Use of Chitin for Protection by Nudibranchs

Sydney Nichols, MacEwan University

Rainer, M., S. Hild, P. Walther, K. Ploss, W. Boland, and K. Tomaschko. 2007. Granular Chitin in the Epidermis of Nudibranch Molluscs. Biological Bulletin 213: 307-315.

A defining characteristic of the opisthobranchs (including nudibranchs) is the loss or reduction of a shell produced by the mantle, which is a source of physical protection in many molluscs. Due to this lack of physical protection, many nudibranchs will retain nematocysts from their cnidarian prey inside their cerata (external projections for gas exchange) and use them as a defensive mechanism. Even though these nudibranchs are capable of sequestering these nematocysts, they are still exposed to their toxins while feeding on cnidarians. Eolid nudibranchs (family Aeolidiidae) have a specialized epidermis containing spindles that protect them from the stinging nematocysts while feeding on cnidarians. Rainer et al. (2007) asked if these spindles are made up of chitin and how it compares to the chitin of arthropods, and how they protect the nudibranch from the stinging nematocysts of their cnidarian prey.

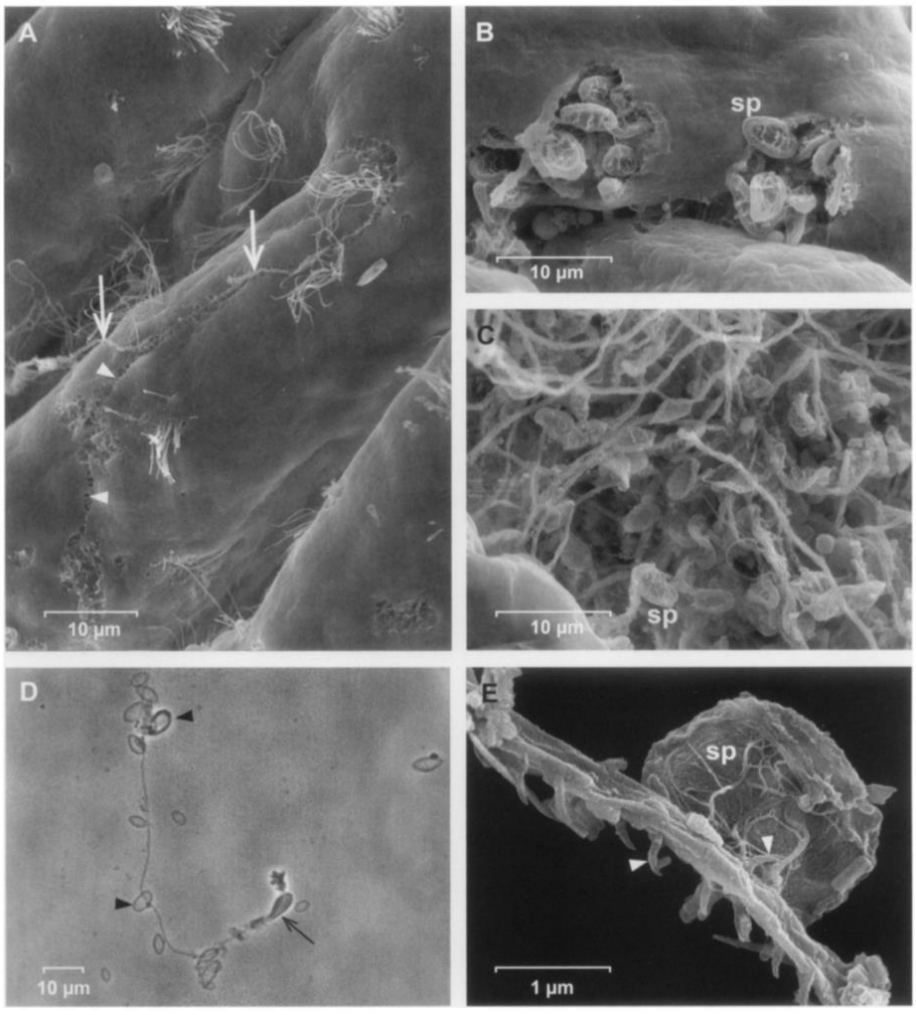
Rainer et al. (2007) collected eolid nudibranchs *Cratena peregrina* and *Flabellina affinis* off the coast of islands Giglio and Elba in Tuscany, Italy, and exposed them to various tests alongside crab chitin. The cerata of the nudibranchs were frozen, chemically fixed, and dehydrated for transmission and scanning electron microscopy. The spindles from the epidermis were then isolated and either boiled in KOH, or exposed to trypsin, proteinase K, or chitinase enzymes. An immunoblot was also done on the spindles using an antibody against crab chitin. A confocal Raman microscope was then used to record a Raman spectra of the nudibranch spindles boiled in KOH and crab chitin.

The authors were able to confirm the presence of chitin in the radula, alimentary canal, and the epidermis of the skin and gut epithelium (it was previously known that the radula contained chitin). The spindles of the nudibranchs were found to resist boiling in KOH suggesting the presence of chitin, as chitin is one of very few biological materials able to withstand boiling in KOH. The spindles were also degraded by chitinase (and not effected by trypsin and proteinase K) and reacted to an antibody against crab chitin. Finally, confocal Raman microscopy confirmed that the spectra of the nudibranch spindles were extremely similar to that of crab chitin.

They found that when contacted with the nematocyst, the nudibranch skin would tear from the toxins released (Focus Figure 1A), however, this tear would release large quantities of spindles (Focus Figure 1B). The nematocysts contained hooks that the spindles would attach to (Focus Figure 1E), and ultimately due to the large number of spindles released, the nematocyst would be forced to detach from the nudibranch skin as the spindles take up all available hooks (Focus Figure 1D). Overall, this reaction prevents further damage to tissues deeper in and under the epidermis. It is assumed that the spindles in the stomach lining protect the tissues in the

same way. This suggests that the nudibranch have found a very efficient use of chitin as protection when compared to arthropods because, although not as robust as chitin armor, the nudibranchs are able to maintain a flexible body shape and have no need to molt or be exposed to the risks associated with molting.

In conclusion, chitin is indeed present in the epidermis of eolid nudibranchs *C. peregrina* and *F. affinis,* but it is present in a very different way than that of arthropods. In arthropods, chitin is present as a rigid exoskeleton that must be molted in order for growth and renewal to occur, where in eolid nudibranchs, it is in a specialized epidermis to protect them from their stinging prey, but still allow for flexibility and fluid movement. Nudibranchs have a variety of defence mechanisms and it leads one to wonder what other ways they protect themselves and how the cost of reducing or losing their physical shell compares to the cost of developing new mechanisms of protection.



Focus Figure 1. Ceras of *Flabellina affinis* contacted by a nematocyst from *Eudendrium racemosum*. (A) SEM of cnidarian nematocyst (arrow) leaving a tear in the epidermis of the ceras (arrow head). (B) SEM of spindles (sp) exiting tears in the skin. (C) SEM of spindles (sp) and nematocyst tubules. (D) Light micrograph of spindles (arrow head) attaching to a nematocyst (arrow). (E) SEM of a single spindle (sp) attached to spines on a nematocyst (arrow head). From Rainer et al. (2007).