Anat Embryol (1985) 172:325–329

Anatomy
and Embryology

An ultrastructural topographical study on myofibrillogenesis in the heart of the chick embryo during pulsation onset period

Tamiko Hiruma and Reiji Hirakow
Department of Anatomy, Saitama Medical School, Moroyama-cho, Iruma-gun, Saitama, Japan

Summary. Ultrathin sections of the chick embryonic heart at the 8-, 9- and 10-somite stage were cut serially at an interval of 20 μm and mounted for transmission electron microscopic examination on a copper grid with a sufficiently large hole to survey the entire section area. The grid was supported by a formvar film. Thick filaments were first found to assemble into well-defined bundles in several cells composing the caudal region of the newly formed heart just before onset of the pulsation at the 8-somite stage. Then, at the 9-somite stage when pulsation commences, the cells possessing nascent myofibril(s) increase in number, slightly more in the right side of ventricular region. At the 10-somite stage, the rhythmical contraction is established and striated myofibrils become distinctly discernible. Right side dominance is more conspicuous at this stage than previously. Then, myofibrillogenesis gradually progresses toward the cranial or bulbar region.

Key words: Cardiogenesis – Chick embryo – Beating stage – Myofibril formation

Introduction

Recently, employing an optical recording technique, Kamino and his co-workers described the early developmental events of cardiac function in the chick embryo. By this method, they clearly demonstrated (i) that rhythmic action potentials appeared at the 8-somite stage and a couple of hours later at the middle period of the 9-somite stage, the first contractions were detected (Fujii et al. 1980, 1981a, 1981b), (ii) that the initial contractions were limited to the right side of the bulbo-ventricular portion, and (iii) that the spread of contractile area became larger as development proceeded (Hirota et al. 1983; Hirota et al. 1985). Considering these detailed physiological findings, it is unfortunate that there is no adequate ultrastructural information about the cells constituting the heart at corresponding stages. Manasek (1968) presented morphological descriptions of the development of the chick heart, but did not include detailed observations of the cells during the early period of initiation of heart beat. The developmental changes of interest in the present study occurred during a brief period spanning formation of only 1–2 somites. Therefore, we undertook an investigation of the spatial sequence of myofibrillogenesis in the embryonic heart during the relatively short period when the heart was commencing its proper function, by means of transmission electron microscopy (TEM) combined with scanning electron microscopy (SEM), as well as ordinary light microscopy (LM).

Offprint requests to: Tamiko Hiruma, Department of Anatomy, Saitama Medical School, 38 Morohongo, Moroyama-cho, Iruma-gun, Saitama, 350-04 Japan

Materials and methods

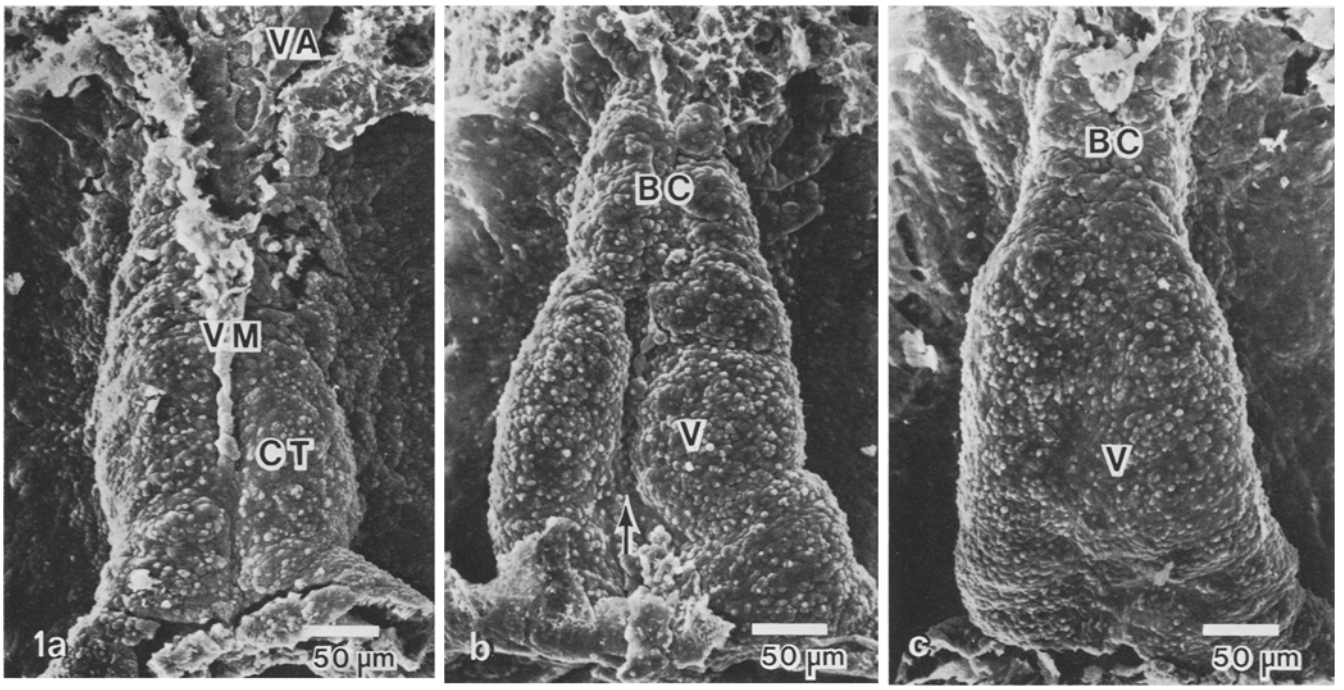
Fertilized eggs of the White Leghorn fowl (*Gallus gallus domesticus*), supplied by Saitama Prefectural Poultry Experiment Station, were incubated at 38 ± 0.5° C for 35 to 40 h to yield embryos with 8, 9 and 10 somites. These correspond to stage 9+, 10− and 10 after Hamburger and Hamilton (1951).

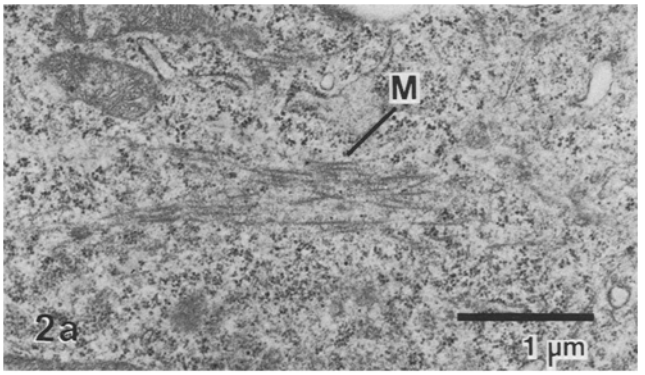
For the TEM examination, the dissected cardiac region was fixed in 2.5% glutaraldehyde mixed with 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.6, for 2 to 12 h at 4° C. After brief washing in 0.1 M cacodylate buffer, the specimens were postfixed in 1% OsO_4 in 0.1 M cacodylate buffer for 1–2 h at 0° C. They were then dehydrated in a graded series of ethanol and embedded in Epon.

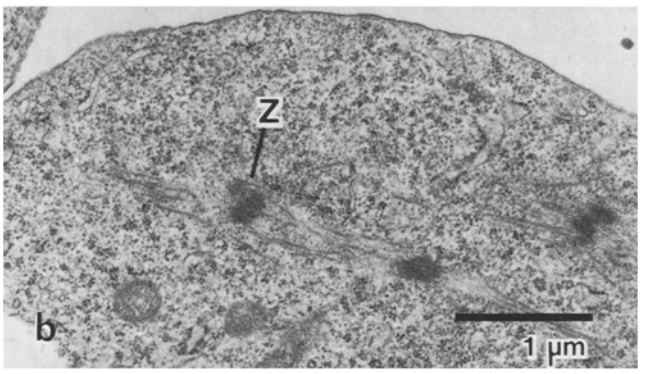
Serial sections 1 μm thick were cut from the caudal to cranial region of the primitive heart; ultrathin sections with silver interference colour were taken after every 20 thick sections, i.e. at intervals of 20 μm. The thick sections were mounted in sequence on glass slides and stained with toluidine blue for LM. Each ultrathin section for TEM was mounted on a copper grid with a sufficiently large hole (ca. 1 × 0.5 mm) to allow examination of the entire section. The grid was supported by a formvar film 70 to 90 nm thick. These sections were stained with an aqueous solution of uranyl acetate and lead citrate and observed in a transmission electron microscope, JEM 100C.

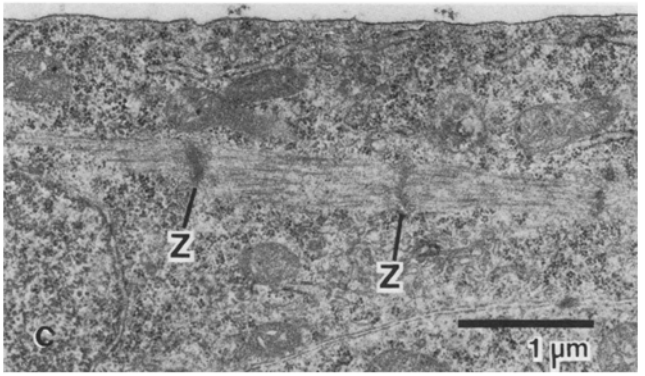
The SEM observations were made to provide better understanding of the exact form of the primitive heart under study. The initial fixation was carried out with the same procedure as that for TEM. The ectoderm and pericardium were removed in fixative with tungsten needles to expose the heart. After a brief rinse in 0.1 M cacodylate buffer, these specimens were refixed in buffered 1% OsO_4 for 1 or 2 h. They were dehydrated through ethanol, dried at critical point in liquid CO_2, coated with Pt and observed in a scanning electron microscope, Hitachi S-550, at 20KV.

To describe the sites of myofibrillogenesis in the cardiac wall, toluidine blue stained thick sections were enlarged



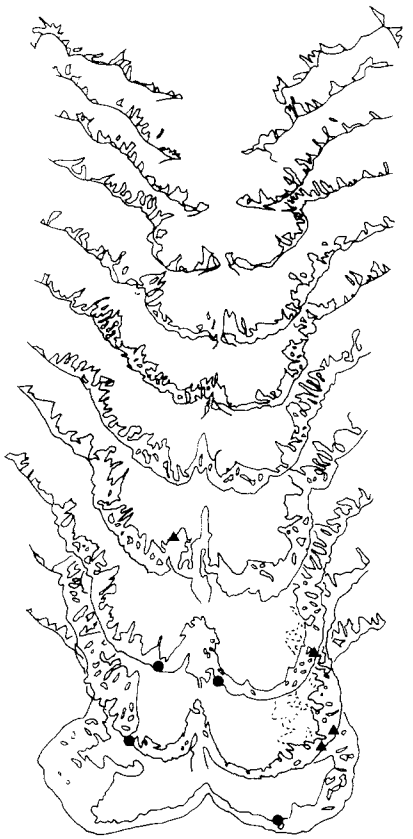




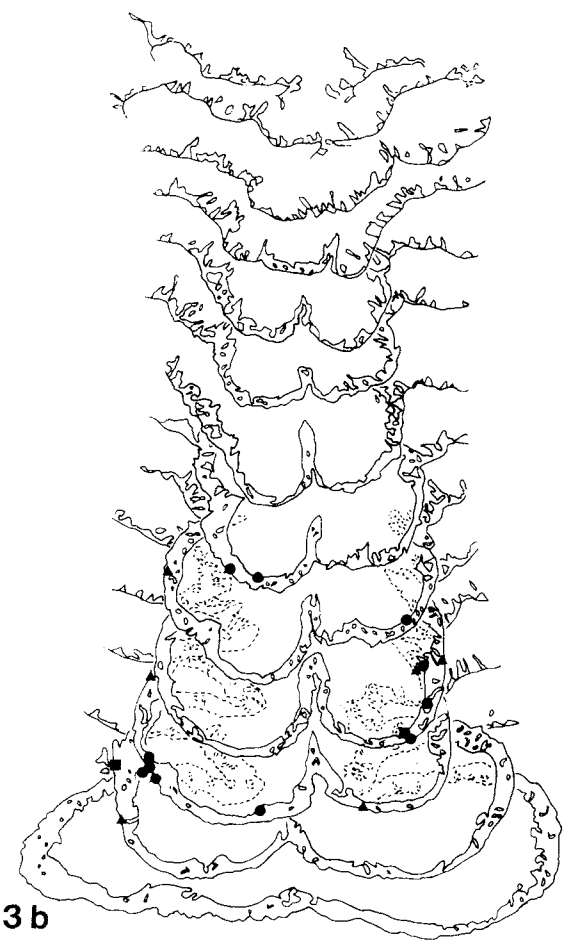


ig. 1 a–c. Scanning electron micrograph to show ventral aspects f the primitive heart at the 8- (**a**), 9- (**b**) and 10- (**c**) somite stage. *3C* presumptive bulbus cordis; *CT*, cardiac tube (actually trough); *', presumptive ventricle; *VA*, ventral aorta; *VM*, ventral mesocarium; an *arrow* shows fusion furrow. All figures, × 200

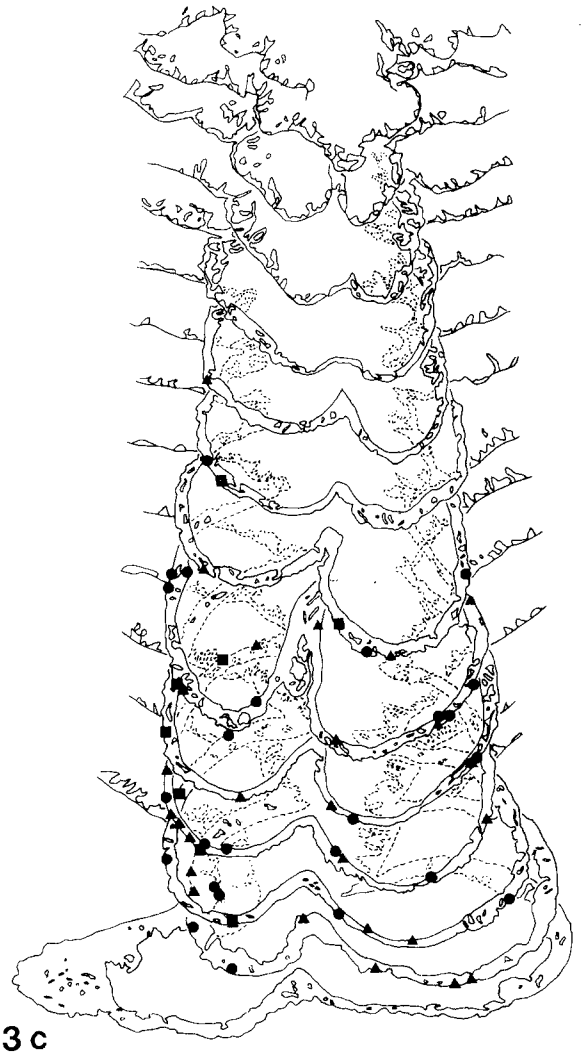
ig. 2 a–c. Transmission electron micrographs to show three phases f myofibrillogenesis. Phase I myofibril represents a bundle composed of loosely-assembled filamentous structures (**a**). At phase I, filamentous structures are aggregated to amorphous dense materials or presumably precursors of Z bands (**b**). A myofibril at phase II is defined as regularly striated nascent fibrils, which are still mmature and lack distinct A and I bands (**c**). All figures, × 18,000



3a



3b



3c

Fig. 3a–c. Topographical representation of the sites of myofibrillogenesis in the chick embryo heart at the 8- (**a**), 9- (**b**) and 10- (**c**) somite stage. Profiles of the transverse sections of the cardiac wall were arranged at 40 μm intervals in a caudal (*lower*) to cranial (*upper*) direction. The position of cells was plotted in which the myofibril(s) at phase I (▲), phase II (●) and phase III (■) were found by transmission electron microscopy on a thin section immediately adjacent to the thick section traced for the figure. Normally, a single myofibril was present in a cell profile. Compare these drawings with scanning electron micrographs at equivalent stages (Fig. 1)

510 times on a light microscopic projection device, Reichert Visopan. The profiles of the transverse sections of the embryonic heart were traced on transparent paper. The sections traced were those which immediately preceded the ultrathin sections. The sites of a cell in which forming myofibril(s) was found by TEM were marked on these traced figures. The figures were then aligned for reconstruction of the entire heart at each stage. One complete reconstruction model was made for each stage with supplementary data obtained from another two to three samples at the same stage.

Results

First, to provide a general view of the embryonic hearts dealt with in this study, their SEM images are presented in Fig. 1. At the 8-somite stage, the newly-formed heart has an almost symmetrical barrel-like shape, and the ventral mesocardium can still be seen at the fusion site of the bilateral cardiac mesodermal rudiments (Fig. 1a). At the next stage, a slight bulge is seen in the presumptive ventricular region that is distinct from the bulbus cordis and the ventral mesocardium disappears leaving a median fusion furrow (Fig. 1b). When regular contractions are well recognized, at the 10-somite stage, a complete primitive heart is established (Fig. 1c). The heart at this stage is usually called a cardiac "tube", but it is actually a "trough" with broad dorsal mesocardium.

The wall of the primitive heart is known to consist of two different simple epithelial cell layers, an inner endocardium and an outer myocardium with intervening cardiac jelly in between. There is no epicardium at these stages as demonstrated by Manasek (1969) and by Ho and Shimada (1978). Myofibrils were formed gradually in the cells composing the outer myocardial layer, while the majority of the other subcellular elements did not change much during the developmental period investigated in present study. Hence, our attention was concentrated on the myofibril formation. In addition, a preliminary freeze fracturing study had revealed the apical tight junction typical of epithelium as well as small punctate gap junctions (about 0.1 μm in diameter). No regional difference was found in junctional structure.

For the following descriptions, we tentatively define three phases of myofibrillogenesis according to the state of organization of the component filamentous and dense materials as shown in Fig. 2. Phase I represents the state in which thick and thin filaments assemble loosely to make an irregular bundle (Fig. 2a). Phase II is considered to be an intermediate state at which the filamentous elements are attached to a dense substance, which is presumed to be a precursor of the Z line (Fig. 2b). The myofibril at phase III are arranged in regularly striated bundles, although they have not yet reached a completely mature state with distinctly recognizable A and I bands (Fig. 2c).

The position of a cell provided with filamentous bundle(s) or myofibril(s) at each of these phases was plotted on the profiles of the transverse section of the cardiac wall. Mapping was made at 20 μm intervals and the tracings were aligned in order from the caudal to cranial region, to reconstruct the original heart as shown in Fig. 3. This figure was made in abbreviated form, arranging the tracings at 40 μm interval to avoid confusion from overlapping of too many profiles. At the 8-somite stage, only a few cells containing myofibril(s) were found, and these were confined to the caudal region. No phase III myofibrils were detected at this stage (Fig. 3a). At the 9-somite stage, cells possessing myofibril(s) increased in number, mainly on the right side (Fig. 3b). At the 10-somite stage, myofibrillogenesis had considerably progressed. Striated myofibrils at phase III were more frequently encountered. The right side dominance became more prominent. However, in the median region myofibril formation appeared to be retarded (Fig. 3c). Although the results are not based on complete serial sections, the sequence of myofibril formation depicted here seems to be accurate.

Discussion

The onset of cardiac function in the chick embryo has attracted the attention of many embryologists (Sabin 1920; Patten and Kramer 1933; see also DeHaan 1965). In early investigations, cardiac pulsation was observed with a microscope, or sometimes with the aid of microscopic cinematography. These methods are inadequate to recognize electrical activity that is not accompanied by mechanical beating, which must be investigated by electrophysiological techniques. Recently, an optical recording method has been devised, which has demonstrated unequivocally the appearance of the pace-maker action potentials prior to the onset of excitation-contraction coupling in the developing chick embryonic heart (Fujii et al. 1980, 1981a, 1981b). This developmental sequence took only about 8 h in the chick embryo. To date, there has been no comprehensive work revealing the ultrastructural events that occur during this same period.

In the present report, we have examined the morphological characteristics correlating the development of cardiac function with formation of myofibrils. To date, no reasonable explanation of mechanism of myofibrillogenesis has been put forward, nor has exact ultrastructural evidence of the excitation-contraction coupling been presented. Markwald (1973) has illustrated diagramatically his concept of myofibril formation based on TEM findings in the developing rat and hamster heart, emphasizing the role of the dense body in sarcomere formation. However, molecular mechanisms are still unknown. It is also difficult to define structural correlates between impulse generation and conduction (DeHaan and Hirakow 1972).

Although many questions remain unsolved concerning ultrastructure and function in the development of the heart, our topographical findings of the myofibril-forming sites are consistent with the results of multi-channel optical recordings concerning the sites of the excitation-contraction coupling in the developing chick heart (Hirota et al. 1985). In addition, it is interesting to note that the conductive delays recorded at the median region of the chick heart at the 8- and 9-somite stage (Hirota et al. 1983) may be ascribed structurally to the presence of the fusion furrow that persists for a while at these stages (Fig. 1a, b), where cells possessing myofibril(s) were rarely found (Fig. 3). However, intercellular junctional structures were not different from those found in other active regions.

The primary purpose of the present study was to correlate structural and functional aspects of the embryonic heart during the onset of beating. As far as the contractile

element is concerned, this goal has in major part been fulfilled. However, with regard to other ultrastructural features of plasma membrane specializations and/or organelles relating to cellular metabolism, no marked changes were seen in this study. Further information needs to be obtained, using techniques such as quick-freezing and immunocytochemical methods, with special focus on the relationship of the cytoskeletal system to membrane structure at the fine-structural level.

References

DeHaan RL (1965) Morphogenesis of the vertebrate heart. In: DeHaan RL, Ursprung H (eds) Organogenesis. Holt, Rinehart and Winston, New York, pp 377–419

DeHaan RL, Hirakow R (1972) Synchronization of pulsation rates in isolated cardiac myocytes. Exp Cell Res 70:214–220

Fujii S, Hirota A, Kamino K (1980) Optical signals from early embryonic chick heart stained with potential sensitive dyes: evidence for electrical activity. J Physiol (Lond) 304:503–518

Fujii S, Hirota A, Kamino K (1981a) Optical recording of development of electrical activity in embryonic chick heart during early phases of cardiogenesis. J Physiol (Lond) 311:147–160

Fujii S, Hirota A, Kamino K (1981b) Optical indications of pacemaker potential and rhythm generation in early embryonic chick heart. J Physiol (Lond) 312:253–263

Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. J Morphol 88:49–92

Hirota A, Sakai T, Fujii S, Kamino K (1983) Initial development of conduction pattern of spontaneous action potential in early embryonic precontractile chick heart. Dev Biol 99:517–523

Hirota A, Kamino K, Komuro H, Sakai T, Yada T (1985) Optical studies of excitation-contraction coupling in the early embryonic chick heart. J Physiol (Lond) (in press)

Ho E, Shimada Y (1978) Formation of the epicardium studied with the scanning electron microscope. Dev Biol 66:579–585

Manasek FJ (1968) Embryonic development of the heart. I. A light and electron microscopic study of myocardial development in the early chick embryo. J Morphol 125:329–366

Manasek FJ (1969) Embryonic development of the heart. II. Formation of the epicardium. J Embryol Exp Morphol 22:333–348

Markwald RR (1973) Distribution and relationship of precursor Z material to organizing myofibrillar bundles in embryonic rat and hamster ventricular myocytes. J Mol Cell Cardiol 5:341–350

Patten BM, Kramer TC (1933) The initiation of contraction in the embryonic chick heart. Am J Anat 53:349–375

Sabin FR (1920) Studies on the origin of blood-vessels and of red blood-corpuscles as seen in the living blastoderm of chicks during the second day of incubation. Contrib Embryol 9:213–262

Accepted May 21, 1985