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; Knappes
and Carlsen, 1962; Reedly, 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 desmo-
somes (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 sig-
ificance 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% OsO4 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 elec-
tron 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 myo-
blasts undergoes striking changes. The mitochon-
dria 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 devel-
opning 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 prob-
able 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 fila-
ments 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). Al-
though evidence for the continuity of Z line sub-
stance 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-
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. X 57,000.

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)
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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