Is there intracellular cellulose in the appendicularian tail epidermis?
A tale of the adult tail of an invertebrate chordate

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All tunicates produce a cellulosic extracellular matrix external to the epidermis; this is the most remarkable character of the subphylum (Tunicata = Urochordata). In Asciidiacea and Thaliacea, two of the three classes of Tunicata, cellulose is the main component of the tunic matrix that covers the whole body. In Appendicularia, however, the animals do not possess a tunic, and instead secrete a feeding apparatus called a “house” that is composed of cellulose. In the appendicularian Oikopleura dioica, we found a unique intracellular matrix in the apical cytoplasm of the adult tail epidermis that appears to form a tough shell supporting the tail. Although some experimental data suggest that this matrix may contain cellulose, the composition is still unknown. The significance of the tunicate capacity for cellulose synthesis is briefly discussed.

Does the Appendicularian Epidermis Contain Intracellular Cellulose?

Tunicata (=Urochordata) is the only metazoan taxon whose members can synthesize cellulose fibrils.1-3 This unique animal group is a relative of the vertebrates; it is one of the three subphyla of the phylum Chordata, and recent molecular phylogenies based on genome sequencing have shown that Tunicata is the closest sister group to Vertebrata within the extant chordates (Fig. 1).4 Tunicata comprises three classes: Asciidiacea, Thaliacea and Appendicularia (= Larvacea). Appendicularia is a unique group with peculiar features such as the absence of a cellulosic integument (tunic), the production of a feeding apparatus made of cellulose (the house), and the presence of a permanent tail with a notochord and epidermal fins (Fig. 2). Appendicularians undoubtedly form a monophyletic group, but their exact phylogenetic position within the tunicates is a subject of debate. They are often proposed to be basal to the other tunicates,5-9 but several studies have suggested that appendicularians are more closely related to stolidobranch ascidians10,11 or aplousobranch ascidians.12,13 Therefore, whether the unique features in appendicularians were shared with the common ancestors of tunicates (and vertebrates) or they originally emerged in the appendicularian lineage remains an open question.

The epidermal cells of ascidians and thaliaceans secrete cellulose fibrils to form an extracellular matrix called a tunic that completely covers the epidermis of larvae and adults. Ascidian larvae have an outer and inner tunic; the outer tunic is shed during metamorphosis from the swimming larval to sessile juvenile stages.14 Although appendicularians lack a tunic, they are able to secrete two types of cellulosic matrices,3,15,18 the larval envelope and the house, which may be equivalent to the outer and inner tunics, respectively, of ascidian larvae. While a single locus of the cellulose synthase gene (CesA) has been found in the genomes of two ascidian Ciona species,16,17 the appendicularian Oikopleura dioica has two CesA isoforms.
and each of the two genes are respectively involved in the synthesis of the larval envelope during embryogenesis, and the house in adults. The larval envelope is shed during metamorphosis from the swimming larva to the pelagic adult, at which point the animals start forming the house.

The appendicularian house is secreted by epidermal cells in the anterior part of the trunk (Fig. 2). In contrast, the apical cytoplasm of epidermal cells in the tail and the posterior part of the trunk of appendicularians contain a moderately electron-dense matrix (Fig. 3). Accordingly, the appendicularian epidermis can be categorized into two groups: (1) the epidermis involved in house production and (2) the epidermis with the intracellular matrix. In the appendicularian Oikopleura dioica, thin sheets consisting of polygonal scale-like structures remain after alkaline treatment of the animals, and an ultra-structural study showed that the scale-like structure matches the intracellular matrix within each squamous epidermal cell (Fig. 4). In a related study, the matrix was always found in the tail epidermal cells except at the edges of the fins, and was never found in the larvae. Because the matrix layers on the right and left sides of the tail were not connected at the fin edges, flat sheets were always observed in the tail following the alkaline treatment of an individual, but no tubular envelope was observed.

In another study on O. dioica, the scale-like structures resulting from alkaline treatment were marked with a carbohydrate-binding module (CBM) family 3 with a strong affinity for cellulose. Within the scale-like structures, a mesh marked with the CBM appeared to be embedded in the amorphous materials, and the mesh retained the CBM marker even after acetic/nitric acid hydrolysis (following Updegraff), a process during which most organic compounds other than cellulose are broken down. These results support the hypothesis that the intracellular matrix in the tail epidermis contains cellulose; however, some of the other evidence does not support this idea. First, expression of the cellulose synthase gene(s) has not been detected in the tail epidermis. Second, neither electron diffraction nor Fourier transform infrared spectroscopic microscopy has demonstrated the presence of cellulose in the matrix, although amorphous materials in the matrix may have interfered with such analyses. Finally, CBM also binds to chitin, and whereas Updegraff’s treatment theoretically decomposes chitin, carbohydrate-binding proteinaceous materials are sometimes protected from complete degradation during treatment.

The cellulosic tunic completely covers the epidermis in ascidians and thaliaceans, suggesting that all epidermal cells are capable of cellulose synthesis. Interestingly, the production of the house and the presence of the matrix are mutually exclusive except within the cells at the edges of the fins in appendicularians. Therefore, the presence of intracellular cellulose in the tail epidermis is a compelling reason to consider the matrix to be equivalent to the tunic, but...
we lack sufficient evidence from which to draw a firm conclusion.

The Tail Epidermis as a Tough, Elastic Shell Supporting Active Movement

We proposed that the intracellular matrix layer acts to make the epidermis a tough and elastic shell for the permanent tail. The possession of a post-anal tail is a chordate synapomorphy (Fig. 1), but appendicularians are the only tunicate group with a permanent tail. In the other tunicates, the larval tail is absorbed into the body during metamorphosis into the sessile or planktonic adult forms. Appendicularians are planktonic and continuously beat their tails to move water into the house enclosure to collect food particles (Fig. 2). Moreover, they are swift swimmers when they shed the house, actively beating their tails. Therefore, the adult tail epidermis should be tough and elastic for withstanding the shear stress caused by active tail movement, a factor that cannot be ignored in an aquatic milieu with a low Reynolds number. In the adult tail, epidermal cells laterally bind via tight junctions, zonulae adherentes, and occasionally gap junctions (Fig. 4).21-24 The zonulae adherentes provide sufficiently tough attachments among the epidermal cells, and they appear to be connected to the intracellular matrix with microfilaments (Figs. 3 and 4).24 In ascidians as well as appendicularians, epidermal cells in the larval tail have only tight junctions.12,14,24 This may indicate that multiple cell junctions and the intracellular matrix layer have evolved to facilitate the function of the adult tail in appendicularians.

What had Tunicate Lost Instead of Tunic, a Cellulosic Extracellular Matrix?

Tunicates are one of the most successful taxa of both sessile and pelagic communities, and they are one of the major suspension feeders in the marine ecosystem. Blooms or outbreaks of tunicates can sometimes seriously impact local communities, particularly in the case of non-indigenous/invasive species. The capacity for cellulose synthesis was likely a principal influencing factor for tunicate diversification and their subsequent prosperity in marine environments. The tunic is a multi-functional integument providing or facilitating innate immunity, protection from predators and solar radiation, self-nonself recognition, algal symbiosis, and more.27 However, it is often bulky and unsuitable for active movement: all ascidians are sessile and thaliaceans usually drift with the current. Appendicularians are exceptional due to the absence of a tunic, and are consequently capable of dynamic motility. With the post-anal tail supported by the notochord, chordate ancestors were probably able to swim, crawl or dig into the seabed. Apart from appendicularians, the tunicates lost the active motility that may have been retained by the ancestors of vertebrates.

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