

## Formation of the Ultrastructural Components of *Ciona intestinalis* Tadpole Test by the Animal Embryo

The ascidian *Ciona intestinalis* tadpole is enclosed in an extracellular coat, called the test. In a previous paper the formation of the test and the ultrastructural changes of the ectoderm cells associated with the test formation have been reported (MANCUSO<sup>1</sup>). Cuticle and hypocuticular filaments are the ultrastructural test components shown by the electron microscope.

The cuticle is a thin layer limiting the test externally. It appears at the tail bud stage tight against the external plasmalemma of the ectoderm cells. Shortly before the hatching of the tadpole, an irregular space, the hypocuticular space, appears between the external plasmalemma of the epidermal cells and the cuticle. In this space, embedded in an amorphous background substance, there are hypocuticular filaments, long filaments with a diameter not exceeding 80 Å. Their chemical nature is unknown and studies are at present in progress.

Concomitantly with the test formation, the ectoderm cells show ultrastructural aspects typical of the secretory cells (MANCUSO<sup>1</sup>). They appear involved in the formation

of the test components; but experimental confrontation at the electron microscope is lacking. Morphological evidence alone cannot rule out other possibilities such as that some of the test components may be produced elsewhere, inside or outside the embryo by the accessory cells, and polymerized on the surface of the ectoderm cells. The present report provides evidence that the ectoderm cells of the *Ciona* embryo produce the test components.

Eggs were demembrated with needles before fertilization and so deprived also of the accessory cells. At the 8-cell-stage they were cut in order to separate the 4 animal blastomeres, that is the presumptive territory of the ectoderm. The 4 isolated animal blastomeres were allowed to develop until the controls, eggs fertilized at the same time, reached the tadpole stage. Then controls and animal embryos obtained from the isolated animal blastomeres were fixed according to the technique already reported (MANCUSO<sup>1</sup>) and studied at the electron microscope.

Detailed descriptions, by the light microscope, of the development of the 4 isolated animal blastomeres of ascidian eggs have been made (REVERBERI and MINGANTI<sup>2</sup>). They give rise to an animal embryo formed by a vesicle with the superficial cellular wall covering a large cavity.

The cells of the superficial wall are arranged in a single layer and show a similar ultrastructural organization of the epidermal cells of the tadpole (MANCUSO<sup>1</sup>). Both the cells of the wall of the animal embryo and the cells of the epidermis of the tadpole, beside the cellular components of the younger stages such as yolk granules and mitochondria, differentiate a well developed rough endoplasmic reticulum with flattened cisternae (Figure 1).

In the cavity of the animal embryo, lined with a basement membrane-like structure (Figure 1), degenerating cells, in strands or loosely arranged, cellular debris and flocculent material are found. Like the tadpole, the animal embryo is also enclosed in the test with the cuticle separated from the outer surface of the cells of the wall by the hypocuticular space (Figure 1) containing the hypocuticular filaments (Figure 2).

The data previously reported show that the presumptive territory of the ectoderm of the *Ciona* egg, even if precociously isolated, is able to synthesize the test components and differentiates cells ultrastructurally similarly to the cells of the tadpole epidermis.

**Riassunto.** Nelle uova di *Ciona intestinalis* allo stadio di 8 blastomeri il territorio presuntivo dell'ectoderma, rappresentato dai 4 blastomeri animali, è isolato e fatto sviluppare; l'embrione animale così ottenuto è studiato al microscopio elettronico. I dati qui riportati mostrano che il territorio ectodermico presuntivo dell'embrione di *Ciona*, anche se precocemente isolato, è capace di sintetizzare i componenti della membrana testacea e di dare origine a cellule con organizzazione ultrastrutturale simile a quella della cellule dell'epidermide della larva.

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<sup>1</sup> V. MANCUSO, *Acta embryol. exp.* (1973), 247.

<sup>2</sup> G. REVERBERI and A. MINGANTI, *Pubbl. Staz. zool. Napoli* 20, 135 (1946).

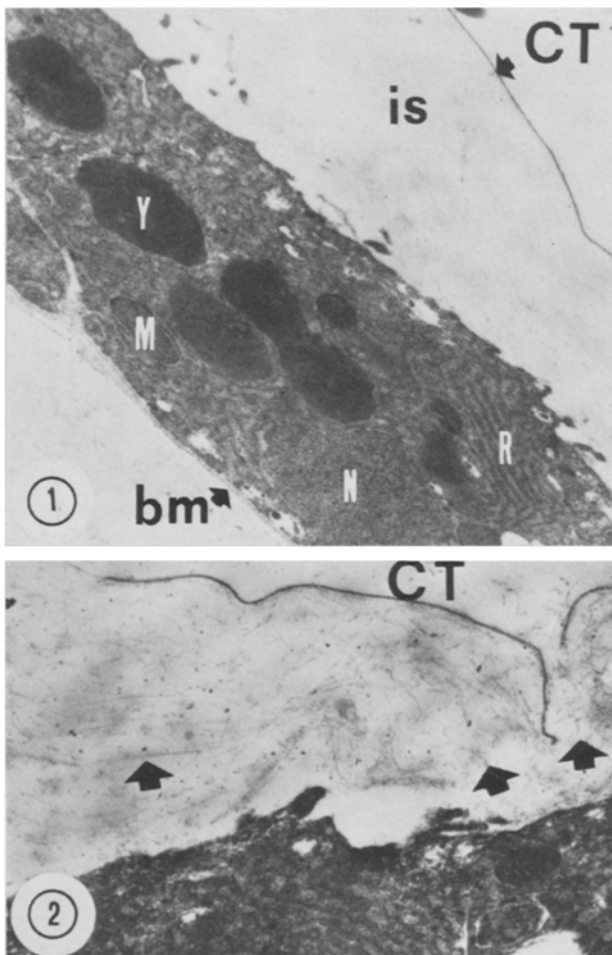


Fig. 1. Cellular wall of the animal embryo. bm, basement membrane-like structure; CT, cuticle; is, hypocuticular space. M, mitochondrion; N, nucleus; R, rough endoplasmic reticulum; Y, yolk granule.  $\times 12,000$ .

Fig. 2. External surface of the animal embryo showing the cuticle (CT) and the hypocuticular space with hypocuticular filaments (black arrows),  $\times 40,000$ .