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REVIEW ARTICLE

Energy sources that fuel metabolic processes in protruding finger-like organelles

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Cells possess a variety of organelles with characteristic structure and subcellular localization intimately linked to their specific function. While most are intracellular and found in virtually all eukaryotic cells, there is a small group of organelles of elongated cylindrical shapes in highly specialized cells that protrude into the extracellular space, such as cilia, flagella, and microvilli. The ATP required by intracellular organelles is amply available in the cytosol, largely generated by mitochondria. However, such is not the case for cilia and flagella, whose slender structures cannot accommodate mitochondria. These organelles consume massive amounts of ATP to carry out high energy-demanding functions, such as sensory transduction or motility. ATP from the nearest mitochondria or other reactions within the cell body is severely limited by diffusion and generally insufficient to fuel the entire length of cilia and flagella. These organelles overcome this fuel restriction by local generation of ATP, using mechanisms that vary depending on the nutrients that are available in their particular external environment. Here, we review, with emphasis in mammals, the remarkable adaptations that cilia and flagella use to fuel their metabolic needs. Additionally, we discuss how a decrease in nutrients surrounding olfactory cilia might impair olfaction in COVID-19 patients.

Introduction

Organelles are subcellular structures that play very specific vital functions in cells. Some, common to most eukaryotic cells, are cytoplasmic and enclosed by their own membrane that determines their structural integrity and identity, such as mitochondria, lysosomes, endoplasmic reticulum, and others. In contrast, a few others, including cilia and flagella, display finger-like shapes delimited by a membrane that is contiguous with the plasma membrane and possess a lumen that can communicate directly with the cytoplasm (after passing through a specialized ciliary gate, the transition zone [1]) (Fig. 1). These organelles generally perform high levels of energy-demanding activity. Functional and structural aspects related to various types of cilia and flagella have been extensively reviewed; however, the very interesting and important issue of how ATP reaches these organelles has not been discussed systematically in an integrated

Abbreviations
AK, adenylate kinase; CK, creatine kinase; CNG, cyclic nucleotide-gated channel; COVID-19, coronavirus disease 2019; GLUT, glucose transporter; IS, inner segment; OS, outer segment; OSN, olfactory sensory neuron; TCA, tricarboxylic acid.

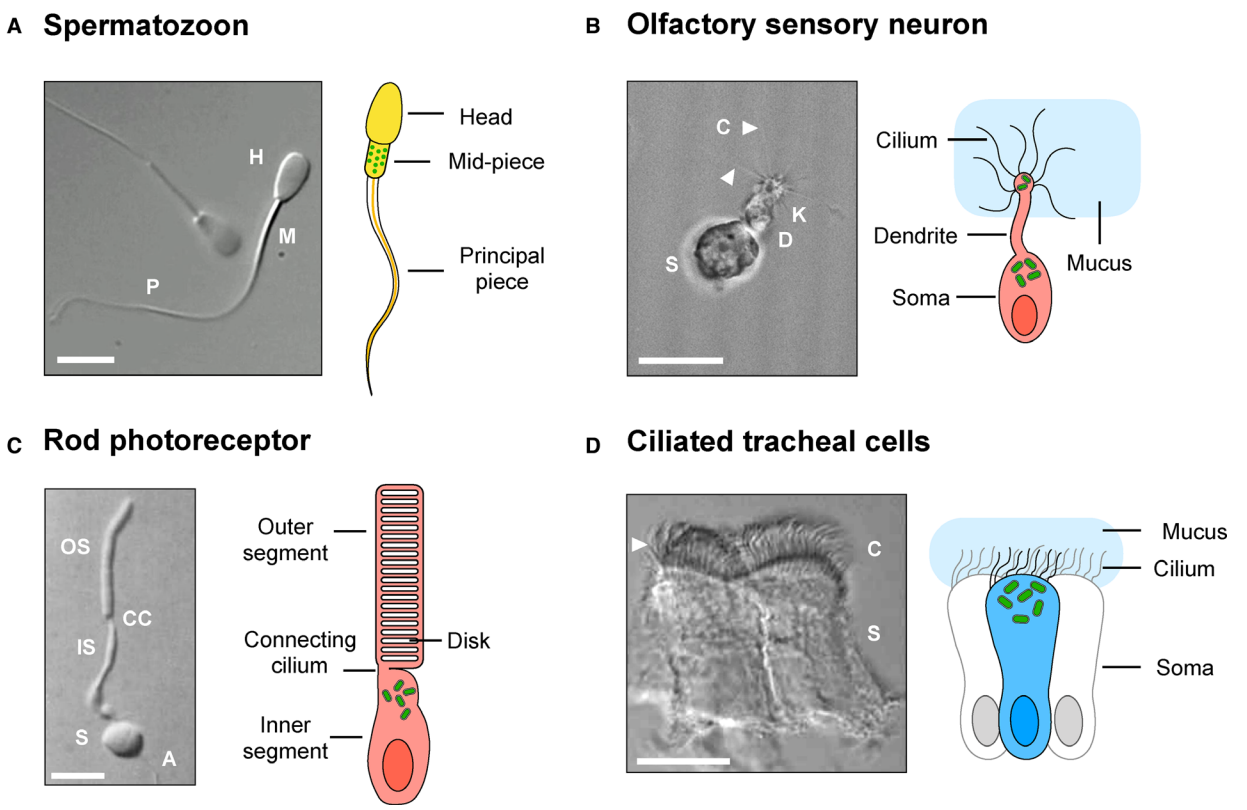

Fig. 1. Morphology of ciliated and flagellated cells. (A) Left, light micrograph of a bovine sperm cell (courtesy of Dr Puey Ounjai). Right, diagram of a sperm. The head (H) contains the nucleus and a small amount of cytoplasm. The sperm flagellum is composed by the mid-piece (M), which accommodates the mitochondria (green circles), and the principal piece (P) that propels the cell. (B) Light micrograph of an isolated rat olfactory sensory neuron (OSN). Several chemosensory cilia (C, arrowheads) protrude from the dendrite (D) apical knob (K), (S) soma. Diagram of an OSN. Two or three mitochondria (green ovals) fit in the dendritic knob. Mucus (light blue area) covers the surface. S, soma. (C) Light micrograph of a mouse rod photoreceptor (taken from Lolley *et al.* [86]). Diagram of a rod photoreceptor. Mitochondria reside in the inner segment (IS) of the cell. OS, outer segment; A, axon; S, soma; CC, connecting cilium. (D) Light micrograph of mouse tracheal ciliated cells (taken from Lorenzo *et al.* [87]). Abundant motile cilia (C) populate the apical face of the cells (arrowhead). S, soma. Diagram of tracheal ciliated cells. Mitochondria cluster next to the apical membrane. Mucus (light blue area) covers the surface. Bar = 10 μm.

approach. Their lengths vary from a few to hundreds of microns, and their width is around 0.2 μm, implying that they cannot accommodate mitochondria in their lumen. Raff and Blum [2] showed that, if cilia receive ATP from a cytoplasmic point source and this ATP is hydrolyzed along the length of the cilia, its concentration would fall below the physiological range for structures longer than 20 μm. Therefore, long structures need mechanisms to generate ATP locally. These slender organelles possess the ability to metabolize nutrients that they incorporate from their surrounding media, such as glucose, fructose, mannose, or other carbohydrates, brake down glycogen, or mobilize ATP produced by the nearest mitochondria by means of ATP-shuttles.

The local ATP production implies that the organelles possess a battery of transporters and enzymes whose identification is key for establishing the corresponding substrates and underlying mechanisms. Particularly important for this review are several members of the glucose transporter families (GLUT), whose K_m for glucose vary between 0.2 and 17 mM. GLUT7 and 10 have the highest affinity (~ 0.3 mM), affinities for GLUT3, GLUT8, and GLUT1 range from 1 to 7 mM, and GLUT2 presents low affinity (~ 17 mM) but high transport capacity. GLUTs also differ in their selectivity, GLUT5 and GLUT7 being essentially fructose transporters while GLUT9 transports both fructose and glucose [3]. The most common techniques used to detect these transporters are immunoblots,

immunochemistry, and proteomics (mass spectroscopy). Some controversies over the participation of a given substrate may be related to differences in techniques used.

Cilia and flagella have very similar cytoarchitecture. Both contain an axoneme consisting of a stereotyped array of microtubules that extends throughout their full length, determining their peculiar cylindrical shape. In sperm cells, the axoneme is crucial to produce the characteristic waveform beating that propels them over considerable distances, allowing them to reach and fertilize the oocyte [4] (Fig. 1A). Among cilia, some are motile and others are not, and some are specialized in sensory transduction while others play mechanical roles. In sensory receptor cells, the cilia greatly expand the plasma membrane area well beyond the soma, providing them with a well-defined subcellular compartment where the proteins involved in sensory transduction, such as ion channels, receptors, and transporters, are confined in vast amounts. The chemosensory cilia of olfactory sensory neurons (OSN) and the outer segments (OS) of photoreceptor cells belong to this category of cilia (Fig. 1B,C). A distinct type of motile cilia populates the apical surface of various types of non-sensory epithelial cells, where they constantly beat in a coordinated manner [5] (Fig. 1D). Motile cilia play important roles removing the pathogen-rich fluid that covers the epithelial surface, as occurs in the trachea and respiratory tract, or transporting the egg in the oviduct and sperms in the seminal ducts [6,7].

Structures of sperm flagella and cilia

In order to fertilize an egg, the sperm has to swim vigorously, propelled by sustained beating of the flagellum. The sperm head contains the nucleus and a minimal volume of cytoplasm lacking organelles. The proximal part of the flagellum, termed midpiece, is shorter and wider than the distal principal part and contains tightly packed mitochondria. The principal part is a long slender structure also devoid of organelles (Fig. 1A). The principal piece varies widely in length across species; for instance, it is about 48 μm in human, 58 μm in bull, 120 μm in mouse, 177 μm in rat, and 356 μm in honey possum [8,9].

An axoneme extends along the flagellum and consists of nine peripheral longitudinal microtubule doublets surrounding a central pair of singlets. Next to tubulins are dyneins, which are ATPases responsible for the mechanical force for motility. Between the outer microtubules doublets and the membrane runs a filamentous structure called fibrous sheath that serves as scaffold to glycolytic enzymes [10].

Sperm motility results from the coordinated sliding of the microtubule doublets, an energetically costly process mainly due to extensive dynein-dependent ATP hydrolysis along the entire flagellum. Therefore, ATP must be supplied efficiently along the flagellum as an absolute requirement to avoid fatigue while sustaining its intense activity.

Cilia and flagella have similar structures. Their axonemes are alike, although a fibrous sheath has not been described in cilia. Some cilia are involved in sensory transduction, such as the chemosensory cilia of the OSNs, which generate electrical signals in response to odorants, and the OS of rod and cone photoreceptor cells, which transduce light into electrical responses. A remarkable characteristic of sensory cilia is their extreme specialization that relies on a sophisticated molecular machinery within each cilium.

OSNs possess around 10–20 cilia that protrude from their single dendrite into a thin (~ 30 μm) layer of mucus embedding the olfactory epithelial surface in the nasal cavity (Fig. 1B). Incoming airborne odorant molecules bind to membrane receptors of the cilia, triggering chemotransduction. The ciliary length varies widely across species; for example, mammalian cilia are ~ 20 μm (range 10–110 μm) [11,12], while amphibian cilia can be a few hundred micrometers. Furthermore, although their proximal part (~ 2 μm) displays a 9 × 2 + 2 microtubule axoneme, the two central singlets are lost along the way as the cilium diameter drops to about half [11].

Vertebrate photoreceptors, rods and cones, present two parts termed the inner and the outer segment (OS) (Fig. 1C). The inner segment contains the nucleus and the intracellular organelles, with many mitochondria packed near the OS. The OS is a modified cilium whose initial region is a short (1.5 μm) canonical cilium, called connecting cilium. Beyond the connecting cilium, it abruptly widens to a diameter around 2 μm in mammals. The OS lacks axoneme and organelles and is almost fully occupied by a single pile of nearly 2000 disk-like membrane structures that are very close to each other and to the plasma membrane, leaving a minimal cytoplasmic space. The disk membranes contain all the phototransduction enzymes, except for the ion channels and the $Na^+/K^+/Ca^{2+}$ transporter that reside in the OS plasma membrane.

Cilia present in non-sensory epithelial cells have mechanical roles, as in the respiratory epithelium and reproductive tracts. Ciliated cells of the tracheal epithelium are covered by approximately 200 cilia, of ~ 6 μm long and 300 nm wide [13] (Fig. 1D). In some

species, they present a wider proximal part with the peripheral microtubules organized in triplets, from where it tapers to a smaller diameter with only singlets forming the peripheral microtubule array [13,14]. The coordinated beating of the tracheal epithelial cilia allows for mucociliary clearance, which is an important mechanism of airways protection [5]. Beating frequencies range from 8 to 16 Hz and are sensitive to external conditions, including extracellular ATP. The epithelia lining the oviducts in females and efferent ducts in males, together with the ependymal cells covering the brain ventricles, contain multiciliated cells alike those in the trachea [5,15].

ATP sources for sperm flagella and cilia

Finger-like organelles need to fuel energy-costly biochemical processes over long distances and extensive periods of time. However, in flagella and sensory cilia ATP generated in the cytoplasm is insufficient, because ATP diffusion is too slow to supply the entire organellar length. This serious constraint necessarily implies the need for locally obtaining ATP. The diffusion coefficient within a cell (0.9×10^{-6} $cm^2 \cdot s^{-1}$) is such that an ATP molecule is estimated to advance 10 μm in ~ 0.5 s [12]. In addition to its slow diffusion, ATP hydrolysis further decreases its luminal availability. Recent measurements in sea urchin sperm (~ 50 μm long, 0.2 μm diameter) show that ~ 2×10^5 ATP molecules are consumed per flagellar beat [16]. Assuming 1 mM ATP in a resting flagellum (~ 1×10^6 molecules of ATP), around 10% the available ATP would be hydrolyzed in every beat, implying the requirement of a highly efficient ATP-generating system. Molecules like glucose, pyruvate, or other carbohydrates have diffusion rates comparable to that of ATP. However, protruding organelles are generally embedded in fluids rich in some of these forms of metabolizable substrates; they express specific transporters and enzymes that allow them to incorporate these substrates into the ciliary lumen and catabolize them to obtain ATP [17–19].

Sperm cells

The diversity of mechanisms used by mammalian sperm cells to supplement the ATP provided by the cell body relates their metabolic machinery and the available nutrients in the extracellular medium. The seminal and female tract fluids have plenty of sugars that sperms incorporate and metabolize glycolytically [20,21].

In most mammals, the main process that generates ATP in the principal piece of the flagellum is glycolysis. Several glycolytic enzymes, such as hexokinase 1 [10], glyceraldehyde 3-phosphate dehydrogenase [22], lactate dehydrogenase, aldolase [23], and pyruvate kinase [24], are anchored to the fibrous sheath scaffold located underneath the membrane, which is rich in A-kinase anchoring protein [10]. Importantly, several glucose transporters of the GLUT family have been found in the principal piece of mammalian sperm cells [25]. GLUT1, GLUT2, GLUT5, GLUT8, and GLUT9 localize to the plasma membrane of the entire cell, including the principal piece, consistent with the notion that glucose might be taken up from the external medium into the axoneme to be processed by glycolysis to make ATP. Importantly, GLUT9 effectively transports fructose as well, which is the main carbohydrate available in the surrounding medium in some cases, such as human and mouse.

It is also possible that other strategies are used to fulfill energy requirements in cases where metabolizable substances are not readily available in the surrounding medium, for example, mobilizing ATP along the lumen with ATP-shuttles or metabolizing glycogen. In the phosphocreatine shuttle system, the enzyme creatine kinase (CK) transfers a phosphate group from ATP to creatine turning it into the highly diffusible intermediate phosphocreatine, from which ATP is formed by CK-dependent phosphorylation of ADP (Fig. 2), creating in this manner a phosphagen flux wave that travels faster than diffusion of other reactants, including ATP [26]. The importance of the CK shuttle is well established in sea urchin sperm [27]; however, its contribution in mammalian sperms is unclear [28]. Adenylate kinase (AK), another common ATP-shuttle, catalyzes the conversion of two molecules of ADP into ATP and AMP, thus also allowing the mobilization of mitochondrial ATP.

We chose a few examples to illustrate how different sperm cells approach their energy needs.

Human

Glycolytic enzymes have been localized along the fibrous sheath of the human sperm flagellum (~ 48 μm, [9]) and shown to be necessary for normal motility [29]. Glycolytic ATP represents their primary source of ATP [30]. Flagellar glycolysis predominantly hydrolyzes fructose and glucose (Fig. 2), which are abundant in the seminal fluid [21]. Human sperm cells uptake these hexoses by GLUT1, GLUT3, and GLUT5 [17]. Low-affinity GLUT1 allows for basal uptake of glucose and high-affinity GLUT3 is most efficient at lower glucose concentrations, while GLUT5 is a high-affinity fructose transporter. Fructose is

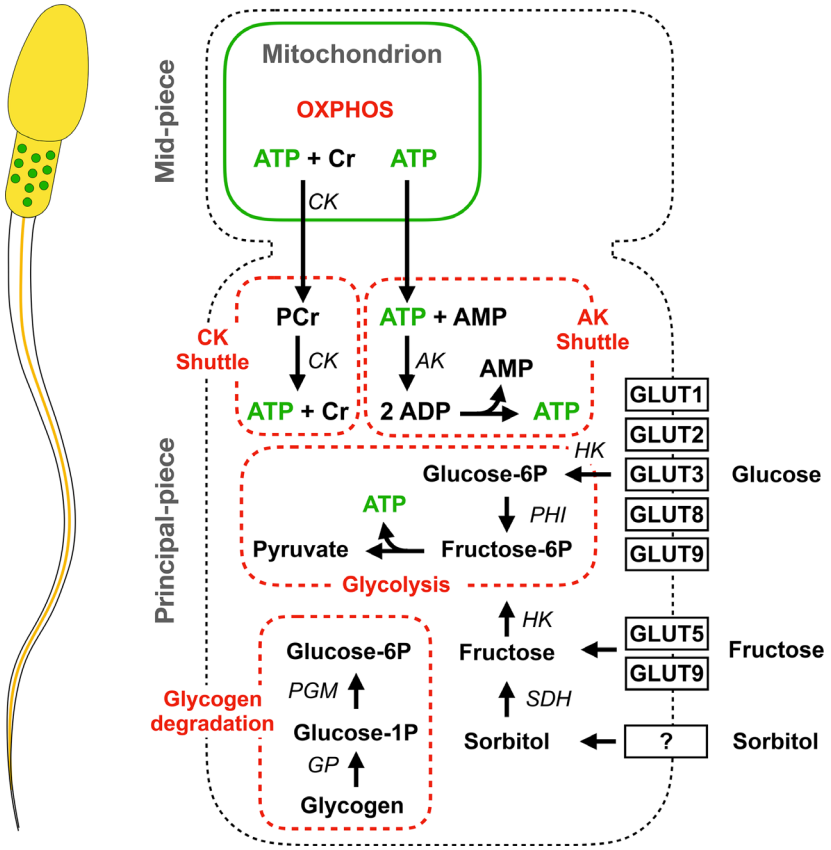

Fig. 2. Energy production in the flagellum of sperm cells. Left, diagram of a sperm cell; mitochondria (green) localize to the mid-piece. Right, integrated metabolic strategies for energy production reported for different mammalian species. Mitochondrial oxidative phosphorylation (OXPHOS) generates ATP in the mid-piece; ATP-shuttle creatine kinase (CK; Cr, creatine; PCr, phosphocreatine) and adenylate kinase (AK) mobilize mitochondrial ATP. According to extracellular availability, fructose, glucose, sorbitol, or other carbohydrates can be incorporated into the principal piece and metabolized by glycolysis. GLUT5 and 9 preferentially transport fructose, GLUT1, 2, 3, 8, and 9 transport glucose and an unidentified transporter transports sorbitol. Glycogen is degraded into glucose by glycogen phosphorylase (GP) and channeled into glycolysis by phosphoglucomutase (PGM). HK, hexokinase; PHI, phosphohexose isomerase; SDH, sorbitol dehydrogenase.

channeled to glycolysis by either hexokinase, which has a higher affinity for glucose than for fructose, or fructokinase, with higher fructose affinity (Fig. 2). The cervical mucus contains several hydrolyzable nutrients such as glucose, fructose, mannose, galactose, and maltose. Interestingly, the amount of glucose in the cervical mucus varies with the different stages of the menstrual cycle, rising from an average of 0.15 mM during preovulatory stage to 2 mM during the postovulatory stage [20]. Considering the importance of glycolytic ATP for normal human sperm motility, it is possible that glucose contained in the cervical mucus contributes to fuel sperm cells during migration.

The CK and AK ATP-shuttles have been identified in human sperm cells. Some evidence supports a role of CK in the human sperm flagella, since motile activity of demembranated flagella could be restored by the addition of phosphocreatine plus ADP, but not to the extent produced by adding ATP [31]. Conversely, CK enzymatic activity in human sperm does not seem to be correlated with successful *in vitro* fertilization [32]. Further studies will be needed to clarify the relevance of the CK shuttle in the human flagellum principal piece.

Mouse

Mouse sperm flagella (~ 120 μm; [9]) express GLUT8 and the high-affinity glucose transporter GLUT9 [33]. Sorbitol has been shown to be metabolized by these sperms, in addition to glucose and fructose. Sorbitol dehydrogenase, also existing in their flagella, oxidizes sorbitol to fructose allowing for glycolytic metabolization [34] (Fig. 2). Genetic disruption of the sperm-specific isoform of the regulatory glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase produces infertility and motility defects in mouse sperm cells [35]. Similarly, pharmacological inhibition of glycolysis, but not oxidative phosphorylation, drastically decreases sperm motility [36], highlighting the relevance of the glycolytic metabolism for normal sperm cellular function.

Dog

The seminal fluid of these animals contains only small amounts of fructose, glucose, and sorbitol [37]; however, their sperm cells (~ 55 μm flagellum [9]) are able to maintain motility for several days after released [38]. This is believed to rely on the metabolism of

glycogen, since the enzymes glycogen phosphorylase and phosphatase have been detected together with glycogen granules [39] (Fig. 2). Similar to humans, the transporters GLUT3 and GLUT5 were found to be functional in dog sperm cells [40]. Thus, extracellular hexoses could be used to complement the synthesis of ATP by glycolytic metabolism or to accumulate glucose in the form of glycogen previous to high activity periods.

Fish

In externally fertilizing fish, sperm cells remain immotile in the seminal fluid until released to the water. After spawning, sperms swim at high speed propelled by a highly motile flagellum. The mechanism for sperm motile initiation varies across species, but common factors that can trigger it are changes in ionic concentration, osmolarity, and pH. Fish sperm cells intense activity lasts only for seconds to few minutes, after which they become energetically exhausted [41]. In most fish sperm cells, creatine phosphate is the main metabolizable compound available to the flagella. ATP is generated aerobically in mitochondria located at the base of the sperm flagellum from fatty acid stores available at the time of spawning. CK has been detected in sperms of several fish species, such as herring (*Clupea harengus*) [42], rainbow trout (*Oncorhynchus mykiss*) [43], and salmon (*Oncorhynchus tshawytscha*) [44]. CK activity in fish is 10- to 100-fold higher than in mouse and human, likely reflecting its pivotal role in facilitating the diffusion of ATP along the flagella during the hyperactivity period [44].

In rainbow trout, prior to spawning, immotile sperm cells use both glycolysis and catabolism of triglycerides to synthesize ATP. After motility begins, glycolysis stands for the first ~ 30 s, after which intra-flagellar ATP levels rapidly drop leading to a decrease in sperm motility [45].

Sea urchin

This echinoderm's sperm has been a longstanding favorite model for the study of sperm physiology. In sea urchins, fertilization takes place externally posing it in quite a challenging situation, since there are virtually no nutrients in sea water. A phosphocreatine shuttle transfers ATP to the tail for motility [27,46], unlike mammals where flagellar glycolytic metabolism predominates [44]. In addition, the AK shuttle has been shown to be important for flagellar motility in sea urchins [47].

Together, the study of the approaches used by sperm cells to fulfill their metabolic needs reveals that a wide range of strategies evolved as solutions to this crucial problem. Thus, sperm conservation procedures, such as cryopreservation, should take into account the species-specific metabolic requirements to optimize cellular viability.

Cilia of olfactory sensory neurons

The cilia of olfactory sensory neurons enclose the molecular machinery for transduction of airborne odor molecules that bind to specific receptors in the olfactory cilia membrane. Odor receptors couple to G-proteins that in turn activate adenylyl cyclase generating a transient increase in cAMP, which directly opens cyclic nucleotide-gated nonselective cationic channels (CNG). Ca^{2+} entering through these channels opens Ca^{2+}-activated Cl^- channels, generating a Cl^- efflux that amplifies the depolarization initiated by the CNG-dependent current [48]. Multiple regulatory kinases that are activated in this process regulate odor chemotransduction at various levels. Thus, ATP demanded by these enzymes and adenylyl cyclase must be readily available in the lumen of the cilia in order for olfactory transduction to proceed. Yet, only two or three mitochondria are allowed within the apical dendrite swelling that project the cilia, termed dendritic knob [49] (Fig. 1). The ATP supply in the distal segment of the cilia only partly depends on passive diffusion from the dendritic knob, and the rest is largely generated by local synthesis [12,18].

A polarized distribution of glucose transporters was found across the rat olfactory mucosa, where the low-affinity GLUT1 was amply expressed in the basolateral side of the sustentacular cells and in the blood vessels, while the high-affinity GLUT3 localized to the apical epithelia membrane [50,51]. This differential distribution of transporters suggested that glucose delivered from the blood vessels in the lamina propria of the olfactory mucosa could cross the epithelium through the supporting cells and reach the mucus, from where the cilia internalize it to fuel chemotransduction (Fig. 3). Consistent with this model, millimolar glucose was measured in the mucus. Olfactory cilia internalize glucose in a GLUT-dependent manner and use it to sustain the odor response, as shown by electrophysiological recordings. Moreover, regulatory glycolytic enzymes were detected in olfactory cilia by immunoblotting and, importantly, blocking glycolysis greatly impaired odor transduction, highlighting a primary role of this metabolic pathway in olfactory transduction [12]. Further supporting this model, the

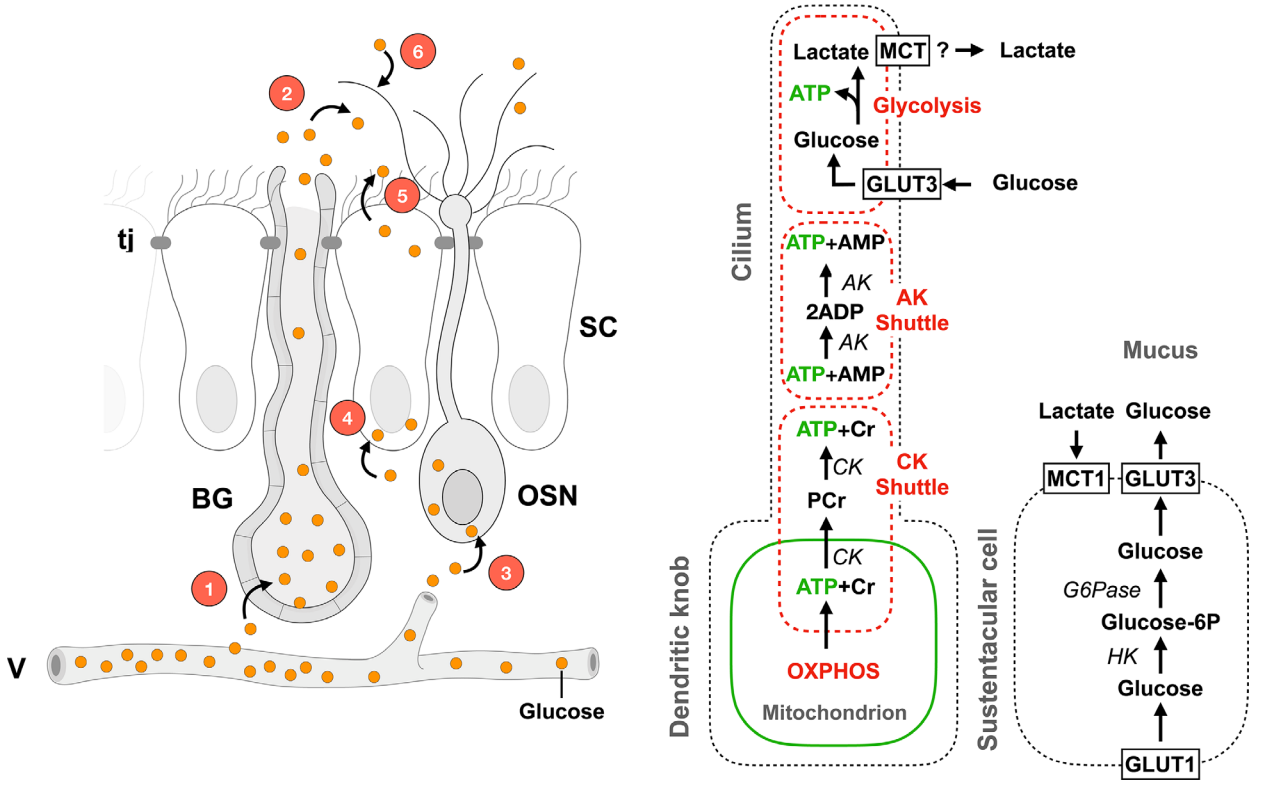

Fig. 3. Energy production in the cilia of olfactory sensory neurons. Left, diagram illustrating the mobilization of glucose (orange circles) across the olfactory mucosa. Plasmatic glucose delivered from the blood vessels (V) is incorporated into the Bowman's gland (BG) cells (1) and subsequently released into the mucus (2). Similarly, olfactory sensory neurons (OSN) uptake extracellular glucose to fuel their metabolism (3). Glucose is also incorporated by the basolateral membrane of sustentacular cells (SC) (4) and released into the mucus by apical glucose transporters (5). Glucose contained in the mucus is incorporated by the cilia of the olfactory sensory neurons (OSN) (6). Right, metabolic pathways for energy production in the cilia of OSNs. Mitochondrial ATP passively diffuses from the dendritic knob toward the cilium and can also be mobilized by Cr and AK ATP-shuttles. The cilia, by local glycolysis, obtain ATP from glucose incorporated from the mucus via GLUT3. In the SCs, glucose is converted into glucose-6P, which is turned back to glucose by glucose-6P-phosphatase (G6Pase) in their apical region and released through the microvilli membrane to the mucus via GLUT3. The monocarboxylate transporter 1 (MCT1) located in supporting cells (and possibly olfactory cilia) might contribute to remove from the cilia lactate accumulated during glycolysis. tj, tight junction.

enzyme glucose-6-phosphatase, which is required to dephosphorylate glucose prior to release, was detected by immunochemistry in the apical region of the supporting cells and in the Bowman's glands of the mucosa [18]. Interestingly, the key ATP-shuttle enzymes adenylate and creatine kinases were reported in the olfactory cilium by immunolabelling and proteomics [18,52]. Altogether, the evidence strongly suggests that olfactory cilia use a combination of complementary strategies to obtain the ATP needed for chemotransduction (Fig. 3) [12,18,50].

Recently, it has been shown that olfaction is compromised in patients of the acute respiratory illness COVID-19, and this is indeed accepted as a salient symptom that allows early detection of the disease [53]. The cause for this condition is unclear, although one gene encoding the angiotensin-converting enzyme 2 (ACE2) membrane receptor to which the spike proteins of the SARS-CoV-2 coronavirus bind previous to cell penetration is expressed in the sustentacular, Bowmann's gland and basal stem cells of the olfactory epithelium, but not in the olfactory sensory neurons [54]. In patients of COVID-19, the olfactory epithelium loses SCs as well as OSNs, but the epithelium recovers after the patient recovers. Based on the aforementioned evidence that SCs provide OSNs with metabolic support by releasing glucose to the mucus which is essential for fueling chemotransduction in the cilia [12,18], it is highly possible that the death of SCs might lead to anosmia because the odor transduction process is compromised by the decrease in energy sources available to the cilia. There are other ways in

which the virus infection could impair olfaction, among them OSNs death as a result of a structural alterations of the olfactory epithelium upon the disappearance of SCs and changes in the ionic medium that surround the neurons.

Outer segments of rod photoreceptor cells

The retina, one of the tissues with the highest energy consumption, principally by light transduction in the photoreceptors and, accordingly, the mechanisms used by these cells to obtain ATP must be extremely efficient. Several pathways have been proposed, although the overall picture is as yet controversial [55–57].

In darkness, vertebrate photoreceptors are depolarized by an inward Na^+/Ca^{2+} current constantly flowing through cyclic GMP-gated channels confined to the OS plasma membrane, in this case opened by cyclic GMP. Light causes a drop in cGMP levels, the consequent closing of the channels and concomitant hyperpolarization by a reduction of the light-dependent current. The light response is triggered upon the absorption of a photon by rhodopsin residing in the OS disk membranes. Activated rhodopsin couples to the G-protein transducin, which in turn activates a phosphodiesterase that decreases cGMP closing the CNG channels. Also in the plasma membrane are the transporters that remove Ca^{2+} at the expense of the Na^+ and K^+ transmembrane gradients, called the $Na^+/K^+/Ca^{2+}$ transporters [58] (Fig. 4).

Multiple processes orchestrating phototransduction utilize ATP, including Ca^{2+}-dependent light adaptation and deactivation of the transduction cascade, which require fine regulation of calcium, regeneration of cGMP by guanylate cyclase, phosphorylation of rhodopsin, the maintenance of the ionic gradients and others.

Glucose is utilized as a general energy source over the whole retina. It is delivered from the blood vessels, taken up by the retinal pigment epithelium (RPE) engulfing the tips of the OSs, released by the RPE through GLUT1 and finally incorporated and processed by the photoreceptors. There is general agreement that GLUT1 is expressed in the IS, but its presence in the OS has been in question.

GLUT1 along with the glycolytic enzymes were detected in rod OSs by immunohistochemistry of bovine and chicken retinas [59,60]. These observations were confirmed by western blotting of isolated rod OSs and measurements of glycolytic enzymes activities, as well as functional experiments with GLUT inhibitors of the uptake of a glucose analog (3-*O*-methylglucose). It was proposed that local glycolytic catabolism of glucose directly incorporated by the OS is one important source of ATP, although most likely not the only one; similar observations were done in bovine OSs [19]. A proteomics study of bovine rod OSs reported the presence of GLUT1 [61], although this protein was not among those detected in another proteomics study [62]. In a more recent immunochemical study of rat and *Xenopus* retinas and isolated *Xenopus* rods, immunoreactivity to an antibody against *Xenopus* GLUT1 was detected in the rod plasma membrane, but not in the OS [63]. Notably, their images revealed GLUT1 immunoreactivity of the microvilli of the pigment epithelial cells capping the distal ends of the photoreceptors, while the proximal part of the OSs was not labeled; these findings were supported by immunohistochemistry on rat retina. The spatial distribution of a GFP-GLUT1 fusion protein in transgenic *Xenopus* tadpole retinas supported the absence of GLUT1 in the OS. A segment in the C-terminal end of GLUT1 retains this protein to the IS; accordingly, the fusion protein was found only in the IS. In contrast, the GLUT1-GFP fusion protein in which the transporter C-terminal was truncated invaded the OS. This study conflicts with the immunochemistry detection of GLUT1 in the OS reported previously [61], which might reflect contamination with pigment epithelium membranes.

Nevertheless, the OS presence of glycolytic enzymes is generally accepted and the use of glucose as source of ATP is not in question. Also, it seems most likely that a significant fraction of the ATP used to support phototransduction is provided by the IS [63].

ATP-shuttles have been proposed to have a relevant role mobilizing ATP from the inner to the OS in some species (Fig. 4). AK and CK activities were reported in frog OSs [64], and CK was immunolocalized in chicken photoreceptors and in the retina by electrophoresis, suggesting a possible role in ATP trafficking to the OS [65]. The brain CK isoform is expressed in the mouse IS but not in the OS [66]; however, it was detected in the bovine OS [67].

Another mechanism that might generate ATP in the OS involves oxidative phosphorylation occurring directly in the rod disks, which are reported to contain the electron transport chain protein complexes. These proteins would be transferred in its correct topological organization from the Golgi apparatus to the newly formed OS membrane forming the disks. Evidence for the presence of all respiratory chain protein complexes is based on proteomic studies in purified bovine disks [68–71]. Importantly, tricarboxylic acid (TCA) cycle enzymes were also detected in OSs by mass spectrometry [71] and electron transport chain activity

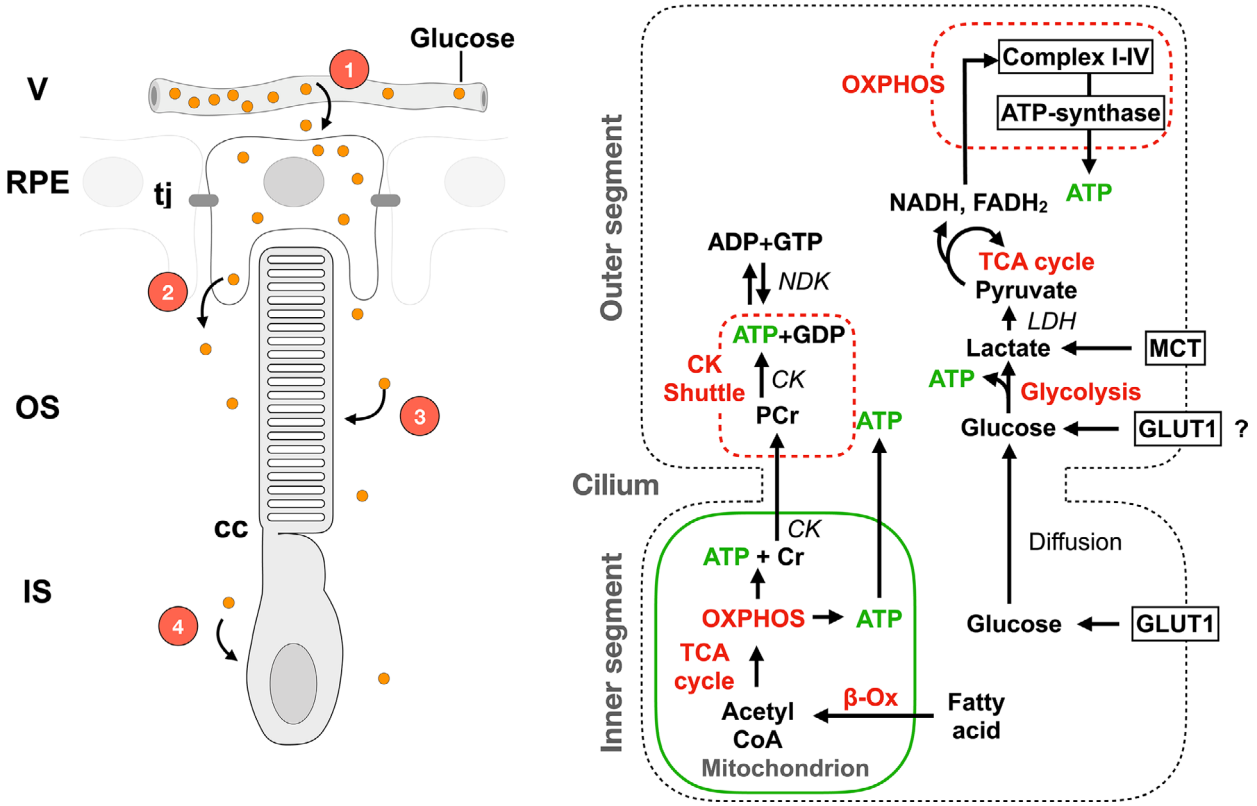

Fig. 4. Energy production in the vertebrate photoreceptors. Left, diagram illustrating the facilitated diffusion of glucose (orange circles) from the blood vessels (V) into the retinal pigment epithelial cells (RPE) (1). Glucose is released from RPE cells into the extracellular medium (2) and taken by the outer (3) or inner (4) segment of photoreceptor cells. Right, metabolic pathways for energy production reported for the vertebrate rod photoreceptors. Mitochondria, localized in the inner segment (IS), metabolize fatty acids that enter the tricarboxylic acid (TCA) cycle, followed by oxidative phosphorylation (OXPHOS) to synthesize ATP. Mitochondrial ATP can passively diffuse to the outer segment (OS, upper box) or be transferred by the creatine kinase (CK) ATP-shuttle. GLUT1 transports glucose into the inner segment and perhaps also into the outer segment. Glucose is catabolized in the outer segment by glycolysis, followed by the TCA cycle. Extramitochondrial electron transport chain would synthesize ATP by OXPHOS in the outer segment. Nucleoside diphosphate kinase (NDK) would convert ATP to GTP, required by the G-protein transducin. tj, tight junction; cc, connecting cilium.

measurements revealed its functionality in isolated OSs [70]. This evidence suggests that OSs might generate ATP by extramitochondrial oxidative phosphorylation (Fig. 4).

The high demands of ATP by phototransduction make it unlikely that glucose is sufficient as the only energy source; rather, it is thought that its metabolism only generates only about 50% of its energy demands [72,73]. Other substrates appear to contribute as well, such as lactate [74] and intermediate metabolites of the TCA cycle of different origins [75]. Additionally, fatty acids (palmitate) appear to be oxidized into acetyl-coenzyme-A, which would enter the TCA cycle and subsequently oxidative phosphorylation to generate ATP in the IS mitochondria [57] (Fig. 4). Importantly, the yield of ATP from a palmitate molecule is much higher than from glucose when fully oxidized by cellular respiration (106 vs. 32 ATP molecules).

One major open question is how ATP and substrates for generating ATP in the IS can diffuse throughout the OS, which in mammals measures 20–30 μm long, particularly considering the extremely small cytoplasmic space left by the closely apposed disks.

Cilia of other ciliated epithelia

Among the ciliated epithelia performing mechanical functions, those of the trachea and the oviduct have been investigated in greatest detail regarding their energy supplies. Tracheal cilia beating require high levels of ATP. The cells contain a dense array of mitochondria at the apical region [76] and are sufficiently short (~ 5 μm) to think that ATP produced by oxidative phosphorylation would be enough for the entire cilium; nonetheless, it cannot be ruled out that they

also use additional means to power motile functions. Consistent with this idea, a recent study showed that an isoform of the glycolytic enzyme enolase-4 specifically expressed in ciliated cells is actively transported along the motile cilia in the mouse [77]. However, whether glycolysis actually takes place in these structures remains unknown.

Apical tight junctions separate the surface mucus embedding the cilia from the fluid surrounding the basolateral membranes, yet glucose can diffuse paracellularly across these junctions from plasma or lung interstitium to the mucus. In the human airway, glucose in the surface fluid is ~ 0.4 mM, 10 to 20 times lower than the plasmatic concentration [78]. Glucose is efficiently removed from the airway surface liquid via glucose transporters, and the epithelial cells rapidly utilize it via glycolysis and to synthesize glycogen. This mechanism and the constant beating contribute to minimizing the proliferation of airborne pathogens favored by extracellular glucose, and to the clearance of noxious particles [79,80]. Similarly, beating also plays important roles in other ciliated epithelia, such as transporting the oocytes in the oviduct and the sperms in the efferent ductules [81].

Interestingly, ATP also has an autocrine function on the airway ciliated cells by regulating the frequency of their coordinated ciliary beating, which depends on the cilia luminal Ca^{2+} concentration [82]. ATP is released to the mucus through hemichannels (connexin/pannexin) of the apical membrane of these cells and binds to purinergic receptors releasing Ca^{2+} from internal stores via phospholipase C activation [83]. Hence, considering the crucial dual role of ATP in dynein-dependent ciliary beating and autocrine signaling, its abundance in ciliated cells is key for normal epithelial function.

Motile cilia also play important roles in the epithelia of the reproductive tracts, transporting the ova along the oviduct and producing turbulence that agitate immotile sperm keeping them in suspension within the lumen of efferent ductules and controlling fluid resorption. In the ventricular cavities and spinal cord of the central nervous system, ependymal ciliated cells generate complex fluid streams that spread nutrients and signaling elements facilitating their interaction with the different targets and clearing cellular debris from the cerebrospinal fluid. As in airway ciliated cells, ATP induces an increase in the beating frequency of oviductal cells mediated by intracellular Ca^{2+} [84]. However, the exposure of ciliated brain ependymal cells to ATP reduces the beating frequency in a Ca^{2+}-independent cAMP-mediated pathway [85].

Conclusion

The morphology of protruding organelles such as cilia and flagella poses serious constrains to fuel their high energy-demanding functions. These organelles employ many strategies that vary with specific physiological features and available ATP sources. Such sources can exist in their cell bodies or external environments, from where they can be internalized and locally metabolized. Although substantial progress has been made toward a characterization of the energetics of cilia and flagella, important questions still remain to be addressed to achieve a comprehensive view of this important biological problem.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

PSV and JB conceived the review and drafted the first manuscript. PSV, JB, and CV wrote and revised the final version. JB grant supported the work.

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