

ALTERNATIVE METHODS FOR DETERMINING THE LOCALIZATION OF THE UNC-82 PROTEIN
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UNC-82 is a serine/threonine kinase required for maintenance of thick filament and M-line organization in the striated body-wall muscle of the nematode *C. elegans*. Antibody staining experiments have shown that animals homozygous for *unc-82* mutations exhibit severe disruption of the thick filament proteins myosin and paramyosin, as well as the M-line protein UNC-89/obscurin. Consistent with these known defects, an UNC-82::GFP fusion has been shown to localize at or near the M-line. In addition, the fusion protein revealed UNC-82 was expressed in other cell types where its role is not yet known. We are taking two approaches to determine the location of the UNC-82 protein with more precision. First, we have created an RFP-tagged UNC-82 construct to enable co-localization of UNC-82 with existing GFP-tagged protein markers in living animals. An existing UNC-82::GFP construct was restriction digested and a PCR fragment containing the RFP sequence was ligated in its place. Second, antibodies have been raised against two UNC-82 epitopes: one adjacent to the N-terminal kinase domain, and one that includes the native C-terminus. Two GST fusion proteins were created to perform affinity purification of the antisera. One GST fusion contains a portion of exon 6, amplified from the cosmid clone B0496; the other was amplified from Yugi Kohara's cDNA clone yk47c5, which contains exons 10-30. The GST fusion proteins were expressed in bacteria, affinity purified, and covalently bound to a matrix. The matrix was used to make affinity columns to purify the anti-UNC-82 antibodies. These purified antibodies will be used in immuno-EM experiments to determine the precise location of the UNC-82 protein in muscle and nonmuscle cells. The antisera may also be used for immuno-precipitation experiments to identify other proteins that interact with UNC-82.