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Evolution

Early gills exchanged ions before hosting gas transfer

Dorit Hockman

During evolution, key physiological changes enabled vertebrates to achieve a more active lifestyle. A comparison between living animals challenges current ideas on the timing of one such change in our ancestors. **See p.699**

Gas exchange and ion regulation are vital biological processes. In vertebrates, gas exchange transfers life-supporting oxygen to the blood from the air and removes carbon dioxide. Ion regulation controls the movement of essential ions, such as sodium and calcium, into and out of cells. The relative levels of ions regulate the pH in cells, affecting cellular function. Changes in how these functions were performed were crucial to the evolution of modern vertebrates, but the timing and nature of the changes remain unclear. On page 699, Sackville *et al.*[1] provide insights that adjust some major pieces in this puzzle.

The current view is that, in our pre-vertebrate marine ancestors, ion and gas transfer was initially performed by the skin[2] (Fig. 1). Gases and ions could easily reach all parts of the body from the skin in these small, relatively inactive creatures. In this scenario, the first gills were used chiefly for filter-feeding – sieving edible particles from the water. Subsequently, early in vertebrate evolution, around 500 million years ago, the gills became the main site for both gas exchange and ion regulation[2,3]. This shift from the skin to the gills as the site of these processes removed a constraint on animal size, because blood circulating through the gills offered a way to deliver oxygen to the rest of the body. This enabled vertebrates to become the large, active creatures that exist today.

This theory needs to be tested, but, without travelling back in time to examine the first vertebrates, this is a difficult task. Sackville *et al.* took on the challenge by using the power of comparative biology. Their approach assumes that if an aspect of biology is shared between two living animal species, it was probably also present in their last common ancestor. By comparing where in the body gas exchange and ion regulation probably occur in living vertebrates and their non-vertebrate cousins, the authors built a model of where in the body these key processes were located during vertebrate evolution.

The authors turned first to lampreys (*Entosphenus tridentatus*), which are often referred to as living fossils because they are thought to closely resemble the first vertebrates[4]. Young lampreys, called ammocoetes, are freshwater, burrow-dwelling animals that use their gills for filter-feeding. To measure the levels of gas and ion exchange taking place in different parts of the body, the authors put ammocoetes of varying sizes into a divided chamber, separating their gills from the rest of their bodies. As they tested larger and larger ammocoetes, the authors found that

From the archive

A mysterious plague in frogs, and consideration of the consumption of oxygen after running.

50 years ago

A curious and so far unexplained ailment which killed off millions of frogs in the United States last winter ... may be showing itself again this year ... The symptoms of the ailment seem to indicate that infection of some sort is involved, but it has also been suggested that agricultural chemicals may have done the damage ... [H]eavy rains fell last autumn, and again this year, increasing agricultural runoff just at the time when the frogs entered the waters to hibernate ... [E]vidence to implicate agricultural chemicals comes from [the] finding that frogs caught in September, before they entered the ponds, have hibernated normally and seem to be quite healthy, while those caught in October have died ... So far, however, analyses of lake water samples have failed to turn up evidence of excessive amounts of agricultural chemicals and in any case the bacterial infection itself could have come from the lake waters.
From *Nature* 27 October 1972

100 years ago

In attempting to analyse the factors which underlie muscular efficiency, most observers have been content to concern themselves with a consideration of the oxygen supply. They have devoted themselves to a study of the means by which fuel arrives at the engine rather than to a study of the behaviour of the engine itself ... Prof. A. V. Hill ... was at some pains to point out the errors into which various observers have fallen by neglecting the oxygen consumption which takes place after running stops. ... [I]t is precisely *because* the oxygen consumption can to a certain extent lag behind the development of energy, it is *because* the isolated muscle can exert its full strength in the absence of oxygen, that a man can run 100 yards at a much greater speed than he can run 1 mile.
From *Nature* 28 October 1922

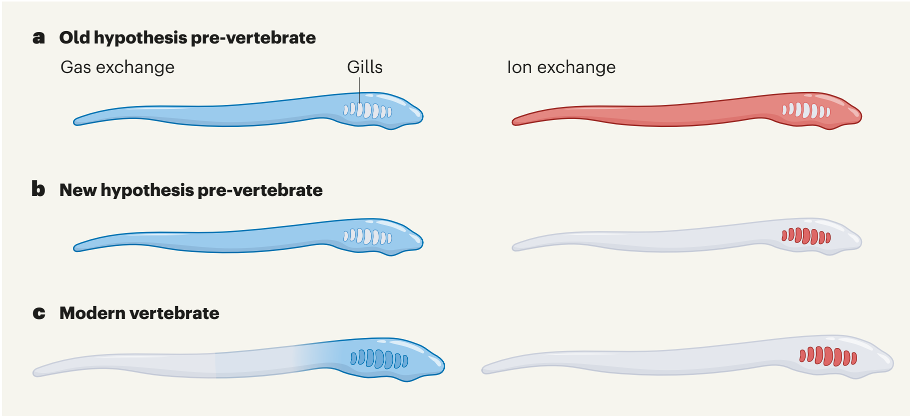

Figure 1 | An early role for gills in ion exchange. a, Small burrow-dwelling animals offer a model for understanding key steps in the early evolution of vertebrates. It has long been thought that gas exchange (the taking up of oxygen and the release of carbon dioxide) and ion regulation (transfer of ions between the body and its environment) occurred through the skin, across the length of the body, in our pre-vertebrate ancestors. **b**, Sackville *et al.*[1] present evidence for a new hypothesis, in which ion regulation was occurring predominantly at the gills much earlier in evolution, perhaps as far back as the first pre-vertebrates with gills. **c**, The authors report data suggesting that, as vertebrates evolved to become larger, more-active organisms, the gills became the dominant site for gas exchange.

gas exchange shifted towards occurring in the gills. This finding supports the theory that increased gas exchange at the gills occurred as vertebrates evolved larger body sizes. Surprisingly, the same was not true for ion regulation – which always occurred at the gills, no matter how big the ammocoetes were.

This led the authors to look further back in the animal family tree to find the roots of this unexpected characteristic. For those experiments, Sackville and colleagues used the acorn worm (*Saccoglossus kowalevskii*) and a species of amphioxus (*Branchiostoma floridae*), which are both close relatives of vertebrates. These small marine creatures have similar lifestyles to ammocoetes, living in burrows where they filter-feed with their gills.

The authors took advantage of the acorn worms' regenerative capacity and cut them in two. When they measured gas exchange over the still-living front and back halves of the worms, they found that the capacity for gas exchange was the same in the two halves. This finding supports the existing theory that, before vertebrate evolution, the dominant site of gas exchange was not the gills. When exploring ion regulation in acorn worms and amphioxus, the authors were hindered by the high ion content of seawater, which meant that they could not directly measure ion changes in the animals' environments. Instead, the authors examined the presence and location of known molecular markers for specialized cells called ionocytes, which control ion regulation in vertebrates[5]. Sackville and colleagues found that in both acorn worms and amphioxus, the levels of ionocyte markers were higher in the gills than in the skin.

The authors' beautiful microscopy analysis of the gills showed that the suspected ionocytes were found in a location similar to where they occur in fish gills. Together with the team's lamprey work, these results dash the theory that ion regulation moved to the gills along with gas exchange in early vertebrates. Instead, the findings suggest that ion regulation shifted to the gills much earlier in animal evolution, maybe even as far back as the first organisms with gills (Fig. 1). The authors speculate that the original regulatory function of the ionocytes in the gills might have been linked to feeding, with ionocytes controlling the secretion of a gel-forming substance to trap food particles.

Although Sackville and colleagues' findings are compelling, sceptics might point out that the presence of ionocyte markers does not prove that these cells function as ion regulators, and that direct measurements of ion transfer in acorn worms and amphioxus are needed. Another issue is whether the ionocytes present in vertebrate gills, as well as suspected ionocytes in the gills of non-vertebrates, are indeed evolutionarily related. It is possible that these similar cells evolved independently. This possibility could be explored by investigating how the ionocytes develop in each of these animals. If they come from the same part of an embryo and require the same genetic instructions for their development, then we will have even stronger support for a deep origin of gill ionocytes.

Vertebrate evolution is a certainly a puzzle that researchers will continue to explore, being driven by a curiosity to understand our own origins. Sackville and colleagues have taken a creative approach to challenge our current understanding of this process, and have provided new avenues for exploration.

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Immunology

How immune cells ignore harmless gut bacteria

Mark A. Travis & Chiara Romagnani

The immune system must be actively controlled to prevent inflammatory bowel disease. Cell populations have been found that promote immunosuppressive regulatory T cells of the immune system in the gut. **See p.737, p.744 & p.752**

Our immune system must respond quickly to dangerous disease-causing agents but also needs to ignore our own cells and anything benign. This balancing act is precarious in the gut, which is home to a multitude of harmless microorganisms, termed the microbiota. These microbes should not attract the attention of the immune system, but if the immune system fails to ignore them and targets them instead, this can cause inflammation and the development of inflammatory bowel disease[1]. Writing in *Nature*, Lyu *et al.*[2] (page 744), Kedmi *et al.*[3] (page 737) and Akagbosu *et al.*[4] (page 752) shed light on a key process that is required to dampen immune responses directed against the microbiota.

Immune cells called regulatory T (T_{reg}) cells have a crucial role in ensuring that the