THE FINE STRUCTURE OF THE CONNECTIONS BETWEEN MUSCLE CELLS IN ASCIDIAN TADPOLE LARVA

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The tadpole larvae of ascidians swim by means of a chordate tail. The tail consists of a central notochord, a muscle band on each side, a small neural tube above the notochord, and an enclosing epidermis. The notochordal and muscle tissue differentiate precociously during development and consequently consist of small numbers of exceptionally large cells, as reported by Conklin (1) and Berrill (2). The muscle cells are about 120 μ in length and 30 μ in width with centrally placed nuclei. These cells are arranged in a linear manner with their long axis parallel to the long axis of the tail. Of particular interest is the fact that in the fully developed tadpole larva the myofibrillae of each muscle band appear to be continuous from one end of the band to the other, passing across cell boundaries as though boundaries did not exist. This was first described in detail by Grave (3) for the tadpole larva of *Amaroucium constellatum* as follows:—

"A single layer of cross-striated contractile fibrillae are differentiated in the cortical layer of each muscle cell. The fibrillae take a general course, but are inclined about 18° to the right of the longitudinal axis of the tail. The fibrillae of adjacent muscle cells of each muscle band join end to end and thus convert the entire series of muscle cells of each muscle band into a single muscle. A further indication that the muscle band, rather than the individual muscle cell, is the morphological as well as the physiological unit, is afforded by the fact that the alternate light and

dark segments of the fibrillae are so placed that they form continuous straight transverse rows or lines across the muscle bands, which are not in any way interrupted or interfered with by the muscle cell walls. Each muscle band functions as a unit in a way that indicates its origin is located at the anterior end of the notochord, its insertion at the posterior end. With each muscular contraction the tail makes a propeller blade-like strike, due to the oblique or spiral course of the contractile fibrillae in the muscle bands."

These observations have been confirmed by others in *Amaroucium constellatum* (4), and in *Stolonica socialis* and *Distomus variolosus* (5), and all the authors have remarked that no cell membrane is discernible, with light microscopy, between adjoining muscle cells, at least in the ectoplasmic zone in which the fibrillae are located. From these observations several questions arise: Is the peripheral continuity of the myofibrillae real or merely apparent? What degree of cell continuity exists? How does the structural and functional continuity of the myofibrillae develop? The present paper concerns the first of these questions.

MATERIALS AND METHODS

Ascidian tadpole larvae of *Dendrodoa* (*Styelopsis*) *grossularia* (van Beneden) were fixed in 1 per cent osmium terroxide in sea water for about 1 hour. They were then dehydrated in graded alcohols and embedded in Epon 812 according to the method of Luft (6). Thick sections were cut, mounted on glass slides, and examined with a phase contrast microscope to ascertain the orientation of the cells in the tail. Thin sections were cut with a glass knife on a Porter-Blum microtome, mounted on copper grids, stained with lead hydroxide (7), and examined in an RCA EMU 3E.

OBSERVATIONS

In the light microscope, even with the use of phase contrast optics, it is difficult to discern where one muscle cell ends and the next one begins; the fibrils give the impression of being continuous and the cell boundaries are difficult to delineate (Figs. 1 and 2).

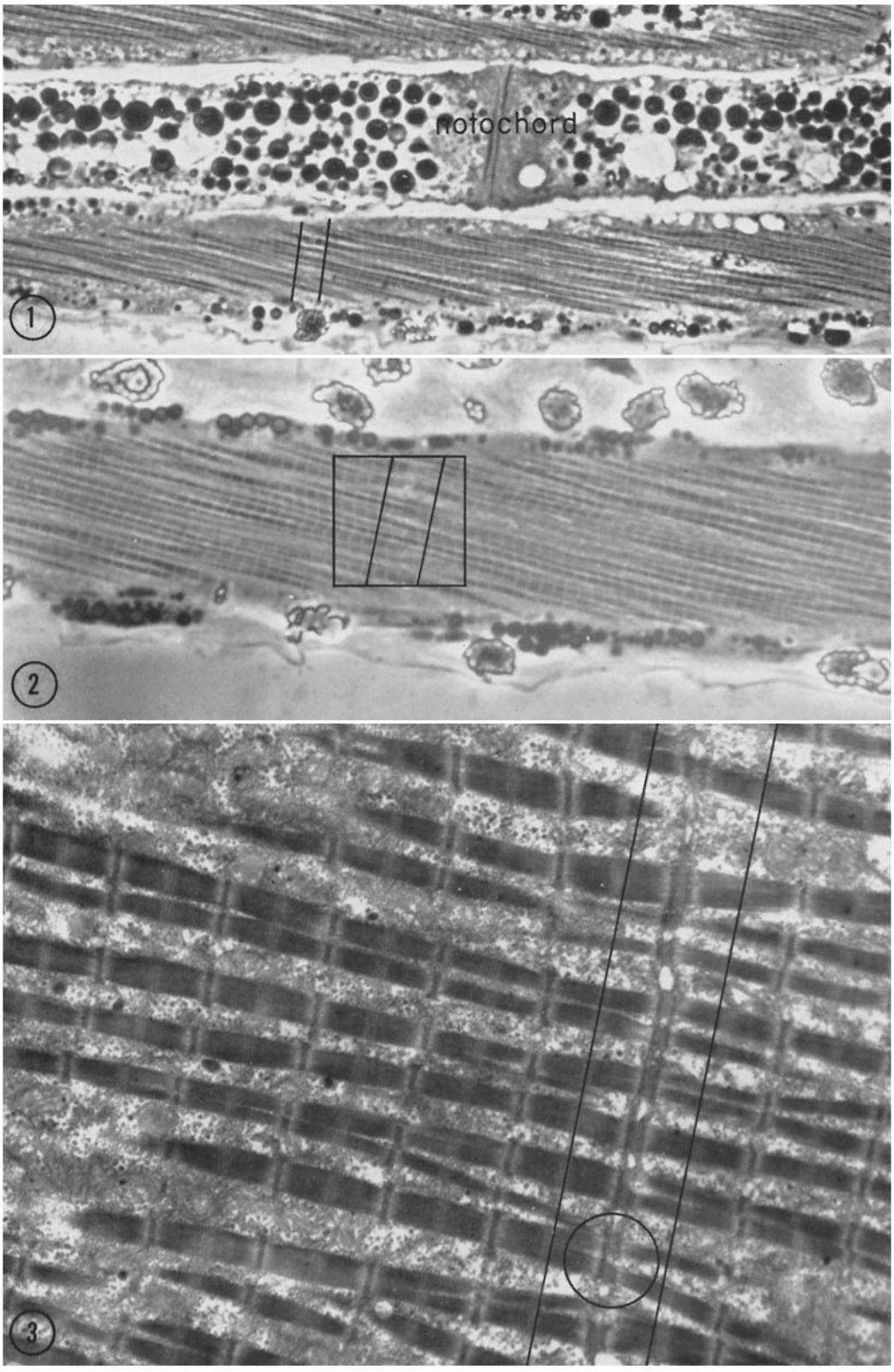
Electron microscopic observations demonstrate that each cell is delimited by a membrane. Where muscle cells lie in apposition, the membrane of each cell follows a more or less parallel course (Fig. 3) except at several points at which the membrane is denser and precisely parallel (Figs. 4 and 5). Wherever the muscle fibrils occur there are junctions between the cells (Figs. 4 to 6) of a more complex nature. One's over-all impression is that the muscle fibre is continuous from one cell to the next despite the interposition of cell membranes. The beginning of a split in one myofibril continues into the next cell, for example see Fig. 3. Closer examination demonstrates that the total width of the I band is nearly doubled in the regions in which the two cell ends lie in apposition. The final half of the I band in this region is the same width as half the I band in normal positions; the extra width can be accounted for by the material at the junction of the two cells. At this juncture between the myofibrils there is a moderately dense material within the potential intercellular space (Fig. 5). At the resolution achieved in the present study, it is not possible to say whether myofilaments do, in fact, cross this space in which the dense substance lies.

This juncture between the myofibrils most closely corresponds to the designation of a junctional complex between epithelial cells which has been called the *zonula adhaerens* by Farquhar and Palade (8). Another type of junction, which can be seen in Figs. 1, 4, and 5, most closely corresponds to the juncture called by them the *zonula occludens*.

DISCUSSION

The myofibrils of the cells of the pair of muscle bands of the tail of an ascidian tadpole larva lie in the cell cortex and superficially appear to be continuous from cell to cell. Each myofibril extends throughout the length of the muscle band and takes a somewhat spiral course which is out of kilter with the mutual arrangement of the muscle cells comprising a muscle band. There is no doubt that the myofibrils have functional continuity.

Structural continuity of some sort is also evident. No discrete structural discontinuity at cell boundaries is discernible even with phase optics. A myofibrillar bundle is often seen to split into two strands, the division being first apparent in one cell and extending with the same angle of divergence into the adjoining cell. Whatever may be present in the intercellular space between the end of the myofibril in one cell and the end of the corresponding myofibril of the adjoining cell must serve functional continuity at least. Some electron micrographs suggest, although they do not prove, that filaments either pass from cell to cell without a break or else are joined end to end by extracellular material organized in such a manner that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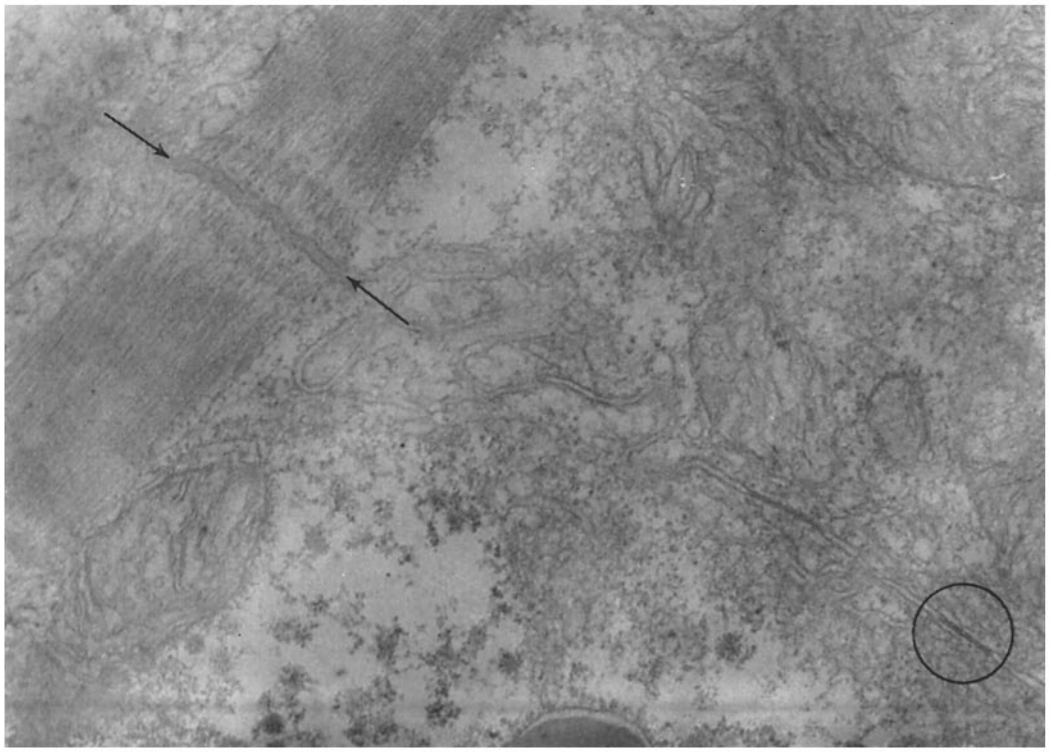

FIGURE 4 This electron micrograph of portions of two muscle cells from the tail of an ascidian tadpole larva shows the junction between the myofibril in one cell and the myofibril in the neighbouring cell (between arrows). At the lower right (circle) another type of junction between the two cells can be seen where the myofibril is not involved. × 25,000.

FIGURE 1 Phase contrast photomicrograph of ascidian tadpole tail showing a junction between notochord cells. The muscle bands lie at each side of the notochord and the muscle fibrils course at a slight angle along the longitudinal axis of the tail. The junctions between muscle cells which appear to be traversed by the muscle fibrils are difficult to see, but one such juncture lies between the parallel lines in the bottom muscle band.

FIGURE 2 Phase contrast photomicrograph of ascidian tadpole tail showing the muscle band cells and illustrating the distribution of the muscle fibrils within the cells. A juncture between two cells similar to that which appears more clearly at higher mangification in Fig. 3 can be seen within the parallel lines in the square.

FIGURE 3 A survey electron micrograph of the interface between two cells similar to that shown in Fig. 2. In addition to the well defined muscle fibrils which lie among glycogen particles and mitochondria in the cytoplasm of the cell, the junctures between about ten muscle fibrils can be seen between the parallel lines superimposed on the micrograph. Small extracellular spaces can be seen in the areas where the muscle fibrils are not apposed. In some instances, a splitting of a fibril in one cell appears to continue into the next cell (circle).

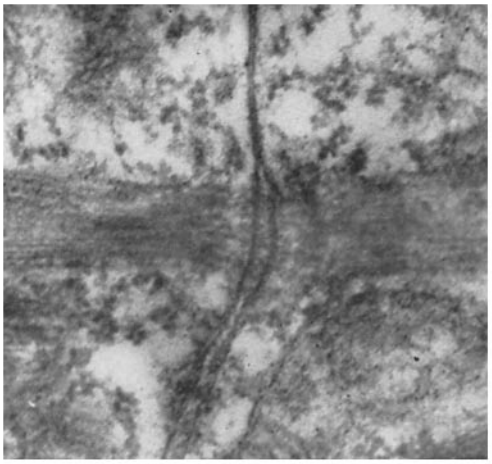

FIGURE 5 Electron micrograph of a junction between myofibrils from two adjacent cells of a younger, prefunctional *Styelopsis* larva, showing a larger gap between the apposed myofibrils. × 25,000.

the effect is the same. In any case, the relatively great separation of adjacent cell membranes at the position of myofibrils and their close apposition elsewhere is clearly significant.

We are left with one of two problems. If myofibrillar continuity, either potentially or in actuality, exists from the first, *i.e.* precedes cell division in the differentiating embryonic muscle band, how can cell membranes form in dividing cells so as to leave the filaments of the myofibrils intact? Alternatively, if myofibrils differentiate within each definitive muscle cell, following the cessation of cell division in the embryonic muscle band, how do they attain the end-to-end orientations finally observed, including the special cases of split fibrils and also the spiral configuration overriding the cellular pattern? Is it possible that cell divisions in and myofibril differentiation of a muscle band as a whole can proceed simultaneously without interfering with one another, comparable to the formation of intercellular walls penetrated by

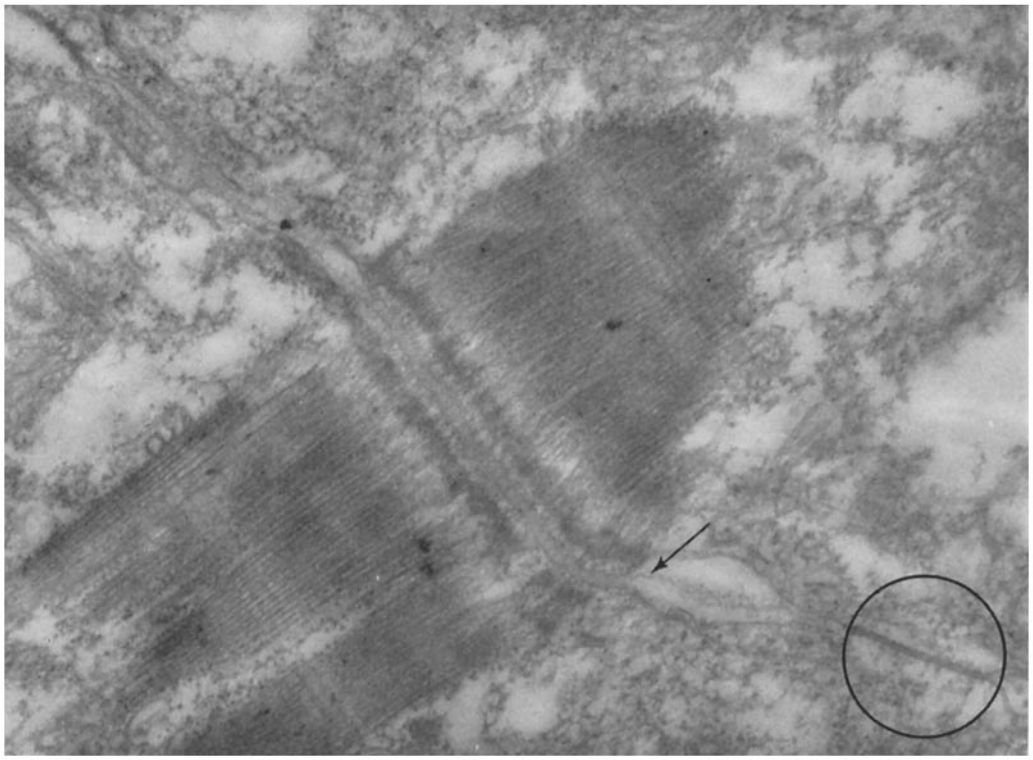

FIGURE 6 This electron micrograph of portions of two muscle cells from an ascidian tadpole larva shows the appearance of the junctional complex which most closely resembles the *zonula adhaerens* of Farquhar and Palade and demonstrates some amorphous material (arrow) in the extracellar space. At one end of the complex the type of junction called *zonula occludens* by Farquhar and Palade can be seen in the lower right corner (circle). × 25,000.

plasmodesmata in plants? Investigation of critical early developmental stages of tail muscle band differentiation is called for.

SUMMARY

The myofibrils of the muscle bands of the tail of ascidian tadpole larvae, which appear to be continuous from cell to cell by ordinary light or phase optics, exhibit intercellular junctures in electron micrographs. The junctures contain moderately dense material within the potential intercellular space. The submicroscopic basis for functional continuity across the junctional complexes requires further elucidation.

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