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Review

Composition and function of the Crumbs protein complex in the mammalian retina

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Abstract

The Crumbs proteins (CRBs) are transmembrane proteins, homologous to *Drosophila* Crumbs, with a key role in defining the apical membrane domain in photoreceptors as well as in embryonic epithelia. Crumbs proteins are conserved between species and their intracellular domains are involved in organizing a conserved macromolecular protein scaffold with important roles in cell polarity as well as morphogenesis and maintenance of the retina. Mutations in the gene encoding human CRB1, the first one identified out of the three human orthologs, have been associated with a number of retinal dystrophies including Leber amaurosis and retinitis pigmentosa type 12. Although no other mammalian Crumbs complex members as of yet have been associated with retinal degeneration, disruption of different zebrafish and fruitfly orthologs can lead to various retinal defects. The core Crumbs complex localizes apical to the outer limiting membrane, where photoreceptors and Müller glia contact each other. Correct functioning of Crumbs ensures adhesion between these cells by an unknown mechanism. This review summarizes the current view on the composition and function of the Crumbs prsotein complex in the mammalian retina. Recently, a number of new members of the Crumbs protein complex have been identified. These include most members of the membrane palmitoylated protein family (MPP), involved in assembly of macromolecular protein complexes. Some components of the complex are found to exert a function in the photoreceptor synapses and/or at the region of the connecting cilium. Studies using polarized cell cultures or model organisms, like *Drosophila* and zebrafish, suggest important links of the Crumbs protein complex to several biological processes in the mammalian eye, including retinal patterning, ciliogenesis and vesicular transport.

Keywords: Crumbs; CRB1; CRB2; CRB3; MPP; MAGUK; retinal degeneration; cell adhesion

1. Introduction

The *Crumbs* gene was initially identified in *Drosophila* and encodes a large transmembrane protein that defines the apical membrane of embryonic epithelial cells (Tepass et al., 1990). Homozygous loss-of-function mutations in *Drosophila crb* cause an almost complete absence of the cuticle of the embryos, leaving visible only a few grains (or crumbs) of cuticle remainder (Tepass et al., 1990). Crumbs plays an important role in the maintenance of apico-basal cell polarity and adherens junctions in embryonic epithelia, that form the adhesion sites where cells connect with each other. In the adult fly retina, Crumbs has a similar function (Izaddoost et al., 2002). For epithelial cells and neurons like photoreceptors, separation of their apical and basal compartments is critical for correct functioning in cell-to-cell adhesion, intercellular signaling, directional transport of (secreted) molecules and correct tissue formation, indicating the processes that are dependent on a correct Crumbs function. The protein was found to organize a macromolecular protein scaffold at the intracellular face of the membrane to exert its function. In this review we describe

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the components of this intracellular Crumbs protein complex and we focus on their (putative) links to different cellular processes in the mammalian retina.

1.1. Crumbs characteristics

As well as in flies, Crumbs molecules are found in many species, ranging from invertebrates to mammals (Richard et al., 2006b). In humans and mice, three genes encoding Crumbs orthologues can be distinguished (respectively human/mouse): *CRB1*/*Crb1*, *CRB2*/*Crb2* and *CRB3*/*Crb3* (Fig. 1). *Crb1* is only expressed in the brain and retina (den Hollander et al., 2002), whereas *CRB2*/*Crb2* is also expressed in the kidney, RPE/choroid and at low levels in heart, lung and placenta (van den Hurk et al., 2005). *Crb3* is ubiquitously expressed in different (epithelial derived) tissues, including the retina (Makarova et al., 2003). In zebrafish, there are up to five Crumbs orthologues, likely due to the whole genome duplication event of teleost fish (Kasahara et al., 2007): crb1, crb2a/ome, crb2b, crb3a and crb3b (Omori and Malicki, 2006).

CRB1/Crb1 (containing respectively 1406/1405 amino acids) and CRB2/Crb2 (1285/1282-aa) comprise of a large extracellular part (1345/1344-aa and 1224/1221-aa) and several epidermal growth factor (EGF)-like repeats (some are predicted to bind calcium) and laminin A globular domain (G)-like repeats, which are also identified in *Drosophila* Crumbs (Fig. 1). Differential splicing of *CRB1*/*Crb1* and *CRB2*/*Crb2* leads to truncated isoforms that lack the transmembrane and intracellular part, and are hypothesized to be secreted, e.g. CRB1b (Fig. 1) (van den Hurk et al., 2005). In mice, an additional short isoform, Crb1s, is detected (Watanabe et al., 2004). CRB3/Crb3 proteins (120/113 aa) lack the large extracellular region, but do contain the transmembrane and intracellular part.

On protein gels, CRBs migrate at a larger molecular weight than predicted, likely due to post-translational modifications. The extracellular domain contains conserved N-linked glycosylation motifs, allowing N-glycosylation of CRB1 and CRB3 (Kantardzhieva et al., 2005; Makarova et al., 2003). An actual function for this decoration with carbohydrates is unknown, although transmembrane proteins generally do not protrude uncovered from the exterior of a eukaryotic cell. Whether or not carbohydrate binding proteins such as lectins have affinity for Crumbs is not known, since no binding partners for the extracellular domain have been identified so far.

The C-terminal intracellular part of the protein contains two protein binding motifs, one that binds FERM domains (first identified as a homologous domain in protein band *4*.1, *e*zrin, *r*adixin and *m*oesin), and a PDZ domain binding motif

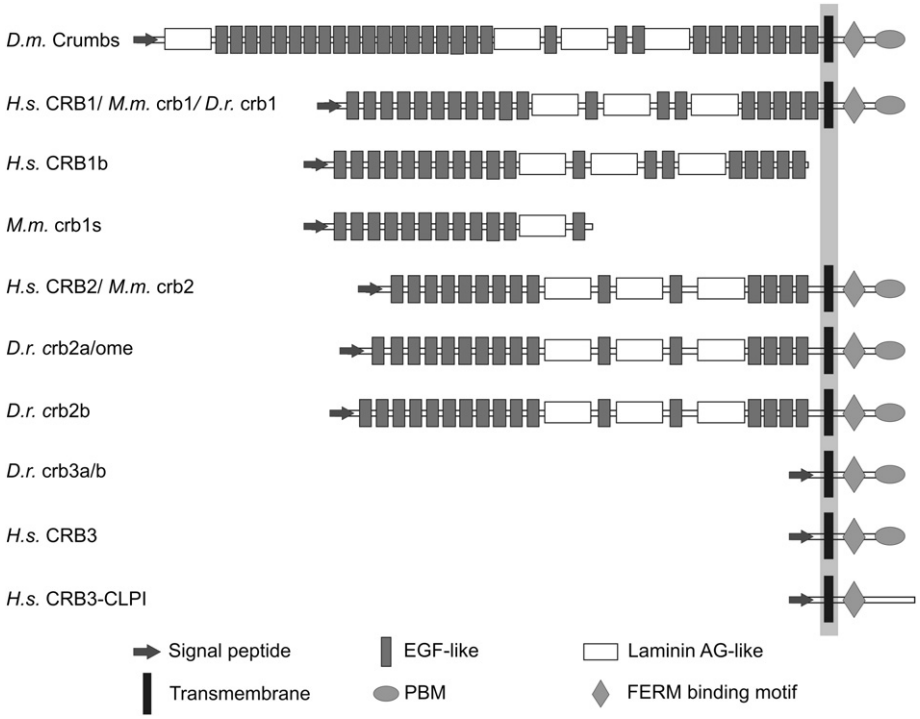

Fig. 1. Crumbs orthologs and paralogs in several species. *Drosophila* (*D.m.*) Crumbs contains a signal peptide, 30 EGF-like domains, four laminin AG-like repeats, a transmembrane (TM) domain, and an intracellular part that contains the conserved FERM binding and PDZ binding motif (PBM). Human (*Homo sapiens*, *H.s.*), mouse (*Mus musculus*, *M.m.*) and zebrafish (*Danio rerio*, *D.r.*) CRB1/crb1 have an identical domain composition of 19 EGF-like repeats, three laminin AG-like repeats, a TM domain and the conserved intracellular motifs. A splice form that lacks the TM and intracellular domain is detected in human (*H.s.* CRB1b) and is predicted to be secreted. In mice, an additional small isoform is detected (crb1s) in skin. Human, mouse and zebrafish CRB2/crb2 all contain a transmembrane and intracellular conserved binding motif, but differ slightly in their extracellular domain composition in comparison to each other and to CRB1. CRB3/crb3 lacks the extracellular EGF-like and laminin AG-like domains, but the intracellular binding motifs are retained. An alternate CRB3 splice form is detected in humans and other mammals including rodents with a divergent C-terminal sequence ending in the amino acids CLPI (CRB3-ELPI).