CHAPTER 1

Myofibrillogenesis in Cardiac Muscle

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INTRODUCTION

Cardiomyocytes are among the first cells in an embryo to become organized into a functioning organ. During heart formation, these cells form myofibrils, contract, and undergo cell division. In this chapter, we will discuss the process of myofibril assembly in cardiomyocytes, based mainly on experiments and observations of primary cultures of embryonic chick cardiomyocytes. On the basis of the arrangement of specific cytoskeletal proteins in these cells, there appears to be a hierarchical progression of fibril formation leading to myofibrillogenesis, beginning with premyofibrils, followed by nascent myofibrils and mature myofibrils (Figure 1.1).

PREMYOFIBRIL MODEL FOR THE ASSEMBLY OF MATURE MYOFIBRILS

In the embryonic chick model system, hearts can be removed from 5- to 8-day-old embryos and treated with enzymes to isolate cardiomyocytes (reviewed by Dabiri et al., 1999a, 1999b). The freshly isolated heart cells, when placed in tissue culture, attach to the substrate, spread, and assemble myofibrils (Figure 1.2). The cells are capable of cell division, repeating in culture processes detected in the intact heart, namely the disassembly of myofibrils, chromosome separation, assembly of a cleavage furrow, cytokinesis, and the subsequent reformation of myofibrils in the two contractile daughter myocytes (Chacko, 1973; Kaneko et al., 1984; Dabiri et al., 1999a).

Z-Bands

The premyofibril model for the stepwise assembly of myofibrils in cardiac muscle cells illustrated in Figure 1.1 was deduced from immunofluorescent images of cardiomyocytes (Figure 1.3) that had been fixed and stained during different stages of spreading in tissue culture (Rhee et al., 1994). The α-actinin antibody delineates the boundaries of the sarcomeres, the smallest repeating units of a myofibril (Figure 1.1). When an anti-α-actinin antibody was used to stain the spreading cells, different populations of striated fibrils were evident (Figure 1.3). At the peripheral spreading edges of the cells, linear arrays of closely spaced bodies of α-actinin could be detected.
These localized concentrations of $\alpha$-actinin we called Z-bodies. The Z-bodies mark the repeating units of minisarcomeres that compose the premyofibrils (Figures 1.1 and 1.3). Probes that specifically stained F-actin demonstrated that actin filaments were present between these Z-bodies in a pattern suggesting that the filaments overlapped (Figure 1.1). Toward the central region of the cell, Z-bodies were spaced further apart and aligned laterally across the fibrils. Adjacent to these fibrils in the central region of the spreading cells, myofibrils with mature solid-staining Z-bands, spaced about two microns apart, were readily visible (Figure 1.3). The continuous $\alpha$-actinin staining of Z-bands composed of fused Z-bodies could be detected in immunofluorescently stained cells (Figure 1.3). It appeared from the patterns of $\alpha$-actinin staining in the cardiomyocytes that these mature Z-bands resulted from fusion of Z-bodies (Figures 1.1 and 1.3).

**Myosin II**

Double labeling of these spreading cardiomyocytes with the muscle-specific $\alpha$-actinin antibodies and two different isoforms of myosin II antibodies (nonmuscle myosin IIB or muscle myosin II antibodies) revealed distinctive patterns of myosin II localization in the spreading cardiomyocytes (Figures 1.1, 1.4, and 1.5). Premyofibrils stained only with the nonmuscle myosin IIB (Figure 1.4), whereas mature myofibrils exhibited only muscle myosin II staining (Figure 1.5). These myosins were localized in banded patterns, with their fluorescence concentrated between the concentrations