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

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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 rhythmic 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).

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% OsO4 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, the specimens were refixed in buffered 1% OsO4 for 1 or 2 h. They were dehydrated through ethanol, dried at critical point in liquid CO2, 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

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

Fig. 2 a–c. Transmission electron micrographs to show three phases of 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 immature and lack distinct A and I bands (c). All figures, × 18,000