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