

Partial characterization of zeugmatin indicates that it is part of the Z-band region of titin  
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#### Keywords

Z-bands; Z-bodies; myofibrillogenesis; stress fibers; alpha-actinin

#### Abstract

Zeugmatin is a muscle specific protein discovered by Maher et al. [1985: J. Cell Biol. 101:1871-1883] to be in Z-Bands of muscle and in the dense bodies of smooth muscle. Maher et al. [1985] generated a zeugmatin specific monoclonal antibody, McAb20, and then used immunoaffinity chromatography to isolate a 600-800 kD protein. During myofibrillogenesis of embryonic cardiac muscle, zeugmatin is detected in fully formed Z-bands in the mature myofibrils but not in the Z-bodies of premyofibrils [Rhee et al., 1994: Cell Motil. Cytoskeleton 28:1-24]. Rhee et al. [1994] have postulated that zeugmatin may be responsible for the fusion of the alpha-actinin containing Z-bodies to form the solid Z-Bands of the mature myofibrils. The current studies were undertaken to characterize the properties of zeugmatin. The McAb20 was used to probe a chicken heart lambda gt11 expression library, and three unique positive clones of 1.1, 1.4, and 1.7 kB were isolated. These were inserted into pcDNA3, sequenced, and assembled into a 1.8 kB ORF. A 60% identity with the N-terminal region of the human cardiac titin sequence was revealed at the amino acid level. This region of the 1.8 kB zeugmatin sequence is located entirely in the Z-band region of the human cardiac titin molecule. The 1.1 kB clone of zeugmatin was subcloned into pTrcHisC and expressed in bacteria. Bacterial lysates were prepared and run over nickel columns to isolate a 46 kD fusion protein. This fusion protein formed a complex with purified alpha-actinin that could be immunoprecipitated with the zeugmatin specific antibody, McAb 20. The 1.1 kB sequence was transfected into non-muscle cell lines, PtK2 and REF. Twenty-four hours after transfection, the 46 kD zeugmatin peptide, not present in control non-muscle cells, was localized in focal adhesions and in a punctate pattern along the stress fibers. Double immunofluorescence staining revealed that zeugmatin colocalized with the alpha-actinin in the dense bodies and focal contacts of the stress fibers. At longer time points, as the transfected cells accumulated more truncated zeugmatin molecules, the cells lost adhesion plaques and stress fibers, and became detached from the substratum. Our results indicate that zeugmatin is part of the titin molecule that is located within the Z-band and that this section of the titin molecule anchors the actin cross-linking alpha-actinin molecules. © 1996 Wiley-Liss, Inc.

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Received: 22 December 1995; Accepted: 16 February 1996

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Funding Agency: American Heart Assoc

Funding Agency: Muscular Dystrophy Assoc of America; Grant Number: HL 48954, HL 14142