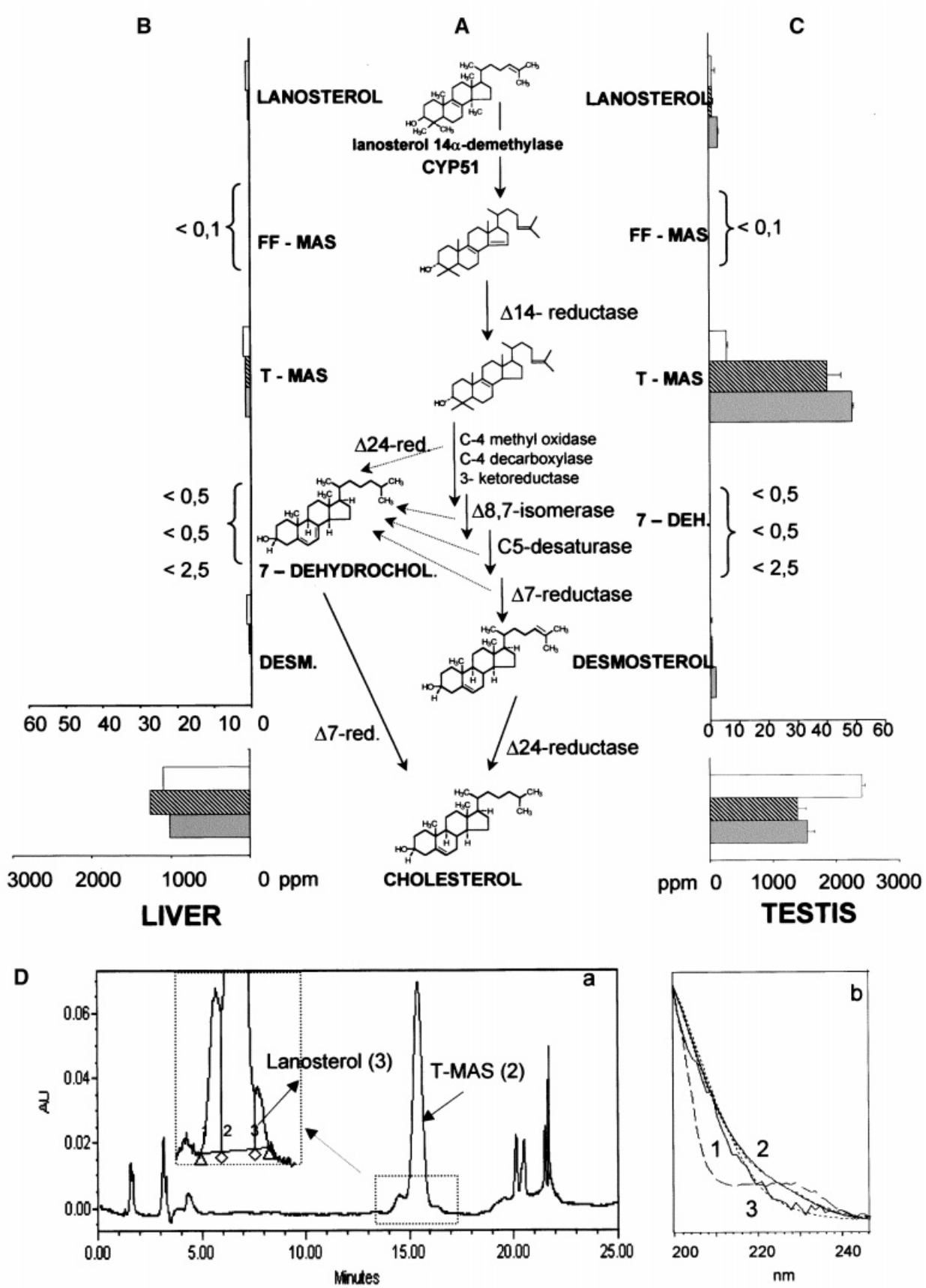

Fig. 1.

western Medical Center, Dallas, TX). Inserts were excised by double restriction with *Eco*RI.

Separation of human germ cells by elutrial centrifugation

Testicular material was obtained from organ donors in connection with donation of multiple organs after brain death according to a procedure approved by the local ethical committee. A cell suspension was prepared by treatment with trypsin and DNAse (34). Cells were further fractionated by centrifugal elutriation (35) into five fractions enriched in germ cells.

Northern analysis and quantification

Total RNA from SREBP transgenic mice liver was prepared as previously described (32, 36). Rat liver and testis were obtained from Sprague-Dawley rats (Møllegaard Laboratory, Ry, Denmark). Isolation of total RNA and Northern analysis were performed by standard procedures (31). ^{32}P-labeled cDNA probes were prepared with a Prime-It II random primer labeling kit (Stratagene, La Jolla, CA). Filters were hybridized for 2 h with QuikHyb® hybridization solution (Stratagene) and washed twice for 15 min at room temperature under nonstringent conditions (0.1% SDS-2× SSC; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). To detect 28S RNA signal, filters were exposed overnight after nonstringent washing. To detect specific probe signal only a stringent wash with 0.1% SDS-2× SSC at 60°C for 5–30 min followed. Autoradiograms were scanned and quantified by densitometry, using Un-Scan-I™ gel version 5.1 (Silk Scientific, Orem, UT). Blots were normalized to signals generated by cyclophilin mRNA (membranes from liver of SREBP transgenic mice) or 28S rRNA (membranes from testis and germ cells).

RESULTS

Expression of cholesterogenic genes during development of rat testis

To investigate mRNA expression of cholesterogenic genes in maturing germ cells, Northern blots were made from whole testis of rats 6, 10, 15, 20, 25, 30, 40, 50, 60, and 70 days old. Expression of pre-MAS genes (SYN, RED, FPP, SS, and CYP51) is upregulated during the sexual development of rats (**Fig. 2**). For RED (37), FPP (38), and CYP51 (16) the increase is due to the appearance of testis-specific transcripts that were observed also for SYN (Fig. 2C). Testis-specific transcripts of SYN, RED, and FPP arise earlier (30, 25, and 25 days, respectively; Fig. 2A and 2C) and increase gradually with time, in contrast to a sudden increase in the testis-specific transcript of CYP51 at day 40 (Fig. 2C). This is in accordance with higher levels of CYP51 mRNA in germ cells from 44-day-old rats compared with 32-day-old rats (16). There is a discrepancy between expression levels of CYP51 and 4,4MO, an enzyme catalyzing the first step of conversion of T-MAS toward cholesterol. 4,4MO is also barely detectable in adult testis (Fig. 2D). Similarly, Δ^7-reductase expression is low and declines with age, being highest in 19-day-old testis, decreas-

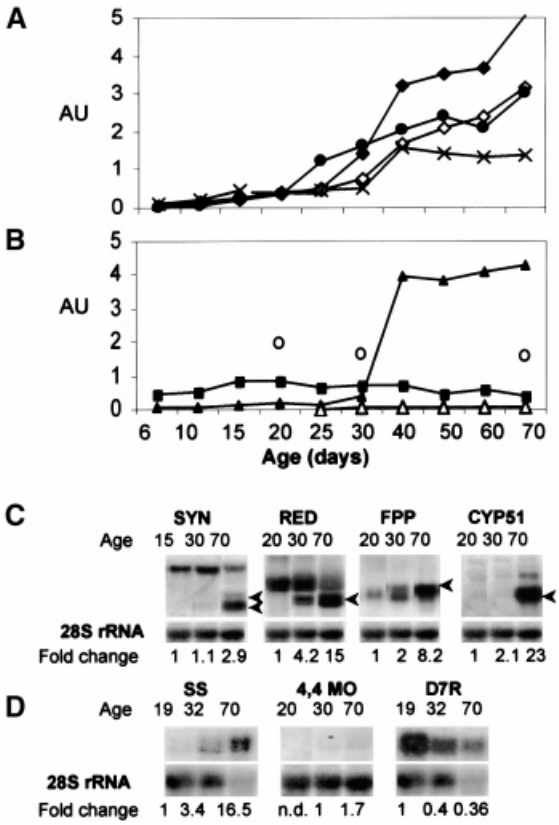

Fig. 2. Discordant regulation of cholesterogenic genes during development of rat testis. A: Expression of pre-MAS genes: SYN (multi sign), RED (solid diamonds), FPP (open diamonds), and SS (solid circles). B: Expression of CYP51 (solid triangles) and of post-MAS genes: 4,4MO (open triangles), Δ^7-reductase (solid squares), and S1R (open circles) in testes of rats age 6, 10, 15, 20, 25, 30, 40, 50, 60, and 70 days. Autoradiograms were normalized to 28S rRNA. C: Representative Northern blots showing expression of SYN, RED, FPP, and CYP51 in testes of 15- and 40-day-old rats. Arrowheads indicate testis-specific transcripts. D: Representative Northern blots showing developmental expression of SS, 4,4MO, and Δ^7-reductase in rat testis of various ages. Fold change in arbitrary units (AU). n.d., Not detectable.

Fig. 1. Postlanosterol part of cholesterol biosynthetic pathway (A) and measurement of sterols in liver (B) and in testis (C). After lanosterol is formed, two parallel pathways develop to produce cholesterol. A series of enzymatic reactions include C-14 demethylation (CYP51), two demethylations on C-4 (C-4 sterol methyl oxidase, C4-decarboxylase, and 3-keto reductase), reductions of Δ^{14}, Δ^{24}, and Δ^7 double bonds, a $\Delta^{8,7}$ isomerization, and a C-5–C-6 desaturation. Enzymes can use substrates with either a reduced or nonreduced Δ^{24} bond, leading to 7-dehydrocholesterol or desmosterol, both being ultimate precursors of cholesterol. Postlanosterol intermediates and cholesterol (different scale) were measured in liver (A) of 20-day-old rats (open bars), 30-day-old rats (hatched bars), and 70-day-old rats (shaded bars) and in testis (C) of 19-day-old rats (open bars), 32-day-old rats (hatched bars), and 70-day-old rats (shaded bars). Sterol values are expressed in micrograms of sterol per gram of tissue (wet weight) (parts per million, ppm). D: Identification of sterols by HPLC analysis. Windows 1–3 from all straight-phase injections were run separately on reversed phase (RP) in order to resolve the sterol analytes of interest, exemplified here by 4,4-dimethylsterol analysis. Panel a displays window 1 from a 19-day-old rat testis rechomatographed on RP. Peaks 2 (T-MAS) and 3 (lanosterol) are eluted over time with the respective standards. The UV spectra between 200 and 300 nm (solid lines, panel b) were confluent with corresponding standards (dotted lines). Eluent 1 is an unidentified component easily distinguishable from 4,4-dimethylsterols by UV absorption pattern (panel b). Other analyzed sterols from other SP windows were identified similarly.

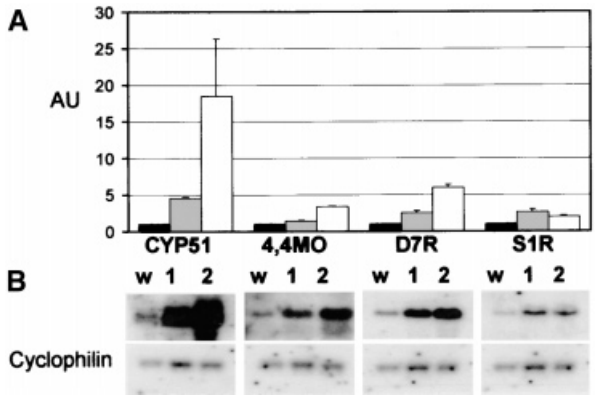

Fig. 3. Postlanosterol cholesterol biosynthetic genes are upregulated by SREBP-1 and SREBP-2 in the liver. A: Expression of CYP51, 4,4MO, Δ^7-reductase, and S1R mRNAs in liver of SREBP-1a (shaded columns) and SREBP-2 (open columns) transgenic mice compared with wild type (solid columns). Autoradiograms were normalized to cyclophilin. Fold upregulation in arbitrary units (AU). B: Representative Northern blots. Lane w, wild type; lane 1, SREBP-1a; lane 2, SREBP-2.

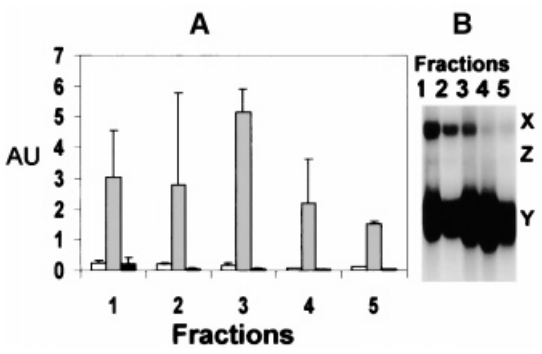

Fig. 4. Expression of two postlanosterol genes, CYP51 and Δ^7-reductase, during spermatogenesis in humans. A: Expression of somatic CYP51 transcript (open columns), testis-specific CYP51 transcript (shaded columns), and Δ^7-reductase (solid columns) in different fractions of human male germ cells that were separated by elutrial centrifugation. Fractions 1 and 2 are 75–90% enriched in haploid cells, fraction 1 containing primarily elongated and elongating spermatids and fraction 2 containing round and elongating spermatids. Fraction 3 is a mixture of round spermatids and blood cells, whereas fraction 4 consists of spermatids (mostly binucleated), secondary spermatocytes, interstitial cells, spermatogonia, and blood cells. Fraction 5 is enriched in primary spermatocytes (approximately 50%) contaminated with multinucleated spermatids and Sertoli cells. Autoradiograms were normalized to 28S rRNA. Results are the means with standard deviations of two separate experiments from two individuals. AU, Arbitrary units. B: Representative Northern blot showing hybridization with identical amounts (cpm/μg) of CYP51 and Δ^7-reductase labeled probes. x, CYP51 somatic transcript; y, CYP51 testis-specific transcript; z, Δ^7-reductase. Note the difference in expression level of the two genes.

ing to 60% by day 32 and even more (63%) in 70-day-old testis (Fig. 2B and D). Expression of S1R does not change significantly with the age of the testis (Fig. 2B).

To evaluate whether mouse EST clones that have been used as probes in expression studies of the developing rat testis indeed represent cholesterogenic genes, RNA from livers of wild-type and SREBP-1a (32), and SREBP-2 (36) transgenic mice was subjected to Northern analysis. CYP51 and three additional genes potentially involved in the postsqualene portion of cholesterol biosynthesis [4,4MO, S1R (potential sterol $\Delta^{8,7}$-isomerase) and Δ^7-reductase] have been tested. **Figure 3** shows that CYP51, 4,4MO, and Δ^7-reductase are upregulated to a greater extent by SREBP-2 compared with SREBP-1. Such a pattern is characteristic for all genes involved in cholesterol biosynthesis (32, 36). S1R showed a weak increase of expression in SREBP-1a transgenic animals and an even lower increase in SREBP-2 transgenic livers, suggesting it is not a cholesterogenic gene.

Expression of late cholesterogenic genes during spermatogenesis in humans

Germ cell development in human seminiferous tubules occurs in a spiral arrangement with cells in different stages of development in close contact (15). In contrast to that, rodent germ cells mature in a wavelike fashion, allowing isolation of cells at a single stage (39). Because of these differences, it is important to determine whether phenomena observed during sexual maturation of rodents are also conserved in humans. We isolated RNAs from the germ cells of two organ donors, transferred them to a membrane, and monitored the relative expression levels of two late cholesterogenic genes, CYP51 and Δ^7-reductase. Membranes were hybridized at the same time with both probes having the same specific activity (2×10^6 cpm/μg). Expression of human Δ^7-reductase is up to 10-fold lower as compared with expression of CYP51 (**Fig. 4**), which is in accordance with results obtained in rats. A combination of in situ hybridization and Northern analysis studies of CYP51 mRNA expression during spermatogenesis in rats (16) and in mice (14) has indicated high level expression of the shortest CYP51 transcripts only in haploid germ cells (round and elongating spermatids). On the basis of similarities in the expression pattern of human, mouse, and rat CYP51 mRNAs and the conserved regulatory elements in the promoters of these genes (40), it is likely that the shortest CYP51 transcripts also appear in haploid germ cells of humans.

Quantification of cholesterol and of late intermediates of the cholesterol biosynthesis pathway in liver and testis of rats

Cholesterol and sterols of the postlanosterol part of the biosynthetic pathway were measured in the liver, as the main organ of cholesterol homeostasis, and in the testis, as the organ where the presence of T-MAS has been described (23, 24). As seen in Fig. 1B, cholesterol is the main product in livers of rats at three different ages. The amount of cholesterol does not vary significantly with the age of normally fed animals. Intermediates of cholesterol biosynthesis are barely detectable in the liver. In contrast to the liver, the amount of testis cholesterol (Fig. 1C) decreased by up to 43% with increasing animal age and sexual maturation of the testis, from 2,415 ppm in 19-day-old rats, to 1,382 and 1,544 ppm in 32- and 70-day-old rats, respectively. Detection of the postlanosterol intermediate T-MAS

in testis presents another difference from the liver. An 8-fold increase in T-MAS is observed with increasing age, being 6.1 ppm in 19-day-old rats, 40.3 ppm in 32-day-old rats, and 49.0 ppm in 70-day-old rats. Other intermediates were observed in much lower amounts, suggesting that T-MAS is indeed the dominant precholesterol sterol of the male gonad (Fig. 1C).

DISCUSSION

Our data provide evidence that male germ cells lack a coordinate transcriptional control over the cholesterol biosynthetic pathway. The tissue-specific expression of pre-MAS and post-MAS genes is the primary regulatory checkpoint that leads to accumulation of the meiosis-signaling sterol T-MAS during maturation of male germ cells. It seems possible that transcription factors of the SREBP family are not involved in upregulation of cholesterogenic genes in male germ cells. In the liver, where regulation of cholesterol homeostasis takes place, a coordinate SREBP-dependent transcriptional upregulation of all involved genes does exist (33, 36). In addition to early cholesterogenic genes (32, 36), CYP51, 4,4MO, and Δ^7-reductase are also upregulated primarily by SREBP-2 and to a lower extent by SREBP-1 (Fig. 3). In both liver and epidermis (41), regulation of cholesterol biosynthesis seems to follow the cholesterol feedback loop. No coordinate transcriptional upregulation is observed in male germ cells, where mobility shift fails to show the presence of SREBP in nuclear extracts (14). In mature 70-day-old rat testis, the expression of early cholesterogenic genes that represent steps in cholesterol biosynthesis leading to synthesis of MAS (SYN, RED, FPP, SS, and CYP51; Fig. 2) is much higher as compared with expression of late cholesterogenic genes encoding enzymes responsible for bioconversion of MAS toward cholesterol (4,4MO and Δ^7-reductase). A direct comparison of CYP51 and Δ^7-reductase expression levels in human spermatogenesis shows the same discrepancy (Fig. 4). In addition to differences in expression levels, the trend in expression of pre-MAS and post-MAS genes differs during maturation of the testis, that is, expression of pre-MAS genes is upregulated whereas expression of post-MAS genes is unchanged or even downregulated with increasing age of the animals (Fig. 2). As in germ cells, transcriptional regulation of cholesterol biosynthesis also seems to be independent of cholesterol levels in endocrine tissues under conditions of increased steroid hormone synthesis. Orphan nuclear receptor steroidogenic factor 1 is able to overcome the negative cholesterol feedback loop and upregulates transcription of SYN also in the cholesterol well-fed state (42).

The SREBP-independent transcriptional upregulation of genes involved in cholesterol homeostasis acquired the term "sterol-independent," but molecular mechanisms leading to such regulation seem to differ with each individual gene. Nonsterol stimuli, such as onkostatin M (43), and phorbol esters and cycloheximide (44), induce transcription of the LDL receptor gene through distinct promoter elements that do not require the presence of SREBP. Cholesterogenic CYP51 is upregulated in male germ cells (16) by a CREM-τ-dependent mechanism (14). CREM-τ is a master activator of cAMP-dependent gene expression in haploid male germ cells (45). However, CREM-τ is not a master activator of cholesterogenic gene expression in spermatogenesis. In addition to CYP51, CREM-τ might upregulate SYN and FPP, which both show increased expression on days 30–40 of testis development, when haploid germ cells appear (Fig. 2A) (15), but does not seem to control RED and SS expression, which are increased already at the spermatocyte stage (Fig. 2A) or are not diminished in testis of CREM$^{-/-}$ animals (14). In addition, CREM-τ does not control 4,4MO and Δ^7-reductase, which remain expressed at low levels during testis development (Fig. 2B).

A lack of coordinate transcriptional regulation and a discrepancy between pre-MAS and post-MAS gene expression indicate that the primary role of male germ cells may not be to synthesize cholesterol but to produce MAS. The HPLC profile shows a correlation between the quantity of sterol intermediates in maturing rat testis and the expression level of genes encoding enzymes of the cholesterol biosynthetic pathway. Increasing amounts of T-MAS are detected with increasing testis age, being 8-fold higher in sexually mature, 70-day-old rats as compared with immature, 19-day-old rats. T-MAS is able to trigger meiotic progression of the oocyte ex vivo (23, 46) but the in vivo role of T-MAS synthesized by testis remains to be demonstrated. Most mature spermatids of the testis have the potential to synthesize MAS in situ because of the expression of the CYP51 system in the acrosome (17). However, an MAS-specific receptor has not yet been found (47). Data suggest that the MAS receptor is not a nuclear-type receptor but rather an oocyte plasma membrane-associated protein with high affinity for FF-MAS (48), and that a G protein-coupled mechanism is involved in MAS signaling (46).

Our data suggest that accumulation of T-MAS is achieved primarily by regulation at the level of transcription, where genes encoding pre-MAS enzymes are upregulated during male germ cell development and genes encoding post-MAS enzymes are not. We propose that the highly expressed CYP51 mRNA that is translated into the functionally active lanosterol 14α-demethylase protein (16, 17) produces FF-MAS, which is readily converted to T-MAS by the sterol Δ^{14}-reductase. The sterol Δ^{14}-reductase gene, which is not yet characterized in mammals, is likely highly expressed in the testis. The absence of immediate metabolites of T-MAS in the testis suggests that insufficient amounts of the 4,4MO enzyme are produced from the observed low level of 4,4MO mRNA. Besides transcriptional regulation, regulation at posttranscriptional levels, such as enzyme inhibition (49, 50), may also be important in the production of MAS. Development of a tissue-specific transcriptional regulatory system to produce a cholesterol biosynthetic pathway intermediate in the male gonad suggests that T-MAS has an important physiological role. It remains to be elucidated whether T-MAS contributes to completion of the second meiotic division of the oocyte

also in vivo or is involved in other, yet unknown signaling processes.

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REFERENCES

1. Simons, K., and E. Ikonen. 2000. How cells handle cholesterol. *Science.* **290:** 1721–1726.
2. Yokoyama, S. 2000. Release of cellular cholesterol: molecular mechanism for cholesterol homeostasis in cells and in the body. *Biochim. Biophys. Acta.* **1529:** 231–244.
3. Repa, J. J., S. D. Turley, J. A. Lobaccaro, J. Medina, L. Li, K. Lustig, B. Shan, R. A. Heyman, J. M. Dietschy, and D. J. Mangelsdorf. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science.* **289:** 1524–1529.
4. Brown, M. S., and J. L. Goldstein. 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell.* **89:** 331–340.
5. Russell, A., R. A. DeBose-Boyd, M. S. Brown, W. P. Li, A. Nohturfft, J. L. Goldstein, and P. J. Espenshade. 1999. Transport-dependent proteolysis of SREBP: relocation of site-1 protease from Golgi to ER obviates the need for SREBP transport to Golgi. *Cell.* **99:** 703–712.
6. Nohturfft, A., R. A. DeBose-Boyd, S. Scheek, J. L. Goldstein, and M. S. Brown. 1999. Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmatic reticulum and Golgi. *Proc. Natl. Acad. Sci. USA.* **96:** 11235–11240.
7. Goldstein, J. L., and M. S. Brown. 1990. Regulation of the mevalonate pathway. *Nature.* **343:** 425–430.
8. Ness, G. C., and C. M. Chambers. 2000. Feedback and hormonal regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase: the concept of cholesterol buffering capacity. *Proc. Soc. Exp. Biol. Med.* **224:** 8–19.
9. Ness, G. C., S. Eales, D. Lopez, and Z. Zhao. 1994. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression by sterols and nonsterols in rat liver. *Arch. Biochem. Biophys.* **308:** 420–425.
10. Simonet, W. S., and G. C. Ness. 1989. Post-transcriptional regulation of 3-hydroxy-3-methylglutaryl-CoA reductase mRNA in rat liver. Glucocorticoids block the stabilization caused by thyroid hormones. *J. Biol. Chem.* **264:** 569–573.
11. Chambers, C. M., and G. C. Ness. 1997. Translational regulation of hepatic HMG-CoA reductase by dietary cholesterol. *Biochem. Biophys. Res. Commun.* **232:** 278–281.
12. Scallen, T. J., and A. Sanghvi. 1983. Regulation of three key enzymes in cholesterol metabolism by phosphorylation/dephosphorylation. *Proc. Natl. Acad. Sci. USA.* **80:** 2477–2480.
13. Ravid, T., R. Doolman, R. Avner, D. Harats, and J. Roitelman. 2000. The ubiquitin-proteasome pathway mediates the regulated degradation of mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* **275:** 35840–35847.
14. Rozman, D., M. Fink, G. M. Fimia, P. Sassone-Corsi, and M. R. Waterman. 1999. Cyclic adenosine 3′,5′-monophosphate (cAMP)/cAMP-responsive element modulator (CREM)-dependent regulation of cholesterogenic lanosterol 14α-demethylase (CYP51) in spermatids. *Mol. Endocrinol.* **13:** 1951–1962.
15. Russell, L. D., R. A. Ettlin, A. P. Sinha Hikim, and E. D. Clegg. 1990. Histological and Histopathological Evaluation of the Testis. Choche River Press, Clearwater, FL.
16. Strömstedt, M., M. R. Waterman, T. B. Haugen, K. Taskén, M. Parvinen, and D. Rozman. 1998. Elevated expression of lanosterol 14α-demethylase (CYP51) and the synthesis of oocyte meiosis-activating sterols in postmeiotic germ cells of male rats. *Endocrinology.* **139:** 2314–2321.
17. Majdič, G., M. Parvinen, A. Bellamine, H. J. Harwood, Jr., W. W. Ku, M. R. Waterman, and D. Rozman. 2000. Lanosterol 14α-demethylase (CYP51), NADPH-cytochrome P450 reductase and squalene synthase in spermatogenesis: late spermatids of the rat express proteins needed to synthesize follicular fluid meiosis activating sterol. *J. Endocrinol.* **166:** 463–474.
18. Potter, J. E., C. F. Millette, M. J. James, and A. A. Kandutsch. 1981. Elevated cholesterol and dolichol synthesis in mouse pachytene spermatocytes. *J. Biol. Chem.* **256:** 7150–7154.
19. Flesch, F. M., and B. M. Gadella. 2000. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. *Biochim. Biophys. Acta.* **1469:** 197–235.
20. Visconti, P. E., X. Ning, M. W. Fornes, J. G. Alvarez, P. Stein, S. A. Connors, and G. S. Kopf. 1999. Cholesterol efflux-mediated signal transduction in mammalian sperm: cholesterol release signals an increase in protein tyrosine phosphorylation during mouse sperm capacitation. *Dev. Biol.* **214:** 429–443.
21. Iborra, A., M. Companyo, P. Martinez, and A. Morros. 2000. Cholesterol efflux promotes acrosome reaction in goat spermatozoa. *Biol. Reprod.* **62:** 378–383.
22. Strömstedt, M., D. Rozman, and M. R. Waterman. 1996. The ubiquitously expressed human CYP51 encodes lanosterol 14α-demethylase, a cytochrome P450 whose expression is regulated by oxysterols. *Arch. Biochem. Biophys.* **329:** 73–81.
23. Byskov, A. G., C. Y. Andersen, L. Nordholm, H. Thogersen, G. Xia, O. Wassman, J. V. Andersen, E. Guddal, and T. Roed. 1995. Chemical structure of sterols that activte oocyte meiosis. *Nature.* **374:** 559–562.
24. Byskov, A. G., M. Baltsen, and C. Y. Andersen. 1998. Meiosis-activating sterols: background, discovery, and possible use. *J. Mol. Med.* **76:** 818–823.
25. Edwards, P. A., and J. Ericsson. 1999. Sterols and isoprenoids: signaling molecules derived from cholesterol biosynthetic pathway. *Annu. Rev. Biochem.* **68:** 157–185.
26. Waterham, H. R., and R. J. Wanders. 2000. Biochemical and genetic aspects of 7-dehydrocholesterol reductase and Smith-Lemli-Opitz syndrome. *Biochim. Biophys. Acta.* **1529:** 340–356.
27. Bae, S. H., and Y. K. Paik. 1997. Cholesterol biosynthesis from lanosterol: development of a novel assay method and characterization of rat liver microsomal lanosterol Δ^{24}-reductase. *Biochem. J.* **326:** 609–616.
28. Waterham, H. R., J. Koster, G. J. Romeijn, R. C. Hennekam, P. Vreken, H. C. Andersson, D. R. FitzPatrick, R. I. Kelley, and R. J. Wanders. 2001. Mutations in the 3β-hydroxysterol Δ^{24}-reductase gene cause desmosterolosis, an autosomal recessive disorder of cholesterol biosynthesis. *Am. J. Hum. Genet.* **69:** 685–694.
29. Baltsen, M., and A. G. Byskov. 1999. Quantitation of meiosis activating sterols in human follicular fluid using HPLC and photodiode array detection. *Biomed. Chromatogr.* **13:** 382–388.
30. Lennon, G. G., C. Auffray, M. Polymeropoulos, and M. B. Soares. 1996. The I.M.A.G.E. Consortium: an integrated molecular analysis of genomes and their expression. *Genomics.* **33:** 151–152.
31. Sambrook, J., E. F. Fritsch, and T. M. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
32. Shimano, H., J. Horton, R. E. Hammer, I. Shimomura, M. S. Brown, and J. L. Goldstein. 1996. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J. Clin. Invest.* **98:** 1575–1584.
33. Shimano, H., I. Shimomura, R. E. Hamer, J. Herz, J. L. Goldstein, and M. S. Brown. 1997. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for the targeted disruption of the SREBP-1 gene. *J. Clin. Invest.* **100:** 2115–2124.
34. Meistrich, M. L. 1977. Separation of spermatogenic cells and nuclei from rodent testes. *Methods Cell Biol.* **15:** 15–54.

35. Blanchard, Y., M. T. Lavault, D. Quernee, D. Le Lannou, B. Lobel, and D. Lescoat. 1991. Preparation of spermatogenic cell populations at specific stages of differentiation in the human. *Mol. Reprod. Dev.* **30:** 275–282.
36. Horton, J. D., I. Shimomura, M. S. Brown, R. E. Hammer, J. L. Goldstein, and H. Shimano. 1998. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J. Clin. Invest.* **101:** 2331–2339.
37. Ness, G. C., and S. J. Nazian. 1992. Developmental expression of multiple forms of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in rat testes. *J. Androl.* **13:** 318–322.
38. Teruya, J. H., E. C. Salido, P. A. Edwards, and C. F. Clarke. 1991. Testis-specific transcripts of rat farnesyl pirophosphate synthetase are developmentally regulated and localized to haploid germ cells. *Biol. Reprod.* **44:** 663–671.
39. Parvinen, M., and T. Vanha-Perttula. 1972. Identification and enzyme quantitation of the stages of the seminiferous epithelial wave in the rat. *Anat. Rev.* **174:** 435–450.
40. Debeljak, N., S. Horvat, K. Vouk, M. Lee, and D. Rozman. 2000. Characterization of the mouse lanosterol 14α-demethylase (CYP51), a new member of the evolutionarily most conserved cytochrome P450 family. *Arch. Biochem. Biophys.* **379:** 37–45.
41. Harris, I. R., A. M. Farrell, C. Grunfeld, W. M. Holleran, P. M. Elias, and K. R. Feingold. 1997. Permeability barrier disruption coordinately regulates mRNA levels for key enzymes of cholesterol, fatty acid, and ceramide synthesis in the epidermis. *J. Invest. Dermatol.* **109:** 783–787.
42. Mascaro, C., A. Nadal, F. G. Hegardt, P. F. Marrero, and D. Haro. 2000. Contribution of steroidogenic factor 1 to the regulation of cholesterol synthesis. *Biochem. J.* **350:** 785–790.
43. Liu, L., D. M. Scolnick, R. C. Trievel, H. B. Zhang, R. Marmorstein, T. D. Halazonteis, and S. L. Berger. 1999. p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage. *Mol. Cell. Biol.* **19:** 1202–1209.
44. Makar, R. S., P. E. Lipsky, and J. A. Cuthbert. 2000. Multiple mechanisms, independent of sterol regulatory element binding proteins, regulate low density lipoprotein gene transcription. *J. Lipid Res.* **41:** 762–774.
45. Sassone-Corsi, P. 1997. Transcriptional checkpoints determining the fate of male germ cells. *Cell.* **88:** 163–166.
46. Grondahl, C., M. Lessl, I. Faerge, C. Hegele-Hartung, K. Wassermann, and J. L. Ottesen. 2000. Meiosis-activating sterol-mediated resumption of meiosis in mouse oocytes in vitro is influenced by protein synthesis inhibition and cholera toxin. *Biol. Reprod.* **62:** 775–780.
47. Janowski, B. A., P. J. Willy, T. R. Devi, J. R. Falck, and D. J. Mangelsdorf. 1996. An oxysterol signalling pathway mediated by the nuclear receptor LXRa. *Nature.* **383:** 728–731.
48. Faerge, I., C. Grondahl, J. L. Ottesen, and P. Hyttel. 2001. Autoradiographic localization of specific binding of meiosis-activating sterol to cumulus-oocyte complexes from marmoset, cow, and mouse. *Biol. Reprod.* **64:** 527–536.
49. Metherall, J. E., K. Waugh, and H. Li. 1996. Progesterone inhibits cholesterol biosynthesis in cultured cells. Accumulation of cholesterol precursors. *J. Biol. Chem.* **271:** 2627–2633.
50. Lindenthal, B., A. L. Holleran, T. A. Aldaghlas, B. Ruan, J. George, J. Schroepfer, W. K. Wilson, and J. K. Kelleher. 2001. Progestins block cholesterol synthesis to produce meiosis-activating sterols. *FASEB J.* **15:** 775–784.