

## Morphogenesis of the larval and juvenile tunic in *Halocynthia papillosa*

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The highly organized adult tunic of the ascidian *Halocynthia papillosa* (VAN DAELE, 1990) calls for a study elucidating the formation of such complex structures. In order to survey tunic morphogenesis from the onset of tunic secretion it is necessary to rear embryos, larvae and juvenile ascidians under confined experimental conditions. Since the reproductive cycle of *Halocynthia papillosa* has not yet been described, the maturation of the gonad was monitored for a whole year. During their main breeding season (September, October) adult animals were collected in the vicinity of the Marine Biological Station at Banyuls (France, Mediterranean Sea). Individuals were kept in separate tanks supplied with running seawater.

Spawning of gametes is light-controlled. Under natural light conditions, it takes place shortly after sunset. Naturally spawned eggs were fertilized with non-self sperm and reared in filtered seawater at 14-15°C. Embryogenesis was completed within 42h. Although the tadpole larvae failed to attach to all offered substrata, they started metamorphosis after a short free-swimming period. Juveniles were kept until they reached the age of three months.

Until tunic secretion is initiated at the early tail-bud stage, the embryos have a smooth epidermis. Then, patches of tunic material are seen on the embryonic surface. In TEM micrographs, these patches appear as a fine discontinuous ribbon which gives rise to the outer cuticle of the larval tunic. Soon after, the embryo is completely covered by the larval tunic and the test cells settle onto the tunic. By the middle tail-bud stage, when melanin is synthesized in the sensory vesicle, a ventral, a dorsal and a short caudal fin have developed. At this stage, the larval tunic consists of the outer cuticle, a rigid fibrous sheet of medium electron density, and the outer compartment. Patches of dark-staining material are irregularly distributed over the tunic surface. This material has a fibrous appearance in SEM micrographs and seems to be associated with test cells. Seconds only before the larva hatches, the test cells detach from the larval tunic. A second fibrous sheet then develops above the apical epidermal membrane of the trunk, the inner cuticle. Some hours later, the trunk tunic consists of the outer cuticle, the outer compartment, the inner cuticle and the inner compartment while the tail epidermis is enveloped only by the outer cuticle and the outer compartment. The outer and inner compartments are composed of granular and fibrous material embedded in a hydrated ground matrix. SEM shows a swimming larva with a few small fins around the trunk and bigger fins, one dorsal and one ventral, lining the tail. Most striking is the hexagonal pattern of the trunk tunic.

Radical metamorphosis starts with elongation of the anterior part of the trunk, retraction of the adhesive papillae, immobilization of the larva, and retraction of the tail. During tail retraction, the first cells pass through the epidermis and enter the inner compartment of the tunic. This process is confined to a small region just anterior to the sensory vesicle. Once the tail is retracted, gaps form between the inner cuticle and the outer compartment. In the following three days the outer cuticle and the outer compartment of the trunk tunic as well as the tail tunic are shed. Only two days after the onset of metamorphosis, the tunic forms the first spines, a feature characteristic of the adult tunic. In the course of tunic morphogenesis, the cuticle forms plates, each carrying a spine. Once the juvenile has lost the outer layer of the larval tunic, the tunic consists of a thin fibrous cuticle and a fundamental layer. There is only very little fibrous material distributed throughout the whole fundamental layer. Some intratunical cells lie directly underneath the cuticle. They all belong to the same cell type. As tunic morphogenesis proceeds, the ultrastructural complexity increases. The diffuse net of fibrous material is progressively replaced by fibres oriented parallel to the epidermal surface except in regions where spines have developed. There, bundles of fibres form the core, supporting the spines. At least one new cell type is then found in the fundamental layer of the tunic. Observations with the light microscope show that intratunical cells are motile.

In juveniles three months old, the tunic has yet not attained the structural complexity of the adult tunic.

### REFERENCE

VAN DAELE Y., 1990.- Organisation du tégument des ascidies *Halocynthia papillosa* Gun. et *Phallusia mammilata* Cuv. (Urochordata, Ascidiacea). Morphogénèse, ultra-structure et composition chimique de la tunique en relation avec ses propriétés physiques. *Extr. Bull. Soc. Roy. Sci. Liège* 59 (5),1-93.

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