

TROPONIN T ISOFORM EXPRESSION AND CHIMERIC
FIBERS IN REGENERATING MUSCLE

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Using antibodies against anti-fast troponin T and anti-slow troponin T, we examined the regenerating muscle cells obtained by transplantation of anterior latissimus dorsi (slow muscle) into pectoralis major (fast muscle) and found by 2-D SDS-PAGE and immunoblotting that they expressed slow type troponin T isoforms. Thus, the pattern of skeletal muscle troponin T isoform expression is fixed in cell lineage. Furthermore, the chimeric muscle fibers composed partly of fast and partly of slow fibers were found in regenerating muscle blocks, suggesting that the fast and slow myoblasts fused during regeneration.

EXPRESSION OF MYOGENIC FACTORS IN
DENERVATED CHICKEN BREAST MUSCLE: ISOLATION
OF THE CHICKEN MYF5 GENE.

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We have isolated chicken Myf5 gene in addition to cDNAs for MyoD1 and myogenin, and analyzed their mRNA levels during chicken breast muscle development. We found that chicken Myf5 and MRF4 genes are tandemly located on single genomic DNA fragment, and that chicken Myf5 gene is organized into at least three exons. The analyses of expression revealed that myogenin expression is restricted to in ovo stages in breast muscle, and is not detectable in neonatal and adult stages. On the other hand, Myf5 expression is detectable until day 7 post-hatching and is not found in adult muscle, whereas high levels of MyoD1 and MRF4 are detectable at all stages. To further understand the roles of innervation during muscle maturation, we analyzed the expression of the myogenic factors in denervated adult breast muscle. We found that MyoD1, myogenin, and MRF4 are induced at high levels in denervated muscle, whereas no change occurs in the level of Myf5.

CAN COLLAGEN SYNTHESIS TRIGGER THE
EXPRESSION OF MYOGENIN IN CULTURED MUSCLE
CELLS?

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It has been known that the myogenic regulatory factor (MyoD family) triggers muscle cell differentiation. In cultured skeletal muscle cells, the decrement of serum or fibroblast growth factor concentration is crucial to activate MyoD family genes. On the other hand, the extracellular matrix (ECM) is known to be an important environmental factor to support muscle cell differentiation. To study the effect of ECM formation on the expression of MyoD family gene in cultured BC3H1 cells, we added 200 μ M of ascorbic acid 2-phosphate (a co-factor for collagen synthesis) into growth medium and cultured for 4 days. The cells continued to proliferate and formed well developed ECMs and multiple cell layers. Immunofluorescent study revealed that only the cells on the bottom of the culture dish expressed myogenin. The result suggests that the enhanced formation of ECM is one of the important factors to up-regulate the expression of MyoD family gene during muscle development.

Involvement of Protein Tyrosine Kinase in the
Regulation of Myogenin Expression

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Myogenin is one of the muscle-specific transcription factors that regulate myogenic differentiation accompanying growth arrest. We are focusing on the role of tyrosine phosphorylation in the process of myogenic differentiation of cultured myoblast C2C12 cells.

Using an affinity-purified anti-myogenin antibody, we revealed the expression pattern of myogenin during the course of differentiation of C2C12 cells. Myogenin was first detected by immunoblot analysis as a pair of bands prior to myotube-formation and expression levels remain high in myotubes. To examine the role of tyrosine phosphorylation in myogenesis, the effect of genistein, an inhibitor of protein tyrosine kinase, was examined. When C2C12 cells were cultured for 2 days in differentiation medium containing various concentrations of genistein, myogenin expression was suppressed in a dose-dependent manner. Furthermore, myotube-formation was also completely inhibited in the presence of 50 μ M genistein. Taken together, these results indicate that genistein blocks myogenesis through the suppression of myogenin expression. The present study suggests that tyrosine phosphorylation plays a key role in the regulation of myogenin expression and myogenesis.