

An N-terminal fragment of titin coupled to green fluorescent protein localizes to the Z-bands in living muscle cells: overexpression leads to myofibril disassembly
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Cultures of nonmuscle cells, skeletal myotubes, and cardiomyocytes were transfected with a fusion construct (Z1.1GFP) consisting of a 1.1-kb cDNA (Z1.1) fragment from the Z-band region of titin linked to the cDNA for green fluorescent protein (GFP). The Z1.1 cDNA encodes only 362 amino acids of the approximately 2000 amino acids that make up the Z-band region of titin; nevertheless, the Z1.1GFP fusion protein targets the alpha-actinin-rich Z-bands of contracting myofibrils *in vivo*. This fluorescent fusion protein also localizes in the nascent and premyofibrils at the edges of spreading cardiomyocytes. Similarly, in transfected nonmuscle cells, the Z1.1GFP fusion protein localizes to the alpha-actinin-containing dense bodies of the stress fibers *in vivo*. A dominant negative phenotype was also observed in living cells expressing high levels of this Z1.1GFP fusion protein, with myofibril disassembly occurring as titin-GFP fragments accumulated. These data indicate that the Z-band region of titin plays an important role in maintaining and organizing the structure of the myofibril. The Z1.1 cDNA was derived from a chicken cardiac lambda gt11 expression library, screened with a zeugmatin antibody. Recent work has suggested that zeugmatin is actually part of the N-terminal region of the 81-kb titin cDNA. A reverse transcriptase polymerase chain reaction using a primer from the distal end (5' end) of the Z1.1 zeugmatin cDNA and a primer from the nearest known proximal (3' end) chicken titin (also called connectin) cDNA resulted in a predicted 0.3-kb polymerase chain reaction product linking the two known chicken titin cDNAs to each other. The linking region had a 79% identity at the amino acid level to human cardiac titin. This result and a Southern blot analysis of chicken genomic DNA hybridized with Z1.1 add further support to our original suggestion that zeugmatin is a proteolytic fragment from the N-terminal region of titin.

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