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Hypothesis Paper

Special relationship between sterols and oxygen: Were sterols an adaptation to aerobic life?

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ABSTRACT

A fascinating link between sterols and molecular oxygen (O_2) has been a common thread running through the fundamental work of Konrad Bloch, who elucidated the biosynthetic pathway for cholesterol, to recent work supporting a role of sterols in the sensing of O_2. Synthesis of sterols by eukaryotes is an O_2-intensive process. In this review, we argue that increased levels of O_2 in the atmosphere not only made the evolution of sterols possible, but that these sterols may in turn have provided the eukaryote with an early defence mechanism against O_2. The idea that nature crafted sterols as a feedback loop to adapt to, or help protect against, the hazards of O_2 is novel and enticing. We marshal several lines of evidence to support this thesis: (1) coincidence of atmospheric O_2 and sterol evolution; (2) sterols regulate O_2 entry into eukaryotic cells and organelles; (3) sterols act as O_2 sensors across eukaryotic life; (4) sterols serve as a primitive cellular defence against O_2 (including reactive oxygen species). Therefore, sterols may have evolved in eukaryotes partially as an adaptive response to the rise of terrestrial O_2, rather than merely as a consequence of it.

Introduction

Different sterols occur in different life forms: cholesterol is the predominant sterol in vertebrates, ergosterol in yeast and other fungi, and phytosterols are the major sterols in plants (Fig. 1). In this review/hypothesis paper, we will focus mainly on cholesterol, bringing in fungal systems when relevant, and drawing on selected examples from the plant kingdom. This paper sets out to examine the special relationship between sterols and molecular oxygen (O_2). Of course, O_2 is an absolute requirement in the formation of sterols. However, we hypothesize that the relationship is reciprocal, posing the question: Were sterols evolved, at least in part, as an adaptive response to the rise of O_2 in the environment? We gather evidence that supports the idea that natural selection crafted sterols as a feedback loop to adapt to, or help protect against, O_2 and/or reactive oxygen species (ROS) (Fig. 2).

The Rise of O_2

Three billion years ago, the earth was a third of its present age, and would have been unrecognisable to its current inhabitants. Life consisted of anaerobic microbes eking out an existence in an anoxic environment. The electron economy required to support their metabolism was a simple trade conducted through a set of well-structured anaerobic pathways [1]. The largest pool of electrons, water, was untapped until sometime before 2.5 billion years ago, when cyanobacteria emerged with photosynthesis. Utilizing the energy of the sun and using water as a reductant to fuel metabolism, these ancient organisms produced O_2 as a metabolic by-product [2,3]. Of course, this waste product had life-changing consequences. O_2 eventually overwhelmed the reducing atmosphere and, over the eons, levels gradually rose to the 21% O_2 that we currently breathe. O_2 drove the evolution of single-cell eukaryotes and eventually pushed the development of the more complex life forms that populate the planet today (Fig. 3A) [4]. Aerobic metabolism was far more efficient in extracting energy from fuels such as glucose, but this came at a cost. The reactivity of O_2 means that it can be extremely toxic to life [5]. Therefore, the accumulation of O_2 also stimulated the development of metabolic refinements that protected organisms from oxidative damage [6]. We argue that the evolution of sterols may represent one of these protective mechanisms.

Abbreviations: CHO, Chinese hamster ovary; DHCR24, 3β-hydroxysterol-Δ24-reductase; EPR, electron paramagnetic resonance; ESR, electron spin resonance; H_2O_2, hydrogen peroxide; HIF-1α, hypoxia-inducible factor 1α; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; O_2, molecular oxygen; RBC, red blood cell; ROS, reactive oxygen species; Seladin-1, Selective Alzheimer's Disease Indicator-1; SREBP, sterol regulatory element binding protein.

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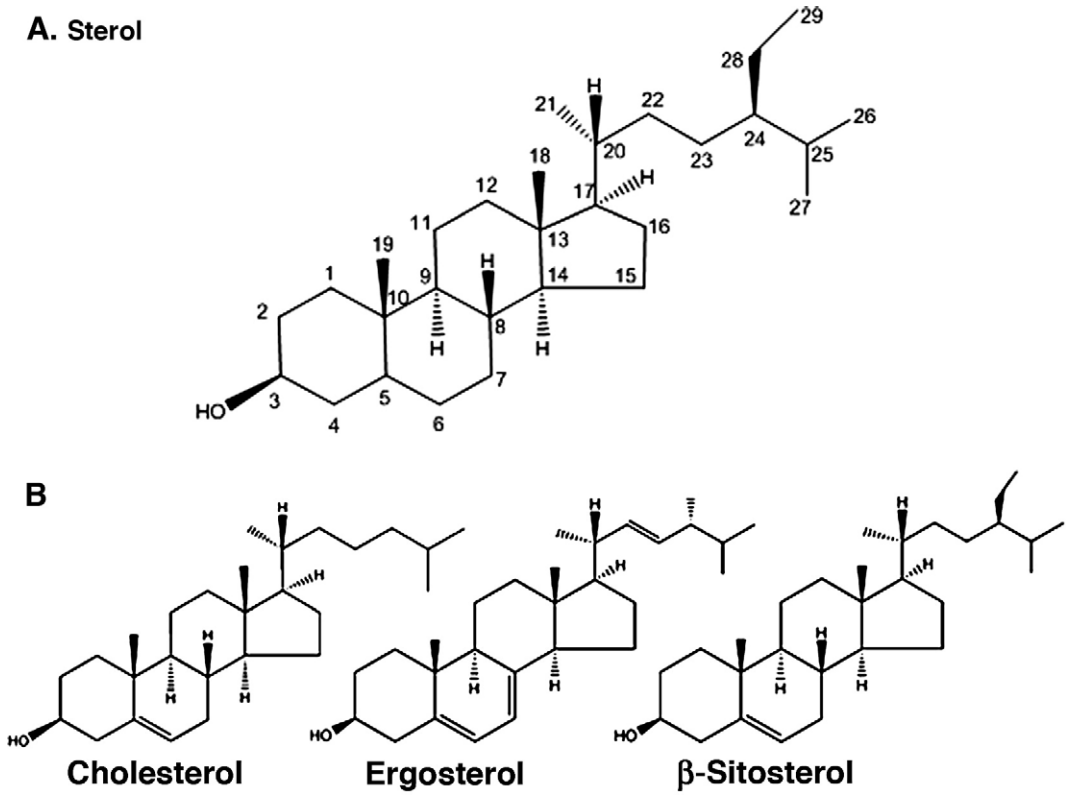

Fig. 1. Sterol structures. (A) A generalised sterol structure with carbons numbered (C1–29). (B) Structures of the major sterols found in animals (cholesterol), fungi (ergosterol), and plants (β-sitosterol).

Coincidence of the appearance of O_2 and the evolution of sterols

Sterols, like cholesterol, could only be produced after the advent of aerobic metabolism, since their synthesis is dependent on O_2 [7]. The synthesis of one molecule of cholesterol, ergosterol, and phytosterol requires 11, 12, and 11 molecules of O_2, respectively (Fig. 4) [8]. For cholesterol synthesis, squalene monooxygenase (EC 1.14.99.7) introduces the first oxygen atom, which becomes the 3β-hydroxyl group of cholesterol. Oxidosqualene cyclase (EC 5.4.99.7) then transforms the flexible hydrocarbon chain of monooxidosqualene into the rigidly locked structure of lanosterol [7]. Further oxidations are required to remove the three additional methyl groups of lanosterol. Jahnke and Klein [9] quantified the O_2 needs of squalene monooxygenase in yeast and found the half optimal concentrations of O_2 to be 1–2% of present-day levels. Bloch [7] noted that this together with the increasing O_2 needs of the sequential demethylation of lanosterol supports the idea that "the stepwise progression of the sterol pathway from squalene via lanosterol demethylation was driven by the gradually rising O_2 levels in the terrestrial atmosphere" (Fig. 3B).

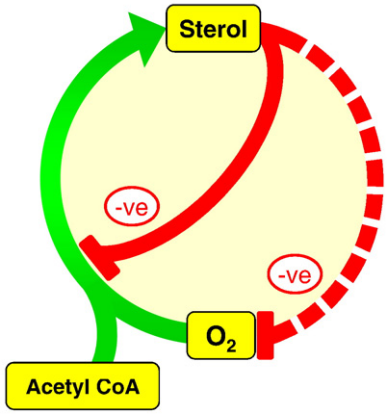

Fig. 2. A schematic representation of our hypothesis: sterols negatively feed back on one of its key inputs, O_2, by limiting its entry into the cell. This hypothesis is built on the known requirement of O_2 in sterol synthesis and the recognized feedback mechanisms whereby sterols inhibit their own synthesis. The evidence for this hypothesis is discussed in the text.

Prokaryotic hopanoids as sterol surrogates

Hopanoids can be considered to be the prokaryotic prototype of sterols. They are also ring-structured products of the mevalonate pathway, but do not require O_2 for their synthesis. As a result, they lack the 3β-hydroxyl group of sterols (Fig. 5A). Nevertheless, it has been speculated that hopanoids fulfill a unique role in acidophile membrane structure, namely, the prevention of inward leakage of protons [10]. In addition, hopanoids are the major class of lipids comprising the membranes that envelope the nitrogen-fixing soil bacterium, *Frankia* spp. Here, hopanoids have been suggested to exclude O_2 by compacting the lipid bilayer [11]. This may be one of several strategies the organism has to protect the O_2-labile, nitrogen-fixing enzyme, nitrogenase. Therefore, just as hopanoids may have fulfilled an evolutionary need in allowing acidophilic bacteria to cope with their environment or nitrogen-fixing bacteria to protect nitrogenase, we propose that sterols may have fulfilled a similar need in eukaryotes as O_2 levels rose.

Sterols as highly evolved eukaryotic hallmarks

Sterols are often considered a defining characteristic of eukaryotes, absent from prokaryotes. However, there are exceptions. The lanosterol cyclizing enzyme, Oxidosqualene cyclase, can be found in bacteria such as *Methylococcus capsulatus*, where it may have been acquired via a lateral transfer from eukaryotes soon after the divergence of eukaryotes and bacteria [12]. Conversely, not all eukaryotes

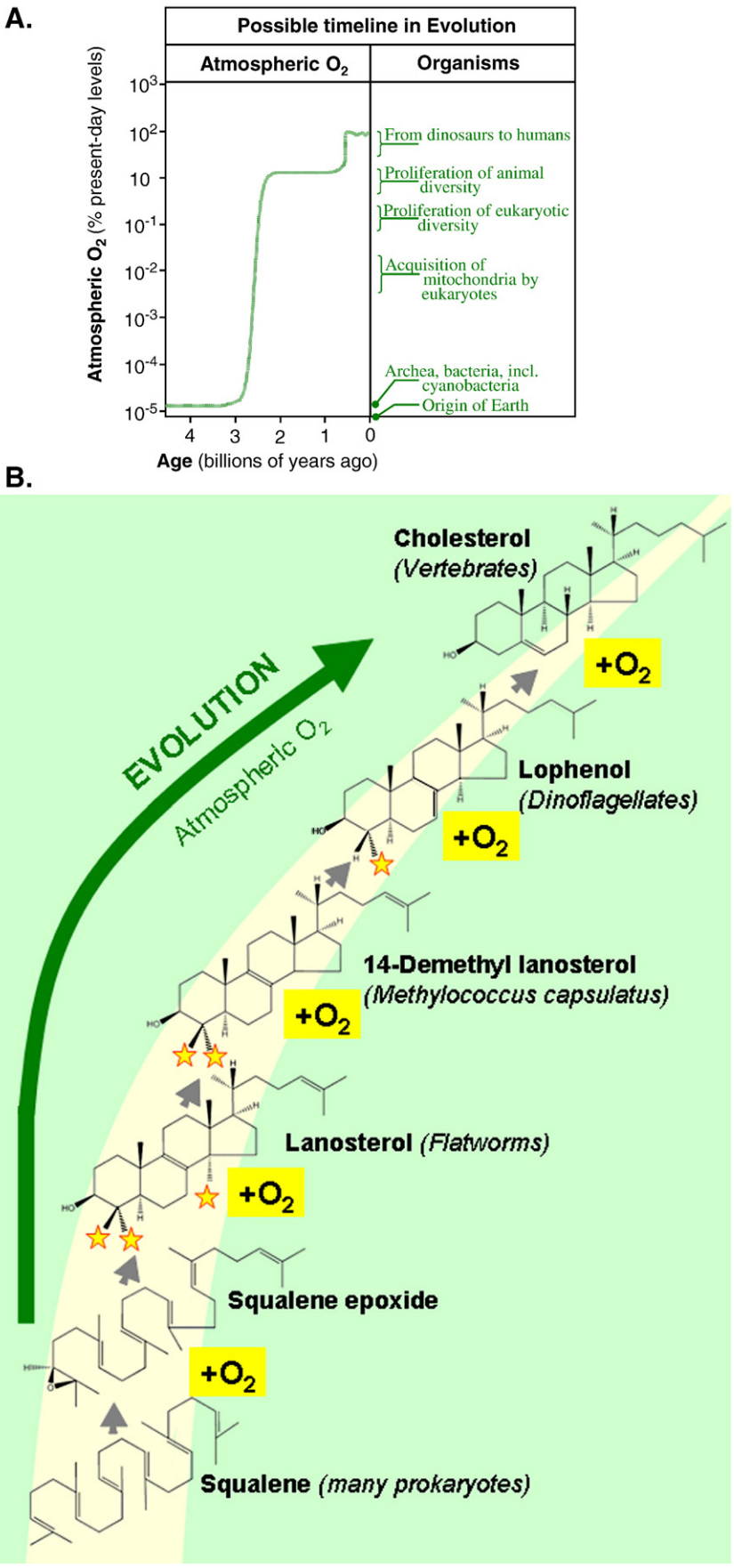

Fig. 3. Coincidence of the appearance of O_2 and the evolution of sterols. (A) An estimate of the rise of atmospheric oxygen over time indicating some evolutionary milestones (after [2,4]). (B) Evolutionary chronology of the sterol structure (after [7]). The stars indicate the methyl groups which are lost during sequential oxidations in the conversion of lanosterol to cholesterol.

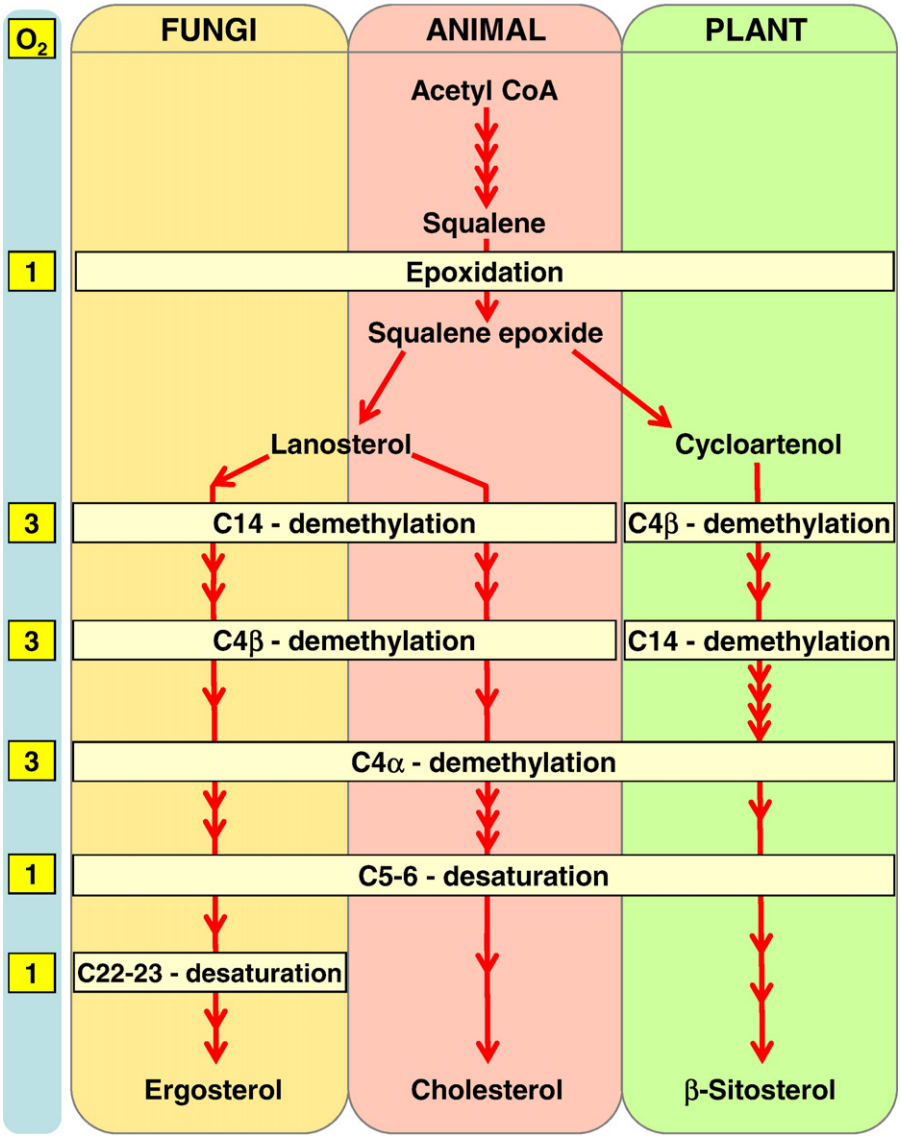

Fig. 4. The key oxygen requiring steps of sterol synthesis in fungi, animals, and plants. The arrowheads indicate the approximate number of intervening steps. (After [8]).

synthesize cholesterol (or related tetracyclic sterols). Numerous squalene cyclization products known as pentacyclic triterpenes, which are generated anaerobically, are found in plants and some protozoans. One example is tetrahymanol, isolated from the ciliated protozoan *Tetrahymena pyriformis* (Fig. 5B). Tetrahymanol seems to play a physiological role akin to that played by cholesterol in other eukaryotes, that is, as a membrane reinforcer. Notably, tetrahymanol can be readily replaced with added cholesterol with no impairment of growth of *Tetrahymena* that cease to produce tetrahymanol [7]. Therefore, although sterols tend to be a characteristic feature of eukaryotes, there are other molecules which may fulfil a similar function in eukaryotes and prokaryotes.

Konrad Bloch argued that cholesterol represents evolutionary perfection, in terms of its functional fitness as a membrane reinforcer. The first sterol structure, lanosterol, has three extra methyl groups protruding from the planar face of the molecule, giving it a rough, knobbly structure. By contrast, the structure of cholesterol is smooth and streamlined, conferring vastly superior membrane properties to lanosterol. Thus, Bloch [7] noted that each sequential demethylation (see Fig. 3B) results in functional improvement of the sterol molecule when tested for effects on membrane fluidity in a liposomal model or growth in *Mycoplasma capricolum* (which require sterols for growth). Indeed, Bloom and Mouritsen [13] proposed that the biosynthesis of cholesterol in an aerobic atmosphere removed a bottle neck in the evolution of eukaryotes.

Are sterols and O_2 linked in a feedback loop?

The central thesis of this review is that sterols and O_2 are intricately entangled. There are many examples across eukaryotic life where sterol levels rise and fall as a function of O_2. For instance, prolonged exposure to O_2 at pressure (hyperbaric O_2) increased cholesterol content of red blood cell (RBC) membranes in human subjects [14]. Other examples are noted in later sections. Beyond the dependence of sterol synthesis on O_2, we propose that there is a reciprocal arrangement whereby sterols in turn help to protect the cell against O_2. Here, as an argument in favor of this proposition, we invoke the concept of feedback control.

An evolutionary imperative is that organisms need to adapt to their changing environment in order to survive. A key strategy employed is feedback control, a recurring theme in biology. Feedback control helps to maintain homeostasis and manage uncertainty in the face of changing metabolic demands. At its simplest, feedback control can be defined as a process in which the level of one

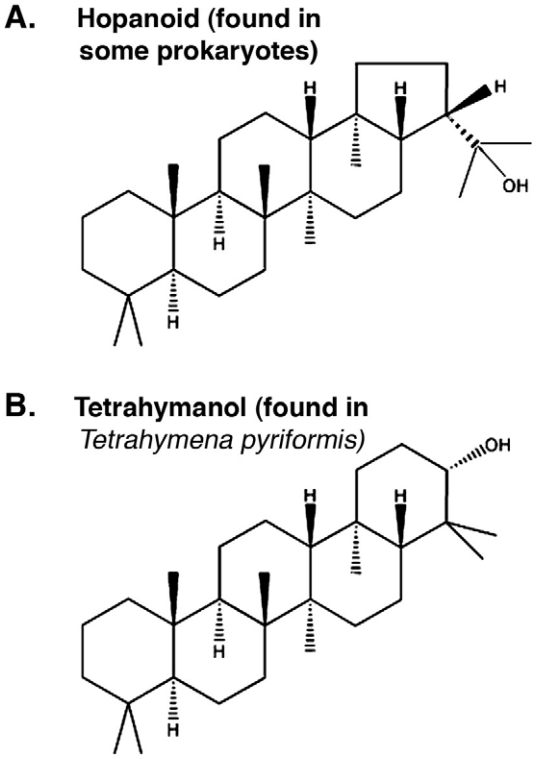

Fig. 5. Structures of two polcyclic triterpenes. (A) A hopanoid (diplopterol) and (B) tetrahymanol. These molecules are formed anaerobically from squalene directly. Their hydroxyl groups are derived from water not O_2. (After [7]).

substance influences the level of another substance. Indeed, cholesterol was one of the first examples of feedback control in biochemistry, when Schoenheimer and Breusch [15] found that mice produced less cholesterol when placed on a cholesterol-rich diet. Subsequently, huge advances have been made in our understanding of the molecular basis of mammalian cholesterol homeostasis through elucidation of the SREBP (sterol regulatory element binding protein) pathway [16], and regulation of a key flux controlling enzyme in cholesterol synthesis, HMG (3-hydroxy-3-methyl-glutaryl)-CoA reductase [17].

In negative feedback regulation, a metabolic end-product can down-regulate its own synthesis. Hence, in cholesterol synthesis, accumulation of cholesterol (or other sterols) will feed back on and inhibit this pathway [18]. It should be remembered that O_2 represents a raw material in the construction of sterols, as captured in the signature 3β-hydroxyl group. Because O_2 is required for cholesterol synthesis, feedback control may go beyond end-product inhibition to encompass the following scenario: the accumulation of cholesterol in the plasma membrane also serves to limit the availability of O_2 in the cells (Fig. 2). There is a corollary for this in cholesterol homeostasis, in that sterols feed back on production of the cofactor NADPH, which is needed as reducing power in the generation of cholesterol, since the three enzymes capable of producing NADPH are all SREBP target genes [19]. Therefore, sterols influencing cellular O_2 levels may signify another loop in feedback control (Fig. 2).

Sterols as membrane gatekeepers for O_2

The majority of cholesterol in the mammalian cell is located in the plasma membrane and therefore a major role attributed to sterols is their barrier function. Accordingly, cholesterol functions as a molecular "polyfiller," plugging gaps in the phospholipid bilayer. By thickening and condensing the membrane, cholesterol prevents polar molecules from leaking across the membrane. Cholesterol's ability to suppress passive permeability has been found for a wide variety of compounds, suggesting that its barrier function is generic [20]. However, it should be remembered that O_2 is not a polar molecule. Therefore, this barrier property cannot be automatically extended to the permeation of small solutes like O_2 [21]. Moreover, the ease with which O_2 dissolves in a bulk hydrocarbon solvent, such as olive oil, does not necessarily reflect its diffusion through a lipid bilayer, as was the customary practice [22]. So what evidence is there that sterols restrict O_2 entry into the cell?

There are several examples in the literature where the sterol content of cell or organelle membranes affects O_2 transport across it. We will highlight several of these.

Red blood cells (RBC)

Buchwald and co-workers [23] found that plasma cholesterol concentration is in equilibrium with RBC membrane cholesterol levels, and this in turn affects O_2 transport in and out of the RBC. In a study on venous blood collected from 22 volunteers, they reported that the plasma cholesterol and RBC membrane cholesterol levels were negatively correlated with the percentage changes in O_2 saturation (Fig. 6A). Therefore, high cholesterol content in the RBC membrane was associated with reduced blood O_2 transport [23]. Subsequently, a key experiment was conducted by the same group employing a

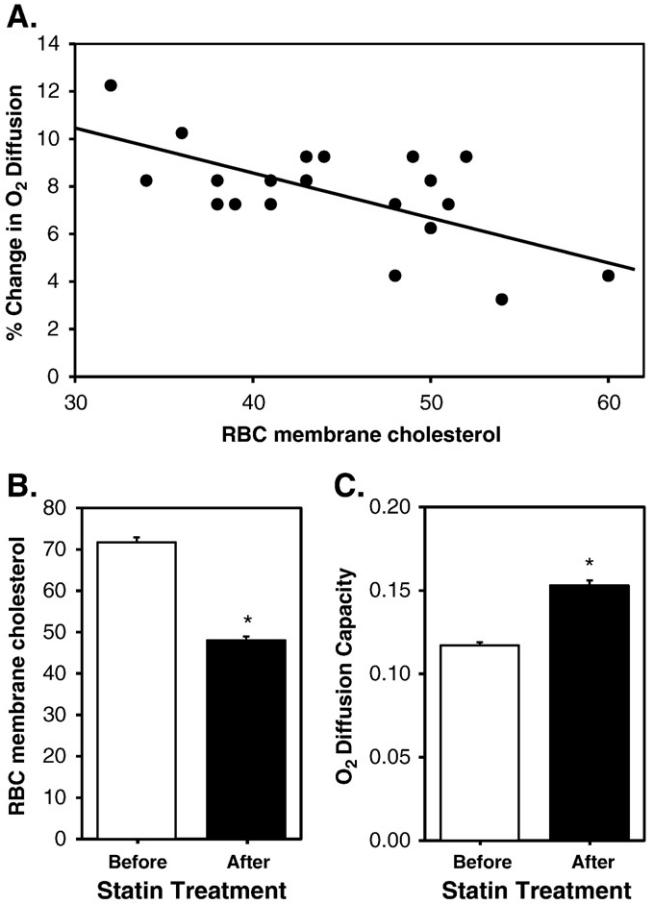

Fig. 6. The relationship between the cholesterol content of red blood cell (RBC) membranes and the O_2 diffusion capacities across it. (A) The correlation of percentage change in O_2 diffusion with RBC membrane cholesterol (mg/dl) from 22 human subjects ($R^2 = 0.39$; $P < 0.005$; adapted from [23]). (B) RBC membrane cholesterol levels (mg/dl) and (C) Blood O_2 diffusion capacity (μl/ml/s) before and after statin treatment in 18 human subjects (values expressed as mean ± SEM; * $P < 0.05$ from Student t tests; adapted from [24]).

cholesterol-lowering drug (statin) which showed that this effect was reversible. Statin treatment decreased blood cholesterol and the content of cholesterol in the RBC membranes decreased by ~30%, while there was a corresponding increase in the blood O_2 diffusion capacity (Figs. 6B and C) [24].

Lens fiber cells

The lens is responsible for focusing of the eye and absorbs much of the ultraviolet irradiation to which the eye is exposed. Fiber cells which comprise the mammalian lens are packed in a highly ordered array. The plasma membrane of fiber cells is particularly rich in cholesterol, usually in excess of the ratios of 0.5 to 1 mole of cholesterol per mole of phospholipid that typify most mammalian cells [25]. Hence, fiber cells are very sensitive to disturbances in cholesterol homeostasis. Consequently, cataracts are common in genetic disorders of cholesterol metabolism and are associated with some no longer prescribed or experimental cholesterol-lowering drugs [25]. An increase in O_2 concentration is thought to be responsible for cataract formation following hyperbaric O_2 treatment or vitrectomy, the surgical removal of the vitreous gel from the middle of the eye [26,27]. Widomska and colleagues used electron paramagnetic resonance (EPR, equivalent to electron spin resonance (ESR)) to compare O_2 transport across model membranes made from calf lens lipids, phospholipids alone, or an equimolar mixture of phospholipids and cholesterol [21]. They concluded that cholesterol at high concentrations reduced O_2 permeability three- to fivefold. In addition, they found that resistance to O_2 transport is located around the polar head group region of the membrane to a similar depth to where the steroid-ring structure of cholesterol reaches into the membrane. This suggests that cholesterol may be ideally placed in the lipid bilayer to retard O_2 transport.

Chinese hamster ovary (CHO) cells

Khan and colleagues [28] studied O_2 diffusion (by EPR oximetry) across the cell membrane of CHO cells with genetically altered capacities to synthesize and take up cholesterol, in addition to biochemical manipulations to alter cellular cholesterol status. They concluded that the concentration of cholesterol in the plasma membrane can be an important determinant for the magnitude of the O_2 gradient observed across the plasma membrane.

Mitochondria

Consistent with the idea that the cholesterol-rich plasma membrane is the cell's outermost defence, there is a decreasing cholesterol gradient from the plasma membrane to the beginnings of the secretory pathway in the endoplasmic reticulum [29]. However, the mitochondrion has the lowest cholesterol content of any organelle membrane in keeping with its likely prokaryotic origin, but also in line with its high consumption of O_2 as the cell's powerhouse. In support of this contention, several studies have observed close correlations between increases in the cholesterol content of mitochondrial membranes and decreases in mitochondrial respiratory function [30–32]. For example, Parlo and Coleman observed markedly reduced respiration in Morris hepatoma mitochondria which become enriched with membrane cholesterol endogenously during tumorigenesis [31]. Their investigation extended to normal mitochondria enriched with membrane cholesterol *in vitro*, which exhibited a reduced capacity to perform oxidative phosphorylation that was similar to that observed in the hepatoma mitochondria. More recently, Modi and colleagues demonstrated that an ageing-induced increase in the mitochondrial cholesterol of brain and liver tissues from rats was also associated with markedly impaired respiratory function [33].

Thylakoid membranes from plants

There are interesting parallels between mitochondria and the key energy-generating system in plants. The *thylakoid* is a membrane-bound compartment inside chloroplasts which also normally contains little or no sterols [34]. Popova and colleagues observed changes in the fluidity and oxygen evolving capacity of isolated thylakoid membranes enriched with the plant sterol, stigmasterol [35]. Structural alterations in the sterol-modified membranes included a decrease in the degree of lipid head group packing and an increase in the rigidity of the hydrophobic phase of the lipid bilayer. The investigators proposed that these alterations reduced O_2 levels by decreasing the number of O_2-evolving centers in the membranes. Although not directly addressing O_2 diffusion across the membranes, this work nonetheless supports the central tenet of our thesis: sterols act to decrease O_2 levels within the cell/organelle.

Therefore, there is abundant evidence across a variety of membrane models indicating that an increased cholesterol content of the membrane impedes O_2 transport across it. Together, these studies imply that a balance between membrane cholesterol content and cellular oxygen requirements has been finely tuned throughout evolutionary processes in order to optimize cellular metabolism.

Sterols: The antioxidant connection

Cholesterol as an antioxidant?

Cholesterol's abundance in the cell membrane places it in an ideal position to encounter ROS like hydrogen peroxide (H_2O_2) that cross this permeability barrier [36]. Therefore, sterols like cholesterol are usually thought of as the victims of O_2. Accordingly, oxidation of cholesterol produces oxysterols which have been noted in certain pathologies such as atherosclerosis [37]. However, Smith [38] argued that the presence of oxysterols signifies past oxidant interception by cholesterol, and together with efficient liver metabolism of oxysterols, constitutes an antioxidant protection system. This "cholesterol as antioxidant" idea remains contentious. Nonetheless, a protective effect of cholesterol against peroxidation, due either to its antioxidant action or to its capacity to stabilize the plasma membrane, has been reported for a variety of model systems. For example, Lopez-Revuelta and colleagues [39] found that depletion of cholesterol from RBCs increased their vulnerability to peroxidation which could be reversed by cholesterol repletion.

Cholesterol and renal cell injury

In experimental models of acute renal failure, cholesterol levels rise significantly in renal tubular cells following diverse forms of injury, including free radical-mediated oxidation [40]. Interestingly, this cholesterol accumulation is thought to contribute to "acquired cytoresistance," an effect that protects the kidney cells from further damage, possibly by stabilizing the plasma membrane [41]. When Zager and colleagues experimentally reduced cholesterol levels in proximal tubule cells using several different approaches, their susceptibility to injury was dramatically enhanced in each case [41]. Furthermore, biochemical perturbations of the plasma membrane cholesterol by low-dose cholesterol esterase or cholesterol oxidase treatment rendered these cells highly vulnerable to various challenges, including iron-mediated oxidative stress [41,42].

Sterols and H_2O_2

In budding yeast (*Saccharomyces cerevisiae*), the diffusion rate of H_2O_2 through the plasma membrane decreases during adaptation to H_2O_2. Sterols are implicated in this adaptive response, since disruption of ergosterol synthesis increases the sensitivity of the cells to H_2O_2

[43]. Interestingly, addition of cholesterol to *Acholesplasma laidlawii*, an unusual type of bacterium lacking a cell wall and being bound by only a plasma membrane, also protected this organism against oxidative damage caused by H_2O_2 [44].

A cholesterol biosynthetic enzyme and H_2O_2

Intriguingly, the enzyme that catalyzes the penultimate step in cholesterol synthesis in mammalian cells has been shown to directly scavenge H_2O_2 [45]. 3β-Hydroxysterol-Δ24-reductase (DHCR24) is a *flavin* adenine dinucleotide-dependent oxidoreductase that converts desmosterol to cholesterol. The gene was initially identified in plants (*DIMINUTO*/*DWARF1*) with the human orthologue originally described in neuronal cells and named *Seladin-1*, the *Sel*ective *A*lzheimer's *D*isease *I*ndicator-*1*. DHCR24 is down-regulated in affected neurons in Alzheimer's disease and has therefore been proposed to play a role in neuroprotection, possibly by increasing lipid raft formation [46]. This H_2O_2 scavenging ability provides a link, albeit indirect, between cholesterol synthesis and antioxidant properties.

Cholesterol in lung surfactant

Accumulation of cholesterol has been noted within the O_2-filled swim bladder of deep sea fish, consistent with a protective role [47]. The lungs of terrestrial animals are similarly the first line of defence against the hazards of O_2. Lungs are particularly susceptible to ozone, a less stable, more toxic allotrope of O_2. Ozone reacts rapidly with cholesterol forming a complex array of products [36]. It is interesting to note that lungfish and other primitive air-breathers have very high cholesterol contents in their pulmonary surfactant, and it has been proposed that these may represent "the vertebrate protosurfactant" [48]. Furthermore, cats exposed to O_2 at high pressure showed altered surfactants with a 150% increase in intra-alveolar cholesterol levels [49]. Therefore, cholesterol in lung surfactant may represent a primitive defence mechanism against the risks associated with oxygen-breathing.

Taken together, there are a few facets of sterols which may confer antioxidant properties. Apart from the direct H_2O_2 scavenging ability of the cholesterologenic enzyme, DHCR24, there is some evidence that sterols may exert protection against oxidant attack, most likely by reducing entry of a ROS like H_2O_2. It is noteworthy that cholesterol in the plasma membrane also reduces passage of the reactive nitrogen/oxygen species, nitric oxide, through the bilayer, diminishing its subsequent signaling [50]. Alternatively, highly reactive ROS like hydroxyl radicals, peroxynitrite, hypochlorite, and perhaps singlet oxygen, would be expected to react before they could cross the membrane.

Sterols as O_2 sensors

In a landmark study, Espenshade and colleagues described a novel eukaryotic O_2-sensing mechanism involving sterols in the fission yeast *Schizosaccharomyces pombe* [51]. They uncovered the *S. pombe* equivalent of the SREBP transcription factor that is involved in cholesterol homeostasis in mammals, which they named Sre1. Remarkably, they found that the molecular targets for Sre1 only partially overlapped with those in mammals. Notably, Sre1 did not control the early rate-limiting step in sterol biosynthesis (equivalent to HMG-CoA reductase)—instead, the molecular targets relate to O_2. Activation of Sre1, by the depletion of either sterols or O_2, induced genes involved in the late, O_2-dependent parts of the sterol synthesis pathway, and in the biosynthesis of heme (an O_2-binding molecule). Since the synthesis of sterols is absolutely dependent on O_2, employing sterol synthesis as a surrogate indicator of O_2 levels is an apt strategy, allowing cells to scavenge O_2 in a hypoxic environment [52]. This strategy exists in more than one yeast species, with SREBP-like proteins shown to operate in an analogous manner in the human fungal pathogens, *Cryptococcus neoformans* [53] and at least to some extent in *Aspergillus fumigatus* [54].

Similarly, in the budding yeast *Saccharomyces cerevisiae*, both sterols and heme depend on O_2 for their synthesis, and thus the levels of both have the potential to act as indicators of the O_2 environment. However, *S. cerevisiae* lack an equivalent of Sre1/SREBP. Davies and Rine [55] found that the depletion of sterol, not heme, was the signal responsible for the hypoxic activation of two sterologenic transcription factors (Upc2p and Ecm22p). This work indicates a conserved role for sterol levels in O_2 sensing in *S. cerevisiae*, although via an alternative molecular mechanism.

A connection has also been made between O_2 sensing and cholesterol in animal cells [17]. Hypoxia causes the accumulation of lanosterol and 24,25-dihydrolanosterol, intermediates preceding the most O_2-intensive demethylation steps in cholesterol synthesis. These sterols feed back on cholesterol synthesis by accelerating the degradation of the committed enzyme, HMG-CoA reductase. This proteasomal demolition of HMG-CoA reductase also requires the action of the O_2-sensitive metazoan transcription factor, HIF-1α (hypoxia-inducible factor 1α) [17].

Therefore, O_2 sensing by sterols across eukaryotic life is a striking demonstration of the special relationship between sterols and O_2. It is but a short step from this surveillance role of sterols to our proposition that sterols actively guard against excess O_2 crossing cell membranes.

Medical implications

The special relationship between sterols and O_2 detailed above has clinical ramifications.

Cholesterol in RBC

Cholesterol, as an integral part of the RBC membrane, has a role in RBC O_2 transport and hence may help to predict the extent of tissue oxygenation [23]. Blood cholesterol lowering therapy could be expanded to include the relief of tissue hypoxia, such as angina pectoris [23,24].

Cholesterol, O_2, and cataract formation

As noted, the fiber cells that comprise the lens have a high cholesterol content. Reduced lens cholesterol may increase exposure to O_2 and ultraviolet radiation and hence precipitate cataract formation. Therefore, in the pursuit of more efficacious cholesterol-lowering agents, it is important to establish that lowering lens cholesterol is not an unwanted side-effect. Consequently, new therapies which alter cholesterol metabolism should be evaluated for their cataractogenic potential. In light of this, it is reassuring that the usage of the most common blood cholesterol lowering treatment, the statins, is associated with reduced risk of cataract development [56].

Mitochondrial cholesterol

Elevated mitochondrial cholesterol levels are associated with a variety of pathological states, including heart dysfunction, liver disease, Alzheimer's disease, Parkinson's disease, ageing, and various cancers [30,57]. The Warburg Effect describes the increased reliance of cancer cells on glycolysis for energy rather than oxidative phosphorylation [58,59]. This change in the mode of energy production has been proposed to disrupt and alter other biochemical pathways such as those involved in the synthesis of cholesterol. High rates of cholesterologenesis may thus have led to the abnormally elevated mitochondrial cholesterol levels and impaired oxidative phosphorylation observed in many tumor cells [57,60,61]. More specifically, Montero et al.'s work [61] suggests that tumor cells use elevated mitochondrial

cholesterol to diminish potentially dangerous oxidative phosphorylation in favor of safer glycolysis. Furthermore, they showed that increased mitochondrial cholesterol increases resistance of hepatocellular carcinoma cells to mitochondrial-targeting chemotherapy [61]. Therefore, chemotherapeutic strategies aimed at reducing mitochondrial cholesterol, restoring optimal membrane composition, and increasing oxidative phosphorylation levels may be of significant therapeutic benefit.

New fungicides

Many of the currently available fungicides target O_2-dependent steps in ergosterol synthesis (e.g., terbinafine, ketoconazole). Garza and Hampton [52] pondered if the sterol-O_2 sensing link, now found in many fungi, may provide an Achilles' heel that can be exploited to develop new antifungal drugs.

Caveats and other considerations

Our hypothesis that the evolution of sterols is teleologically related to the appearance of O_2 resonates with the evidence discussed above. Below we discuss certain provisos and qualifications of the argument, as well as other noteworthy issues.

Multipurpose sterols

The functions of cholesterol and other sterols are unusually diverse [7]. Just as the human arm is more than a swimming aid when viewed telescopically from its evolutionary precursor, the fish fin; we do not argue that the protection of cells against O_2 and ROS is the sole, or even a major, function of sterols in organisms today. Sterols are also precursors for steroid hormones in multicellular organisms (animals, insects, and plants). In animals, cholesterol (or sterol precursors) is also metabolized further to bile acids and vitamin D. As is clear from defects in the cholesterol biosynthetic pathway, cholesterol is also crucial for development in multicellular organisms ranging from flies to humans.

From a teleological perspective, alternative functions may have exerted evolutionary pressures in the shaping of cholesterol. These include cholesterol's "fitness" for forming lipid rafts, which are critical in signaling within and between cells. Cholesterol, often via its association with lipid rafts in the cell membrane, is vital for endocytosis/exocytosis [62,63], processes believed to be a critical driving force for the evolution of single eukaryotic cells to multicellular organisms [13]. It is likely that more than one selective pressure was brought to bear to create this multifunctional molecule.

Superior antioxidant systems

The concept that sterols may display antioxidant properties is certainly controversial and still somewhat underdeveloped. Nevertheless, it is noteworthy that molecules more readily recognized as antioxidants, such as vitamin E and carotenoids (in plants) and ubiquinol (in respiration), are, like sterols, also produced in the mevalonate pathway. A myriad of enzymic antioxidant systems, including peroxidases, superoxide dismutases, and catalases, have presumably superseded sterols in terms of overall protection of cells against O_2/ROS toxicity.

More to membrane permeability than sterols

Widomska and colleagues [21] proposed that under physiological conditions, membranes are not usually barriers to O_2 transport into the cell and mitochondrion. However, they conceded that O_2 diffusion across membranes is severely reduced if membranes contain a high cholesterol concentration (and are dense with integral membrane proteins) [21]. The cholesterol content of cell membranes is high across a diverse range of animal cell types (30–50% of lipid [25,29]) and influences O_2 transport in various cell types and organelles. Protein content in the membrane can also affect O_2 permeability since integral membrane proteins are practically impermeable to O_2 [21]. Interestingly, like the rise of sterols, the oxygen content of transmembrane proteins increased during the evolution of eukaryotes as atmospheric O_2 levels rose [64].

An assumption in our argument is that O_2 and ROS cross the cell membrane primarily by passive diffusion. However, we cannot exclude the idea that active transporters may also contribute. For example, one aquaporin acts as a gas channel that increases permeability to carbon dioxide [65]. There is also some evidence that H_2O_2 can diffuse through specific members of the aquaporin family [66], while the superoxide anion may penetrate the cell membrane through anion channels [67]. Whether or not a channel protein(s) contributes to the transport of O_2 remains to be established, as does the relative contribution of different channel proteins in transporting specific ROS such as H_2O_2 and superoxide.

Besides sterols, another influence on membrane fluidity, and perhaps permeability, is the extent of unsaturation of the fatty acids that constitute the membrane phospholipid bilayer. Increasing fatty acid unsaturation by introducing double bonds is a common strategy employed for temperature adaptation. In yeast and some protozoa, desaturases are also induced in an O_2-sensitive fashion [68,69]. But can our argument that cholesterol evolved as a eukaryotic adaptation strategy against the rise of O_2 be extended to the desaturation of fatty acids? Probably not. First, it is unclear how the degree of membrane unsaturation would influence O_2 transport, if at all. Second, unlike the case for sterols, anaerobic desaturation (stearate to oleate) also occurs. Moreover, aerobic desaturation is not a eukaryotic invention, operating in some mycobacteria [70].

The heme connection

It is worth noting that there are several parallels among heme, sterols, and O_2. Synthesis of sterol and heme both require O_2, and are major adaptive responses to switch yeast to aerobic conditions [55]. Many heme-related proteins, such as heme oxygenases, cytochrome P450 enzymes, and components in the mitochondrial electron transport chain, are thought to play a role in O_2 sensing [71]. Furthermore, it is probably no coincidence that many of the enzymes involved in sterol synthesis and conversion to steroid hormones, bile acids, or oxysterol regulators are heme-containing proteins.

Conclusions

As we commemorate the 200th anniversary of the birth of Charles Darwin and 150 years since the publication of *On the Origin of Species* [72], this hypothesis paper may offer a tantalizing glimpse of our earliest origins—of the selective pressures brought to bear by the advent of O_2 for the fashioning of this class of essential eukaryotic molecules, the sterols. We have reviewed the tangled relationship between sterols and O_2, and offered a novel teleological rationale for cholesterol evolution with the rise of terrestrial O_2.

As stated by Leland Smith in his article entitled "Another cholesterol hypothesis: cholesterol as an antioxidant" (also published in *Free Radical Biology & Medicine*): "Studies of cholesterol and O_2 share other characteristics, including polemic disputation with oft repeated biases for which fresh viewpoints are desirable" [38]. We have constructed a fresh viewpoint—a hypothetical yet plausible scenario whereby sterols requiring O_2 for their synthesis may have also provided an early defence against this vital but potentially dangerous molecule. The idea that nature crafted sterols as a feedback loop to adapt to, or help protect against, the hazards of O_2 is enticing. However, while second-guessing how natural selection gave rise to a molecule like cholesterol

may be instructive, it does not lend itself readily to experimental verification. We have assembled several lines of evidence to support our thesis, including: (1) coincidence of atmospheric O_2 and sterol evolution (Fig. 3); (2) sterols regulate O_2 entry into eukaryotic cells and organelles; (3) sterols act as O_2 sensors across eukaryotic life; (4) sterols serve as a primitive cellular defence against O_2 and ROS. Therefore, sterols may have evolved in eukaryotes partially as an adaptive response to the rise of atmospheric O_2 (Fig. 2), rather than merely as a consequence of it. Our hypothesis will hopefully stimulate others to consider fresh viewpoints on the special relationship between sterols and O_2.

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