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The role of the crumbs complex in vertebrate rod morphogenesis and its regulation by a novel FERM protein mosaic eyes

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Abstract

Mutations in zebrafish mosaic eyes result in the disrupted retina lamination and other abnormalities. The moe locus encodes a FERM protein. In this study I sought to determine in which molecular pathway moe acts. We propose that Moe forms a complex with the Crumbs (Crb) proteins which are key determinants of the apical cell polarity. I identified zebrafish crb genes and found that expression of crb2a resembles the moe expression. Injection of crb2a antisense morpholinos phenocopies the moe mutations. Moe and Crumbs proteins colocalize in the photoreceptors. I showed Moe and Crumbs proteins, Pals1, and aPKCA form a complex by pulldown assays and coimmunoprecipitation. I demonstrated that Moe can directly interact with the Crumbs proteins. Using genetic mosaic analyses, I showed that moe is required for rod morphogenesis and *moe*⁻ rods have greatly expanded apical structures, suggesting that Moe is a negative regulator of Crumbs protein function in photoreceptors. Next I sought to determine the function of each domain of Crb2a/b proteins in rod morphogenesis. I constructed nine Crb2a constructs and made stable fish lines to express each of them specifically in rods. I also made lines that overexpress a Moe peptide that contains the predicted Crumbs proteins binding motif. I showed that Crb2a^{ΔFBD}, Crb2a^{ΔFBDΔPBD}, Crb2a^{IntraDD}, Crb2a^{IntraAA}, and Crb2a^{TM-Extra} proteins mostly go to the outer segment. Crb2a^{IntraWT}, Crb2a^{FL}, and Crb2a^{ΔPBD} localize mostly to the inner segment and cell body. Binding assays showed that GST-Crb2a^{ΔFBD}, GST-Crb2a^{IntraDD}, and GST-Crb2a^{IntraAA} do not bind HIS-Moe_FERM as well as GST-Crb2a^{IntraWT}. Overexpression of Crb2a^{FL} and Crb2a^{ΔPBD} causes Rhodopsin mislocalization. Crb2a^{Intra} expression causes mislocalization of endogenous Crumbs proteins, indicating a dominant effect of transgene expression. I also showed that Crb2a^{Intra} expression causes an increase in the size of the outer segment by over 50%, and Crb2a^{IntraAA} produces the largest increase. These data suggest that targeting of transgene products to the outer segment is likely due to the impaired binding ability to Moe and that the apical membrane adding activity of Crb2a^{Intra} proteins can be inhibited by Moe. Further, my data show that the interaction of Moe and Crumbs proteins depends on the phosphorylation state of Crumbs proteins.^

Subject Area

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