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DEVELOPMENTAL HISTORY OF THE TWO M-LINE PROTEINS MM-CREATINE KINASE AND MYOMESIN DURING MYOGENESIS

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INTRODUCTION

The terminal differentiation of myogenic cells represents a system extremely well suited for the study of regulatory processes going on during muscle development. When myogenic cells become postmitotic and fuse into multinuclear muscle fibers, large amounts of "new" proteins necessary for the specialized functions of muscle tissue are produced. The components of the contractile organelles, many enzymes involved in the metabolic pathways of energy production as well as membrane constituents bringing about the specialized functions of the muscle cell surface are among the most abundantly synthesized proteins during this time period. This in turn means that the genes and/or the messages for these proteins must become very active in a well-coordinated way at that time. At present, however, not much is known about the regulatory mechanisms underlying the "switching on" of this muscle-specific program. Moreover, the sequence of events leading to the highly ordered structure of a myofibril is still unclear. In spite of the fact that the most abundant proteins of the contractile apparatus have been characterized in some detail the factors controlling the appearance and determining the organization of these proteins during myofibrillogenesis are still unknown. In this report the developmental characteristics of two proteins will be described: creatine kinase (CK), the dimeric enzyme catalyzing the regeneration of ATP from ADP and creatine phosphate, and myomesin, a protein located in the M-line region of the myofibrillar sarcomeres.

RESULTS AND DISCUSSION

The Creatine Kinase Isoenzyme Switch

In chicken embryonic myogenic cells as well as in many adult chicken tissues like brain, smooth muscle and heart BB-CK represents the predominant form of creatine kinase (Eppenberger et al. 1964, Morris et al. 1972, Turner et al. 1974, Perriard et al. 1978b). During terminal differentiation of skeletal muscle cells, however, the muscle-specific M-CK subunit gradually replaces the B-subunit. In fact, the large increase in total cellular CK activity found in differentiating skeletal muscle cells in culture and in vivo is mostly due to a drastic accumulation of MM-CK within these cells (Turner et al. 1974, Perriard et al. 1978a). This isoenzyme switch which in vivo leads to an almost complete replacement of BB-CK by MM-CK has been shown to be entirely due to newly synthesized CK-subunits (Caravatti et al. 1979). The levels of total cellular and polysomal mRNA coding for the two different CK-subunits have been determined in cell-free translation systems using sensitive immunoprecipitation methods discriminating between the produced M- and B-subunits (Perriard et al. 1978b, Perriard 1979). From these experiments the conclusion could be drawn that the increased synthesis of M-CK is mostly due to the new appearance of translatable specific mRNA for this protein. Currently experiments are underway to isolate and characterize the M- and B-CK genes. The comparison of the arrangement and structural details of these genes might bring some important information about the possible mechanisms underlying the switching on of muscle-specific genes.

It is assumed that the large increase in CK-activity taking place during terminal differentiation in myogenic cells is related to the enhanced activity of the energy metabolism. Why, however, should a cell switch on a "new" CK-gene in order to ensure the increased need for this protein? On one side, a higher rate of transcription of the M-CK gene, or a higher stability and/or translational activity of the resulting M-CK mRNA might well be a reason for the observed phenomenon. On the other hand, unique properties of M-CK when compared to B-CK could equally well account for its necessity within a muscle cell. MM-CK has indeed been found to differ significantly from BB-CK with respect to its ability to bind to the contractile organelles. While it became clear that most of the

MM-CK exists as soluble enzyme in the cytoplasm a small, yet significant, amount of this protein (at least 5 % of the total MM-CK) is firmly and specifically bound to the center of the myofibrillar sarcomeres (Fig. 1) where it makes up for the bulk of electron-density of the M-line (Wallimann et al. 1977, 1978; Strehler et al. 1980). In contrast, BB-CK is never present in the M-line during any stage of myogenesis even though it is present in small amounts in the I-band during the early stages (Wallimann et al. 1977).

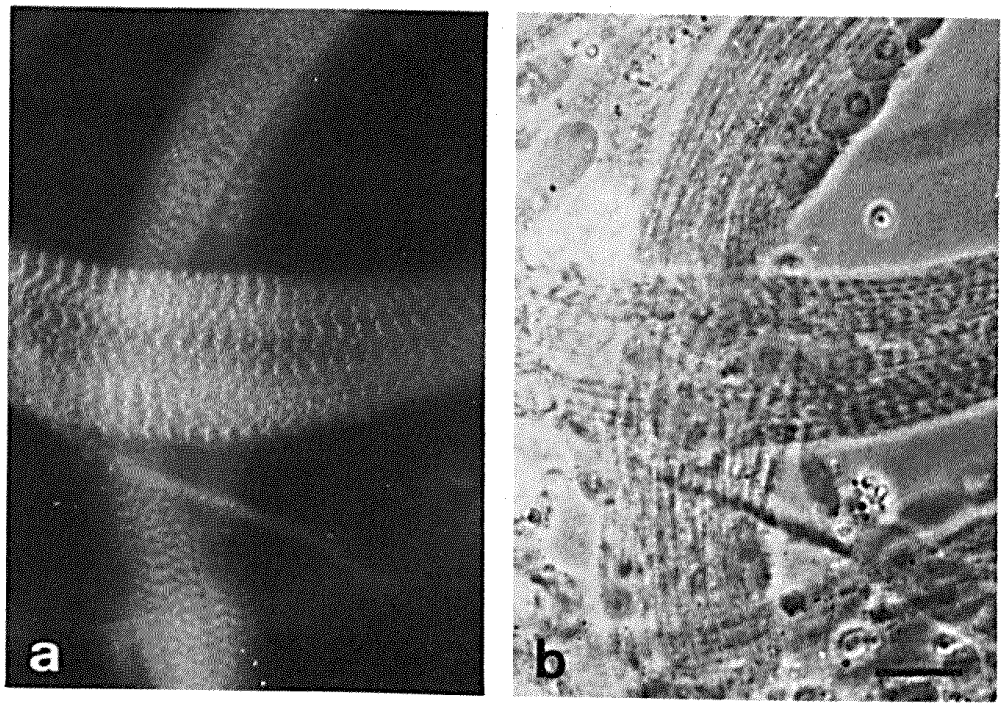

FIGURE 1: Indirect immunofluorescence localization of M-line bound MM-CK in differentiated skeletal muscle cells cultured for 5 days. Cells were permeabilized with Triton X-100, washed extensively to remove the bulk of the soluble MM-CK, and then stained with affinity-purified anti-MM-CK IgG (Eppenberger et al. 1981). By superimposing fluorescence and phase contrast images staining can be localized within the middle of the A-band of the sarcomeres. (a) Fluorescence; (b) phase contrast picture of the same culture area. Bar, 10 µm.

In recent experiments we not only have been able to show that the M-line-bound MM-CK is enzymatically active but also that the amount and specific activity of this enzyme fraction might be sufficient to regenerate a considerable amount of the ADP produced during contraction (T. Schlösser and T. Wallimann, in preparation). It is thus quite possible that the presence of MM-CK within a myofibril provides an effective way of ATP regeneration during contraction. The properties enabling MM-CK to bind to the middle of the myofibrillar sarcomeres are still unknown. The fact that different muscle types differ with respect to the amount of M-line MM-CK and that MM-CK may be incorporated into this structure after other components are already present suggests that other properties of a muscle cell, e.g., the myosin type present in the myofibrils, determine whether and at what time MM-CK will be incorporated in the M-line.

Myomesin, a Myofibril-specific Protein of Cross-striated Muscles

The M_r = 165,000 M-protein (Masaki and Takaiti 1974, Trinick and Lowey 1977, Strehler et al. 1979, 1980), now called myomesin (Eppenberger et al. 1981), is another example of a developmentally regulated muscle-specific protein As observed for MM-CK the synthesis of myomesin starts with the onset of terminal myogenic differentiation (Eppenberger et al. 1981). In contrast to CK where a switch from the more ubiquitous embryonic to the muscle-specific form of CK takes place during myogenesis, no embryonic form of myomesin has been detected up to now. Using an antibody against chick myomesin, myomesin has been identified only in the M-line region of the cross-striated muscles of a wide range of vertebrate species indicating the highly conserved nature of its strict tissue specificity (Table 1). Not much is known about the level at which the synthesis of this protein is controlled, this to some extent being due to the difficulties encountered in isolating the mRNA coding from myomesin, a protein which constitutes at most 0.04 % of the total muscle proteins synthesized during that time (Eppenberger et al. 1981).

TABLE 1: Cross-reactivity of rabbit polyclonal antibodies against chicken skeletal muscle myomesin (immunofluorescence).

Species	Skeletal muscle myofibrils	Heart muscle myofibrils	Skeletal muscle cells	Heart muscle cells
Chick	+	+	+	+
Quail	+	ND	+	ND
Rat	+	+	ND	ND
Mouse	+	ND	ND	ND
Hamster	+	+	+	+
Cow	ND	+	ND	ND
Cat	ND	+	ND	ND
Sheep	ND	+	ND	ND
Man	+	+	ND	ND
Xenopus	+	+	ND	ND
Trout	+	+	ND	ND
Crab	-	-	ND	ND
Drosophila melanogaster (flight muscle)	-	ND	-	ND

ND, not determined

Even though myomesin is present in low amounts relative to other myofibrillar proteins, its exclusive localization within the narrow M-line region makes it detectable by the indirect immunofluorescence technique (Fig. 2). Myomesin can already be identified in early postmitotic myoblasts (Eppenberger et al. 1981) or suspended cells (Puri et al. 1980) in a regularly cross-striated pattern. If young myotubes are stained for myomesin by incubation with specific antibodies and then processed for electron microscopy the localization of myomesin in the M-line region of nascent myofibrils can easily be demonstrated (Fig. 3b). Incubation of young myotubes with preimmune IgG shows clearly that an electron-dense M-line (to which MM-CK makes the most essential contribution) is often not yet or only partially developed in the myofibrils at that time (Fig. 3a). Myomesin thus appears to be an attractive candidate for a protein playing a role in the assembly process of cross-striated myofibrils.

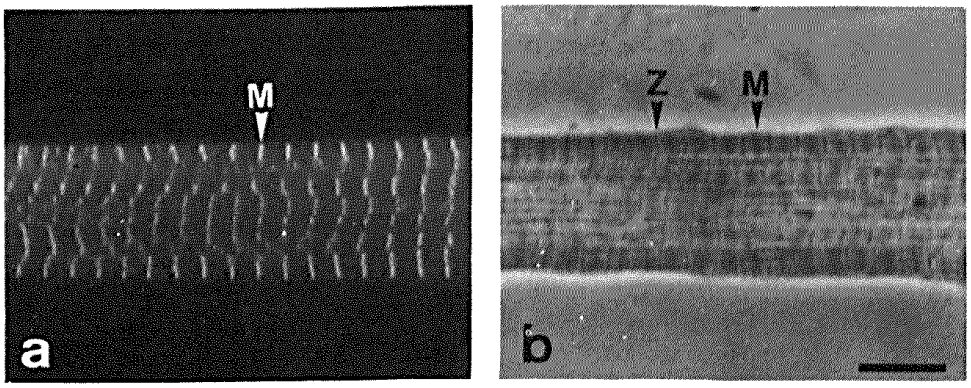

FIGURE 2: Indirect immunofluorescence using affinity-purified anti-myomesin IgG. Localization of myomesin in a myotube of a chicken embryonic breast muscle cell culture 13 days after plating. (a) Fluorescence; (b) phase contrast. Z, Z-line; M, M-line region. Bar, 10 μm.

Although an interaction of myomesin with myosin has been reported to occur in vitro (Mani and Kay 1978), the exact nature and the functional significance of this phenomenon have not yet been established. Experiments designed to study the influence of myomesin on the formation of myosin thick filaments as well as on the aggregation of these filaments in vitro are underway in our laboratory. In addition, we will investigate the functions of myomesin and MM-CK with microinjection techniques employing fluorescently labelled myomesin and other myofibrillar components as well as antibodies against these proteins (Fig. 4). By injecting such proteins into living cultured cells of differing origin and age we hope to learn more about the process of myofibrillogenesis within muscle cells.

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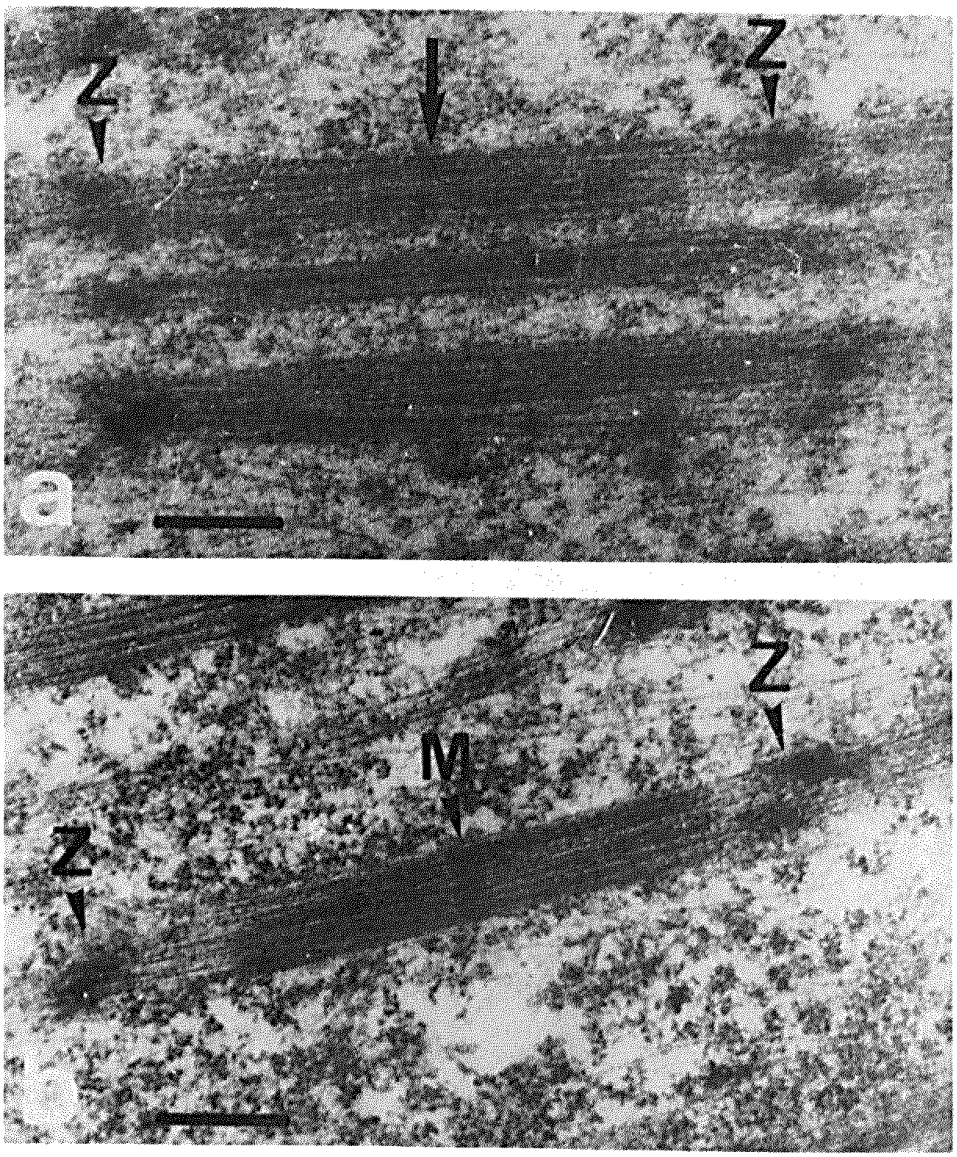

FIGURE 3: Localization of myomesin in nascent myofibrils in small myotubes of chicken breast muscle cells 48 hrs after plating. (a) Incubation with preimmune IgG. Beginning incorporation of electron dense M-line material (MM-CK) into some sarcomeres (arrow). (b) Incubation with affinity-purified anti-myomesin IgG. Heavy antibody decoration of myomesin already present in the M-line region. Z, Z-line; M, M-line region. Bars, 0.4 um.

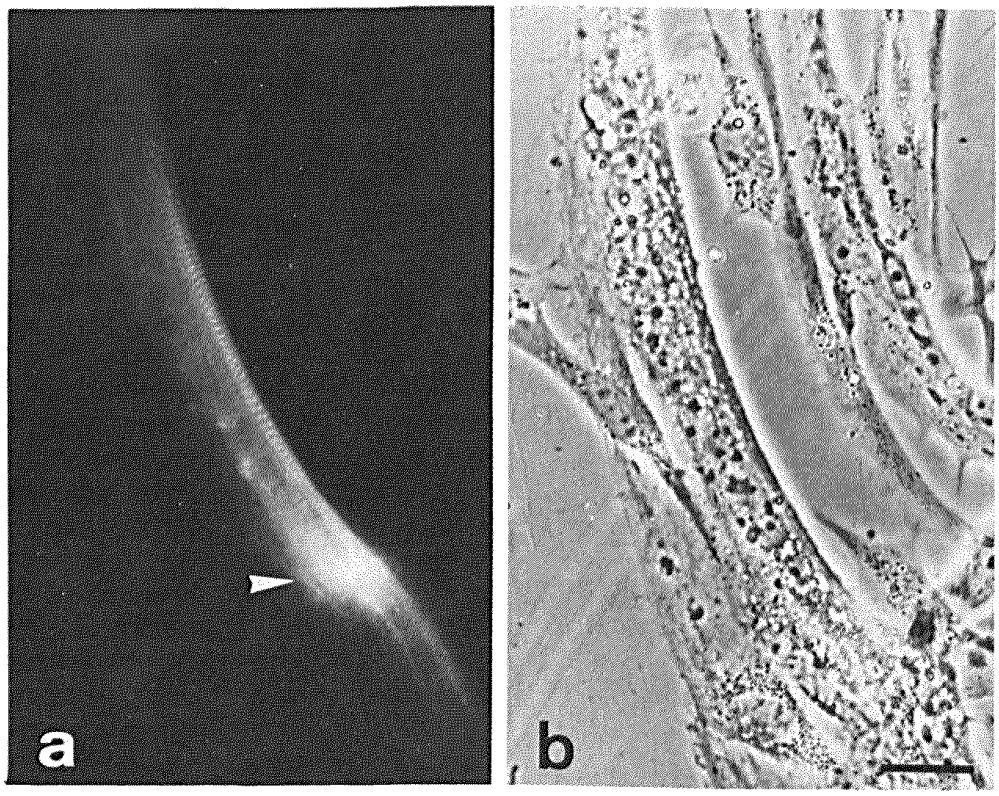

FIGURE 4: Direct immunofluorescence of rhodamine-labelled anti-myomesin IgG microinjected into a living myotube at 3 days of culture. (a) Fluorescence; (b) phase contrast pictures of the myotube 30 min. after injection. Arrowhead shows site of injection. Bar, 20 μm.

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