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Tube synthesis and growth processes in the hydrothermal vent tube-worm *Riftia pachyptila*

Juliette RAVAUX[1], Bruce SHILLITO[1], Françoise GAILL[1], Lucien GAY[2], Marie-Françoise VOSS-FOUCART[3] and James J. CHILDRESS[4]

[1] *Laboratoire de Biologie marine - 7, Quai Saint-Bernard, 75252 Paris Cedex 05, France - Fax : (33) 1 44 27 52 50 et Station Biologique, INSU-CNRS-UPMC Roscoff - e-mail : Francoise.Gaill@snv.jussieu.fr*

[2] *Laboratoire de Biologie Cellulaire Fongique - UMR CNRS 5534 - Université Lyon I, 69622 Villeurbanne, France*

[3] *Institut de Zoologie, Université de Liège - 22, Quai Van Beneden B-4020 Liège, Belgium*

[4] *Department of Biological Sciences and Marine Science Institute - University of California, Santa Barbara, California 93106, USA*

Vestimentiferans, particularly *Riftia pachyptila* Jones, 1981, are representative of the communities associated with deep-sea hydrothermal vents. These organisms, unusual in their anatomy and gigantism, live in tubes which have a skeletal function and allow the branchial region to open out close to the vent fluid. A significant part of *Riftia*'s growth is accounted for by tube production. This tube, which may have impressive dimensions (up to two meters long), is composed of chitin associated with proteins (Gaill & Hunt, 1986). To understand the tube synthesis and growth processes, cellular structures and morphological aspects of worms and tubes were studied. Moreover enzymatic activities involved in synthesis and degradation of the chitinous tube were investigated.

The results have shown that *Riftia*'s tube secretion is achieved by highly specialized structures, the chitin secreting glands, constituting a very efficient secretory system (Gaill et al., 1992). The secreting cells exhibit original microvilli-like structures, named "cups" because of their shape (Shillito et al., 1993). These specialized microvilli are involved in the chitin crystallite secretion (Shillito et al., 1993). The main steps of tube wall formation from *Riftia pachyptila* are summarized in Figure 1 (Shillito et al., 1993; Shillito et al., 1997).

This model of chitin synthesis suggests the existence of a chitin synthase located at the plasma membrane of the secreting cells which polymerizes N-acetylglucosamine (GlcNAc) monomers to form chitin chains. To test this assumption, the enzymatic activity involved in chitin synthesis was studied in chitin secreting tissues (Ravaux et al., 1998). The chitin synthase assays revealed the presence of an activity GlcNAc-transferase associated with membranes. This activity shares some characteristics with chitin synthases activities from fungi and crustaceans, but the *Riftia* enzyme seems to be quite different from other enzymes regarding some parameters tested, for example the high affinity of the enzyme for its substrate (i.e. low K_M). This could play a role in the high rate of chitin synthesis recorded (Gaill et al., 1997). Preliminary immunological studies performed on *Riftia* chitin secreting tissues allowed us to identify a 108 kDa protein recognized by an anti-chitin

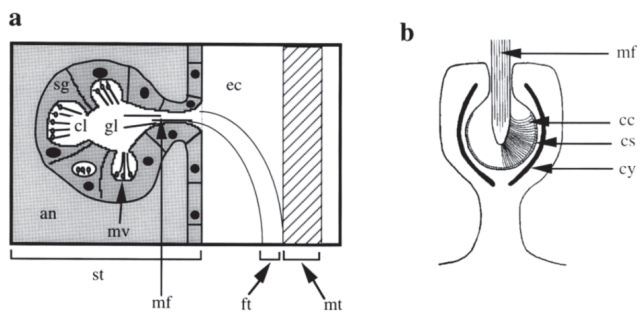

Figure 1. Chitin secretion in the tube-worm *Riftia pachyptila* (redrawn from Shillito et al., 1993 and Shillito et al., 1997). (**a**) Schematic drawing of the secreting tissues *(st)* facing the tube *(mt)* of *Riftia*. On the surface of the animal (an), multicellular chitin secreting glands (sg) are responsible for tube formation. The chitin microfibrils that compose the tube are assembled in cup-shaped microvilli-like structures (mv). After synthesis, the microfibrils (mf) transit through the cell lumen (cl) and the gland lumen (gl), before the whole secretion is extruded outside the animal in the environmental compartment (ec), separating the tube wall from the animal body. This fresh tube secretion (ft) is laid down on the preexistant mature tube wall (mt).

(**b**) Schematic drawing of the cup-shaped microvillus showing chitin chains (cc) that assemble to form a chitin microfibril (mf) in the cavity of the microvillus. The chitin chains synthesis would take place at the plasma membrane surface of the cavity. It is suggested that the chitin synthases (cs) are located in this plasma membrane. Inside the cup-shaped microvillus there is a cytoskeleton-like structure (cy) which is supposed to control the relative orientation of putative chitin synthases.

synthase antibody (unpublished results). Further investigations of this 108 kDa protein would possibly allow the location of *Riftia* chitin synthase in the secreting cells.

Observations on several tubes have shown deposit of freshly secreted tube material, not only at the top of the tube but also at the base, as well as the existence of bifurcate tubes, which allow us to propose a model of tube growth at both ends (Gaill et al., 1997). By remodeling the tube base, *Riftia* can adapt the tube width to its increasing size, in contrast to other vestimentiferans, which inhabit conical-shaped tubes. Such a growth pattern would also allow adaptation to the fluctuating environment. First, the high growth rate can rapidly modify the position of the worm towards the vent fluid. Second, *Riftia pachyptila* are found in clumps where space competition is important for fluid access; this competition will be more acute as newly settled worms are added to the initial population, thus modifying their respective positions towards the vent source. Bifurcate basal shapes may help in space displacement, and in the modification of the position towards access to vent fluid.

This model suggested the existence of enzymatic activities which would be involved in the basal degradation of the tube, i.e. chitinolytic and proteolytic activities. A chitinolytic activity has been demonstrated in the tissues which are in contact with the bottom of the tube, i.e. in the opisthosome, thus supporting the tube growth model proposed above (Ravaux et al., 1998).

In order to estimate the tube apical growth rate and the tube production rate, tube secretion experiments were performed on live animals in pressure aquaria (Gaill et al., 1997). The results showed that the vestimentum may secrete up to 4.22 mg of dried tube material in 1 day, leading to an estimated minimum tube growth rate of 14 cm yr^{-1}. This value is at the lower end of the range reported from in situ experiments, or estimated, which range from 10 cm yr^{-1} (Fustec et al., 1988) to 85 cm yr^{-1} (Lutz et al., 1994). Apart from the possibility that the worm growth rates were influenced by the experimental conditions, this may be explained by the fact that, in these experiments, it was assumed that only the vestimentum was a tube-producing region, thus underestimating the tube production potential of the whole animal. In term of chitin production, it is clear that the rates of production per unit of area in *Riftia* colonies are the highest recorded for any marine ecosystem (Gaill et al., 1997). The chitin production rates at vents (up to 79 g m^{-2} yr^{-1}) approach 100 times those of pelagic (1 to 5.3 g m^{-2} yr^{-1}) and benthic (1 g m^{-2} yr^{-1}) ecosystems.

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