Sterol Regulation of Metabolism, Homeostasis, and Development

Joshua Wollam[1,2,3] and Adam Antebi[1,2,3]

[1]Department of Molecular and Cellular Biology, Huffington Center on Aging, [2]Interdepartmental Program in Cell and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030

[3]Max-Planck-Institute for Biology of Ageing, 50931 Cologne, Germany; email: AAntebi@age.mpg.de

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Abstract

Sterol metabolites are critical signaling molecules that regulate metabolism, development, and homeostasis. Oxysterols, bile acids (BAs), and steroids work primarily through cognate sterol-responsive nuclear hormone receptors to control these processes through feed-forward and feedback mechanisms. These signaling pathways are conserved from simple invertebrates to mammals. Indeed, results from various model organisms have yielded fundamental insights into cholesterol and BA homeostasis, lipid and glucose metabolism, protective mechanisms, tissue differentiation, development, reproduction, and even aging. Here, we review how sterols act through evolutionarily ancient mechanisms to control these processes.

Contents

1. INTRODUCTION

Sterols are important for innumerable biological processes essential to reproduction and survival. The major source of sterols in mammals, cholesterol, is both synthesized de novo and acquired from the diet, and plays a crucial role in modulating cell membrane dynamics (1). In addition, cholesterol is metabolized into many physiologically important molecules, including the triumvirate of oxysterols, bile acids (BAs), and steroid hormones. It has become increasingly clear that sterols act in key signaling events to regulate both metabolic and developmental processes. Accordingly, the synthesis and metabolism of cholesterol is tightly regulated, reflecting the essential nature of these molecules.

Oxysterols, BAs, and steroid hormones have distinct structures and functions. Oxysterols are oxygenated forms of cholesterol produced through both autoxidation and enzymatic reactions (2). Enzymatically derived oxysterols are important intermediates in steroid and BA synthesis, and they are considered indicators of endogenous cholesterol levels. BAs are highly oxidized amphipathic molecules composed of a hydroxylated sterol core and a side chain carboxylic acid moiety (3). Produced in the liver and stored in the gall bladder as bile, they are required for dietary fat absorption. Finally, cholesterol is also metabolized into steroid hormones, including the classical steroids: mineralocorticoids, glucocorticoids, androgens, estrogens, and progestins. These molecules, derived from the common precursor pregnenolone in the adrenal glands and gonads, control diverse aspects of animal physiology, including ion balance, stress response, metabolism, development and reproduction, reviewed extensively elsewhere (4). Related molecules, the secosteroids, have a broken B ring structure catalyzed by photolysis in the skin. The most important secosteroid is 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], which acts to modulate calcium homeostasis as well as various cell growth, differentiation, and developmental processes (5).

Nuclear hormone receptors (NHRs) are transcription factors that regulate gene transcription in response to lipophilic ligands and are major players in sterol regulation and homeostasis. These receptors are highly conserved from invertebrates to mammals, characterized by having conserved paired zinc-finger DNA-binding domains as well as C-terminal ligand-binding domains that bind ligands and dock coregulators (6). Oxysterols, BAs, and steroid hormones activate NHRs to exert many of their effects. In particular, mammalian NHRs include a subcluster of evolutionarily related, metabolically active receptors, which partition cholesterol into oxysterol, BA, and steroid hormone pathways, and coordinate energy homeostasis, reproduction, and survival. These include the liver X receptor (LXR), farnesoid X receptor (FXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR), and the vitamin D receptor (VDR), which act together with the heterologous binding partner retinoid X receptor (RXR) to transactivate target gene expression. In addition, model invertebrate organisms, such as *Drosophila melanogaster* and *Caenorhabditis elegans*, have conserved counterparts (**Table 1**). In *Drosophila*, the ecdysone receptor (EcR), activated by 20-hydroxyecdysone (20E), is responsible for morphological changes during larval development and for directing the life cycle (7). Fly DHR96 binds cholesterol and participates in cholesterol, lipid, and perhaps ecdysteroid metabolism (8, 9). In *C. elegans*, the NHR DAF-12 is activated by BA-like dafachronic acids (DAs) and regulates developmental progression, controlling components of the heterochronic circuit and the decision to undergo normal reproductive development or enter the dauer diapause, a state of arrested development (10). In particular, invertebrate studies have revealed unique insights into roles of sterol signaling on development, reproduction, and life span.

Across phyla, sterol-activated NHRs link energy homeostasis with developmental programs in response to changing nutritional and environmental status. By acting as metabolic and environmental sensors, they govern the choice of programs geared toward survival and somatic endurance versus growth and reproduction. Here, we discuss the regulatory processes mediated by sterols through these conserved NHR pathways.

2. STEROL-MEDIATED CONTROL OF MAMMALIAN ENERGY HOMEOSTASIS

2.1. Oxysterol Activation of the Liver X Receptor Regulates Cholesterol Metabolism

In mammalian cells, cholesterol levels are tightly controlled through regulation of endogenous biosynthesis, absorption from the diet, storage, catabolism to other sterols, and elimination. One component of this regulation includes the well-studied sterol regulatory element binding proteins (SREBPs), involved in regulation of cholesterol and fatty acid metabolism. Other key components include the LXRs, LXRα and LXRβ, which maintain cholesterol homeostasis through transcriptional control of genes involved in sterol dynamics and transport (11, 12). LXRα is expressed in the liver and many other metabolically active tissues, including adipocytes, gonads, intestine, macrophages, and kidneys, whereas LXRβ is expressed in most tissues (12). These receptors are activated by oxysterols, the most potent of which are 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol, and 24(*S*),25-epoxycholesterol (12, 13). Other compounds that reportedly activate LXRs include isoprenoid precursors of cholesterol, BAs, and high concentrations of phytosterols and D-glucose.

Clear evidence of a role in cholesterol homeostasis came from analysis of LXRα knockouts, which display severe hepatomegaly owing to an accumulation of liver cholesterol esters when fed a high-cholesterol diet, as well as defects in BA metabolism (14). In mice, the LXRs positively regulate transcription of cholesterol 7α-hydroxylase (*CYP7A1*), which carries out the

Differentiation: the programmed production of specified cell types from less specific parental cells, occurring through regulatory signaling events

EcR: ecdysone receptor

20E: 20-hydroxyecdysone

DA: dafachronic acid

Dauer: a diapause state occurring in *C. elegans* during the L3 larval stage, characterized by stress resistance and extended longevity

Diapause: a physiological state of arrested development or dormancy, typically induced by adverse conditions to enable survival

Table 1 The sterol-sensing nuclear hormone receptors

Nuclear hormone receptor	Systematic name	Physiological role(s)	Notable ligands[a]	References
Liver X receptor (LXR)	NR1H3 (LXRα) NR1H2 (LXRβ)	Cholesterol sensor and a key regulator of cholesterol, bile acid and fatty acid homeostasis, in addition to roles in differentiation and immunity	Oxysterols, including: 24(*S*),25-epoxycholesterol, 22(*R*)-hydroxycholesterol, 24(*S*)-hydroxycholesterol Bile acids (LXRα), including hyodeoxycholic acid, taurohyodeoxycholic acid, cholestenoic acid Synthetic ligands include T0901317, GW3965	12, 13, 179
Farnesoid X receptor-α (FXRα)	NR1H4	Bile acid sensor and master regulator of bile acid metabolism as well as cholesterol and fatty acid homeostasis. Also involved in hepatoprotection, liver regeneration, and inflammation	Bile acids, including chenodeoxycholic acid, cholic acid, deoxycholic acid, lithocholic acid Synthetic ligands include GW4064 and 6E-CDCA	37
Pregnane X receptor (PXR)	NR1I2	Xenobiotic receptor responsive to many compounds including bile acids, involved in hepatoprotection in addition to roles in the inflammatory response and metabolism	Xenobiotics, including pregnenolone-16α-carbonitrile (PCN), phenobarbtol, rifampicin, and coumestrol Bile acids, including lithocholic acid and 3-ketolithocholic acid Various steroid hormones	52, 53, 56
Constitutive androstane receptor (CAR)	NR1I3	Xenobiotic receptor involved in hepatoprotection as well as liver regeneration and various metabolic processes	Xenobiotics, including phenobarbitol, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP), and 6-(4-Chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde O-3,4-dichlorobenzyl) oxime (CITGO) Lithocholic acid (in vivo) Various steroid hormones	56–58
Vitamin D receptor (VDR)	NR1I1	Primarily known as the master regulator of calcium homeostasis, but also involved in differentiation, immunity, and metabolic processes	1α,25-dihydroxy-vitamin D3 (calcitriol) and various synthetic analogs Lithocholic acid	5, 60, 61
***Drosophila* ecdysone receptor (EcR)**	CG1765	The *Drosophila* molting receptor, responsible for key processes of larval molting and metamorphosis	20-hydroxyecdysone	7
***Drosophila* hormone receptor 96 (DHR96)**	CG11783	Proposed cholesterol sensor of the fly, regulating cholesterol and fatty acid metabolism	Cholesterol	8, 9

(*Continued*)

Table 1 (*Continued*)

Nuclear hormone receptor	Systematic name	Physiological role(s)	Notable ligands[a]	References
***C. elegans* DAF-12**	CE27584	Regulator of the *C. elegans* dauer diapause decision, governs key developmental and metabolic events and is also required for gonadal longevity	Dafachronic acids: 25(*S*),26-3-keto-4-cholestenoic acid, 25(*S*),26-3-keto-7(5α)-cholestenoic acid Bile acid: 24(*S*)-cholestenoic acid	10, 177, 178

[a]Not a comprehensive list.

rate-limiting step in BA biosynthesis (14, 15). The consequent secretion of BAs provides one avenue for removal of excess cholesterol from the liver, the loss of which could explain several phenotypes of *LXRα* knockouts. More recent studies suggest that LXRα regulates other aspects of BA metabolism, including promotion of the BA-conjugating enzyme UGT1A3, which facilitates BA elimination by conversion into more hydrophilic compounds (16).

In addition to BA production, LXRs also regulate cholesterol homeostasis by controlling cholesterol transport and storage. In response to oxysterols, several members of the ATP-binding cassette (ABC) transporter superfamily are upregulated, stimulating cholesterol efflux (17). In macrophages, LXR activation induces ABCA1 and ABCG1 expression; these mediate the export of cholesterol and phospholipids to lipoproteins during reverse cholesterol transport from circulation back to the liver (18). LXR also induces ABCG5 and ABCG8, which eliminate hepatic cholesterol through secretion into the bile and promote resecretion of absorbed sterols into the intestine. Further suppression of dietary cholesterol absorption by LXR occurs through downregulation of the intestinal sterol transporter Niemann-Pick C1-like 1 (NPC1L1) (19). Additionally, LXR promotes expression of the cholesteryl ester transferase protein CETP, indirectly facilitating uptake of cholesteryl esters, storage forms of cholesterol, by the liver (20). Several cholesterol-accepting apolipoproteins and associated enzymes are upregulated by LXR, including hepatic apolipoprotein A-IV (ApoAIV) and macrophage apolipoprotein E, which promote cholesterol transfer to high-density lipoprotein (HDL) particles and have protective roles in atherogenesis (18). Accordingly, LXR agonists have antiatherogenic effects in mice, making LXR a target for treatment of this disease (21).

LXR also impacts subcellular cholesterol distribution through regulation of Niemann-Pick C1 (NPC1), which transfers sterols from the endosomal/lysosomal compartments to endoplasmic reticulum and plasma membrane. Mutations in *NPC1* and *NPC2* in humans cause Niemann-Pick type C (NPC) disease, a rare autosomal recessive syndrome in which sterols accumulate in late endosomal/lysosomal compartments, leading to progressive neurodegeneration and premature death. LXR activation with nonsteroidal agonists leads to upregulation of *NPC1* (22, 23). Importantly, treatment with an LXR agonist delayed the demise of *NPC1*-null mice, whereas *LXRβ* loss accelerated disease progression (24). The positive effects are presumably caused by upregulation of the ABCA1 and ABCG1 sterol transporters, increased cholesterol flux, and decreased neuroinflammation. Moreover, this suggests that Niemann-Pick pathology could partially arise from failure to activate LXR and that NPC1 normally facilitates production of bioactive LXR ligands (25).

Aside from BA synthesis, LXR may also divert cholesterol toward steroid hormone production. In the adrenal gland, LXR regulates the steroidogenic acute regulatory protein (StAR) and cytochrome P450 11A1

(CYP11A1) (26). Catalyzing the rate-limiting step in steroidogenesis, StAR transports cholesterol from the outer to the inner mitochondrial membrane, and CYP11A1 functions downstream, converting cholesterol to pregnenolone. Treatment of mice with the synthetic LXR agonist T0901317 reportedly increases expression of *StAR* and *CYP11A1* in the adrenal gland, which is dependent on LXRα (26), although a conflicting report using GW3965 showed downregulation of these genes, suggesting agonist-specific effects (27). The regulation of *StAR* by LXR complements other studies showing oxysterols stimulate *StAR* expression in specific cell types (28). LXR activation with T0901317 increases expression of adrenal ABCA1/ABCG1 cholesterol transporters, suggesting that LXR may protect the adrenals from cholesterol excess by increasing efflux as well as steroidogenesis. Paradoxically, *LXR* knockouts display elevated levels of plasma glucocorticoids, which might be caused by derepression of *StAR* (26, 27). Further studies are needed to elucidate the role of LXR in adrenal steroidogenesis.

Evidence suggests that LXR may also influence gonadal steroidogenesis. Both LXRα and LXRβ are expressed in ovaries and testes, and *LXR* deficiency is associated with decreased fertility in mice of both sexes, although only LXRβ affects male fertility (29). LXR agonists reportedly inhibit luteal cell production of progesterone in the ovary and downregulate both *StAR* and *CYP11A1* (30). Nevertheless, mice lacking *LXR* have unaltered progesterone or estradiol levels, but they are defective in resumption of meiosis during oocyte maturation, which may partially explain decreased fertility (31). *LXRαβ* knockouts also exhibit an attenuated response to meiosis resumption by gonadotropins. Interestingly, a precholesterol metabolite that induces meiotic resumption, follicular-fluid meiosis-activating sterol, weakly activates LXR, correlating with a possible role of LXR in this process (32). In addition, LXR may impact fertility through activities in the placenta and uterus, although further in vivo studies are needed to support these functions (29). In males, loss of *LXRβ* leads to impaired Sertoli cell function and decreased spermatogenesis attributed to accumulation of testicular cholesterol droplets (33, 34). *LXRαβ*-null mice display decreased androgen production, and treatment with the T0901317 agonist leads to increased testosterone in the testes of wild-type mice and increased expression of *StAR* (35). Thus, LXRs may act to couple sterol availability with reproductive function and fertility, although whether LXR influences reproductive development itself remains undetermined.

2.2. Bile Acid Sensors Coordinate Cholesterol and Bile Acid Homeostasis

The synthesis of BAs from cholesterol is critical to cholesterol homeostasis and essential to dietary lipid absorption. Moreover, BAs themselves are toxic and therefore must be tightly regulated. The BA pool is maintained through control of synthesis, recycling in enterohepatic circulation, catabolism, and excretion. Synthesis occurs in the liver through two pathways, the classical neutral pathway and the acidic pathway, involving similar steps: initiation by 7α-hydroxylation, ring structure modifications, oxidation and shortening of the side chain, and amino acid conjugation to facilitate export to the gall bladder (36). Upon food ingestion, the gall bladder secretes BA-containing bile into the intestine, where the BAs act in fat absorption. From there, most are reabsorbed by the intestine and return to the liver through the portal vein (3).

The BA sensor farnesoid X receptor-α (FXRα) is the master regulator of BA physiology. Several endogenous BAs bind and activate FXRα, most potently chenodeoxycholic acid and its conjugated derivatives (37). FXRα is highly expressed in tissues of BA transport, including the liver, intestine, and kidney. Other compounds that activate FXRα with lower affinity include farnesol and related metabolites, androsterone, polyunsaturated fatty acids, and oxysterols as well as other intermediates in BA synthesis, although their physiological roles

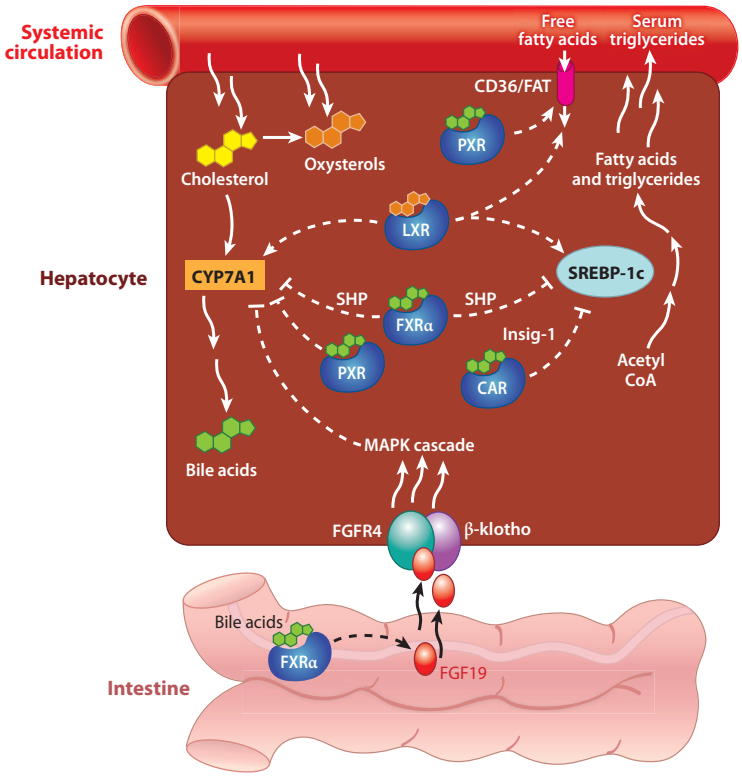
are less defined. Consistent with a role in BA homeostasis, mice lacking *FXRα* display severe alterations in BA metabolism (38–40). Notably, when challenged with dietary cholic acid, they experience hepatotoxicity and wasting. Plasma BA levels are elevated, along with cholesterol and triglycerides, whereas gall bladder levels are reduced relative to wild-type mice. FXRα regulates expression of genes involved in almost every aspect of BA physiology, including promotion of BA conjugation to facilitate secretion (e.g., UDP glucuronosyltransferase *UGT2B4*), export into the bile canaliculli (through transporters including the bile salt export pump *BSEP* and the ABC transporters *MRP2/ABCB4* and *MDR3/ABCC2*), modulation of import into the liver from circulation (e.g., the sodium taurocholate cotransporting polypeptide *NTCP* and the organic anion transporter polypeptides *OATP*s), and control of reabsorption in the intestine (the intestinal BA-binding protein *I-BABP*, apical sodium-dependent BA transporter *ASBT*, and organic solute transporter *OSTα/β*), whereas activation of fibroblast growth factor (mouse FGF15/human FGF19) controls gall bladder filling to regulate bile release into the intestine (41, 42). Additionally, FXRα acts in coordination with PXR, CAR, and VDR in hepatoprotection from BA toxicity through regulation of genes involved in BA detoxification, including hydroxylation, export, and sulfation (43).

A series of elegant experiments over the past decade has yielded molecular insights into how BAs regulate their own synthesis through feedback repression (36). In response to BA excess, FXRα acts to repress BA synthesis primarily through downregulation of cholesterol 7α-hydroxylase (*CYP7A1*) and sterol 12α-hydroxylase (*CYP8B1*) (41), cytochrome P450 enzymes essential for BA synthesis, in a manner opposing regulation by LXRα. Repression is achieved by upregulation of the small heterodimer partner (*SHP*), a noncanonical nuclear receptor, lacking a DNA-binding domain, that works as a transcriptional repressor. SHP inactivates several NHRs responsible for either *CYP7A1* or *CYP8B1* expression, including LXR, liver receptor homolog-1 (LRH-1, NR5A2), and hepatocyte nuclear factor-4α (HNF4α) (44–46). FXRα also orchestrates a hormonal feedback mechanism originating in the intestine and circulating back to the liver, which involves fibroblast growth factor (mouse FGF15/human FGF19) (47–49). In the ileum, FXRα directly activates FGF15/19, which binds to cognate coreceptors in the liver: FGFR4, a receptor tyrosine kinase, and β-klotho, a single-pass transmembrane protein. Downstream, MAP kinase cascades repress hepatic *CYP7A1* expression. Thus, FXRα acts in opposition to LXR in the regulation of BA synthesis (**Figure 1*a***). There is currently intense research aimed at uncovering further details of these fascinating signaling networks.

FXRα also mediates aspects of cholesterol transport, including reverse cholesterol transport and efflux. *FXRα*-null mice display elevated plasma levels of low-density lipoprotein, very low-density lipoprotein (VLDL), and HDL-associated cholesterol and triglycerides (39, 50). Increased HDL levels may be partially due to decreased hepatic levels of the scavenger receptor B1 (SR-B1), which absorbs HDL in liver during reverse cholesterol transport. *FXRα* loss also leads to decreased cholesterol efflux, owing in part to decreased expression of *ABCG5/8*, involved in the elimination of hepatic cholesterol as well as resecretion into the intestine. FXRα also upregulates phospholipid transfer proteins and differentially regulates several apolipoproteins to favor overall reduction of plasma lipids. Accordingly, treatment with FXRα ligands reduces serum HDL and VLDL cholesterol as well as triglyceride levels (51).

Best known for its roles in xenobiotic metabolism, PXR has a flexible ligand-binding domain that binds structurally diverse hydrophobic compounds, including several BAs (52, 53). PXR is expressed in overlapping tissues with LXR and FXRα, including the liver, intestine, and kidneys. PXR has also been implicated in feedback regulation of BA synthesis through repression of *CYP7A1*, but in

a



b

NHR	Process: Bile acid synthesis	Bile acid import	Bile acid export	Bile acid detox	RCT	Cholesterol export	Lipoprotein metabolism	Lipogenesis/ fat uptake	Fatty acid β-oxidation	Gluconeo-genesis	Insulin sensitivity
LXR	↑	–	–	–	↑	↑	↑	↑	↑*	↓	↑
FXRα	↓	↓↑	↑	↑	↑	↑	↓	↓	↑*	↓*	↑
PXR	↓	↓	↑	↑	↓	–	↓	↑	↓*	↓*	↑
CAR	–	–	↑	↑	↑	–	↓	↓	↓*	↓*	↑
VDR	–	–	↑	↑	–	–	–	–	↓*	–	↑

↑ Promotes ↓ Represses ↓↑ Differential regulation * Not well characterized

Figure 1

Pleiotropic actions of mammalian sterol-sensing nuclear hormone receptors (NHRs) on energy homeostasis. (*a*) Differential regulation of select components of bile acid (BA) synthesis and lipogenesis by the sterol-sensing NHRs. LXR and FXRα act in an opposing manner upon expression of the CYP7A1 cholesterol 7α-hydroxylase, impacting BA synthesis, and the SREBP-1c transcription factor, which regulates fatty acid and triglyceride synthesis. LXR and PXR also positively regulate the fatty acid translocase CD36/FAT, promoting uptake of free fatty acids. (*b*) Comparison of the roles of sterol-sensing NHRs in various metabolic processes demonstrates the pleiotropic actions of these receptors. CAR, constitutive androstane receptor; FGF19, human fibroblast growth factor 19 (mouse FGF15); FXRα, farnesoid X receptor-α; Insig-1, an endoplasmic reticulum–associated membrane protein that prevents proteolytic activation of SREBP; LXR, liver X receptor; PXR, pregnane X receptor; RCT, reverse cholesterol transport; SHP, small heterodimer partner; VDR, vitamin D receptor.

a FXRα- and SHP-independent manner (53). In contrast to FXRα, PXR positively regulates lipoprotein levels, as PXR activation leads to increased serum HDL and ApoAI levels in wild-type but not *PXR*-null animals (54). In vitro studies suggest this may be partly mediated by downregulation of *ABCA1* and *SR-B1*, which would result in reduced HDL uptake and efflux (55). Thus, PXR and FXRα both act to inhibit BA synthesis, but in opposition to influence lipoprotein distribution.

Another xenobiotic receptor, CAR, impacts several metabolic pathways and is activated by many of the same ligands as PXR (56). Although not directly binding BAs, CAR is activated in vivo by treatment with lithocholic acid (57). CAR is highly expressed in the liver and at lower levels in the intestine and stomach (58). Similar to FXRα, CAR activation decreases plasma HDL and ApoAI levels in wild-type but not $CAR^{-/-}$ mice. Unlike *FXRα*-null mice, *CAR* knockouts exhibit normal levels of serum lipoproteins, unless fed a high-fat diet (59).

VDR is also activated by lithocholic acid (60) and regulates some of the same xenobiotic detoxification genes as CAR and PXR. VDR is expressed in a wide variety of tissues, where it exerts its many physiological effects (5, 61). However, relatively little is known about the effect of VDR on cholesterol metabolism. *VDR*-null mice demonstrate decreased levels of serum cholesterol and triglycerides relative to wild type, and upon feeding a high-fat diet, show slower growth rates and accumulate less fat (62), although these studies may be complicated by the use of high-calcium diets to avoid hypocalcemia. Additional studies are needed to investigate the possible roles of VDR as well as the other BA-activated receptors in modulating cholesterol and BA homeostasis as well as to learn how they might interact to regulate these processes.

In addition to NHRs, BAs activate several other pathways, including mitogen-activated protein kinase (MAPK) and G protein–coupled receptor signaling. BAs activate all three MAPK signaling pathways (ERK, JNK, and p38/MAPK) (63, 64), and in particular, the JNK pathway may mediate FGF15/19 repression of BA synthesis. BAs also activate AKT, a key protein kinase involved in the insulin signaling pathway, possibly through induction of $G\alpha_i$ receptors. One G protein–coupled receptor activated by BAs is TGR5, which mediates BA-protective processes and promotes insulin secretion (65, 66). Accordingly, BA treatment attenuates diet-induced obesity and insulin resistance in mice and also induces mitochondrial activity and energy expenditure in human skeletal muscle and murine brown adipose tissue. This is dependent upon TGR5 and the thyroid hormone-activating type 2 iodothyronine deiodinase, further implicating BAs in regulating energy mobilization.

2.3. Sterol-Sensing Nuclear Hormone Receptors Modulate Lipid Metabolism

In concert with cholesterol homeostasis, both LXRs also regulate fatty acid metabolism, primarily through regulation of SREBP-1c, a master regulator of fatty acid and triglyceride biosynthesis (67). Excess cholesterol or synthetic ligands of LXR induce *SREBP-1c* expression and fatty acid synthesis, which is absent in *LXRαβ*-null mice. These animals are resistant to diet-induced obesity, display increased metabolic rates and decreased lipogenesis, and fail to induce *SREBP-1c* expression in response to cholesterol or insulin (67, 68). The LXRs also upregulate the carbohydrate-responsive element-binding protein (*ChREBP*), which independently regulates lipogenic gene expression in response to glucose (69). Furthermore, the LXRs directly upregulate enzymes involved in fatty acid synthesis and lipoprotein remodeling, including fatty acid synthase, lipoprotein lipase, and the fatty acid transfer protein, CD36 (70, 71). These studies demonstrate a link between cholesterol uptake and fatty acid synthesis, implicating LXR as a key regulator in this balance.

In contrast, the LXRs may facilitate fatty acid β-oxidation in muscle and adipocytes by upregulating pyruvate dehydrogenase kinase 4 (*PDK4*) (72, 73). PDK4 inhibits the activity of

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the pyruvate dehydrogenase complex, which represses glycolysis in favor of fatty acid oxidation, shifting the balance of energy derived from glucose to fat. LXRα also reportedly promotes hepatic peroxisomal β-oxidation of very long-chain fatty acids, possibly to counter increased triglyceride levels occurring upon its activation, but this remains unclear (74).

FXRα regulates lipid metabolism in a manner opposite that of LXR (**Figure 1*a***). *FXRα*-null mice suffer from increased levels of plasma triglycerides, lipoproteins, and cholesterol, in addition to a fatty liver (50). Notably, activation of FXRα represses *SREBP-1c* expression via SHP, downregulates genes involved in lipoprotein metabolism, and lowers triglyceride levels (51). Similarly in humans, oral intake of chenodeoxycholic acid decreases plasma triglycerides (75). Furthermore, activation of FXRα leads to decreased secretion of VLDL from the liver and, together with the repression of *SREBP-1c* and fatty acid synthesis, indicates the importance of FXRα in the repression of hepatic lipogenesis (76). In short, the phenotypes of *FXRα*-null mice can be described as proatherogenic, and the triglyceride- and cholesterol-lowering effects of FXRα agonists make them attractive therapeutic candidates. Finally, FXRα may also influence β-oxidation in a manner similar to LXR by inducing *PDK4*, possibly through upregulation of the peroxisome proliferator–activated receptor α (*PPARα*), an important regulator of fatty acid oxidation (77).

Other BA-activated receptors also impact lipid metabolism, although the underlying mechanisms are not fully understood. For PXR, activation is generally associated with enhanced lipogenesis and suppressed fatty acid β-oxidation. Expression of constitutively activated PXR leads to increased triglyceride levels, hepatomegaly, fatty liver, and lipogenesis, but in an SREBP-1c-independent manner (78). Instead, activated PXR stimulates expression of fatty acid translocase *CD36* and several other lipogenic enzymes, including fatty acid elongase and steroyl-coenzyme A (CoA) desaturase-1. PXR also directly transactivates peroxisome proliferator–activated receptor-γ (*PPARγ*), a key regulator of adipogenesis and lipogenesis (71). Conversely, PXR represses *PPARα* expression as well as inhibits forkhead transcription factor FoxA2, thereby repressing rate-limiting genes in β-oxidation [e.g., carnetine palmitoyl transferase-1α (*CPT1α*) and ketogenesis (3-hydroxy-3-methylglutaryl-CoA synthase 2 (*HMGCS2*)], respectively (78, 79).

Activation of CAR in mice also appears to modulate genes involved in fatty acid β-oxidation but not in *CAR*-null animals, although conflicting reports describe either repression or induction of this process, making additional studies necessary (80–83). In contrast, both PXR and CAR can suppress lipogenesis through activation of Insig-1, an endoplasmic reticulum-associated membrane protein that prevents proteolytic activation of SREBP (84).

VDR may also inhibit β-oxidation as this occurs at elevated rates in the white adipose tissue of *VDR*-null mice (62). Increased expression of several uncoupling proteins (UCPs) was noted in these animals. Accordingly, treatment of primary brown adipocyte cultures with 1,25-$(OH)_2D_3$ downregulated *UCP* expression. The UCPs dissipate energy as heat in brown adipose tissue during the process of adaptive thermogenesis, and thus presumably lead to higher energy expenditure in the absence of VDR.

2.4. Glucose Metabolism and the Hepatic Fasting Response

Several sterol-activated NHRs also impact energy homeostasis through regulation of glucose metabolism. As discussed above, LXRs regulate SREBPs and ChREBP, which promote the conversion of glucose to fatty acids. LXR agonists improve glucose tolerance in a mouse model of diet-induced obesity in the liver and adipose tissue (85). Suppression of hepatic gluconeogenesis occurs through decreased expression of *PPARγ* coactivator-1α and phosphoenolpyruvate carboxykinase (*PEPCK*), whereas in adipose tissue, the insulin-sensitive glucose transporter *GLUT4* is upregulated (85). In support of these findings, *LXRαβ*-null mice

fail to suppress gluconeogenesis in response to agonist treatment, and mice deficient in *LXRβ* display reduced glucose tolerance (86, 87). Furthermore, in response to glucose or synthetic agonists, the LXRs also promote insulin secretion by pancreatic β-cells, although no changes in insulin sensitivity have been detected in the absence of the LXRs (87, 88). High concentrations of D-glucose and D-glucose-6-phosphate also reportedly activate the LXRs, but the physiological relevance is unclear (89).

In response to fasting or starvation, the liver modulates several metabolic processes to maintain blood glucose levels: Glycogenolysis and gluconeogenesis increase glucose levels, fatty acid oxidation provides energy for gluconeogenesis and substrates for ketogenesis, and attenuation of lipogenesis shifts fatty acids from storage to utilization. *LXRα* knockouts exhibit an impaired response to fasting, with delayed mobilization of hepatic glycogen stores (90). Upon refeeding, induction of hepatic lipogenesis is also reduced, secondary to reduced SREBP activity. Interestingly, *LXRαβ*-null mice display cholesterol-dependent elevation of thyroid hormone levels and a corresponding increased energy expenditure through upregulation of *UCP*s (68). Thus, LXR may coordinate the balance between energy accretion and mobilization depending upon dietary conditions.

FXRα influences glucose metabolism through several targets, including genes involved in gluconeogenesis, glycolysis, and glycogen synthesis (91, 92). *FXRα*-null mice display impaired glucose tolerance and insulin resistance, although reports conflict on the glucose levels and altered expression of gluconeogenic genes in these mice (92–94). In response to fasting, *FXRα*-null mice fail to induce *PEPCK* to the same extent as wild type, suggesting FXRα is required to upregulate gluconeogenesis (93). By contrast, FXRα agonists suppress gluconeogenic genes in FXRα- and SHP-dependent manners (94). Knockout mice also display decreased insulin sensitivity in peripheral white adipose and muscle tissues (92). It is interesting to note that FXRα is expressed in adipose tissue and may promote preadipocyte differentiation. Moreover, treatment of adipocytes with an FXRα agonist enhances glucose uptake in response to insulin (92, 95). Similar to LXR, FXRα agonists also improve glucose tolerance in a diabetic mouse model and promote insulin secretion in pancreatic β-cells (92, 96). As might be expected, *FXRα*-deficient mice exhibit defective fasting responses, characterized by transient hypoglycemia attributed to altered glycogen metabolism and impaired hepatic glucose production (97). Mutants also fail to lower serum triglycerides in response to fasting, possibly because of defects in fat utilization. Finally, fasting *FXRα* knockouts have an increased susceptibility to enter torpor (98), a state of decreased physiological activity induced by stress, characterized by reduced body temperature and metabolic rates to conserve energy. By inference, FXRα may promote adaptive thermogenesis, the regulated production of heat, through increased *UCP* expression. These data suggest that FXRα, like LXR, plays a role in the regulation of energy homeostasis and mobilization in response to changing nutritional conditions.

PXR and CAR also appear to influence glucose metabolism and the fasting response. Treatment of mice with corresponding agonists represses hepatic expression of gluconeogenic enzymes as well as improves insulin sensitivity in both diabetic animal models and human subjects (56, 80, 83, 99). Provision of PXR agonists reportedly decreases serum glucose levels in fasting wild-type mice and decreases expression of β-oxidation and ketogenesis components (79). Suppression of gluconeogenesis may result from PXR inhibition of two transcription factors key to this process, FOXO1 and CREB, whereas repression of β-oxidation and ketogenesis is proposed to arise from inhibition of FoxA2 (56, 79). CAR is thought to oppose gluconeogenesis in a similar manner by competing with the transcription factor HNF4α at response elements in the *PEPCK* promoter (56). Moreover, CAR, like PXR, may bind and inhibit the FOXO forkhead transcription factor, a key mediator of the insulin

Torpor: a state of decreased physiological activity, characterized by reduced body temperature and metabolic rates, promoting energy conservation

Hepatic fasting response: a response to fasting conditions to sustain glucose production

signaling pathway. Interestingly, CAR and its target genes are induced during the fasting response, and *CAR*-deficient mice exhibit a defective response (100, 101). CAR may act in an NHR network that governs the fasting response, as two NRs, PPARα and HNF4α, activate CAR in this context. In addition, dramatic weight loss occurs in *CAR*-deficient animals during long-term caloric restriction, presumably from reduced degradation of the thyroid hormone and a resultant dysregulation of metabolic rates (100). Accordingly, fasting or treatment with a CAR agonist induces thyroid hormone-metabolizing enzymes, depending upon CAR. CAR may thus function to attenuate the fasting response by dampening gluconeogenesis, fatty acid β-oxidation, and energy utilization.

Finally, although little is known about its roles in glucose homeostasis, *VDR* deficiency is associated with decreased insulin production and a corresponding increased risk for diabetes in both animal models and humans (62, 102). In a similar manner, vitamin D deficiency leads to decreased glucose tolerance and pancreatic insulin secretion. These effects may arise from misregulation of pancreatic β-cell insulin secretion and glycolysis by altered calcium concentrations. As mentioned above, VDR may repress adaptive thermogenesis through downregulation of *UCP*s, although its association with the hepatic fasting response and torpor has not been studied.

In summary, these sterol-sensing NHRs have pleiotropic roles governing energy homeostasis and control the balance between energy accretion and mobilization (**Figure 1*b***).

3. STEROL-ACTIVATED NUCLEAR HORMONE RECEPTORS MODULATE INFLAMMATION AND IMMUNITY

The immune system naturally functions to protect the animal throughout life, yet when dysregulated can lead to immunosuppression or inflammation, with deleterious consequences. LXR plays a central role in regulating inflammation and innate immunity in several contexts. In response to bacterial infection, exposure to the bacterial cell wall component lipopolysaccharide or to cytokines, the inflammatory response is induced in macrophages, resulting in expression of interleukins and chemokines. Oxysterol activation of LXR inhibits expression of several of these genes, including iNOS, interleukin-6 (IL-6), and IL-1β in macrophages derived from wild-type but not *LXR*-null mice (103). After challenge with LPS, *LXR* knockouts reportedly display an enhanced systemic inflammatory response and increased hepatic expression of inflammatory mediators, although conflicting studies of primary macrophages from these animals describe decreased expression (104, 105). The possible anti-inflammatory activities of LXR are proposed to stem largely from suppression of NF-κB signaling (104). Recent studies reveal that sumoylated LXR stabilizes NCoR corepressor complexes residing at promoters of NF-κB-dependent genes, thereby preventing their expression (106). Similarly, derepression of the hepatic inflammatory response is seen in *FXRα*-null mice, and ligand activation of FXRα, PXR, or PPARγ also results in transrepression of NF-κB targets via sumoylation and NCoR stabilization. These receptors thus share a common mechanism by which they modulate the inflammatory response (107–111). In addition, the heterodimeric partner of these receptors, RXR, is regulated by inflammatory mediators, possibly an important aspect of the common involvement of sterol-sensing NHRs in this response (112). Corresponding with a role in innate immunity, *LXR*-deficient animals are susceptible to intracellular *Listeria monocytogenes* infection, and *FXRα*-null animals display increased intestinal bacterial levels and susceptibility to development of colitis (111, 113). Moreover, the coupling of xenobiotic receptors with immunity may help explain how compounds such as polyphenols have beneficial effects on inflammation and metabolism (114).

Sterol-sensing NHRs are also involved in regulating acquired immunity. Mice deficient

in *LXRβ* display age-associated lymphoid hyperplasia, and T cells derived from these mice exhibit enhanced proliferation after antigenic stimulus (115). Conversely, activation of LXRβ inhibits the proliferation of cultured lymphocytes by driving ABC-mediated cholesterol efflux, which in turn inhibits the cell cycle (116). In a similar manner, *PXR*-deficient mice display increased T lymphocyte proliferation, whereas PXR activation has the opposite effect (117). VDR also modulates the immune response, targeting multiple cell types of the immune system, extensively reviewed elsewhere (118). It will be interesting to see whether corresponding invertebrate receptors also influence immunity.

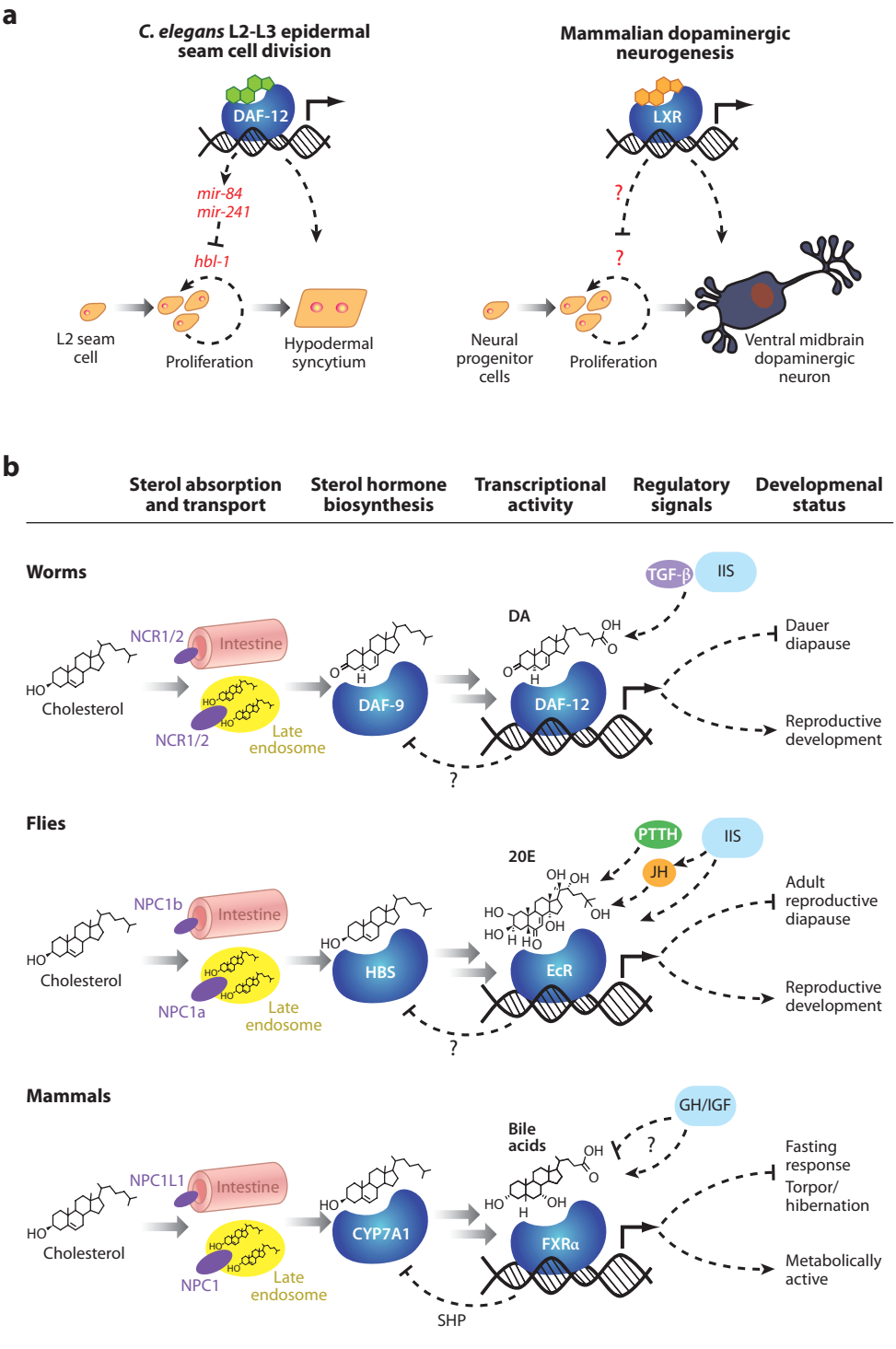
4. DEVELOPMENTAL ROLES OF MAMMALIAN STEROL-SENSING NUCLEAR HORMONE RECEPTORS

4.1. Oxysterols and the Liver X Receptor Regulate Differentiation

In addition to modulation of energy homeostasis, oxysterols promote differentiation in a variety of tissues. Among these processes, the best-described role of oxysterols is the regulation of skin keratinocyte differentiation. Keratinocytes comprise the majority of cells in the epidermis, arising from a stem cell population located in the basal proliferative layer. These cells divide and differentiate into progeny that migrate upward in a continuous process of renewal, eventually dying and forming a cornified outer layer. Oxysterol ligands of LXR induce expression of keratinocyte differentiation markers (e.g., involucrin) and lipid synthesis while suppressing proliferation, depending upon LXRβ, the primary isoform found in the epidermis of the mouse (119). This is due in part to upregulation of components of the AP-1 protein complex (including c-Fos and jun-D), which regulate genes required for keratinocyte differentiation. $LXR\beta^{-/-}$ mice display thin epidermal layers and have reduced levels of proliferating keratinocytes, which are refractory to differentiation after topical treatment with oxysterols. In addition, skin from these mice has similarities in gene expression profiles to photoaged and chronologically aged skin, and treatment with LXR agonists reduces UV-induced skin thickening and wrinkle formation in hairless mice (120). Thus, LXR activation may protect against age-related skin damage, making it a promising antiaging target.

Recent evidence reveals that oxysterols and LXR regulate dopaminergic neurogenesis (121). In *LXRαβ*-deficient mice, the number of progenitor neurons in the ventral midbrain is reduced, leading to a decrease in dopaminergic neurons. Accordingly, overexpression of *LXRβ* or treatment with oxysterol agonists enhances neurogenesis, although this is not seen upon treatment of *LXR*-deficient progenitor cells. Loss of *LXR* leads to defective cell-cycle progression of progenitor cells at the G2/M phase and a corresponding failure of differentiation (**Figure 2*a***). Although LXRs have been implicated in regulation of several cell-cycle genes, the mechanism by which LXR exerts cell-cycle control of neural progenitors, and how it acts specifically on this subset of neurons, remains unclear. In addition, *LXRβ* and double knockout mice exhibit age-associated lipid accumulation in the brain, an abnormal blood-brain barrier, and neural degeneration (122, 123). The LXRs thus appear to both promote neurogenesis during early development as well as prevent neural degeneration with age.

Oxysterols also function in the osteogenic differentiation of multipotent bone marrow stromal cells. When exposed to 20(*S*)-hydroxycholesterol in combination with 22(*S*)- or 22(*R*)-hydroxycholesterol, a strong induction of osteogenic gene expression and cell matrix mineralization are seen (124, 125). This is reportedly mediated through activation of hedgehog signaling (126, 127), while additional studies suggest that activation of LXR may actually inhibit this signaling (128). Further studies in vivo are needed to clarify roles of oxysterols and LXR in osteogenesis.


a
C. elegans L2-L3 epidermal seam cell division
DAF-12
mir-84
mir-241
hbl-1
L2 seam cell
Proliferation
Hypodermal syncytium
Mammalian dopaminergic neurogenesis
LXR
?
?
Neural progenitor cells
Proliferation
Ventral midbrain dopaminergic neuron
b
Sterol absorption and transport
Sterol hormone biosynthesis
Transcriptional activity
Regulatory signals
Developmenal status
Worms
NCR1/2
Intestine
Cholesterol
NCR1/2
Late endosome
DAF-9
DA
DAF-12
TGF-β
IIS
Dauer diapause
Reproductive development
?
Flies
NPC1b
Intestine
Cholesterol
NPC1a
Late endosome
HBS
20E
EcR
PTTH
IIS
JH
Adult reproductive diapause
Reproductive development
?
Mammals
NPC1L1
Intestine
Cholesterol
NPC1
Late endosome
CYP7A1
Bile acids
FXRα
GH/IGF
?
Fasting response
Torpor/ hibernation
Metabolically active
SHP

4.2. Bile Acid-Activated Nuclear Hormone Receptors Promote Liver Regeneration

The liver is unique in its ability to undergo growth and repair after injury to restore normal size, followed by the return of cells to quiescence. Regeneration is induced by several signaling events, including secretion of cytokines and growth factors such as transforming growth factor α (TGF-α), IL-6, and tumor necrosis factor α (129). BAs, acting through FXRα, play an important role in the regeneration process. Notably, diets enriched with 0.2% cholic acid accelerate regeneration, and diets containing a BA-sequestering resin impede it (130). These effects are not observed in *FXRα*-null mice in which liver regeneration is retarded. Thus, BAs, acting through FXRα, are regeneration-promoting signals, which are normally required for this process. One mechanism underlying regeneration is the activation of Forkhead Box transcription factor M1b (FoxM1b), which regulates cell-cycle progression during liver regeneration and is a direct target of FXRα (131). Interestingly, hepatic *FoxM1b* expression declines with age, whereas increased expression of *FoxM1b* or activation of FXRα restores regenerative potential (132). FXRα may thus be a target for therapeutics aimed at enhancing liver regeneration after transplant or surgery and during aging. Paradoxically, *FXRα* knockout mice are more susceptible to spontaneous liver tumors (37). It is thought that FXRα normally plays a role in hepatoprotection from BA toxicity, damage, oxidative stress and inflammation, which when absent could contribute to tumorigenesis. One potential mechanism by which some of these effects might be mediated is through upregulation of *CAR*, which can be tumorogenic in the presense of agonists such as phenobarbitol (133–135).

Other BA-responsive receptors also appear to promote liver regeneration. PXR activation stimulates hepatocyte proliferation and liver growth, and *PXR*-null mice display impaired regeneration after partial hepatectomy (136, 137). Similarly, $CAR^{-/-}$ mice exhibit delayed hepatocyte proliferation after partial hepatectomy, and vitamin D deficiency compromises hepatic regeneration (130, 138). Whether these receptors are specifically regulated by BAs for this process is currently unknown. In addition to liver regeneration, VDR has been implicated in diverse developmental processes governing calcium homeostasis, bone biology, nervous system and skeletal development, immunity, skin differentiation, and hair follicle cycling, which are extensively reviewed elsewhere (5).

←

Figure 2

Sterols act through conserved pathways to mediate developmental processes. (*a*) In specific contexts, both *C. elegans* DAF-12 and the mammalian liver X receptor (LXR) act to inhibit proliferation of precursor cells to promote terminal differentiation. (*b*) Across phyla, similar mechanisms of cholesterol absorption and transport, sterol biosynthesis, and transcriptional regulation by sterol-sensing nuclear hormone receptors mediate decisions between energy-conserving states and normal reproductive development. Upstream endocrine networks, notably insulin/insulin-like growth factor-1 signaling, impact the production of sterol ligands, and thus activities of these receptors, although many mechanistic details remain to be uncovered. CYP7A1, a cytochrome P450, which acts at the rate-limiting step in mammalian BA synthesis; DA, dafachronic acid; DAF-9, *C. elegans* homolog of mammalian BA synthetic enzyme CYP27A1 required for DA production; DAF-12, *C. elegans* DA-activated nuclear hormone receptor; EcR, ecdysone receptor; GH, growth hormone; HBS, hormone biosynthesis; IGF, insulin-like growth factor; IIS, insulin/IGF-I signaling; JH, juvenile hormone; *mir-84*, *-241*, targets of DAF-12 that are involved in developmental timing and seam cell differentiation during the L2–L3 stage in *C. elegans*; NCR1/2, *C.elegans* homologs of the Niemann-Pick C1 and Niemann-Pick C1-like-1 sterol transporters; NPC1a, *Drosophila* homolog of Niemann-Pick C1; NPC1b, *Drosophila* homolog of Niemann-Pick C1-like-1; NPC1, mammalian Niemann-Pick C1 sterol transporter; NPC1L1, mammalian Niemann-Pick C1-like-1 sterol transporter; PTTH, prothoraciotropic hormone; TGF-β, transforming growth factor β.

IIS: insulin/IGF-I signaling

5. MAMMALIAN STEROL-SENSING NUCLEAR HORMONE RECEPTORS AND LIFE SPAN

Aging entails a decline in metabolic, tissue, and organismal homeostasis. As major regulators of these processes, sterol-sensing NHRs are bound to play a role. Few studies have examined their effects on life span, although a growing body of evidence suggests they impact aging and age-related diseases. For example, LXR may play a role in ameliorating Alzheimer's disease (AD). In an amyloid precursor protein transgenic mouse model, loss of *LXRα* or *LXRβ* increased amyloid plaque formation, a key component of AD pathogenesis; however, treatment with an LXR agonist decreased plaque formation and improved cognitive ability, possibly owing to increased cholesterol flux (139, 140). Interestingly, different human *LXR* polymorphisms associate with either increased risk of AD or increased life span, rendering LXR a potential target for treatment of age-related pathologies (141, 142).

Vitamin D production also declines with age, and VDR influences aging (143). Mice deficient in *VDR* exhibit decreased longevity and phenotypes reminiscent of premature aging, including skin wrinkling, alopecia, muscle atrophy, osteoporosis, decreased immunity, and deficiencies in hearing and balance. Knockout of 25-hydroxyvitamin D_3 1α-hydroxylase, required for vitamin D synthesis, results in similar phenotypes (144). Mice deficient in either the transmembrane protein Klotho or FGF-23, which modulate vitamin D homeostasis, also display progeroid phenotypes (145). Interestingly, these mutants have extremely high circulating levels of vitamin D, or hypervitaminosis D, and their phenotypes are rescued by vitamin D-deficient diets or genetic ablation of *VDR*. In contrast, transgenic mice overexpressing *Klotho* exhibit increased longevity and resistance to oxidative stress, possibly through decreased insulin/insulin-like growth factor I signaling (IIS) (146). The adverse effects of both hypo- and hypervitaminosis D reveal that the appropriate balance of vitamin D is essential to avoid age-related pathologies.

Finally, sterol-sensing NHRs may invoke protective mechanisms that extend survival, and perhaps life span. Xenobiotic metabolism protects the animal through elimination of toxic compounds, and several studies suggest that upregulation of these pathways may be beneficial and correlate with longevity. In *C. elegans*, transcriptional profiles of long-lived *daf-2* insulin/IGF-1 receptor mutants revealed upregulation of numerous genes involved in xenobiotic detoxification, postulated to act as a means of longevity assurance (147). Similar upregulation has been reported in several long-lived mouse models, including calorie-restricted mice, Snell dwarf mice, and Little mice (148, 149). In Little mice, deficient in growth hormone releasing hormone receptor, this is associated with increased hepatotoxin resistance, partially dependent upon FXRα (150). Interestingly, these mice display increased BA levels, and treatment of wild-type mice with cholic acid induces a similar profile of xenobiotic detoxification genes. These studies suggest that BAs may act through FXRα to induce the xenobiotic response and impact life span. In the nematode *C. elegans*, BA-like steroids acting through the FXRα homolog, DAF-12, promote increased longevity in the absence of the germ line, discussed in detail below (151). Microarray studies indicate that genes involved in xenobiotic metabolism are indeed altered in long-lived *daf-12*(*rh273*) ligand-binding domain mutants (152). Conceivably, the conserved pathways of BA signaling may stimulate xenobiotic protection and enhance longevity, although causal support for this hypothesis remains to be found (**Figure 2*b***).

6. REQUIREMENT OF STEROLS BY INVERTEBRATE MODEL ORGANISMS

Work in both *C. elegans* and *D. melanogaster* has revealed critical roles for sterols in these model organisms. As cholesterol auxotrophs,

these animals must obtain exogenous cholesterol from the diet. In *C. elegans*, cholesterol depletion for one generation leads to defects in molting, growth, gonadal morphogenesis, and germ line proliferation and differentiation, and depletion for additional generations leads to larval arrest (153). Similarly in the fly, larvae grown in cholesterol-deficient conditions also arrest early in larval development because cholesterol is required for molting, growth, and reproductive development (154). The striking phenotypes seen upon cholesterol depletion in both worms and flies demonstrate that, although these organisms are unable to produce cholesterol, it is an essential component of the diet required for normal development.

Although obviously important molecules, relatively little is known about the sterol metabolome of either nematodes or insects. Both harbor sterol metabolites derived from dietary cholesterol, some of which are also found in mammals, such as 7-dehydrocholesterol (155, 156). The ecdysteroids have been extensively studied in insects, but oxysterols and BAs have not yet been identified. In the worm, the BA-like DAs work through the DAF-12 NHR to regulate dauer diapause, developmental timing, fat metabolism, and life span (10). However, the full spectrum of BAs and their functions remain unexplored, and no oxysterols are yet described.

7. CONSERVED STEROL-SIGNALING PATHWAYS MEDIATE DEVELOPMENTAL PROCESSES

A key role of sterols in *C. elegans* and *D. melanogaster* is the regulation of molting and developmental timing events. Nematodes and arthropods develop through larval stages separated by molts when the exoskeleton is shed and replaced. In *Drosophila*, the cholesterol derivative 20E regulates molting and morphogenesis during larval and pupal development (7). Ecdysteroid pulses during early larval stages induce molting, and later pulses initiate key morphological events of the prepupa and pupa. Mutations in genes of ecdysone synthesis confer severe larval arrest phenotypes, demonstrating the importance of this hormone in development (156). The effects of 20E are mediated by a heterodimer complex of NHRs, the EcR and ultraspiracle (USP, a homolog of mammalian RXR), which initiate transcription of genes critical for life cycle progression (7). The synthesis of ecdysone itself is also hormonally regulated by IIS and prothoraciotropic hormone signaling, which are subject to environmental and nutritional cues, coupling external signals with molting and growth (**Figure 2*b***).

A number of downstream NHRs are activated by the ecdysone cascades in a stage-specific manner to control specific molting programs, including *Drosophila* hormone receptor 3 (DHR3), Fushi tarazu-F1 (Ftz-F1), and DHR38. DHR38, a relative of the mammalian NGF-1B class of NHRs, forms heterodimers with USP/RXR and is regulated by a combination of ecdysteroid and RXR agonists (157). Interestingly, DHR38 lacks a conventional ligand-binding pocket and is thought to be ligand regulated through other structural interactions.

Recent studies suggest that DHR96, the *Drosophila* homolog of DAF-12, binds cholesterol and regulates cholesterol homeostasis and triglyceride metabolism similar to vertebrate LXR (8). Mutants arrest as second instar larvae in low-cholesterol conditions and accumulate cholesterol upon provision of excess. Moreover, numerous genes show cholesterol-dependent regulation through DHR96, although critical evidence confirming cholesterol as a bona fide ligand is lacking. DHR96 targets include the fly ortholog of NPC1-L1, implicated in intestinal sterol absorption, and homologs of NPC2. Mutations in the *NPC1* or *NPC2* genes lead to defective sterol trafficking and decreased ecdysteroid titers (158). Although developmental arrest phenotypes of these mutants can be rescued with 20E, those of *DHR96* mutants cannot, suggesting that DHR96 has a more global role in sterol homeostasis. DHR96 also impacts fat metabolism, as mutants are starvation sensitive and have reduced triglycerides,

Adult reproductive diapause: an arrest of reproductive development, possibly an overwintering strategy in insects, characterized by increased stress tolerance and longevity

whereas overexpressors are starvation resistant and have increased triglycerides (9). These phenotypes arise partly from regulation of a gastric lipase implicated in dietary fat absorption. The similarities between DHR96 and LXR are striking examples of conservation of cholesterol and lipid homeostasis mechanisms.

Although sterols are implicated in *C. elegans* molting, edysteroids have not been identified, and homologs of EcR and USP are not present. Nevertheless, components tied to sterol metabolism or transport impact this process. Intriguingly, mutations in *apl-1*, a homolog of amyloid precursor protein implicated in AD, functions in nematode molting and may work through cell nonautonomous mechanisms via release of its ectodomain (159). The low-density lipoprotein receptor-like protein LRP-1 is required for molting, and loss of proteins homologous to NPC1 and NPC1L1, thought to be involved in sterol transport, induces sensitivity to cholesterol depletion (160, 161). Importantly, two NHRs, NHR-23 and NHR-25, promote molting and are orthologous to the ecdysone-regulated DHR-3 and Ftz-F1 in *Drosophila* (162, 163). Despite the discovery of these components and others, the active hormones, signaling cascades, transport mechanisms, and cognate receptors governing molting remain unidentified.

An important developmental process regulated by sterols in both *C. elegans* and *Drosophila* is diapause, a stress-resistant state of arrested development and reproductive quiescence induced by stressful conditions, possibly analogous to mammalian torpor and hibernation. In flies, adult reproductive diapause (ARD) is important for overwintering (164). *C. elegans* can enter diapause at multiple points including larval L1, L3, and adult stages in response to starvation cues, but the L3 dauer diapause has been most intensively studied (10). Diapause has a major impact on organismal life history, significantly increasing overall life span. Upon return of favorable conditions, animals exit diapause and resume reproductive growth, revealing enormous phenotypic plasticity in response to environmental and nutritional conditions. This physiology may reflect a trade-off between programs geared toward growth and reproduction versus extended survival.

In *Drosophila*, ARD is characterized by arrested ovarian development, decreased metabolism, increased lipid deposition, resistance to stress, and increased longevity (165). A product of sesquiterpenoid biosynthesis, juvenile hormone (JH) regulates many physiological processes, including metamorphosis, all major aspects of reproduction, and ARD. In response to insulin-like signals, JH is produced in the corpora allata gland and is downregulated during ARD. Removal of the corpora allata during adulthood induces ARD, whereas the JH analog methoprene stimulates diapause recovery. Flies in ARD suppress ovarian ecdysteroid synthesis as well as vitellogenesis in the fat body. Reduced insulin signaling may also be responsible in promoting diapause: heteroallelic mutations in the insulin/IGF-I receptor provoke phenotypes reminiscent of ARD, including increased stress resistance and longevity, suppression of JH and ecdysteroid biosynthesis, and arrest of vitellogenesis and reproduction. Moreover, methoprene reverses several phenotypes of these mutants, including longevity. Animals heterozygous for mutations in EcR display increased longevity, revealing that 20E also has life-shortening effects (166). Both JH and ecdysone can restore vitellogenesis of animals in diapause, implying both hormones stimulate diapause exit (165). USP, the RXR-like heterodimeric partner of EcR, has actually been proposed as a receptor for JH, although this remains controversial (167). Overall, JH and 20E evidently act together to regulate a trade-off between reproduction and longevity, promoting increased reproductive capacity at the expense of long life.

A recent discovery suggests that *C. elegans* also has ARD, induced by fasting under crowded conditions (168). Upon removal from food during the mid-L4 stage, resultant adults halt reproduction while harboring two arrested embryos inside the uterus, trim back the germ line, and undergo gonadal regression. Worms in ARD can survive weeks of starvation yet

when returned to favorable conditions will rejuvenate the germ line and other tissues, produce small broods, and live a normal life span. Little is known of the physiologic and molecular events underlying ARD except that it depends partly on the nuclear receptor NHR-49, which functions similarly to mammalian PPARα in regulating fat metabolism, β-oxidation, and response to food deprivation (169). It will be interesting to see if *C. elegans* ARD displays metabolic and molecular features comparable to *Drosophila* ARD or mammalian torpor.

Best understood in *C. elegans* is the dauer diapause, a developmental program executed during the third larval stage in response to stressful conditions of high temperature, low food, sterol depletion, or crowding during early larval development (10). As in the diapausing female fly, dauer larvae display increased lipid accumulation, stress resistance, and longevity. In addition, dauer larvae undergo dramatic morphological changes, including remodeling of the neural system and pharynx, bodily constriction, and secreting a thickened stress-resistant cuticle and cuticle plug over the mouth.

Early evidence for a sterol-derived hormone regulating dauer formation came from the observation that depletion of cholesterol for more than one generation induced dauer formation, revealing that cholesterol promotes nondauer reproductive growth (153, 170). Furthermore, defects in putative sterol transport proteins induced dauer formation, including mutations in *ncr-1* and *ncr-2*, homologs of both *NPC1L1* and *NPC1*, as well as *mrp-1*, a multidrug-resistant protein (161, 171). Perhaps the most critical insights arose, however, from systematic genetic analyses of mutations in genes that govern dauer formation, called *daf* genes, which revealed that the dauer decision is mediated by an endocrine network, including IIS, cGMP, TGF-β, serotonergic, and steroid hormone signaling (10, 172).

Among these genes, *daf-12* encodes an NHR related to mammalian LXRs, FXRα, and VDR, which ultimately mediates the dauer decision (173). *daf-12* null mutants are dauer defective and cannot enter the dauer diapause. Genetic epistasis placed *daf-12* downstream of IIS, cGMP, and TGF-β signaling, as *daf-12* null mutations suppressed dauer-constitutive (Daf-c) mutations in all three pathways (172, 174, 175). In contrast, mutations in the *daf-12* ligand-binding domain confer Daf-c phenotypes, suggesting that loss of the DAF-12 ligand induces dauer diapause (173). Accordingly, animals deficient in DAF-9, a cytochrome P450 related to mammalian steroidogenic enzymes, also constitutively form dauers, which can recover into sterile, stress-resistant adults that are long-lived (170, 176). *daf-12* null mutations completely suppress *daf-9* phenotypes, suggesting that a hormone produced by DAF-9 acts upon DAF-12 to promote reproductive development and prevent dauer formation.

Daf-c: dauer constitutive

Indeed, biochemical analysis revealed that DAF-9 produces BA-like 3-keto steroids called Δ^4- and Δ^7-DA, which work as high-affinity ligands for DAF-12, with Δ^7-DA being more potent (177). A related compound, 25(*S*)-cholestenoic acid, binds DAF-12 as well as its mammalian relative LXR, albeit with lower affinity (178, 179). It is unknown if different ligands have distinct physiological roles and transcriptomes. DA rescues all known *daf-9* phenotypes including constitutive dauer formation, gonadal morphogenesis defects, and longevity. Importantly, by "deorphanizing" DAF-12, these studies identified the first ligands for a NHR in the worm and provided evidence that BAs regulate animal development and longevity.

The current paradigm for dauer formation suggests that, in favorable conditions, environmental and internal signals are integrated via neurosensory processing, resulting in stimulation of IIS and TGF-β signaling (10). These pathways converge in downstream endocrine tissues to induce production of DAs, which activate DAF-12 throughout the body, promoting reproductive development. In unfavorable conditions, DAs are not produced, and DAF-12 works in a corepressor complex with DIN-1, the homolog of mammalian SHARP, to repress reproductive programs and specify dauer formation and longevity (180). This working

model has served as a paradigm for understanding how environmental cues are converted to global hormonal signals that regulate an organismal decision between reproduction and survival, but many questions remain. What is the nature of the hormone biosynthetic pathway? How is it regulated by environmental and signaling inputs? How are inputs integrated to mediate the dauer decision? What are the downstream outputs for stress resistance and longevity?

Further studies have begun to elucidate the DA biosynthetic pathways. On the basis of identified sterols and hormone biosynthetic genes, a branched biosynthetic pathway has been proposed starting with dietary cholesterol and ending with the Δ^7- and Δ^4-DAs (181). The first step in the Δ^7 branch is carried out by DAF-36, a Rieske-like oxygenase, proposed to work as a cholesterol Δ^7-desaturase, converting cholesterol to 7-dehydrocholesterol. Remarkably, the *Drosophila* homolog Neverland works similarly in the first steps of ecdysteroid biosynthesis (182). Although mammalian homologs of *daf-36* do not exist, the first modification in BA synthesis also occurs with hydroxylation by CYP7A1 at the 7 position, suggesting that this residue may play a critical and conserved role in initialization. Downstream, a proposed 5α-reduction to lathosterol and further 3β-dehydrogenation to lathosterone might be achieved by several conserved enzymes present in the worm. HSD-1, a 3β-hydroxysterol dehydrogenase homolog, is proposed to work in the Δ^4 branch, converting cholesterol to 4-cholestene-3-one, although biochemical evidence remains to be found (183). The last step in both of the Δ^4 and Δ^7 pathways is catalyzed by DAF-9, which carries out successive side chain oxidations to create the carboxylic acid moiety, much like its mammalian counterpart CYP27A1 in BA biosynthesis (177). The conservation of these components in nematodes, flies, and mammals suggests an ancient origin for the partition of sterols toward steroid and BA production (**Figure 2*b***).

Another similarity to mammals is the importance of feedback regulation by NHRs. As mentioned above, mammalian LXR promotes feed-forward synthesis of BAs, and FXRα regulates negative feedback through modulation of *CYP7A1* expression. Similarly, DAF-12 regulates DA synthesis through both feed-forward and feedback mechanisms by modulating hypodermal expression of DAF-9/CYP27A1 (184, 185). DAF-9 is expressed constitutively in a pair of neuroendocrine cells, called XXXL/R, from early larval development onward and in the hypodermis from mid-L2 at the point of commitment to reproductive growth. On the basis of DAF-9::GFP expression studies, it is inferred that tonic levels of DA released from XXXL/R are amplified and distributed in the hypodermis upon commitment to reproductive growth. In particular, hypodermal DAF-9 expression is dependent upon *daf-12* and DA. Although modestly expressed in wild-type animals, this expression is absent in *daf-12* and *daf-9* null mutants, whereas such expression is strongly upregulated upon moderately diminished DA production, occurring with mutations in IIS, TGF-β, and *daf-36/Rieske* (181, 184, 185). These observations suggest that when tonic DA levels are absent, DAF-12 represses or fails to activate hypodermal DAF-9, driving dauer development. When levels are low, DAF-12 amplifies hypodermal DAF-9 in a process of positive feedback, promoting reproductive development, whereas when tonic levels are high, DAF-12 decreases hypodermal DAF-9 expression through negative feedback. This complex regulation is thought to tightly control DA levels in a switch-like mechanism, thus ensuring an all-or-none dauer decision and maintaining proper hormone levels for reproductive development. Interestingly, like *daf-12* null animals, *FXRα*-null mice display decreased *CYP27A1* levels and fail to downregulate *CYP27A1* upon BA provision, demonstrating defective feedback mechanisms and the conservation of this regulation (39).

Most nematode species are capable of entering similar diapause states, including pathogenic nematodes. Notably, these states often correspond to the infective stage; thus, targeting entry and exit from this stage is of

interest in creating antihelminthic therapeutics. DAF-12 signaling is remarkably conserved in several parasitic nematodes, and DA treatment of the infective stages of several species, including the canine hookworm *Ancylostoma caninum*, the bacteriovorous beetle parasite *Pristionchus pacificus*, as well as the human pathogen *Strongyloides stercoralis*, promotes diapause exit (186, 187). In *S. stercoralis*, Δ^7-DA prevents the initial formation of infective larvae, suggesting that selective DAF-12 modulators could be used to treat these infections. Evidently DAF-12 signaling has also been co-opted to regulate the dimorphic trait of mouth denticles in *P. pacificus* (188). Upon starvation, these nematodes are predisposed to develop mouth structures used for predation, a switch regulated by DAF-12/DA signaling, revealing an ancient role of this pathway in coupling environmental cues to developmental plasticity. Additionally, a unique feature of nematode sterol metabolism is the production of 4-methyl sterols, an activity ascribed to the STRM-1 methyltransferase thought to partition sterols away from DA biosynthesis (189). Mammals lack this activity, opening the possibility of targeting this enzyme in antihelminthics.

In addition to the dauer decision, DAF-12 regulates the heterochronic circuit, a regulatory hierarchy that specifies the temporal identity of each stage during larval development. DAF-12 generally regulates progression from larval stage L2 to L3, and various *daf-12* mutants repeat L2 programs during the L3 stage in various tissues, most notably affecting the division patterns of epidermal seams, larval stem cells that undergo specific asymmetric divisions at each stage (190). Several direct transcriptional targets of DAF-12 mediate these events, including members of the *let-7* family of microRNAs: *mir-84* and *-241* (191). These microRNAs specify L3 stage programs by downregulating *hbl-1/HUNCHBACK*, which encodes a zinc finger protein that specifies the earlier L2 stage (192). It is proposed that, in the presence of DA, DAF-12 upregulates these microRNAs, which in turn repress *hbl-1* and L2 programs, thereby promoting the L3 fate (191). Conversely, in the absence of DA, these microRNAs are repressed, and animals enter the dauer diapause. Thus, DAs regulate the L2/L3 transition as well as the L2/L3 dauer transition through modulation of DAF-12 activity. By working at the convergence of dauer and heterochronic pathways, DAF-12 couples environmental and global hormonal signals to developmental progression in specific cells and tissues. The role of DAF-12 in this process may be analogous to that of mammalian LXR in promoting developmental progression in dopaminergic neurogenesis or skin differentiation described above (**Figure 2*a***). Interestingly, *let-7* homologs in mammals and flies act in differentiation and metamorphosis, but it remains to be seen whether DAF-12 homologs regulate *let-7* microRNAs to control these processes (193, 194).

Similar to mammalian and *Drosophila* homologs, DAF-12 has also been implicated in lipid metabolism. Mutations in *daf-9/CYP27A1* lead to increased fat storage in L2 animals preparing for dauer (referred to as L2d), dependent upon DAF-12 and its corepressor DIN-1/SHARP (170, 180). Other Daf-c mutants, such as *daf-2* insulin/IGF-I receptor mutants, also display increased fat deposition, but in a *daf-12*-independent manner. Surprisingly, under reproductive growth conditions, mutations in *din-1* alone also lead to increased fat accumulation, dependent upon *daf-12*. Conceivably, the DAF-12/DIN-1 corepressor complex suppresses fat storage in reproductive conditions but promotes fat storage during dauer-inducing conditions. Microarray studies indicate that genes involved in lipid metabolism are misregulated in *daf-12* mutants, but additional molecular and biochemical studies are needed to clarify this role (147).

8. DAF-12 MODULATES *C. elegans* LONGEVITY IN MULTIPLE CONTEXTS

Aside from regulation of worm life span through larval dauer formation, DAF-12 also regulates adult longevity in various contexts. One such context is life span regulation in

response to temperature. *C. elegans* and many other ectotherms live longer at lower temperatures and shorter at higher ones (195). Although a shorter life at higher temperatures is typically ascribed to increased metabolic rates and consequent damage, evidence indicates that this process is regulated in the worm. Temperature sensation in *C. elegans* is mediated by the AFD neurons, a pair of bilateral neurons required for movement to temperatures previously associated with food (196). Loss of AFD function leads to an even shorter life span in response to increased temperature, depending upon *daf-12*, possibly owing to decreased DA production. Accordingly, DAF-9 expression in wild-type animals is increased at higher temperatures, depending upon AFD function, suggesting that thermal sensation by these neurons acts to oppose earlier death at high temperature by upregulating DA and DAF-12 activity. Conversely, at lower temperatures, *daf-9* null mutants are longer lived depending on *daf-12* and *din-1*. DAF-12/DA signaling may thus act in response to temperature to catalyze homeostatic mechanisms that maintain metabolism, and hence life span, within normal bounds.

DAF-12/DA also regulates adult life span in response to signals from the reproductive system, termed gonadal longevity. Animals lacking germ line stem cells, resulting from ablation by laser microsurgery or genetic manipulation, live up to 50–60% longer than wild type (197). Longevity is not the result of sterility alone as animals lacking both somatic gonad and germ line have normal life spans, suggesting that signals originating from these tissues act antagonistically to regulate longevity. Genetic analysis reveals that gonadal longevity depends on the hormone biosynthetic genes *daf-36/Rieske*, *daf-9/CYP27A1*, and *daf-12*, as well as *daf-16/FOXO*, a forkhead transcription factor that promotes longevity and extended survival in response to reduced IIS (170, 181, 197). Germ line ablation further extends the long life of *daf-2/InsR* mutant animals, suggesting that the gonadal pathway and IIS regulate DAF-16/FOXO in parallel. As predicted, treatment of germ line ablated *daf-9* and *daf-36* mutants with DA restores longevity but does not influence wild-type or *daf-12* mutants, indicating that DA works through DAF-12 to promote longevity in animals without a germ line (151). The longevity signal emanating from the somatic gonad is likely DA itself because animals lacking the whole gonad (somatic gonad and germ line) are not long-lived unless supplemented with DA (198).

What mechanisms lie downstream of DA signaling to influence life span? A molecular correlate of gonadal longevity is the translocation of DAF-16/FOXO into the nucleus of intestinal cells, presumably initiating target gene expression (199). DA signaling and DAF-12 appear to facilitate nuclear localization and activity of DAF-16/FOXO because mutants abrogate nuclear localization (151, 199). However, DAF-16 nuclear localization alone is not sufficient for life span extension and requires DAF-12, as expression of a constitutively nuclear-localized DAF-16 does not increase life span of *daf-12* germ line–ablated animals. Interestingly, DAF-16/FOXO targets include fatty acid/cholesterol lipases whose overexpression extends life, whereas loss of function abrogates extension (200). This remarkably suggests that free fatty acids or cholesterol, through hormonal signals or metabolism, impacts organismal life span.

Recent evidence suggests that gonadal longevity may be evolutionarily conserved. In *Drosophila*, ectopic misexpression of *bag of marbles* in the germ line leads to germ line stem cell loss and life span extension (201). Although little is known about the underlying molecular mechanisms, this may arise from impaired IIS because insulin-like peptides and binding proteins are misregulated. In mice, transplantation of ovaries from young mice to older, ovariectomized mice leads to an increased median life span compared with nontransplanted ovariectomized mice or fertile controls, suggesting that the gonad can also control mammalian longevity (202). Further studies should uncover the molecular mechanisms underlying these observations, which include the possibility of sterol signaling pathways. It will be

interesting to see if the mammalian sterol-sensing NHRs are also components of this process to influence longevity. The increasing parallels between invertebrate and mammalian sterol signaling pathways make this an exciting possiblity.

SUMMARY POINTS

1. Sterols are partitioned away from cholesterol and toward biosynthesis of oxysterols, bile acids, and steroid hormones.
2. Conserved sterol-sensing NHR-mediated pathways govern cholesterol partitioning through feed-forward and feedback circuits.
3. Sterol-sensing NHRs couple energy balance with protective and developmental processes, ultimately ensuring survival and reproductive success in the face of changing environments and nutritional states.
4. In vertebrates, sterol-sensing receptors regulate multiple metabolic pathways to impact energy homeostasis, including cholesterol, lipid, and glucose metabolism, as well as modulating the fasting response and possibly torpor.
5. Studies in mammals demonstrate protective roles of these receptors in immunity, hepatoprotection, liver regeneration, and metabolic homeostasis, as well as governing various differentiation processes and possibly influencing longevity.
6. Invertebrate model organisms have provided critical insights into the impact of sterols and corresponding signaling pathways on development, survival, and life span.

FUTURE ISSUES

The vertebrate sterol signaling pathways have illuminated various aspects of metabolism, whereas invertebrate studies have shed light on development and life span. The challenge for the future is to utilize comparative biology and evolutionary conservation to further elucidate the roles of sterol metabolites and their receptors as well as to answer several complementary questions:

1. What functions do vertebrate sterol receptors have in reproduction, differentiation, developmental timing, stress management, and longevity?
2. What is the invertebrate sterol metabolome? And how might their corresponding NHRs couple metabolism with development and reproduction?
3. To what extent does conservation exist between animal models and humans at molecular, mechanistic, and physiologic levels?

The answers to these and other questions are bound to yield fundamental insights into basic biology, with important implications for human health and disease.

DISCLOSURE STATEMENT

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