TRANSVERSE SARCOMERE SPLITTING

A Possible Means of Longitudinal Growth in Crab Muscles

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ABSTRACT

Transversely split sarcomeres are seen in mouthpart muscles of the blue crab in the electron microscope. Sarcomeres split only at the H zone. Two new sarcomeres are formed by a Z disk which appears in the H zone of the splitting sarcomere. Splitting may involve breaking of the thick filaments in the H zone, elongation of these filaments, and formation of both new actin filaments and Z-disk materials. Sarcomere splitting would allow longitudinal growth of muscle cells without lengthening of sarcomeres and concomitant changes in contractile properties.

KEY WORDS transverse sarcomere splitting · growth · muscle · myofilaments · ultrastructure

Increase in length of muscle fibers during the growth of animals can involve both lengthening of sarcomeres (1, 5, 6, 13, 15, 23) and addition of sarcomeres (10, 13, 15, 16, 26). The mechanism by which sarcomeres are added, however, still remains unclear. Auber (5) suggested that Z disks split transversely and that a new sarcomere grows between the two Z-disk fragments during development of blowfly flight muscles. In vertebrate muscle, the sarcomeres are somewhat shorter near the ends of a muscle cell (13, 14) and protein synthesis is greatest there (16, 26). This has led numerous investigators (see reference 14) to suggest that sarcomeres are added at the ends of muscle cells.

Nonuniform sarcomere lengths are known to occur within single muscle cells in both vertebrate and invertebrate muscles (2, 12, 13). As Franzini-Armstrong (12) pointed out: "the possibility should be explored that a continuous addition of new sarcomeres underlies the noticeable variability in A-band length of crustacean fibres."

In this paper we present ultrastructural evidence of transverse splitting of sarcomeres which could underly longitudinal growth as well as nonuniformity of sarcomere length within single muscle cells in blue crabs.

MATERIALS AND METHODS

Blue crabs, *Callinectes sapidus*, were obtained from local suppliers in Toronto and kept in tanks of three-quarter strength artificial seawater at ~15°C. The maxilliped exopodites were removed, and the flagellum abductor muscles (muscles 78, 87, and 102 of reference 9) were exposed by removing most of the overlying exoskeleton on one side of the appendage. The flagellum joint was immobilized and the muscles were fixed *in situ* at rest length for 1 h in 2.5% glutaraldehyde containing 0.2% formaldehyde and 0.15 M sodium cacodylate buffer at pH 7.3. Muscles were then placed in cacodylate (0.15 M)-buffered sucrose (0.3 M) wash containing 0.06 M NaCl and 2 mM $CaCl_2$ for 2–3 h (H. L. Atwood, unpublished observations). Bundles of fibers were dissected from the exopodite and postfixed in 2% OsO_4 (in 0.15 M cacodylate). Other procedures employed were standard for this laboratory (20).

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RESULTS

The flagellum abductor muscle has short sarcomeres (2–4 μm) which are usually well aligned across several adjacent myofibrils. Some aspects of the physiology of this muscle have been described by Burrows and Willows (7) and by Charlton (8). The contractions are fast and the muscle is extremely fatigue resistant. The muscle is well supplied with blood vessels, and each fiber has a thick cortex of mitochondria. This muscle has distinct myofibrils completely separated by sarcoplasmic reticulum elements and T tubules. Myofibrils are well separated into small groups by layers of mitochondria. There is a distinct H zone in the center of the A band.

When longitudinal sections are viewed in the electron microscope, some sarcomeres appear to be partially split at the H zone to form two "new" sarcomeres (Fig. 1). A new Z line forms the apex of the split and is flanked by broken thick filaments in two short A bands whose combined total length is greater than that of the unsplit A bands. The I bands are shorter opposite the apex of the split than at other unsplit regions of the sarcomere. In the partially split sarcomeres, two new sarcomeres gradually taper into one sarcomere and the H zone displays a Y shape pointing in the direction of the split. In each new sarcomere, the H zone is found closer to the apex Z line than to the Z lines at the opposite ends of the new sarcomeres. Evidently, the thin filaments attached to the apex Z line must be quite short because the H zone is close to the Z line in this region. In split sarcomeres, the diads and triads are found at the H zone as in normal sarcomeres of this muscle.

The splitting is sometimes confined to a single sarcomere of one myofibril but can often extend across several adjacent sarcomeres in a group of myofibrils. In the latter case, the split sarcomeres taper toward the apex of the split, and the H zones with their diads or triads display the Y shape. Occasionally, two short sarcomeres of equal length are seen, presumably produced by recent splitting (Fig. 2).

No partially split sarcomeres were seen in which the split did not touch one edge of the sarcomere. Within a group of myofibrils, splitting may occur in the inner myofibrils and advance toward the periphery, or vice versa (Fig. 3). Independent splitting can occur on both sides of a group of myofibrils, i.e., the apexes of splits can point in opposite directions within one group of myofibrils (Fig. 4).

Serial longitudinal sections were examined to determine the appearance of splits at different depths within a group of myofibrils. Fig. 4*a* and *b* shows two splits on each of two serial sections separated by a depth of 2.1 μm. One split traversed more of the group of myofibrils while the other split traversed less of the group in deeper sections.

Splitting occurs randomly along the length of a myofibril. For example, in one group of myofibrils followed longitudinally on a single section for 132 sarcomeres, splits were observed at sarcomere numbers 14, 22, 44, and 80. Splitting in one group of myofibrils was not correlated with splitting in adjacent groups of myofibrils.

DISCUSSION

Mechanism of Splitting

We propose the following tentative scheme for the mechanism of sarcomere splitting:

(*a*) Thick filaments are bisected at the H zone, and the two halves are pulled toward opposite Z lines.

(*b*) New myosin molecules are added to the cut ends of the thick filaments. The heads of the new myosin molecules point in the direction opposite to that of those in the existing half filament to which they become attached.

(*c*) Coincident with the assembly of new thick filaments, cross bridges become available to which actin molecules attach and subsequently form new thin filaments. Thin filaments elongate.

(*d*) New Z line material appears in the split and the new thin filaments are attached to it.

The exact mechanism and the sequence of these steps are unknown. However, actin molecules in the presence of myosin filaments can spontaneously self-assemble into contractile units, and Z-line material is not needed for this assembly (11, 17). Because no partially split sarcomeres were seen in which the split did not reach one edge of the myofibril, we presume that splitting begins at the edge of a myofibril and not in the middle. It is not known what initiates or controls the splitting process.

Origin of Sarcomere Splitting

According to Tiegs (24, 25) and Ruska and Edwards (22), the existence of verniers (regions

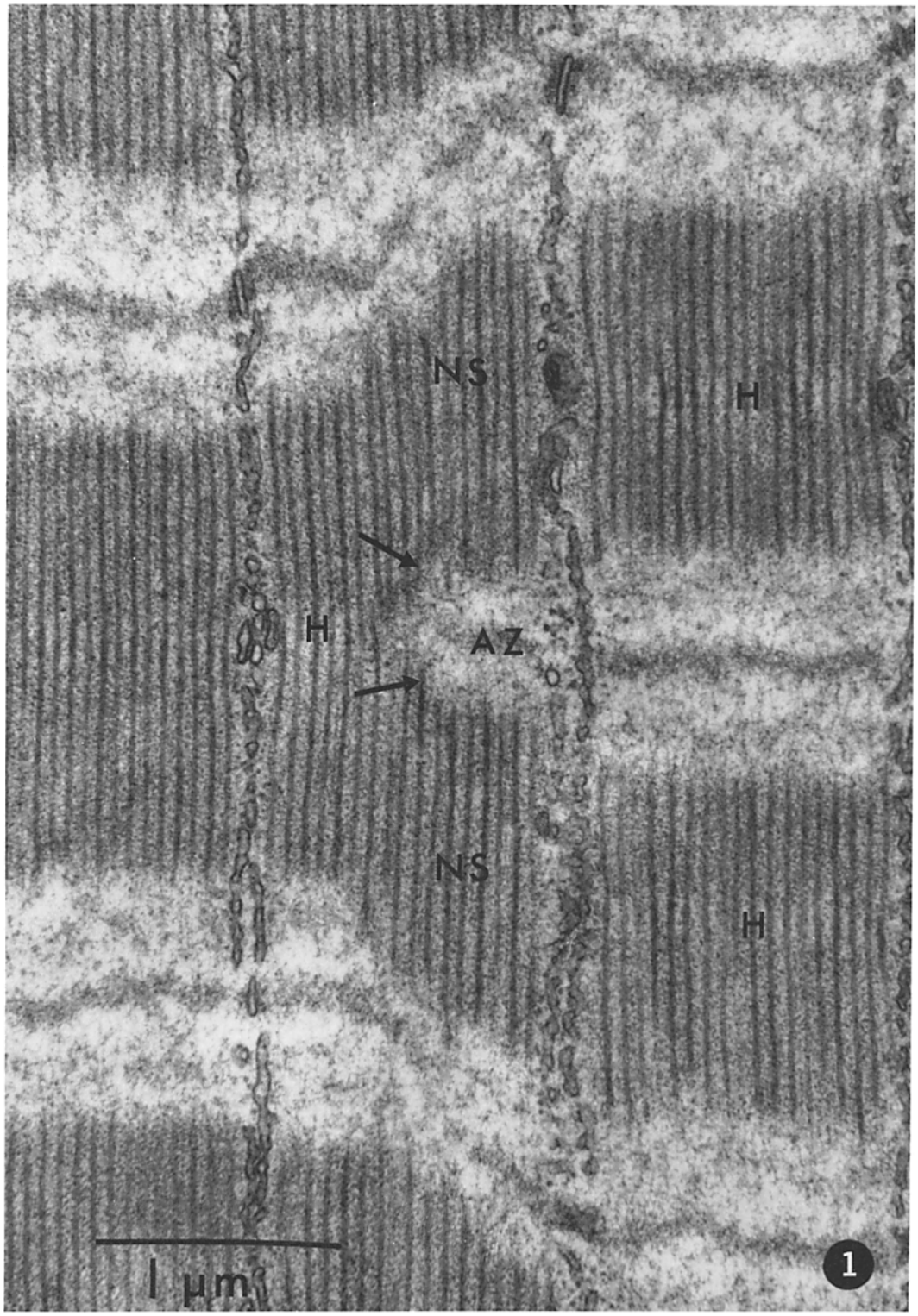

FIGURE 1 Partial transverse sarcomere split seen in a longitudinal section. Thick filaments are broken (arrows) in the region of the apex Z line (*AZ*). The new sarcomeres (*NS*) are formed between the apex Z line and the immediately adjacent Z lines of the splitting sarcomere. *H*, H zone. × 38,100.

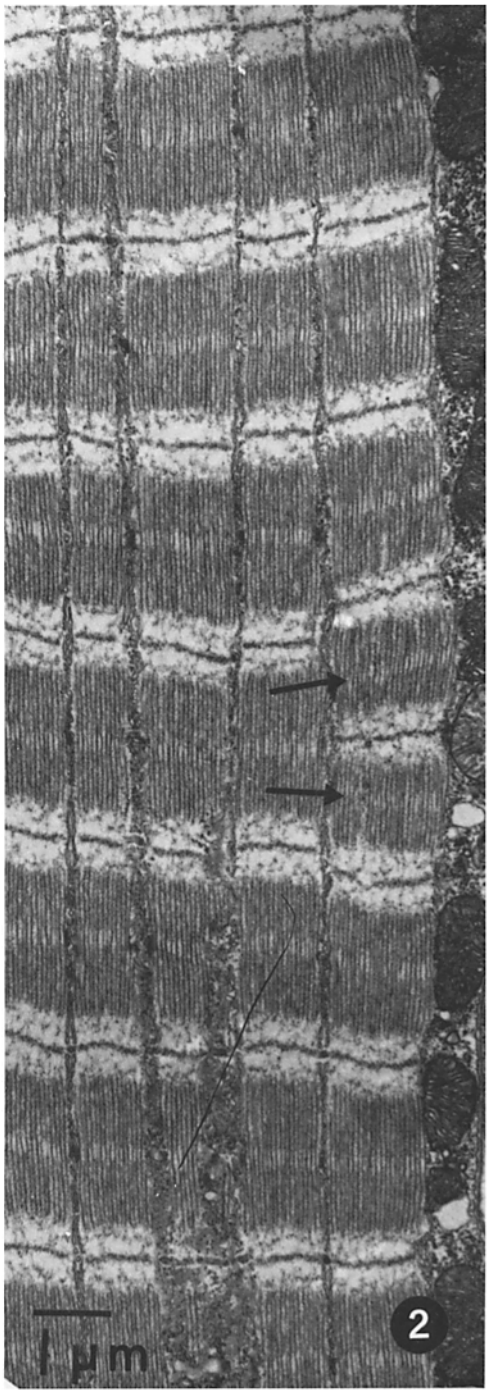

FIGURE 2 A longitudinal section through a group of myofibrils, showing a pair of short sarcomeres (arrows). Except for the two Z lines delineating the short sarcomeres, the Z lines of neighboring myofibrils appear to be nearly in register. × 10,400.

in which n sarcomeres of one myofibril are apposed by n + 1 sarcomeres in the adjacent myofibril) can be explained by an underlying helicoidal arrangement of the Z line. A section cut at an angle through the axis of a Z helicoid would produce a vernier appearance (22). The splitting described in the present work bears a superficial similarity to verniers but in our material n = 1 whereas verniers involve several sarcomeres. Occasionally, verniers in the maxilliped muscle do involve sarcomere splits, but this is not always the case. It is, of course, possible that the sarcomere splitting could produce the verniers.

It is unlikely that split sarcomeres are related to helicoidal muscle structure as described by Tiegs (24, 25), Ruska and Edwards (22), or Peachey and Eisenberg (21) since splitting only occurs at H zones. If splitting were due to a regular helicoid structure, the splits would occur at all sarcomere zones.

Sarcomere Splitting and Growth

Why would muscle growth be accomplished by sarcomere splitting? There is now ample evidence that in crustacean skeletal muscle the speed of contraction is inversely related to the sarcomere length (3, 4, 18, 19). Therefore, the maintenance of a particular mechanical function in a crustacean muscle, barring other structural or biochemical changes, is dependent on the maintenance of a particular sarcomere length. The flagellum abductor is a fast muscle and has short sarcomeres. Increases in the sarcomere length during longitudinal growth of the muscle cells would gradually result in transformation of the fast cells into slow cells. However, sarcomere splitting could prevent such a transformation by reducing the sarcomere length, thus retaining the fast activity of these muscle cells. Obviously, the addition of new sarcomeres would render the lengthening of other sarcomeres unnecessary during growth.

Sarcomere length could also be maintained during growth if sarcomeres were added at the ends of muscle cells (13, 14, 16, 26) or if new sarcomeres were produced between the two halves of split Z lines (5). The scheme presented in this paper might have some advantage over the above methods of sarcomere addition since splitting at the H zone would leave a template (bisected myosin filaments) which would guide the assembly of the new sarcomeres.

The observation of sarcomere splitting is as yet uncorrelated with actual growth of the muscle or

FIGURE 3 A longitudinal section through a group of myofibrils in which the inner myofibrils are split. Splitting is incomplete in one sarcomere (arrow), and the split appears to progress toward the periphery. Notice that in the myofibrils which have split, Z lines form a bulge around the split area. × 13,400.

of the whole animal. At present there are two different views in the literature regarding longitudinal growth of crustacean muscle. Bittner and Traut (6) have reported that lengthening of muscle fibers in various crayfish skeletal muscles occurs by sarcomere lengthening, whereas Govind et al. (15) have found that lengthening of limb muscles in lobsters is accomplished in early stages by sarcomere lengthening and in later stages by sarcomere addition. The sarcomere splitting described herein could underly the addition of sarcomeres reported by Govind et al. (15). In crustaceans, increase in body size occurs after moulting of the exoskeleton. It would be interesting to

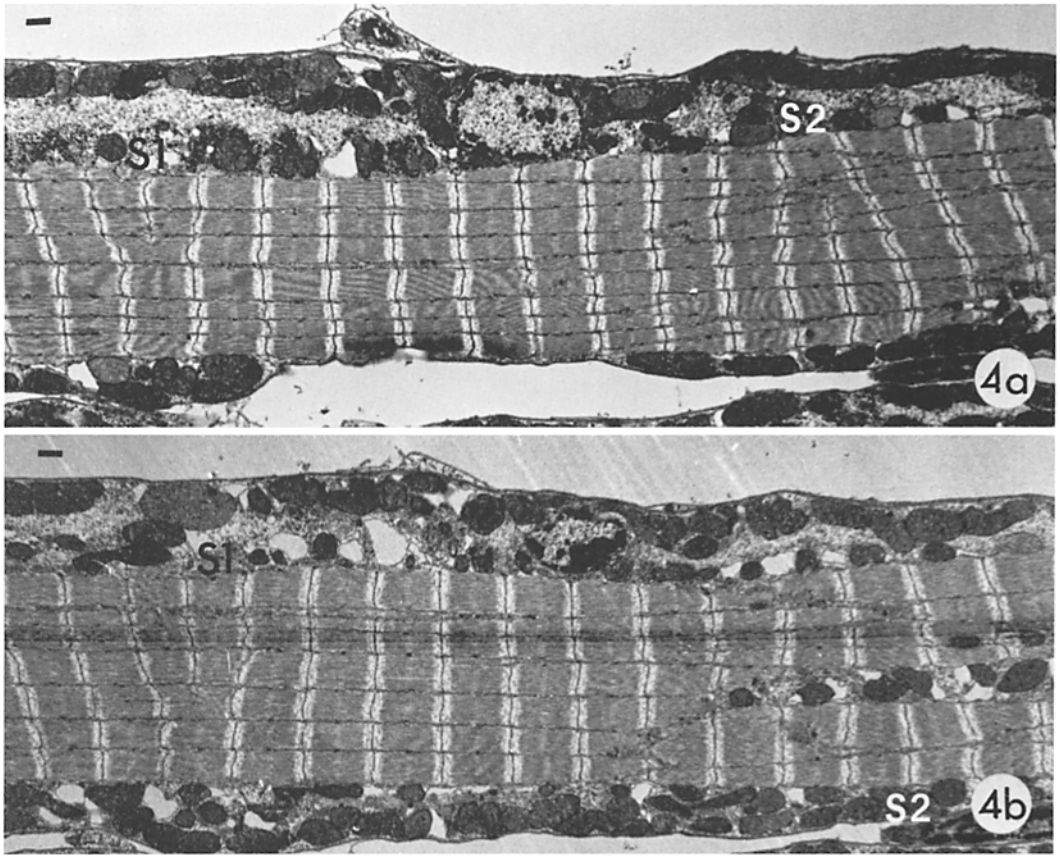

FIGURE 4 Serial longitudinal sections through a group of myofibrils showing two splitting regions nine sarcomeres apart (*S1* and *S2*). The two sections (*a* and *b*) were separated by 2.1 μm. One split area (*S1*) traversed more of the myofibrils while the other split (*S2*) traversed fewer of the myofibrils as the depth of sectioning was increased (*a* to *b*). × 3,200 Bars, 1 μm.

determine whether there is a higher incidence of sarcomere splitting immediately before or after moulting.

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