and the mechanosensory hair cells stem from an ancient population of ectodermal cells with motile cilia and an apical microvillar collar (hence 'collar cell'). This way, the long-standing riddle of nephridia evolution may come closer to a solution. The rich new dataset generated by Gąsiorowski and colleagues[1] enters center stage in exemplifying how molecular and morphological comparison join forces in solving evolutionary mysteries.

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Metabolism: Evolution of dolphin sperm endurance

Lisa Noelle Cooper
Northeast Ohio Medical University, Department of Anatomy and Neurobiology, Rootstown, OH 44272, USA
Correspondence: lcooper@neomed.edu
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Mammalian sperm have long been known to use energy derived from the metabolism of sugars and fatty acids. A new study shows that sperm of dolphins and their relatives lost functionality of the glycolysis pathway and are fueled only by energy-rich fatty acids that are metabolized by extra-large mitochondria, giving them exceptional endurance.

It has long been known that the spermatozoa of humans and domestic animals use sugars and fatty acids as metabolic fuel[1–3]. Fatty acid metabolism is usually a minor component in mammals. However, metabolism of fatty acids yields roughly three times the amount of ATP produced by oxidation of glucose. This evolutionary strategy of a double fuel source allows sperm to metabolize carbohydrates and lipids on their long traverse in the female reproductive tract but depends on availability of glucose and lipids. A new study by Alves, Ruivo and colleagues in this issue of *Current Biology* shows that the sperm of toothed whales (odontocetes, like dolphins and their close relatives) are fueled only by the oxidation of fatty acids within mitochondria[4]. They show dolphin sperm benefit from this rich

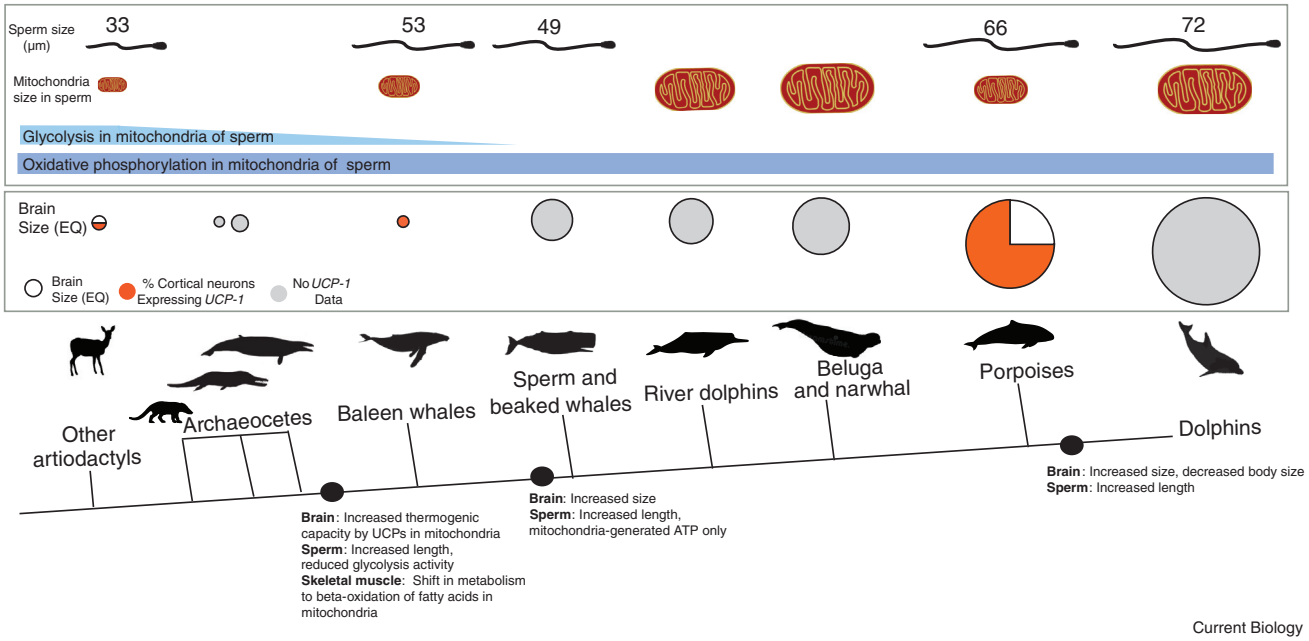

Figure 1. Mitochondrial evolution in cetaceans.
Alves, Ruivo and colleagues add a new organ system, sperm, to our understanding of mitochondrial evolution in cetaceans. Within toothed whales (odontocetes), sperm length and sperm-specific mitochondrial size both increase (top rows). These sperm-specific mitochondria shifted to a metabolism in which ATP is produced solely by oxidative phosphorylation (blue lines). Skeletal muscle of all modern cetaceans is also uniquely fueled by oxidative phosphorylation[10]. Finally, brain size (circles), as shown by encephalization quotient (EQ)[15], a measure of brain:body size, increased dramatically in odontocetes and is associated with increased energetic demands[14]. Cetacean brains are also unique in that cortical neurons display large percentages of neurons with uncoupling proteins (*UCPs*) that likely increase the thermogenic capacity of these cells and potentially keep brain cells warm (orange)[12].

fuel source and swim for days, not hours, in a culture dish.

Mammalian sperm have a tail that is partially wrapped by a helical network of mitochondria. The sperm of odontocetes are long[5,6] and in their new study Alves, Ruivo and colleagues show that their mitochondria are larger than those of the sperm of other mammals, including baleen whales[4]. To address whether cetacean sperm are capable of metabolizing glucose as a fuel source, Alves, Ruivo and colleagues tested for the integrity of genes within the glycolysis pathway of sperm using genomics. Within the sperm transcriptome of odontocetes, they found inactivating mutations in enzymes associated with glycolysis (*Gapdhs, Pgk2*), and disrupting mutations or exon-loss events in genes encoding key aspects of glucose subsidiary pathways (pyruvate and lactate metabolism and ketone body catabolism). Taken together, this genomic evidence suggests a profound restructuring of energy production in the sperm of odontocetes with a dismantling of glucose metabolism. In contrast, glycolysis is probably still partially active in baleen whales (Figure 1).

Alves, Ruivo and colleagues hypothesized that sperm of odontocetes are likely fueled only by oxidative phosphorylation and tested the endurance of sperm motility in a culture system. By supplementing media with known fuel associated with glycolysis (glucose, pyruvate) and an inhibitor of mitochondrial fatty acid β-oxidation (etomoxir), they quantified exhaustion of sperm. Surprisingly, without access to glucose and pyruvate, the sperm of mice and bulls became immobile within hours, but the sperm of dolphins kept swimming for three days. In contrast, the motility of sperm of all dolphins declined markedly two hours after exposure to the inhibitor of mitochondrial fatty-acid β-oxidation. Also, the metabolites associated with oxidative phosphorylation were found to be in greater abundance in the sperm of dolphins compared to bulls. Taken together, these *in vitro* studies of sperm performance show that the sperm of dolphins only use mitochondrial fatty acid β-oxidation as a fuel source (Figure 1).

Novelties in the metabolism of sperm of odontocetes are probably a response to greater needs for aerobic respiration in a carbohydrate-poor environment in which fatty acids are relatively abundant.

This perspective of cetaceans displaying unique metabolic needs is well founded from evolutionary studies of mitochondria. To begin to shed light on this, it is helpful to consider the evolutionary history and ecology of cetaceans. Between 40 and 48 million years ago, archaic cetaceans (archaeocetes) underwent a land-to-sea transition and became completely aquatic. They are descended from even-toed ungulates (artiodactyls), like hippos, boars, and bulls. In this transition from a terrestrial ancestor, all cetaceans underwent an exceptional transformation and show evidence of positive selection in genes associated with favoring oxidative phosphorylation, which may have aided them in adapting to overcome heat loss, hypoxia, and switching to a diet of sea creatures consisting of mostly lipids and amino acids[7–9].

Cetaceans have an inactivated *MSS51* gene, which encodes a protein that is mostly found in fast glycolytic fibers of skeletal muscle and functions in mitochondrial metabolism. Experimental inactivation of *MSS51* within cells resulted in a redirection of muscle energy metabolism toward beta-oxidation of fatty acids[10,11]. This inactivation suggests that the muscles of cetaceans may be fueled largely by fatty acids, which is consistent with their high intramuscular lipid content serving as a local fuel source.

Cetaceans also show modified metabolism of mitochondria within the brain that results in neurons and glia showing potentially increased thermogenic capacity. Living in water creates a massive challenge in overcoming heat loss, and this is especially critical for the brain, which functions only within a narrow range of temperatures. Keeping the brain at a stable temperature is therefore critical for its function and the animal's survival. Compared to artiodactyls, neurons and glia within the brains of cetaceans have expanded expression of uncoupling proteins (UCPs)[12]. These proteins allow mitochondria to generate heat and are common in brown fat. Mutations in the sequence of UCPs in cetaceans were considered inactivating[13], but greater spatial signaling of proteins in the cortex of modern cetaceans suggests their functionality may be unique. Taken together, these data show mitochondrial evolution is a major driver of the unique metabolic capacity of cetaceans, and these mitochondrial alterations were potentially critical for successfully conquering the aquatic habitat.

The study by Alves, Ruivo and colleagues shows that extreme enlargements of sperm and their mitochondria are specific to odontocetes, and several studies have shown similar trends in the odontocete brain. Odontocetes, but not baleen whales, have high relative brain and sperm sizes, and delphinoids (e.g., dolphins and porpoises) display brain[14,15] and sperm sizes greater than some primates. In both the brain and sperm, mitochondria play a key function. Within the brain of cetaceans, mitochondrial function is augmented such that they have greater thermogenic capacity, and this may have evolved in response to a sudden drop in the ocean's temperature in an ancestor of baleen whales and odontocetes about 34 million years ago[12]. Similarly, within sperm, the greater size of mitochondria is associated with greater capacity for ATP production and overall endurance, and this also may have evolved in the earliest fossil odontocetes. But it is not clear what actually drove the evolution of these traits, and it is possible that mitochondrial adaptations for sperm ended up benefitting brain novelties in metabolism or vice versa. But this shows that integrating sciences such as paleontology and molecular biology, especially in tracking the evolutionary history of multiple organ systems, will lead to a deeper understanding of evolution.

A key concept that emerges from these studies is that cetaceans underwent dramatic shifts in mitochondrial physiology that allowed them to enter the water, and probably again when odontocetes evolved. Just as increased mitochondrial performance was critical for the successful radiation of cetaceans into the oceans, it was also essential for the ability of bats to satisfy the energy demands associated with flight[16]. These two lineages of mammals both departed the terrestrial habitat and independently evolved greater metabolic capacities. Within cetaceans, these studies may show that changes in mitochondrial function enhanced sperm, muscle, and brain function and that they were correlated. Evolutionary origins of these traits are only beginning to be understood, and it seems likely that more surprises await as researchers continue to integrate molecular evidence with the fossil record and our understanding of how cetaceans benefit from these changes in their unique habitat.

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