Quick guide

Oikopleura

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What is Oikopleura? *Oikopleura* is a urochordate, with an evolutionary position stemming from the base of the vertebrate tree, and is part of the tunicate order of animals that at some point in their life generate an external, protective tunic. *Oikopleura* is specifically an appendicularian tunicate: appendicularians live their entire lives as free-swimming, pelagic individuals, distinguishing them from the other tunicate families, which either metamorphose into sessile, benthic adults or live in pelagic colonies. For this reason *Oikopleura* is also termed a 'larvacean' tunicate, because it maintains rather than loses the larval body plan.

There are about 20 species of *Oikopleura*. Collectively they are found throughout the world's oceans. Sizes range from a couple of millimeters in length (*Oikopleura dioica*) to nearly a centimeter in length (*Oikopleura longicauda*).

Oikopleura has a relatively simple body plan (Figure 1). The head of the animal, also referred to as the trunk because it harbors both cephalic and somatic organs, contains the mouth, digestive system, and anus, the heart, the cerebral ganglion with several peripheral nerves innervating oral and other peripheral structures, and the gonads. *Oikopleura* is unusual among tunicates in being gonochoric. The male and female gonads mature after several days, and 'broadcast' spawning spells the end of the short, semelparous life cycle, typically 5–7 days in *Oikopleura dioica*.

Movements of *Oikopleura* are generated by the tail, which in addition to the notochord contains 10 bilateral pairs of iterated muscle cells, the caudal ganglion, and a caudal nerve. At metamorphosis, the dorsoventral axis of the tail rotates 90 degrees relative to the dorsoventral axis of the head, such that left–right alternating muscle contractions in the adult give the misleading appearance of dorsoventral flexions, because the tail flexions now occur in the sagittal plane of the head.

What is special about Oikopleura's tunic? The molecular composition of the tunic can startle many an amateur biologist, for one of its major constituents is a form of cellulose called tunicin. How did this archetypical plant and bacterial polysaccharide became a major component of an entire order of multicellular animals? Comparative genomics studies suggest that the gene for cellulose synthase was introduced into the tunicate genome by horizontal gene transfer from a prokaryote, perhaps a bacterial symbiont.

Oikopleura has taken the construction of its tunic to an architectural extreme. Unlike non-appendicularians, it engineers its tunic into a highly faceted, spaceship-like structure by dint of the underlying patterning of the oikoplastic epithelium that secretes it. This becomes *Oikopleura's* 'house', which it uses as both a residence and a food-catching device (Figure 2). Upon secretion, the house is only slightly larger than the head of the animal, but through hydration aided by vigorous head movements it expands to dwarf the animal, providing a roomy shelter within which the animal takes up a specific position. Once situated inside, *Oikopleura* generates sinusoidal tail movements that drive seawater through strategically placed funnels. The food-catching function derives from a corrugated internal structure in the house that filters algae and bacteria out of the water stream and posits them at the animal's mouth.

This dual function gives rise to the name *Oikopleura*, literally ‘house thorax’, from the Greek ‘oikos’ for house and ‘pleura’ for rib or body side. The house is an efficient filtering apparatus, but it invariably becomes clogged, at which point the animal exits the house and elaborates a new one from a rudiment that has already been secreted in advance. The continual production and abandonment of houses, up to several a day, contributes significantly to marine snow, the organic detritus that constantly rains down through the water column. *Oikopleura* plays therefore a pivotal role in delivering carbon from shallow to deep ocean waters, and potentially to the food chain of the aphotic zone (likely mediated by bacterial degradation, given the cellulose content of the house).

Is there anything peculiar about* Oikopleura*’s genome? *Oikopleura* has become a key model organism for studies of genomic evolution. Research into the organization of the *Oikopleura dioica* genome has revealed a number of peculiarities, including a highly compact and small genome — at about 70 Mb it is the smallest known chordate genome and much smaller than the 175Mb of the fruitfly *Drosophila* and the 100Mb of the nematode *Caenorhabditis elegans* — exhibiting a substantial loss of genes relative to the vertebrate lineage, the declustering of *Hox* genes, and the use of operons, sets of cotranscribed adjacent genes which are rare in eukaryotes.

Oikopleura lacks one of the major DNA repair systems, the classical non-homologous end joining (c-NHEJ) system. In its stead, a microhomology-mediated end-joining mechanism is employed to repair double-strand breaks in DNA, and this results in an unusually high incidence of deletions and nucleotide insertions. This is believed to have contributed to the general loss of *Oikopleura* genes that has occurred since diverging from the vertebrate lineage. Among the genes lost on the *Oikopleura* lineage are many that play important roles in developmental patterning in vertebrates, including about 30% of *HOX* genes and key genes involved in retinoic acid signaling. Despite these losses and a remarkable degree of spatial reshuffling of genes, *Oikopleura* successfully develops the same basic body plan as vertebrates, indicating that the link between genomic structure and body morphology is much weaker than once thought.

How 'vertebrate' is* Oikopleura*'s brain? Being a chordate, *Oikopleura* has a notochord, but it does not develop vertebrae or any other of the skeletal structures typical of vertebrates. Its central nervous system (CNS), on the other hand, appears to be built according to the vertebrate bauplan. Specific developmental regulatory genes that pattern the vertebrate CNS into forebrain, hindbrain and spinal cord divisions are expressed in a similar rostrocaudal sequence in the neural anlage of ascidian tunicates, and it is likely that this holds also for appendicularians like *Oikopleura*, although we do not yet have direct proof of this homology. The CNS is organized into two ganglia: the 'cerebral' ganglion, located rostrally within the head, and the 'caudal' ganglion, located in the base of the tail. A slender nerve carrying a handful of axons connects the two ganglia, and a caudal nerve cord extends from the caudal ganglion down the length of the tail along the notochord. The cerebral ganglion receives sensory information from chemosensors in the oral region and from the statocyst for detecting gravity and movement, and there are mechanoreceptors on both the head and tail. But *Oikopleura* has no eye or photoreceptors, so it goes about its business blindly.

Remarkably, the total number of neurons in the *Oikopleura* CNS is extremely low for a chordate: only about 130 in total, well less than half the number in the diminutive nematode *C. elegans*. Twenty of these are cholinergic motoneurons, one for each muscle cell. The number of GABAergic inhibitory neurons has been tallied at about 13 (there is some variability with developmental stage), and preliminary studies indicate the presence of a smaller number of glycinergic inhibitory neurons. Most of the other neurons are likely to be glutamatergic excitatory neurons, based on ongoing *in situ* hybridization and immunohistochemical analyses. Minor neuron populations are also present, including neurons expressing specific neuropeptides, and a single pair of putative dopaminergic neurons in the cerebral ganglion. Unexpectedly, *Oikopleura* contains no serotonergic neurons, which are relatively common in metazoans.

What can you do with a miniaturized brain? The tiny CNS of *Oikopleura* has prompted the hypothesis that the basal chordate anlage that gave rise to the vertebrate brain has undergone an extensive miniaturization in the tunicate lineage, maintaining most of the central neuron types but squeezing their number to the bare minimum. Indeed, the one-to-one relationship between motoneurons and muscle cells, the low number of inhibitory neurons, which are essential elements in vertebrate central motor and sensory circuits, and the presence of only a single pair of dopaminergic neurons, which are of pivotal importance in modulating brain processes in vertebrates, suggest that any further loss of neurons might render the *Oikopleura* CNS dysfunctional.

Despite its diminutive scale, the CNS of *Oikopleura* generates an impressively diverse repertoire of behaviors. *Oikopleura* exhibits several cardinal tail movements with specific functions, including swimming, bending, nodding (used to help expand the house rudiment), and the graceful filtering movements employed to drive seawater through the house. In addition, *Oikopleura* generates more complex movement patterns associated with bringing the tail into the house when taking up residence or leaving the house when the food filters clog or the animal is stressed. Transitions between movement types, particularly abandoning the house at appropriate times, indicate *Oikopleura* has a mechanism for behavioral choice, perhaps even a primitive kind of ‘decision making’. Experiments on decapitated tails indicate the presence of several descending projections from the cerebral to the caudal ganglion that mediate such behavioral transitions. An exciting line of current research involves the comparison of motor circuits and their descending control in *Oikopleura* and vertebrates, as this may reveal essential core elements that all chordates share and depend on for survival.

Are tools and resources available for genomic and functional genetic studies of* Oikopleura*? Yes. Sequencing of the *Oikopleura* genome was initiated in the early 2000s and an annotated genomic sequence database and transcriptomics database was published in 2013. This database is continuously being extended and improved. Several approaches for gene manipulation have been developed, including gene knockdown by dsRNA or dsDNA injection into oocytes or the female gonad, and several laboratories have begun to use CRISPR-Cas9-based approaches for gene editing.

Why study* Oikopleura*? *Oikopleura* is unarguably exotic, not just in appearance, but in the dramatic changes that have taken place since its lineage diverged from its common ancestor with vertebrates. From understanding the consequences of and adaptations to genome compaction and gene loss, to exploring the molecule-to-structure mystery of the house and its ‘just-add-water’ construction, to deciphering the neural circuits of the minimalistic brain with their implications for what is the essential core of vertebrate motor control, *Oikopleura* offers a spectrum of fascinating questions about the origin of chordate diversity — enough for multiple lifetimes of research. And if you’d like to meet *Oikopleura* up close, you need not go far: if you have been swimming in the ocean, you have almost certainly already bumped into it.

Where can I find out more?

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Figure 1. Body structure of *Oikopleura*.

A sexually mature male *Oikopleura dioica*. The head/trunk contains head and visceral structures, including the cerebral ganglion, and the flexible tail contains the notochord, muscle cells, and caudal nervous system (caudal ganglion and caudal nerve cord). Photo credit: Yana Mikhaleva, Sars International Centre for Marine Molecular Biology, Bergen, Norway.

Figure 2. The *Oikopleura* house.

Viewed from above, the house dwarfs the animal, whose head can be seen positioned at the midline about a third from the upper tip of the house structure, between the two concave funnels. The tail is not visible, as in this view it lies below the head, where it undulates to drive sea water through the house to capture food in the convoluted and corrugated filters. Photo credit: Per Flood; image used with permission from Per R. Flood/ArtDatabanken.

In Brief:

The appendicularian tunicate *Oikopleura* epitomizes the degree to which evolution can constrain both genome and cellular composition, while at the same time unleashing fantastic specializations.