Title
Protein interactions in C. elegans muscle attachment structures studied in vivo by fluorescence resonance energy transfer (FRET)

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Abstract

We are interested in deciphering the protein interactions in the focal adhesion-like structures that attach the muscles of C. elegans to its hypodermis and cuticle. To this goal we have created transgenic animals in which the proteins are fused pair-wise to the GFPmutants CFP (cyan) and YFP (yellow). Fluorescence resonance energy transfer (FRET) between the CFP- and YFP-labels will only occur if a direct intermolecular interaction between the host proteins exists. FRET measurements are done in vivo using several microscopy techniques. Using two-photon excitation we applied lifetime imaging, ratioimaging, donor photobleaching kinetics and recovery of the donor intensity after acceptor photobleaching. Using one-photon excitation we applied the intensity-based three-filter set method. To evaluate the accuracy of our results we performed control measurements on a Ca2+-sensitive cameleon construct purified in solution and expressed in bacteria, and on a CFP-YFP fusion construct expressed in C. elegans. The advantages and disadvantages of the different methods are discussed, as well as particular considerations for in vivo experiments. This research was supported by NIH, PHS 5 P41 RRO3155.