DERIVATION OF THE Z LINE IN THE EMBRYONIC CHICK HEART

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INTRODUCTION

Although the fine structure of the Z line in muscle has been described in great detail (Auber and Couteaux, 1963; Franzini-Armstrong and Porter, 1964; Garamvolgyi, 1963; Kelly, 1967; Knappeis and Carlsen, 1962; Reedy, 1964), the origin of the Z line substance has not been definitely established. Our studies of the myocardium in the embryonic chick, however, indicate an association of the dense amorphous substance in the desmosomes (maculae adherentes) or the closely related zonulae adherentes with the Z line areas of developing myofibrils. Some examples of this association are illustrated and their possible significance in myofibrillogenesis is discussed.

MATERIALS AND METHODS

Fertilized eggs from white Leghorn chickens were incubated at 38°C for several days. The embryos were removed from the yolk at different time intervals and placed into 2.0, 4.0, and 6.3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.6) for 3 hr. The embryos were then washed in 0.1 M phosphate buffer for about 18 hr, after which the number of somites was counted (10–38 somite stage), and the hearts were immersed in 2% OsO_4 in Veronal-acetate buffer (pH 7.6) for 1 hr at 4°C. Dehydration in graded concentrations of acetone was followed by embedding in Araldite. Sections were stained with uranyl acetate and lead citrate and were examined with the Philips 200 or the Siemens Elmiskop I electron microscope.

RESULTS

Throughout the stages examined (10–38 somites) the cytoplasm of cardiac myoblasts shows scant endoplasmic reticulum, abundant free ribosomes, prominent Golgi zones, packets of glycogen, and secretion-like granules. Arrays of microtubules are often seen. The myoblasts are polygonal in shape and show specialized junctions, either maculae or zonulae adherentes, on all surfaces between adjacent cells. Cells at any particular stage are always found in various degrees of maturation. At the 10 somite stage the cardiac myoblasts are still relatively small and immature. The cytoplasm of some of these cells, however, shows regions in which collections of thin filaments are distributed in disordered arrays.

Between the 10-and 24-somite stages (30–44 hr) the undifferentiated cytoplasmic matrix of myoblasts undergoes striking changes. The mitochondria become more elongated, and the ribosomes can be classified into the following four types: single, aggregate, helically-arranged, and those attached to the endoplasmic reticulum. Thin and thick myofilaments appear and begin to show some organization into myofibrils. These primitive myofibrils tend to associate at the periphery of the cardiac myoblast, parallel to the sarcolemma, and their Z lines are often in register with the zonulae or maculae adherentes. Fig. 1 shows several such junctions with an underlying developing myofibril. A dense Z line is beginning to appear below the left desmosome. Approximately 1.5 μ to the right, the length of the sarcomere, a discontinuity in the myofibril indicates the probable site of the next Z line. This Z line will also be adjacent to a desmosome. Although these primitive myofibrils lack all the bands of striated muscle except the organizing Z line, when viewed in transverse section they are seen to possess the normal compound lattice of thick and thin filaments characteristic of differentiated sarcomeres.

As myofibrillogenesis proceeds to the 38 somite stage numerous examples suggesting continuity between the dense amorphous substance of desmosomes and zonulae adherentes and the underlying Z lines are observed (Figs. 2–6). Although evidence for the continuity of Z line substance with the desmosome is at most suggestive (Fig. 1), definitive connections with the zonulae are frequently observed. In its electron opacity and finely granular appearance this Z line material is indistinguishable from the dense substance in specialized junctions. Similar specialized junctions at the ends of the muscle fibers are also continuous with Z lines. These junctions at right angles to the long axes of myofibrils will eventually constitute the intercalated disc in the mature muscle.

Even with the development of several con-

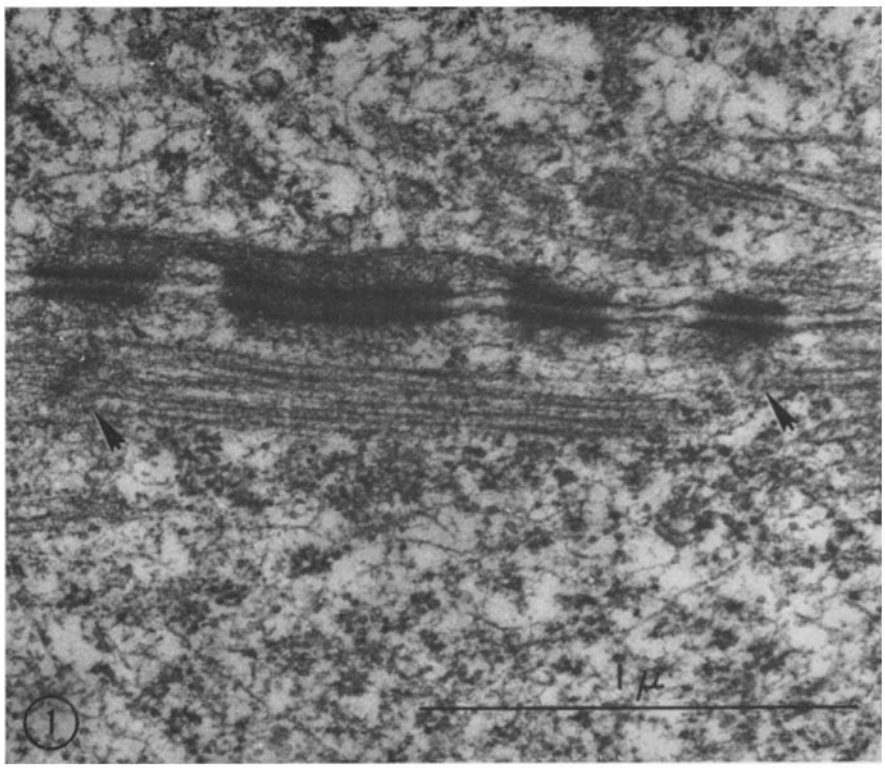


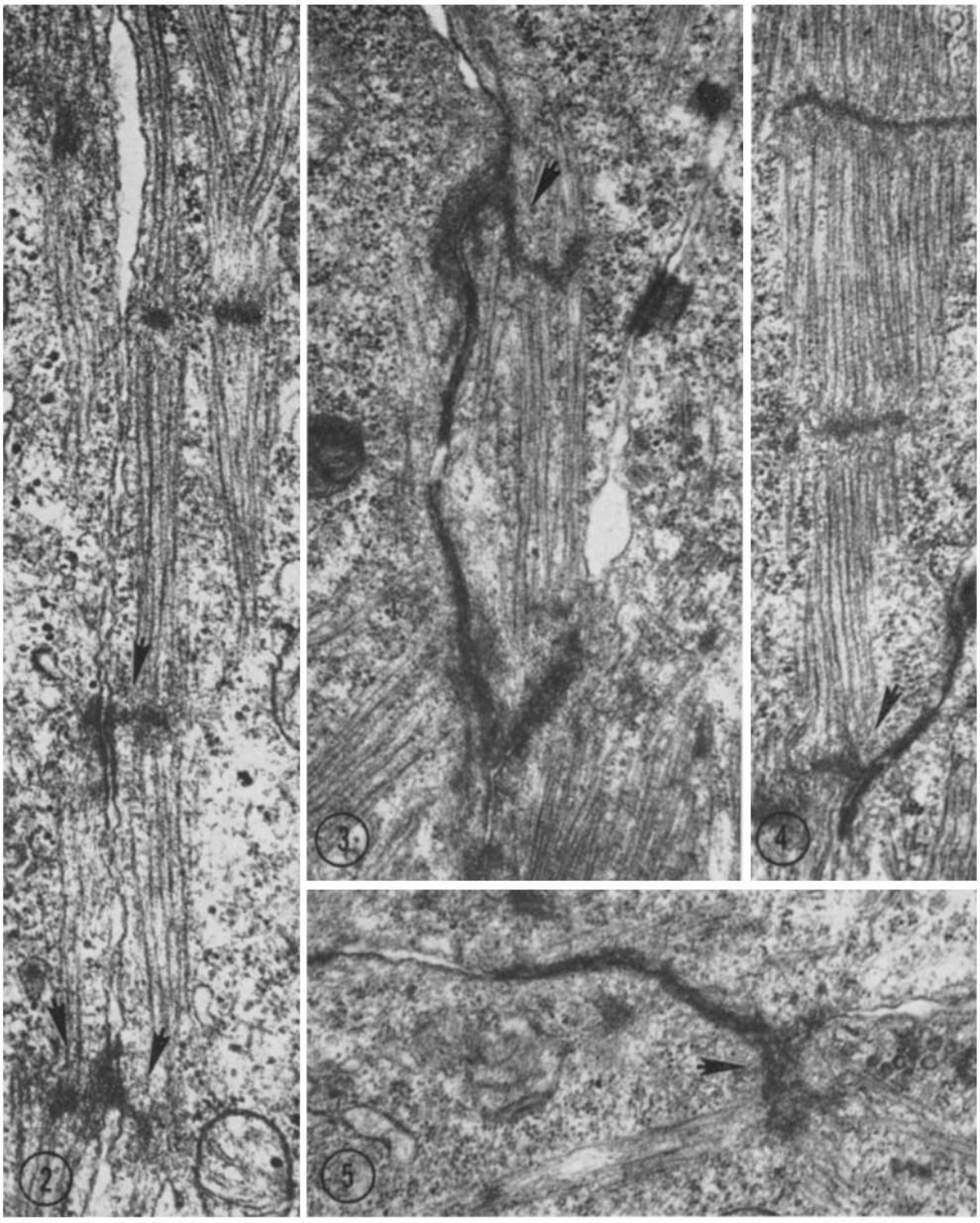
FIGURE 1 Numerous desmosomes are seen parallel to a primitive myofibril subjacent to the cell surface in the embryonic chick heart. Z line is noted in close proximity to one of the desmosomes (left arrow). The right arrow points to a region of discontinuity of myofilaments that is 1.5 μ in distance from the above mentioned Z line and that will be the probable site of another Z line. × 57,000.

tiguous sarcomeres, no I bands or H zones are apparent (Figs. 2, 4). The absence of these bands may be due to the fact that the sarcomeres are uniformly and completely contracted. Myofibrils cannot be experimentally loaded in a stretched state in embryonic muscle. The lengths of the thick and thin filaments comprising the primitive nonbanded myofibrils have not been adequately assessed. However, there are often abrupt changes in the direction of the myofilaments and discontinuities in the filament arrays at intervals of 1.5 μ. These observations strongly suggest that the thick and possibly the thin filaments have attained their ultimate lengths prior to the appearance of the Z lines.

DISCUSSION

The present observations support the concept that the dense substance of Z lines in the cardiac muscle of the embryonic chick originates from or in association with the specialized junctions in the surface of myoblasts. Desmosomes and zonulae adherentes exist before any Z lines are evident. The electron-opaque substance of these junctions is ultrastructurally indistinguishable from Z line substance, and numerous examples of suggestive and direct continuity between these two amorphous materials have been seen. The interpretation of these observations is subject to the limitation that only one type of fixation and one type of staining have been used. However, continuities between Z lines and maculae adherentes have already been reported in the differentiated heart (Grimley and Edwards, 1960; Staley and Benson, 1968). The intercalated disc is structurally composed of specialized junctions in which thin filaments terminate at a level corresponding to the next Z line. Fawcett (1966) has considered the intercalated disc to be a modified Z line.

Other evidence for a correspondence between



FIGURES 2–5 Arrows point to the association of the Z lines with the specialized surface junctions. The Z lines appear to be derived from or associated with these junctions. Fig. 2, × 30,000; Figs. 3–5, × 28,000.

Z line and junctional substance was presented by Heuson-Stiennon (1965) who showed that, in the embryonic rat skeletal muscle, Z lines appear from dense bodies formed at the cell membrane. She speculated that the dense bodies could be compared to desmosomes of epithelial cells, intercalate discs of cardiac cells, and the myoepithelial junctions of insects. Warren and Porter (1969)

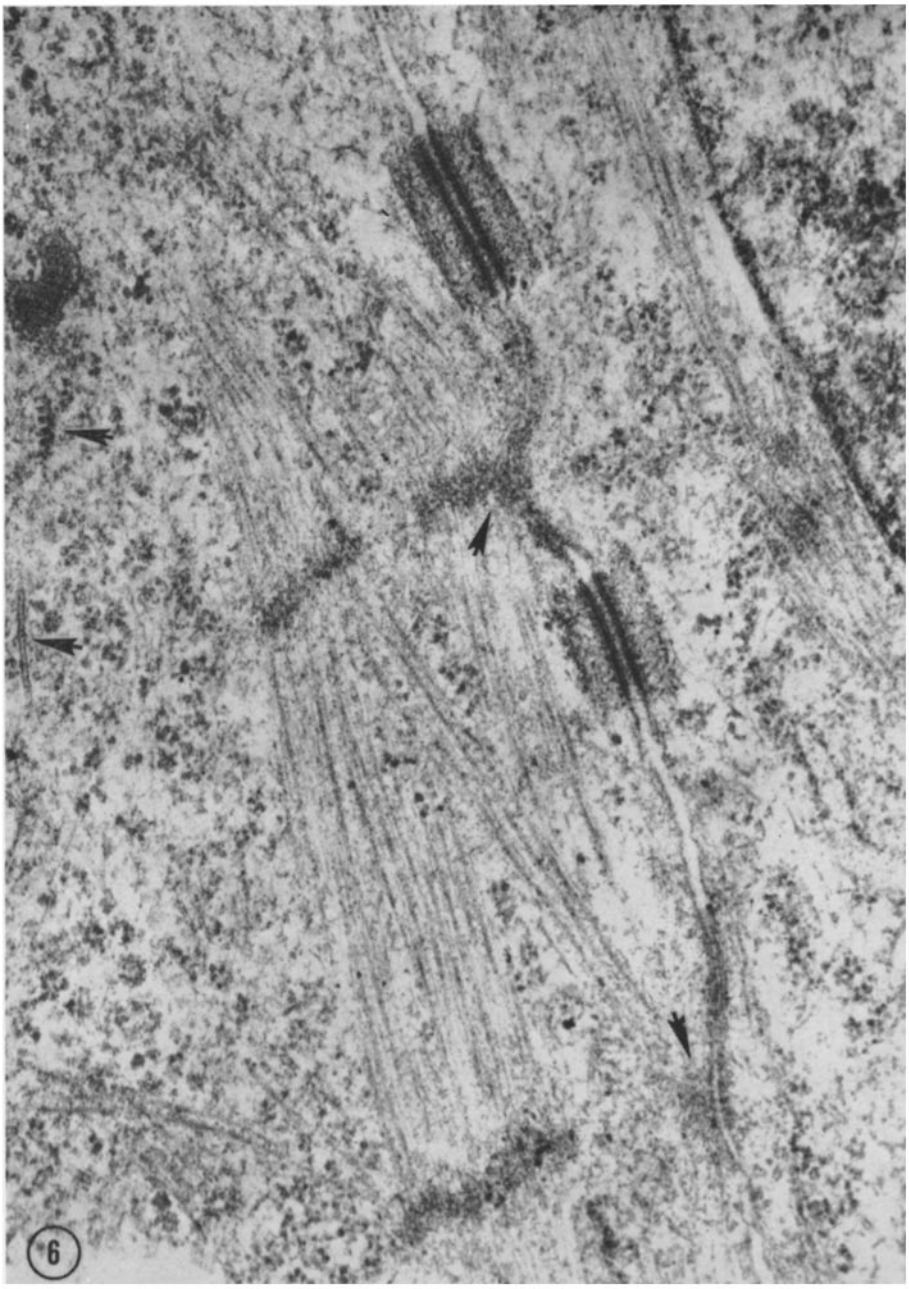


FIGURE 6 Two chick heart myoblasts showing desmosomes and zonulae adherentes. Continuity between Z lines and zonulae adherentes is indicated by the two right arrows. Upper left arrow indicates polyribosome, whereas lower left arrow shows a microtubule. × 54,000.

have reported a developmental continuity between Z line segments and sarcolemmal dense plaques in the abdominal muscle of a molting insect. Surface dense bodies and attaching filaments are also visible in smooth muscles (Pease and Molinari, 1960) and in pericytes (Epling, 1966). Most significantly, Rash et al. (1968) have recently examined muscle cells of embryonic chick

hearts after extraction with urea to remove actin and/or tropomyosin. They found that the extraction removed the dense material of the Z lines, intercalated discs, and desmosomes.

Authors of other studies of myofibrillogenesis have proposed that the Z line might be a centriolar derivative in the indirect flight muscles of *Drosophila* (Shafiq, 1963) or that Z lines are organized under the influence of fenestrated smooth-surfaced tubules of sarcoplasmic reticulum as seen in monolayer cultures of chick embryo leg muscle (Reporter, 1967). In another interpretation, Kelly (1969) speculates that Z bands appear to develop by coalescence of Z bodies, which appear to be related to fine filamentous material in the peripheral cytoplasm. After careful observation, no support for any of these views was discernible in the present study of cardiac myoblasts.

That myofilaments are selectively ordered in the subsarcolemmal regions of myoblasts has been previously observed in developing skeletal and cardiac muscles (Holtzer et al., 1957; Allbrook, 1962; Shafiq, 1963; Przybylski and Blumberg, 1966; Fischman, 1967; Spiro and Hagopian, 1967). Whether the spacing is determined by the cell surface or some property of the myofibril, such as the length of the thick or thin filaments, is a matter of conjecture. The present study, however, suggests that the coincident spacing of specialized intercellular junctions at intervals corresponding to the length of thick filaments (1.5 μ) may be responsible for the initial positioning of the primitive myofibrils and determining of the locations of Z lines. The presence of myofibrils radiating in several directions from areas where Z lines are expected to form or are forming has also been noted. Such observations suggest that the Z line subsequently plays a role in the parallel orientation of the myofibrils (Hay, 1963; Price et al., 1964; Wainrach and Sotelo, 1961). Finally, it has been proposed (Warren, 1968) that the function of microtubules in determining the orientation of myofibrils is only secondary to their primary role in maintaining the elongated cell shape.

SUMMARY

Evidence is presented that Z lines in the embryonic chick heart are derived from zonulae or maculae adherentes.

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