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In vivo visualization of protein-protein interactions in C-elegans muscle attachment structures by FRET microscopy

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Abstract

Dense bodies and M-lines are highly ordered structures that attach actin, respectively myosin filaments in the body wall muscle cells of C. elegans to the basal lamina, hypodermis and cuticle. Using genetic techniques many proteins that localize at dense bodies and M-lines have been identified. To determine which proteins interact with each other in adult muscle attachments, we applied fluorescence resonance energy transfer (FRET) microscopy in living transgenic animals in which the proteins are fused pair-wise to the GFP-mutants CFP (cyan) and YFP (yellow). Occurrence of FRET between the CFP- and YFP-labels indicates a direct intermolecular interaction between the host proteins. FRET was measured by both steady-state and time-resolved two-photon excitation microscopy methods. We show that PAT-4, the C. elegans homolog of integrin-linked kinase, forms multimers and binds to PAT-6, a homolog of actopaxin, confirming results obtained from genetic and yeast two-hybrid studies. We discuss the advantages and disadvantages of the different measurement methods as well as issues pertaining to in vivo FRET measurements such as autofluorescence, heterogeneity in expression levels and labeling, and rapid photobleaching of CFP. This research was supported by NIH.