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Z Disc Ultrastructure in Scutal Depressor Fibers of the Barnacle

Abstract. *The ultrastructure of Z discs in nonglycerinated, striated muscle fibers of the barnacle* Balanus nubilus *Darwin was examined in contracted, resting, and stretched preparations. At all sarcomere lengths, the Z discs are perforated sheets comprised of "Z rims" and "Z perforations," extending continuously across the myofibril; the dimensions of the rims and perforations change with changes in sarcomere length. Accordingly, with shortening of the sarcomere, there was an accompanying decrease in the transverse width of the Z rims and an increase in the diameter of the Z perforations.*

Striated muscle fibrils are composed of a serially repeating structure, the sarcomere. Bounded by transversely situated Z material, each sarcomere contains an ordered array of thick and thin protein filaments. Thick filaments occupy the central region of the sarcomere while thin filaments originate in the Z material and extend into the center of the sarcomere where both sets of filaments interdigitate. Contraction is characterized by the sliding of thick and thin filaments relative to each other.

Physiologically many types of striated muscle do not contract to the extent that the ends of the thick filaments make contact with the Z bands, but other muscles "supercontract" to less than 50 percent of rest length, and the fate of the thick filaments is not fully known. The questions remain whether the thick filaments crumple against the Z material when the sarcomeres shorten, whether they pass through unobstructed, or whether supercontraction occurs in another manner.

Hoyle and his group (*1*, *2*), in their studies of supercontracting fibers of the barnacle *Balanus nubilus* Darwin, believe that the Z discs in resting muscle are comprised of individual dense bodies held together closely by elastic bridges, so that the discs appear as continuous, nonperforated structures. Hoyle *et al.* postulate that, when muscle is activated, the dense bodies become rearranged and discontinuous; perforations now appear in the discs so that the thick filaments from adjacent sarcomeres may pass through unobstructed.

Our work was designed to study the ultrastructure of the Z discs of barnacle fibers, in resting, stretched, and contracted muscle. Our conclusions differ somewhat from those of Hoyle *et al.* in that we believe that, under all conditions, the Z discs of the myofibrils are continuous but perforated sheets; and that during supercontraction the Z perforations enlarge and permit unrestricted passage of the sarcomere filaments.

Relaxed nonglycerinated preparations of scutal depressor fibers were obtained by injecting 5 ml of *d*-tubocurarine chloride (aqueous, 1.5 mg/ml) through the connective tissue layer located between the shell and the operculum (hard platelike valves protecting the soft parts of the barnacle). After 5 minutes, three "windows" were drilled in the shell and an additional 8 ml of the tubocurarine was added through the windows. The depressor muscles were then fixed by 4.0 percent formaldehyde and 0.5 percent glutaraldehyde in 0.1*M* phosphate buffer, *p*H 7.3. The relaxed fibers were fixed in situ.

To obtain "physiologically" contracted preparations, the barnacle operculum was lightly tapped, so that the depressor muscles contracted. The fixative then was injected through the shell windows. To obtain stretched preparations, the operculum of curarized barnacles was forcibly extended from the shell before the fixative was added.

Longitudinal and transverse sections (thickness, 350 to 600 Å and 500 to 900 Å) were examined with an electron microscope (RCA EMU-3).

The longitudinal sections reveal that the Z discs manifest different structural appearances under different physiological conditions. Most frequently in rest-

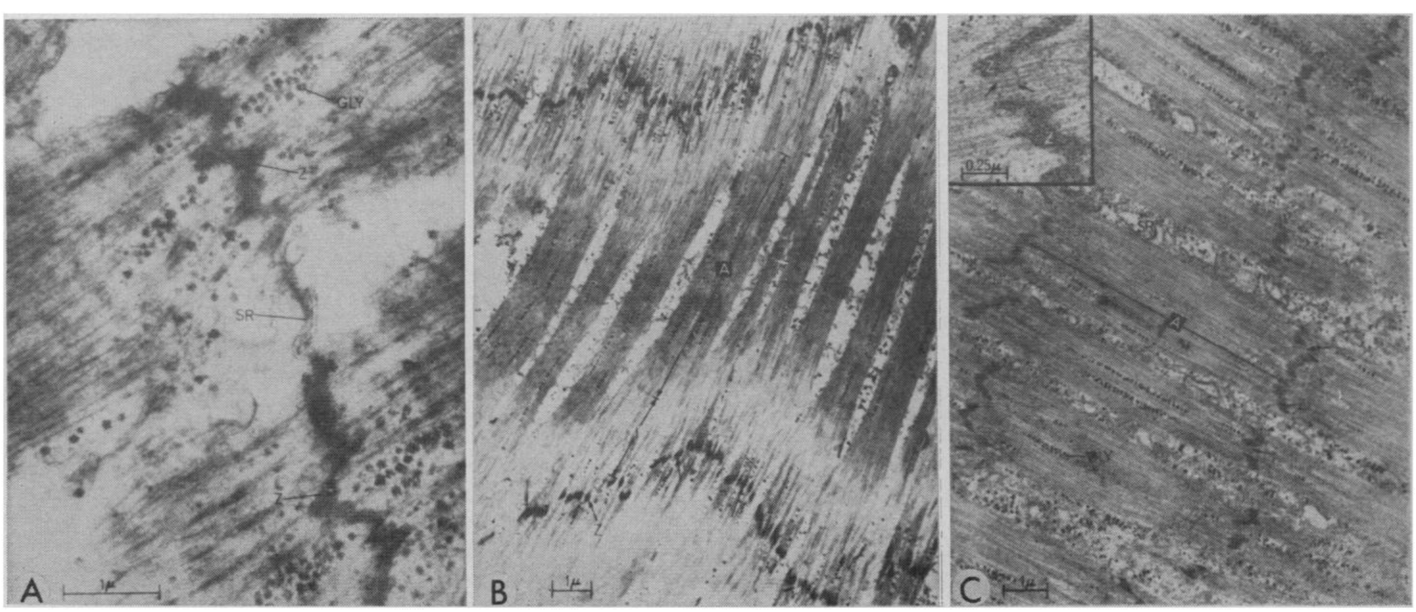

Fig. 1. Longitudinal sections through barnacle fibers. (A) Resting preparation, with continuous Z discs (× 28,000); (B) stretched preparation, with Z discs appearing as separate Z bodies (× 10,000); (C) supercontracted preparation, with absence of I bands. Inset shows intersarcomeric passage of filaments (× 48,000); *Z*, Z discs; *SR*, sarcoreticular tubules; and *GLY*, glycogen.

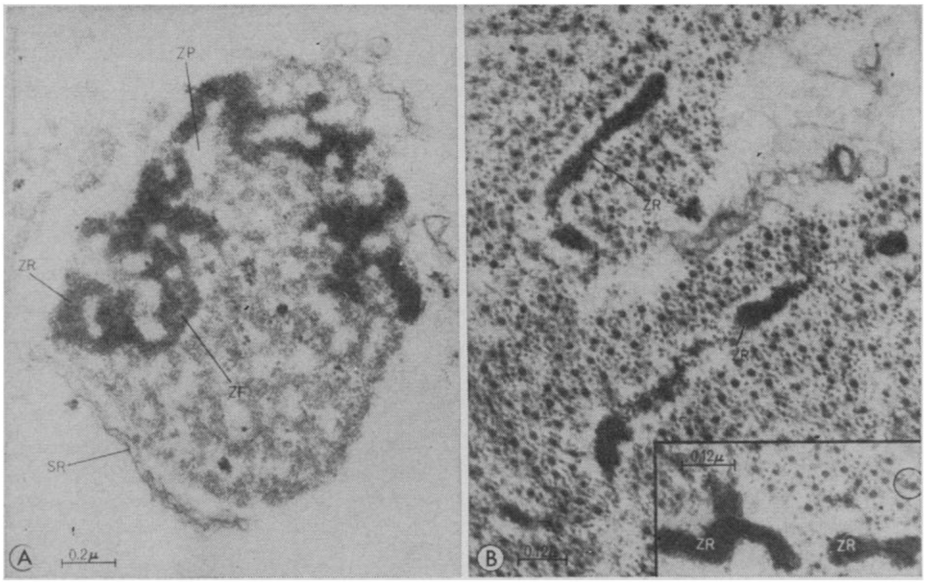

Fig. 2. Transverse sections of barnacle fibers at level of Z discs. (A) Resting preparation, showing Z disc as a continuous sheet comprised of Z rims (*ZR*) and Z perforations (*ZP*) (× 68,000); (B) supercontracted preparation showing enlarged and incomplete Z perforations filled with both thick and thin filaments (× 120,000).

ing preparations (Fig. 1A), the appearance is that of irregularly shaped, continuous bands extending across each myofibril; in stretched preparations (Fig. 1B) the bands appear to be separated into bodies of electron-opaque material; while supercontracted preparations (Fig. 1C) also show the discs extending across the myofibril as discontinuous Z bodies; the spaces between the Z bodies are filled with thick filaments (Fig. 1C inset).

Transverse sections of preparations of resting or stretched fibers reveal that the Z disc structure is a continuous sheet which crosses the myofibril and is comprised of Z rims and Z perforations (Fig. 2A). Z areas were located through serial sections. The thickness of the Z rims in preparations of resting fibers was, from 30 measurements, 660 ± 16(S.E.) Å.

In the preparations of contracted fibers, transverse sections show an enlargement of the Z perforations (Fig. 2B), accompanied in most cases by an apparent disruption of the Z rims so that the rims do not seem to encircle the Z perforations completely. Also, in the contracted preparations, the Z rims were measured and the thickness, compared with that in the resting state, was diminished ($P < .01$; 470 ± 17 Å; 30 measurements). Whereas the perforations of resting and stretched preparations were devoid of filamentous structures, in the contracted preparations the perforations were filled with both thick and thin filaments.

The most reasonable interpretation of our results is that, under all physiological conditions, the Z disc of the scutal depressor muscle is structurally a perforated sheet of material extending completely across each myofibril. When sarcomeres shorten, the perforations become enlarged and permit unobstructed passage of the longitudinal filaments. Thus, longitudinal sections of the resting fiber show that the Z disc is continuous in most cases because the perforations are relatively small, and cutting across a disc reveals mainly Z rim material. However, in preparations of contracted muscle which have enlarged perforations and thin Z rims, longitudinal sections should display a more discontinuous Z line (thus giving the appearance of individual "Z bodies"), with the interspaces being filled with filaments, as in Fig. 2B.

The discontinuous Z line seen in longitudinal sections of the stretched preparations may be thin filaments apparently embedded in the Z rim material. One possibility is that stretching the myofibrils results in a compression and distortion of the Z perforations so that longitudinal sections display a more discontinuous appearance of the Z rims. If perforated Z disc structures are present in all types of muscles where supercontraction occurs, such structure would be compatible with the concept that contraction occurs through the sliding of filaments (*3*). This view is shared by Osborne (*4*) who showed that in the body wall muscles of the blowfly larva the Z discs were continuous perforated sheets at all sarcomere lengths, that is, at rest, stretched, or contracted. We believe this is the case in the scutal depressor muscle of the barnacle. During supercontraction the Z perforations enlarged and permitted the thick filaments from adjacent sarcomeres to overlap through the Z discs. Others have reported "incomplete" Z structures in the form of granules or blocks of material in a variety of muscles, such as the visceral muscles of the rhinoceros beetle (*5*) and the lepidopteran larva (*6*), and in muscles of nematodes (*7*), annelids (*8*), mollusks (*9*), and arthropods (*10*).

Our study does not offer insight into the force generating mechanisms for contraction, nor does it explain how the observed structural changes may be brought about. If the force for shortening of the sarcomere resides at the cross bridges between the thick and thin filaments, then enlarged Z perforations could result from the general widening of the sarcomere lattice structure which occurs with isovolumetric sarcomere shortening. If, on the other hand, the Z material actively undergoes conformational changes with stimulation of the muscle (*2*, *11*), then sarcomere shortening could be a consequence of isovolumetric expansion of the lattice structure of the sarcomere.

There is the additional problem of the regular array of thin filaments becoming "irregular" so that thick filaments, as well as the thin filaments themselves, may pass through the Z perforations during supercontraction.

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