commentary

Worming our way toward multiple evolutionary origins of convergent sterol pathways[1]

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Sterols represent one of the most ubiquitous and diverse classes of biological molecules derived from the common precursor, mevalonic acid. While there are thematically similar modes by which various organisms synthesize sterols, there also are some unique twists in the pathways by which such organisms produce sterols as well as differences in the chemical nature of the dominant resident sterol present at steady-state in a given organism or cell type. In this issue of the *Journal of Lipid Research*, David Nes and colleagues [Zhou et al. (1)] present a compelling and novel story, wherein they have elucidated a previously unknown alternative biosynthetic pathway utilized by nematodes (roundworms, of which *Caenorhabditis elegans* is an exemplar) to generate C4-methyl sterols. This provides an evolutionary "missing link" along the continuum from prokaryotes to eukaryotes or, eventually, a singular divergent evolution of roundworms with regard to diversification of sterols and the metabolic routes accessible to them to achieve such diversity.

After condensation of two molecules of the branch-point intermediate, farnesylpyrophosphate, to form the 30-carbon intermediate, squalene, and its subsequent epoxidation to form the epoxide 2,3-oxidosqualene, sterol biosynthesis proceeds through the cyclization of 2,3-oxidosqualene to form the steroidal tetracyclic $C_{30}H_{50}O$ products, lanosterol or cycloartenol. Decades of sterol research in prominent laboratories have unveiled the core enzymatic and genetic equipment of eukaryotes that shape sterol end-products by isomerization, desaturation, and reduction of double bonds, as well as demethylation reactions of the committed precursors (2–5), with Δ^7, $\Delta^{5,7}$, and Δ^5 unsaturated tetracyclic rings accounting for the major types of sterol structures. For example, cholesterol, a C_{27} monounsaturated (Δ^5) 3β-hydroxy sterol, is the dominant sterol, by far, at steady-state in mammalian cells. An important set of enzymes that increase sterol diversity in eukaryotes are the sterol-C-methyltransferases (hereafter called SMTs), which carry out the addition of exocyclic carbon atoms on the sterol side chain to produce $C_{28,}$ and C_{29} ergostane and stigmastane backbones, respectively, besides the C_{27} cholestane backbone. A powerful diversification in multigenic families of lineage-specific SMTs confers to eukaryotes the capacity to produce epimeric 24α-alkyl-Δ^5 sterols or 24β-alkyl-Δ^5 sterols with distinct functionalities (6). Such diversification of sterol-C24-methyltransferases is causative of the segmented sterol biosynthesis of eukaryotes, especially in viridiplantae, where two distinct enzymes contribute to the production of campesterol (a 24α–methyl-Δ^5 sterol) and sitosterol (a 24α-ethyl-Δ^5 sterol) (**Fig. 1**). Variation in the position and number of double bonds in sterol side chains is common in nonmammalian eukaryotes, although many fungi synthesize ergosterol (a 24β-methyl-$\Delta^{5,7}$ sterol) because of the activity of a single SMT gene (7, 8) (Fig. 1). Although the origin of the lineage-specific sterol pathways remains unclear, it has been established that the earliest eukaryotes (sponges and heterokonts) had evolved sterol side chain alkylation by distinct SMT gene duplication events as far back as the Proterozoic era (about one billion years ago) (9).

In their article in this issue, Nes and colleagues (1) present a comprehensive analysis of the unusual sterol pathway and novel sterol-C-methyltransferase (dubbed "4-SMT" to distinguish it from the SMTs operating at C-24 on the sterol side chain) utilized by the roundworm *C. elegans*. Nematodes such as *C. elegans* are atypical organisms in which to study sterol biosynthesis because they are sterol auxotrophs, exhibiting a strict dependence on dietary cholesterol. Nematodes lack the genetic equipment to make $C_{30}H_{50}O$ precursors, although they autotrophically produce the C_{15} isoprenoid farnesylpyrophosphate, enabling them to carry out other biologically significant reactions unrelated to sterol synthesis, such as protein

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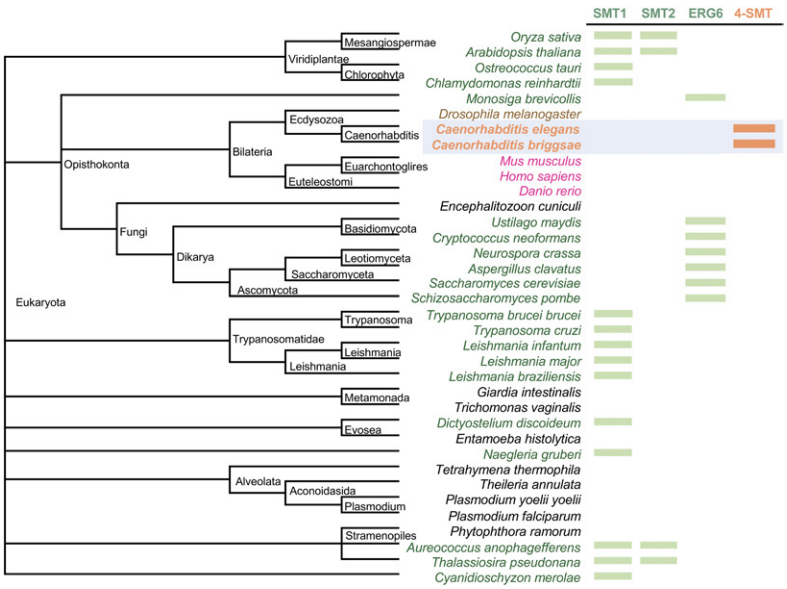

Fig. 1. Orthologous groups of sterol-C-methyltransferases in eukaryotes. Orthologous groups were retrieved from the OrthoDB database (Group 661953at2759 at Eukaryota level). The common tree was based on the NCBI taxonomy database using species names. Sterol autotroph organisms with SMT genes (shown in green), versus organisms without SMT genes (shown in purple). Sterol auxotroph organisms with 4-SMT (shown in orange), versus organisms without 4-SMT genes (shown in brown). Organisms that do not produce sterols are shown in black. 4-SMT, sterol-C4α-methyltransferase; SMT1, cycloartenol-C24-methyltransferase; SMT2, 24-methylenesterol-C24-methyltransferase; ERG6, zymosterol-24-methyltransferase.

prenylation (10). Nematodes are also unusual with regard to other invertebrates. For example, whereas insects convert sterols into the molting hormone 20-hydroxyecdysone, nematodes convert cholesterol into a class of species-specific oxidized derivatives called dafachronic acids (DAs) (11), which in turn afford nematodes the ability to produce lophenol (4α-methyl-cholest-7-en-3β-ol) from cholesterol (i.e., essentially a retro-cholesterol biosynthetic pathway) (**Fig. 2**). [DAs are ligands for the nuclear hormone receptor DAF-12; binding of DAs to DAF-12 inhibits progression to the dauer stage, thereby promoting reproductive development in nematodes (11).] The production of lophenol and 4α-methyl-cholest-8(14)-en-3β-ol as pathway end-products is also mandatory for nematodes, which require 4α-methylsterols at precise stages of their lifespan (11). In fact, the accumulation of these 4α-methylsterols confers upon *C. elegans* the metabolic capacity to minimize the supply of DAs. This metabolic shift causes nematodes to enter a diapause stage typical of so-called "stress-resistant" dauer larvae. Hence, loss-of-function of 4-SMT (also known as STRM-1) promotes DA neosynthesis and blocks entry into the dauer stage (11). The sterol profiles and enzymology of *C. elegans* performed by the Nes group show that the 4-SMT-mediated alkylation at position C4 (ring A of the cholestane nucleus) applies to a cholesterol-derived 3-oxo-sterol substrate (see Fig. 2). Consequently, in agreement with a phylogenomic analysis of sterol-C-methyltransferases (see Fig. 1), the evolutionarily shaped specificity of the 4-SMT active site requires the amphiphilic sterol substrates to rotate for molecular recognition and catalysis. This is reminiscent of other examples in nature involving distinct sterol ligand or substrate recognition sites differing in the same functional groups of proteins;: for example, in the lysosomal cholesterol shuttle, cholesterol binds to the Niemann-Pick C proteins NPC1 and NPC2 in opposite orientation (12), and in the case of certain hydroxylases, sterol nonheme iron-dependent oxidation is performed either at C4 of the tetracyclic ring (as is the case for sterol methyl oxidases) or at C25 of the side chain (as is the case for cholesterol-25-hydroxylase) (13).

Whereas C4-methylsterols generally serve as sterol biosynthetic intermediates (C4-SBIs) in most other organisms and cell types (14), they are pathway end-products in nematodes. This raises questions regarding the evolutionary origin of 4-SMT and, more generally, of other enzymes implicated in the "retro-cholesterol pathway" mentioned above as well as questions regarding the exact function(s) of C4-methylsterols. Nematodes have compact genomes as a consequence of a high rate of large spontaneous deletions, which is responsible, for example, for the loss of steroidogenic genes (15). Nematodes also have a very high rate of evolutionary progression, which is two- to threefold higher than for any other animal group (16). This supports the view that 4-SMT has diverged from remnant SMT genes defining ancient sterol metabolism in LECA (Last Eukaryotic Common Ancestor) (1). Consequently, sterol methylation at C4 and C24 obligatorily relies upon cooption of exapted ancient enzymes. Alternatively, the lophenol pathway in nematodes may have arisen evolutionarily by horizontal gene transfer (HGT). Such processes have been documented in the case of an HGT between bacteria and nematodes to facilitate parasite-host interactions (17). In this regard, the triterpenoid hopane-C2-methyltransferases presumably utilized by ancient cyanobacteria are consistent with the latter speculation (18).

As alluded to above, organisms or cell types with 4α-methylsterols as functional pathway end-products are not frequent, but this is the case for dinoflagellates and the prokaryote *Methyloccocus capsulatus*. Like *C. elegans*, these bacteria synthesize 4α-methyl-cholest-8(14)-en-3β-ol, albeit from lanosterol and not from cholesterol; C4-dealkylation of lanosterol (bacteria) and C4-alkylation of Δ^7-cholestenone (nematodes) converge to the production of 4α-methyl-cholest-8(14)-en-3β-ol (9, 11). In this process, bacteria perform a C4 demethylation reaction of lanosterol (a 4α,4β-dimethylsterol) with an enzyme classified as a Rieske-type oxygenase belonging to a sterol-C4-demethylation complex

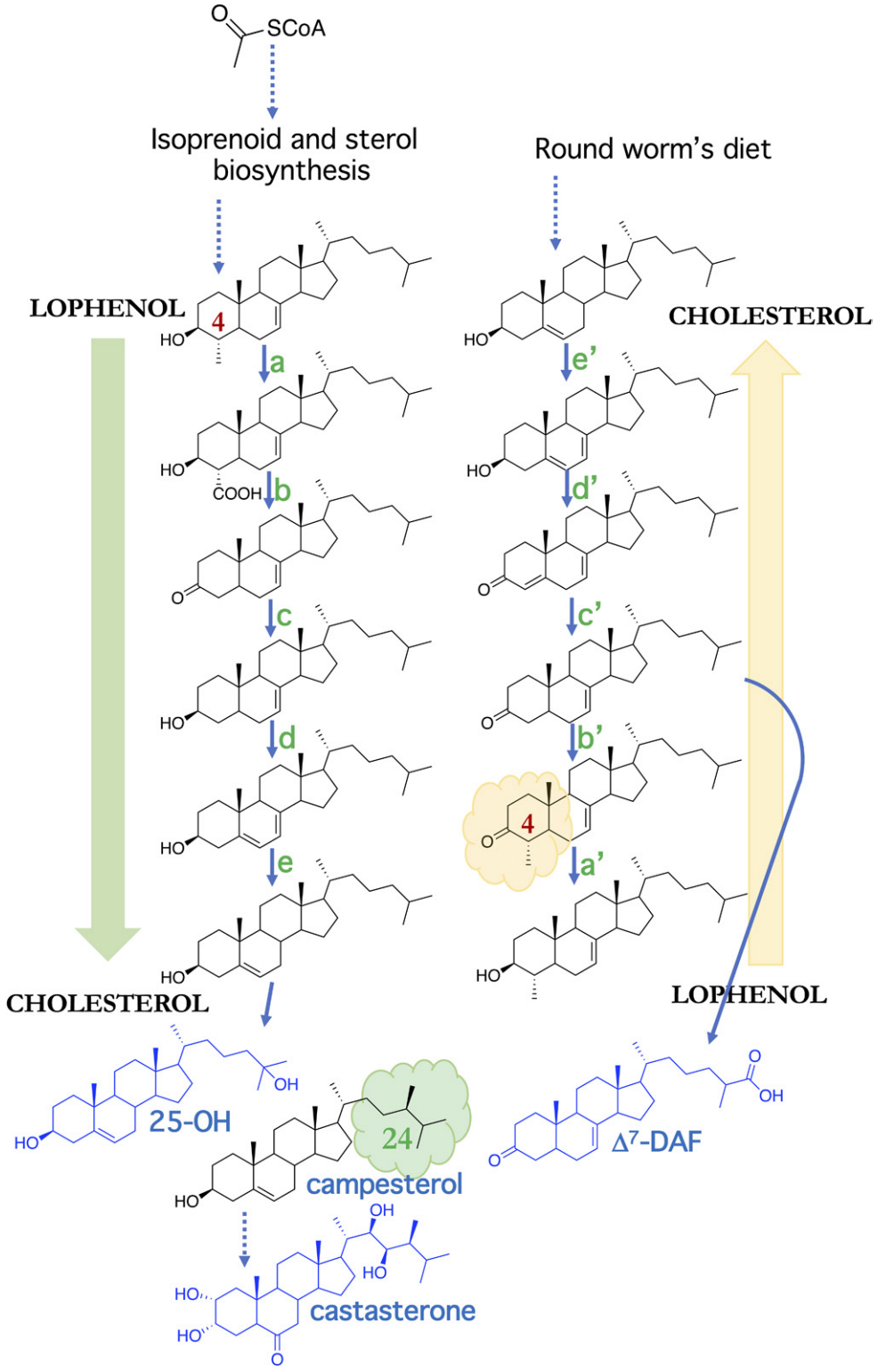

Fig. 2. Simplified divergence of sterol biosynthesis and metabolism in mammals, plants, and other eukaryotes (left) versus nematodes (right). De novo synthesis of cholesterol (cholest-5-en-3β-ol) from acetyl-CoA through the mevalonate pathway proceeds via several isoprenoid and sterol intermediates [left; reviewed in (2–4)]. In this conventional pathway, lophenol (4α-methyl-cholest-7-en-3β-ol) is converted to cholesterol by enzymes a to e. Sterol-C24-methyltransferases (SMTs) when active in this pathway allow the production of 24-alkyl-sterols such as campesterol, bearing a 24-methyl side chain (highlighted in green). Pathway end-products are the precursors of bioactive oxygenated derivatives; e.g., 25-hydroxycholesterol produced from cholesterol, or castasterone, a brassinosteroid plant hormone derived from campesterol. a, sterol 4-methyl oxidase; b, 4-carboxysterol decarboxylase; c, sterone-Δ^3-reductase; d, sterol-Δ^5-desaturase; e, sterol-Δ^7-reductase. Alternatively (right), in nematodes such as *C.elegans*, lophenol is synthesized from dietary cholesterol in their environment [see (1,11)]. Successive putative or demonstrated enzymes are: e', cholesterol-Δ^7-dehydrogenase (DAF36); d', sterol dehydrogenase (DHS16); c', sterol-Δ^4-reductase; b', sterol-4-methyltransferase or 4-SMT; a', sterone-Δ^3-reductase. The 3-oxo-sterol substrate of the 4-SMT is also a precursor of a dafachronic acid (Δ^7-DAF). 25-OH, 25-hydroxycholesterol. C4 of the sterol nucleus is indicated by the red 4.

notably different from the eukaryotic sterol-C4-demethylation complex of unlinked evolutionary origin (19). The presence of 4-methylsterols in dinoflagellates and bacteria has been related to adaptation to water stress, high salt, or low oxygen conditions (14, 19). In *C. elegans*, anaerobiosis-related genes are expressed during the dauer stage, and lipid metabolism is furthermore reduced (20). It is therefore tempting to consider C4-methylsterols of auxotrophic worms as endogenous signals, analogous to what has been proposed for mammals or plants (14, 21). Remarkably, steroidogenic enzymes in *C.elegans* are components of a sterol pathway of a different evolutionary origin than those operating in other sterol autotrophs, but with a clear functional convergence. The contributions of David Nes and his colleagues herein to our current understanding of sterol diversity and the underlying pathways by which such diversity arises represents an artful foray into this topic, and is highly recommended to the reader.

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