Seminars in Cell & Developmental Biology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Role of the crumbs proteins in ciliogenesis, cell migration and actin organization

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ARTICLE INFO

Article history:
Received 11 July 2017
Received in revised form 9 October 2017
Accepted 18 October 2017
Available online xxx

Keywords:
Polarity complexes
Cell polarity
Cell migration
Cytoskeleton

ABSTRACT

Epithelial cell organization relies on a set of proteins that interact in an intricate way and which are called polarity complexes. These complexes are involved in the determination of the apico-basal axis and in the positioning and stability of the cell–cell junctions called *adherens* junctions at the apico-lateral border in invertebrates. Among the polarity complexes, two are present at the apical side of epithelial cells. These are the Par complex including aPKC, PAR3 and PAR6 and the Crumbs complex including, CRUMBS, PALS1 and PATJ/MUPP1. These two complexes interact directly and in addition to their already well described functions, they play a role in other cellular processes such as ciliogenesis and polarized cell migration. In this review, we will focus on these aspects that involve the apical Crumbs polarity complex and its relation with the cortical actin cytoskeleton which might provide a more comprehensive hypothesis to explain the many facets of Crumbs cell and tissue properties.

Contents

1. Introduction

Cell polarity is a general feature of living cells, from bacteria to eukaryotes. Overall cell polarity is linked to the necessity to move, to divide or to function directionally. Multicellularity has however introduced an additional level of organization as cell polarity and movements have to be coordinated at the level of the tissue [1]. This is particularly true for metazoans since morphogenetic events such as gastrulation that are essential for morphogenesis, involve coordinated cell movements and coupling of cell forces while keeping the homeostasis of the developing organism [2]. To achieve these complex morphogenetic events, metazoans have developed a new tissue organization with epithelial layers that are made of a single

Abbreviations: AJ, *adherens* junction; TJ, tight junction; aPKC, atypical protein kinase C; CRB, crumbs; DLG, discs large; ECM, extracellular matrix; FERM, 4.1 ezrin radixin moesin; LGL, lethal giant larvae; MAGUK, membrane-associated guanylate kinase; MUPP1, multi PDZ domain protein; Ome, oko meduzy; PALS, protein associated with Lin seven; PAR, partition defective; PATJ, PALS1-associated tight junction protein; PDZ, PSD-95 discs large ZO-1; SCRIB, scribble; Sdt, stardust; SH3, Src homology domain 3.

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https://doi.org/10.1016/j.semcdb.2017.10.018
1084-9521/

sheet of polarized adherent cells. In epithelia, each cell has a polarity which is integrated in a higher order of polarized organization of the tissue. Several years of research have led to define cell polarity in epithelial cells within two axes: The Planar Cell Polarity (PCP) and the Apico-Basal Polarity (ABP).

PCP coordinates in the plane of the epithelium the asymmetric distribution of several cell features, such as actomyosin cytoskeleton organization or cilia positioning, necessary for movement, feeding or sensing (for review see [3]). This polarity relies on a set of proteins called the PCP core complex made of several transmembrane proteins (Flamingo, van Gogh . . .) and adapters such as Prickle or Disheveled (for review see [4]).

The other polarity system is the one that defines the ABP within epithelial cells. ABP is based on the formation of a free cell surface in contact with the external medium (the apical side), cell–cell contacts in the lateral domain and a basal side that lies most often on a basement membrane, opposite the apical side. The apical side is separated from the lateral domain by a set of specialized cell–cell junctions, which preserve the organism homeostasis (for review see [5]). The integrity of the cell layers, in vertebrates, is mediated by the physical coupling of the cells through different sets of junctions, namely tight junctions, *adherens* junctions, and desmosomes [6]. Apical and basolateral membranes are characterized by the presence of protein and lipid markers such as channels, transporters or enzymes linked to the function of these membranes. While these proteins or lipids are usually strongly associated to a specific polarized domain most of them do not play an instrumental role in the establishment or maintenance of a polarized epithelium. Only a set of few proteins or lipids has been identified to play a role in establishing and/or maintaining epithelial ABP and organization [7,8]. The first set of genes involved was discovered using the *Caenorabditis elegans* model and genetic screens that identified Par proteins (for partitioning defective) including the Par3/Par6/aPKC (atypical protein kinase C) apical complex and the lateral Par1/Par4 complex [9,10]. For the polarity to be established, the Par6/Par3/aPKC and Par1 mutually exclude each other through antagonistic phosphorylation. This will actively drive the segregation of the Par polarity protein into their respective apical and basolateral domains [11]. Once the polarity established, these complexes regulate the actin cytoskeleton and the endocytosis providing thus a mean to maintain distinct apico-basal cortical and membrane subdomains [12]. Another complex involved in ABP is the lateral Scribble complex identified in flies [13] and made of Scribble, Discs large (Dlg) and Lethal giant larvae (Lgl) (for review see [14]). This complex is involved in vesicular trafficking and cell proliferation (for review see [15]).

In addition to these cortical or cytoplasmic complexes, a membrane anchored complex is formed by Crumbs, an apical transmembrane protein [16], stardust (PALS1,Protein Associated to Lin Seven, in mammals), an adaptor of the MAGUK (Membrane Associated GUanylate Kinase) family [17,18] and Patj (PALS1-Associated TJ protein), a protein containing multi PDZ (PSD-95, Discs large, ZO-1) domains [19,20]. This was the first core Crumbs complex identified and later it was shown in vertebrate that CRUMBS itself can bind directly to PAR6 [21] and that in Drosophila aPKC phosphorylates Crumbs cytoplasmic tail [22] suggesting that they might form another complex together. Moreover, it was shown that Stardust/PALS1, PATJ and Par6 also interact together [23,24], blurring the distinction between two distinct Crumbs complexes. The core Crumbs complex is involved in the regulation of the cortical actin cytoskeleton [25], the stabilization of AJs [26], vesicular trafficking [27] and cell proliferation [28,29]. For a more detailed description and functional analysis of the Crumbs complexes we suggest several recent reviews [27,30]. In this review, we will focus on the role of the Crumbs complex in less explored functions or in fast moving aspects of its cell biology.

2. Crumbs complex and ciliogenesis

Cilia are extensions of the apical surface of most quiescent and differentiated cells (for review [31]). In most cases, primary ciliogenesis begins by the gathering of small vesicles originated from the Golgi apparatus that reach the activated mother centriole using a polarized endosomal trafficking [32]. Fusion of these vesicles produces a membranous cap called the ciliary vesicle at the distal tip of centriole. From this distal tip, microtubules grow in a polarized manner under the cap that jointly increases due to the addition of membrane. This nascent axoneme is therefore inserted in a double membrane which fuses with the apical plasma membrane during the emergence of the cilium. In epithelial cells, however, cilia grow directly by extension of the apical membrane around the axoneme (for review see [33]). Like all organelles, the cilium is maintained by polarized vesicular traffic within the cell and along the axonemal microtubule network, with the specific molecular intraflagellar transport machinery [34].

Crumbs proteins and the polarity Par complex that specify apical identity have been involved in epithelial ciliogenesis (Fig. 1). The first Crumbs involved in ciliogenesis was CRB3 and in mammals, the *CRB3* gene codes by alternative splicing for two isoforms: CRB3A with the canonical COOH-terminal ERLI motif and CRB3B with a COOH-terminal CLPI motif. These two isoforms are localized in cilia of MDCK cells (Madin Darby Canine Kidney cells) and are involved in its formation [35,36]. This is also the case for the polarity Par complex (PAR6, PAR3 and aPKC) which co-localizes to the primary cilium in the same cells and it has been proposed that CRB3A and the Par complex interact in the cilium [36]. Previously, we have identified an interaction between CRB3A and PAR6α *via* the PDZ binding domain (ERLI) of CRB3A and the PDZ domain of PAR6α [21] thus providing a direct link between these two complexes involved in ciliogenesis. While CRB3A is involved in the initiation of ciliogenesis, PAR3 (linked to KIF3A/kinesin2/microtubules) seems to participate to the anterograde vesicular transport for the elongation of primary cilia [37] suggesting that CRB3A is required for the delivery of the Par complex to the cilium and acts upstream of it. It is interesting to mention that PAR6γ is also present at the centrosome suggesting that it could act earlier in ciliogenesis than proposed by organizing the pericentriolar domain [38]. CRB3B (also called CRB3-CLPI) does not interact with the Par complex but its targeting to the cilium is mediated by importin β-1, a nuclear import protein, that is essential for cytokinesis but also for ciliogenesis [35]. Despite the fact that CRB3A or B have been involved in ciliogenesis a decade ago the molecular mechanisms at work remain unclear. In addition to be involved in primary ciliogenesis, CRB3 is necessary for the multiciliated airway cell differentiation [39] but a direct role of the Crumbs proteins in multiciliogenesis has not been demonstrated yet. It must be however noted that CRB2B (one of the CRB2 proteins in zebrafish) accumulates at the basis of the ciliary tuft in pronephric cells and that CRB2B knock-down induced a strong reduction in cilium length indicating that it plays a role in the formation or maintenance of cilia in multiciliated cells [40].

Some cells possess cilia with specialized sensory functions and it is the case for retina photoreceptors which bear inner and outer segments on their apical side (Fig. 1). This specialized cilium is in constant renewal throughout life while photoreceptors are not renewable. Several studies from flies to man have shown that Crumbs proteins are essential for proper photoreceptor morphogenesis and survival [41–43] and are involved in the building of this specialized structure both in the zebrafish and in mammals. In zebrafish, CRB2A is expressed in the inner segments of all types of photoreceptors and in the apical domain of Müller cells whereas CRB2B (also called Oko meduzy or Ome) [40] is mainly expressed in the inner segments of green, red and blue cones [44]. CRB2A is involved in the regulation of the inner segment size as over-

Please cite this article in press as: E. Bazellières, et al., Role of the crumbs proteins in ciliogenesis, cell migration and actin organization, Semin Cell Dev Biol (2017), https://doi.org/10.1016/j.semcdb.2017.10.018