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A NOTE ON THE FINE STRUCTURE OF MYOSKELETAL JUNCTIONS IN *ACARTIA TONSA* DANA (COPEPODA, CALANOIDA)

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Introduction

The endoskeleton of the calanoid copepod, *Calanus finmarchicus*, and its muscle attachments were described by Lowe (1935). She reported that the endoskeleton in *C. finmarchicus* consists of two tendinous endosternites and chitinous exoskeletal ingrowths to which muscles are attached. Howse (1960) noted attachments of the main muscles of the thorax to the exoskeleton in *Acartia tonsa*.

Bouligand (1962) described the ultrastructure of muscle attachments to cuticle in three species of freshwater copepods of the genus *Cyclops*. Raymont *et al.* (1974) described the fine structure of muscle attachments to cuticle in *C. finmarchicus*.

Information of the internal anatomy of marine copepods remains sparse. Therefore, we thought it worthwhile to focus our observations on the attachments of muscle to exoskeletal ingrowths in *A. tonsa*.

Materials and Methods

Live specimens of *Acartia tonsa* Dana were fixed overnight in cold phosphate-buffered 3% glutaraldehyde (pH 7.2), washed in cold 0.1 M phosphate buffer (pH 7.2) with 5% sucrose for two hours, and post-fixed in phosphate-buffered 1% osmium tetroxide (pH 7.2) for two hours (Millonig 1961). The specimens were embedded in a Maraglas-Cardolite mixture according to the method of Freeman & Spurlock (1962). Ultrathin sections were cut and doubly stained with uranyl acetate and lead citrate for electron microscopy. These sections were examined and photographed with a Siemens Elmiskop IA electron microscope.

Results and Discussion

Lowe (1935) reported that the muscles in *C. finmarchicus* are attached to the chitinous exoskeletal ingrowths (CEI) by tendinous connections, and that the endosternites are attached to the exoskeleton by "groups of ectodermal tonofibrils." Furthermore, she stated that some of the chitinous ingrowths serve only as attachments for muscle and "may be regarded as true apodemes comparable with those which Manton (1928) has described in *Hemimysis* as being formed by the gradual sinking in of the attachment of a group of muscle." The muscle attachments to the exoskeleton that we observed in *Acartia* appear to fit this criterion for apodemes. Lowe (1935) stated that the endosternites provide support for the muscles of the antennae and mouth parts. The chitinous ingrowths from the exoskeleton provide attachments for the remaining somatic muscles.

Raymont *et al.* (1974) described muscle attachments in *C. finmarchius* to a tendon: "Arising from this are fine tubules which become grouped together into electron-dense bundles of fibers with loss of the tubular appearance." They stated that these fibers bridge a narrow space and insert "...into the cuticle as tonofilaments which can be seen with diminishing density for practically the full thickness of the cuticle." Further, they found no specialized tendinous attachments in some areas. However, the sarcolemma of the muscle cell is apposed to the hypodermal membrane. But there are no tubular fibers in the hypodermis or tonofilaments penetrating the cuticle.

Slight variations occur in the diameter of microtubules (MT) among different species. They are over 280 Å in diameter in the brown shrimp, *Penaeus aztecus* (Talbot *et al.* 1972), about 210 Å in diameter in the horseshoe crab, *Limulus polyphemus* (Sherman 1974), 240 Å in the crab, *Carcinus maenus* (Roosner and Sherman 1976) and about 230 Å in the insects, *Calpodes ethlius* and *Rhodinus prolixs* (Lai-Fook 1967).

Acartia epidermal cells (tendinal cells, TC) are interposed between the chitinous exoskeletal ingrowth (apodeme) and the muscle cell (Figs. 1,2,3) where they form the tendo-skeletal junction with the former and the myotendinal junction with the latter. The gap of the myotendinal junction is from 160 to 230 Å in width (Fig. 1), narrower than a similar gap in the copepod, *Cletocamptus retrogressus*, in which it is 400 Å (Gharagozlou-van Ginneken and Bouligand 1973), and in *P. anemoniae* in which it is 300 to 400 Å wide (Brigg, 1979). A similar gap in the barnacles, *Balanus improvisus* and *B. balanodies*, ranges from 250 to 700 Å depending upon the region (Koulish 1973). The membranes of the cells forming the gap in *Acartia* are electron-dense but no desmosomes or other

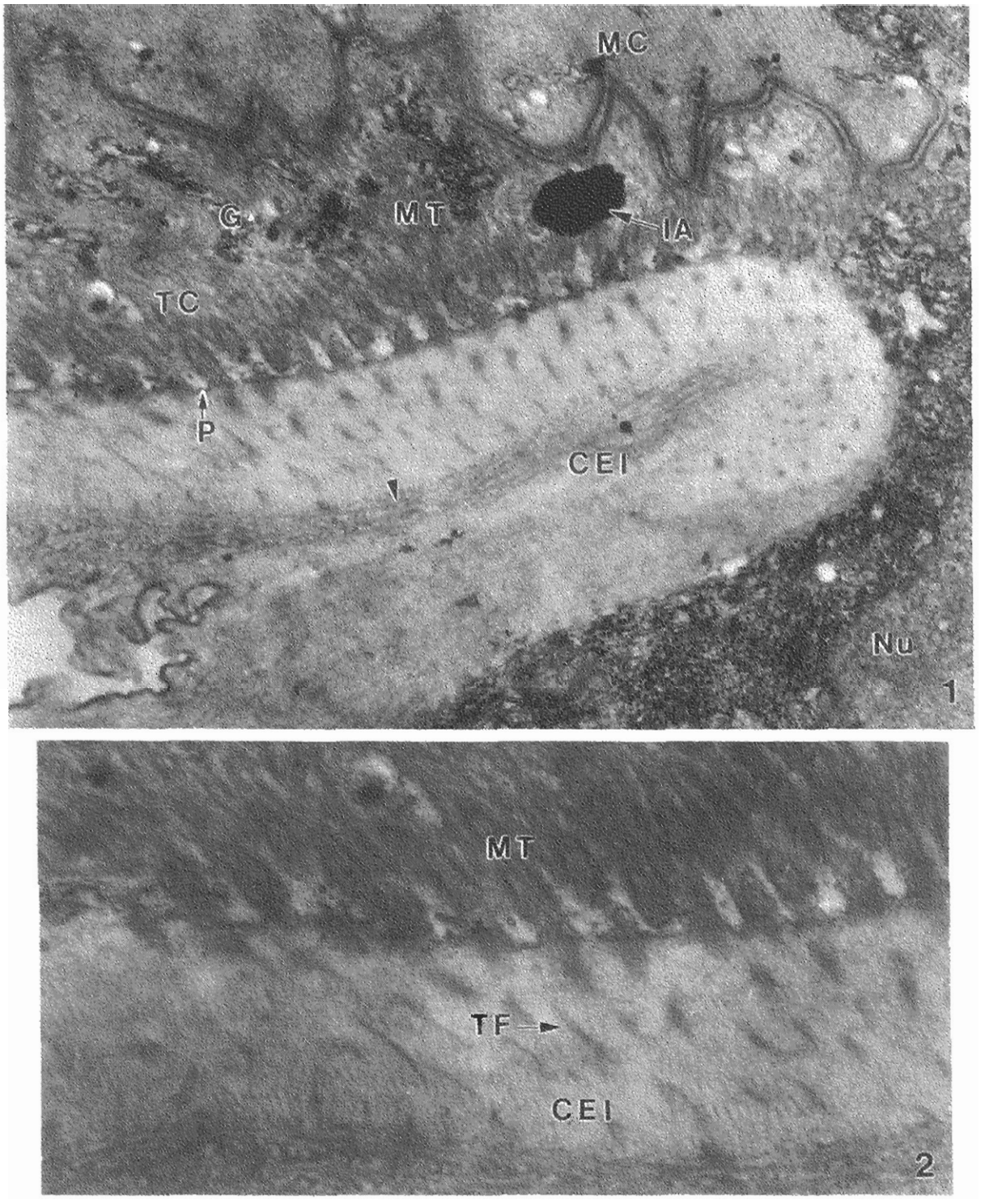

Figure 1. Section through an epidermal tendinal cell (TC) interposed between the distal end of a muscle cell (MC) and a chitinous exoskeletal ingrowth (CEI). The microtubules (MT) are shown in longitudinal view, most of which are bent. Note the several layers of chitin (arrowhead) in the center of the CEI. G-glycogen; IA-irremovable artifact; Nu-nucleus; P-plasmalemma. X 43,500.

Figure 2. Higher power view of the MT as they attach to cuticular projections, and the tonofibrils (TF) penetrate and ramify within the CEI. X 104,400.

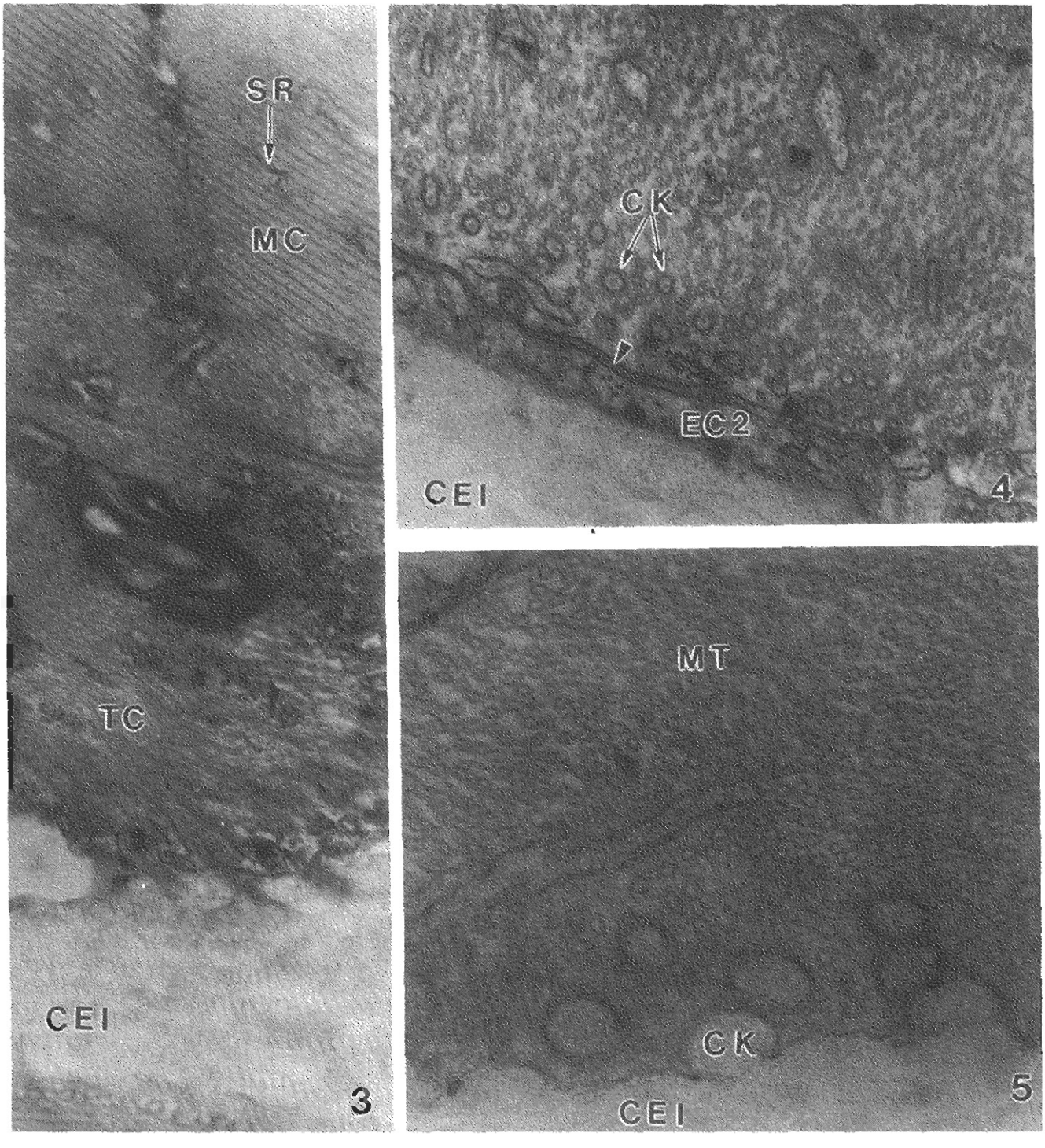

Figure 3. Section of an obliquely attached epidermal tendinal cell (TC) to the exoskeletal chitinous ingrowth (CEI). MC-muscle cell; SR-sarcoplasmic reticulum. X 58,000.

Figure 4. Transverse view of the microtubules (MT). Note desmosomal attachment of MTs to chitinous knobs (CK) and their uniform distribution throughout the cytoplasm. Note the intermediate junction (arrow head) between the tendinal cell and an adjacent epithelial cell (EC2). CEI-chitinous exoskeletal ingrowth. X 58,000.

Figure 5. Transverse view of the MTs and large chitinous knobs (CK) indenting the apical plasma membrane of the epidermal tendinal cell. CEI-chitinous exoskeletal ingrowth. X 108,000.

specialized junctions occur.

The cytoplasm of the TCs in *Acartia* contain numerous MTs that are about 230 Å in diameter and extend from their insertion in the basal end of the TC to their insertion in the apical region (Figs. 4,5). The MTs are larger than those in *Cyclops* in which they are 125 to 150 Å in diameter (Bouligand 1962) and in the cyclopoid copepod, *Paranthessius anemoniae*, in which they are 200 Å in diameter (Briggs 1979). In *Acartia* they are dispersed but closely associated throughout the cytoplasm (Fig. 1). They form groups, each of about 800 Å in diameter, and become electron-dense where they attach by hemidesmosomes to cuticular projections that form invaginations in the apical region of the TC (Fig. 2). In some areas, the attachment of the TC to the cuticle is marked by chitinous knobular projections that arise from the cuticle. They vary in diameter up to 1200 Å (Fig. 5). The microtubules of the TC attach to the chitinous knobs by hemidesmosomes (Figs. 2,5). In other areas, the TC appears to be attached to the cuticle by tonofibrils that pass from the core of the invaginations deeply into the cuticle. This finding differs from that in *C. finmarchicus* which in some areas lack tonofibrils and the muscle cell attaches directly to the innermost layer of the cuticle (supra cit.). In other areas, tonofibrils span a narrow space (Raymont *et al.* 1974), and in *Cyclops* (Bouligand, 1962) there is a space between the epidermal cell and the cuticle through which the tonofibrils cross. In *C. maenus*, cuticular rods arise from the cuticle and insert into conical invaginations of the tendinal cell (Roosner and Sherman 1976). Similar groups in *Cyclops* consist of about 10 tonofibrils (Bouligand 1962), and in *P. anemoniae* they form electron-dense fibers of about 400 nm in thickness (Briggs 1979). The TFs that attach the TC to the cuticle are 0.08 um in diameter in *C. ethlius* and 0.05 to 0.22 μm in *R. maenus* (Lai-Fook 1967).

In one of our preparations, the MTs are bent, a configuration that may reflect the relaxation or severance of the proximal end of the muscle (Fig. 1).

Close association between adjacent TCs occur near the CEI (Fig. 4). The TCs are nucleated and contain pockets of glycogen (Figs. 1,3). The plasmalemma of the adjacent cells are electron-dense and are separated by a gap of about 140 Å which is bisected by electron-dense material forming intermediate junctions.

The chitinous projections and knobs provide a firm anchor for the TC, the microtubules of which may contribute powerful tensile strength to enable the cell to withstand the force of muscle contraction (Lai-Fook 1967). The tendinal cell not only provides a strong tendinous attachment for the muscle to the exoskeleton, but may also absorb the shock of contraction in this constantly swimming and highly active organism. Neither the chemistry of the tonofibrils nor their functional mechanisms are known, but Dustin (1978) stated that "... they appear to have a mechanical role (in tension)..."

ACKNOWLEDGMENTS

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