HHS Public Access

Author manuscript

Gene Expr Patterns. Author manuscript; available in PMC 2016 September 22.

Published in final edited form as:

Gene Expr Patterns. 2015 January ; 17(1): 31–37. doi:10.1016/j.gep.2015.01.002.

Common and distinctive localization patterns of Crumbs polarity complex proteins in the mammalian eye

Jin Young Kim[1], **Ji Yun Song**[1], **Santi Karnam**, **Jun Young Park**, **Jamie JH Lee**, **Seonhee Kim**, and **Seo-Hee Cho**[*]

Shriners Hospitals Pediatric Research Center and Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140, United States

Abstract

Crumbs polarity complex proteins are essential for cellular and tissue polarity, and for adhesion of epithelial cells. In epithelial tissues deletion of any of three core proteins disrupts localization of the other proteins, indicating structural and functional interdependence among core components. Despite previous studies of function and co-localization that illustrated the properties that these proteins share, it is not known whether an individual component of the complex plays a distinct role in a unique cellular and developmental context. In order to investigate this question, we primarily used confocal imaging to determine the expression and subcellular localization of the core Crumbs polarity complex proteins during ocular development. Here we show that in developing ocular tissues core Crumbs polarity complex proteins, Crb, Pals1 and Patj, generally appear in an overlapping pattern with some exceptions. All three core complex proteins localize to the apical junction of the retinal and lens epithelia. Pals1 is also localized in the Golgi of the retinal cells and Patj localizes to the nuclei of the apically located subset of progenitor cells. These findings suggest that core Crumbs polarity complex proteins exert common and independent functions depending on cellular context.

Keywords

Crumbs polarity complex; Pals1; Crumbs; Patj; retina; lens

INTRODUCTION

The establishment and maintenance of epithelial cellular polarity is essential for the development and proper functioning of multicellular organisms. Epithelial cell polarity is

[*]Correspondence author: Shriners Hospitals Pediatric Research Center, Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140, USA. Tel.: +1 (215) 926 9361; Fax: +1 (215) 926-9325. seo.hee.cho@temple.edu (S.-H. Cho).

[1]These authors contributed equally to this work.

CONFLICT OF INTEREST STATEMENT

None

determined by the segregation of the apical and basolateral domains along the plasma membrane. These domains are differentiated by the localization of specific membrane-associated protein clusters known as polarity complexes. Three polarity complexes are critical in establishing apical and basolateral polarity: the Scribble complex, which is composed of Scribble, Dlg and Lgl, forms the basolateral domain; the Par complex, which consists of Par6, Par3 and atypical PKC (aPKC), and the Crumbs complex, which consists of Crb, Pals1 and Patj, localize to the apical domain and help to establish junctions between neighboring cells (Bazzoun et al., 2013; Bulgakova and Knust, 2009; Elsum et al., 2012; Gosens et al., 2008; Knust and Bossinger, 2002; Macara, 2004; Nguyen et al., 2005; Rodriguez-Boulan and Macara, 2014).

In the developing retina, core Crumbs complex proteins localize at the apical side of the epithelium, which will ultimately construct the adhesive belt formed between photoreceptor cells and Muller glia located in outer limiting membrane (OLM). Interactions among core Crumbs polarity proteins are also essential for other properties, including polarization of the epithelium, laminar organization and cell survival (Alves et al., 2014a; Alves et al., 2014b; Alves et al., 2013; Cho et al., 2012). Crb1 is a transmembrane protein with a large extracellular portion composed of EGF-like and Laminin A/G-like domains which allow for homophilic interactions with other cells (Fig. 1; (Thompson et al., 2013)). It also harbors a short, but essential intracellular domain that contains a FERM binding motif for interaction with EPB4.1L5 and an extreme C-terminal PDZ-binding motif responsible for its critical interaction with the PDZ domain of Pals1 (Alves et al., 2014a). Protein associated with lin seven 1 (Pals1/MPP5) is a MAGUK which contains, from N- to C- terminus: an evolutionarily conserved region 1 (ECR1) for association with Par6-aPKC, two tandem L27 domains for interaction with Patj and Lin7 respectively, a PDZ domain, and SH3 and GUK domains (Bit-Avragim et al., 2008a; Hurd et al., 2003; Kamberov et al., 2000). Finally, the Pasl1-associated tight junction protein (Patj/Mupp1) contains an N-terminal L27 domain and ten tandem PDZ domains, which allow for its interaction with multiple partners, including ZO-3 and Claudin1 (Adachi et al., 2009; Roh et al., 2002). In humans, Crb1 mutations induce retinal degeneration with early onset in Leber Congenital Amaurosis 8 and with late onset in Retinitis Pigmentosa 12 (RP12). Importantly, certain features of the human phenotypes are phenocopied in mouse mutants of Crb1, Crb2 and Pals1 (Alves et al., 2014a; Alves et al., 2013; Cho et al., 2012; Park et al., 2011; Pellissier et al., 2013). These features include focal laminar disorganization, pseudorosette formation, photoreceptor cellular degeneration and functional impairment determined by electroretinography.

Although Crb-Pals1-Patj is known to form the tripartite core Crumbs polarity complex in mammalian cells, it is not known whether each component has independent functions that do not rely on binding partners. In developing *Drosophila* photoreceptor cells, Crumbs and Stardust (Pals1 ortholog) are suggested to function independently and in a cell-type specific manner (Hwa and Clandinin, 2012). In addition, *Drosophila* Patj (Dpatj) is not required in all the epithelial cells for which Crumbs complex is essential (Penalva and Mirouse, 2012). Therefore, it is plausible that each component may participate in complex formation in specific cellular contexts while also engaging in complex-independent functions alone or by binding with other unknown partners. This versatility can increase the usage of a protein. For example, in the zebrafish mutant of Pals1, Nagie Oko, Pals1 was shown to be localized

in the nucleus using its two putative conserved nuclear localization signals and association with Par6-aPKC through its ECR1 (Bit-Avragim et al., 2008b). In addition, deletion of Pals1, which is expressed in the apical villi of retinal pigmented epithelium (RPE), induces abnormal formation of RPE, a phenotype not reported in Crb1 mutant, as well as retinal architectural disorganization (Park et al., 2011). Furthermore, mouse Crb3 is expressed not only in the apical junction of the retinal epithelia, but also in the connecting cilium and plexiform layers of the developing and mature retina (Herranz-Martin et al., 2012). CRB1 is expressed in the human cornea and enriched in the nucleus (Beyer et al., 2010). Increasing evidence suggests the functional and structural versatility of the Crb complex in orchestrating cell-contact dependent and/or independent roles in development. In this study we extensively investigated the expression and localization of Crumbs polarity complex proteins during and after the development of ocular tissues, including retina and lens. In addition to confirming their localization at the apical junction, our results suggest that core Crumbs polarity complex proteins play roles in a limited number of dividing cells and that they are intricately regulated in the Golgi of retinal cells.

RESULTS AND DISCUSSION

Expression of Crb polarity complex proteins shows overlapping and distinct patterns in early developing ocular tissues

In developing optic cup, Crb polarity complex gene transcripts are uniformly expressed in retinal progenitor cells whereas the proteins are specifically localized at the apical surface of the epithelia (Fig. 2A – C; (Alves et al., 2014a; Alves et al., 2013; Cho et al., 2012; den Hollander et al., 2002; Zou et al., 2013)). In developing lens, Crb polarity complex proteins show similar apical localization at the interface of the lens epithelium (LE) and fiber cells (LF) (Fig. 2A – C ; Song et al., 2014). This apical localization is essential for maintaining the polarity of the epithelia and adhesion among retinal and lens cells. Therefore, retinas with deficient Pals1 or Crb1, Crb2 and Crb1/Crb2 display laminar disorganization, retinal folding and pseudorosette formation (Alves et al., 2014a; Alves et al., 2013; Cho et al., 2012; Park et al., 2011; Pellissier et al., 2014; Zou et al., 2013). The specificity of the antibodies used in the present study was previously demonstrated. For example, Pals1 signal was specifically abolished or disrupted in the retinal region where Pals1 gene was deleted. Similarly, pan-Crb staining was specifically disrupted or disorganized in the area where Pals1 was ablated (Cho, 2012 #17). Intriguingly, careful examination of Patj antibody staining revealed non-apical expression in the nuclei of the subset of apically located retinal cells (Fig. 2C). This pattern was observed in retinas from early embryonic to neonatal stages (Fig. 2D – E2). The nuclei of the Patj (+) cells were typically condensed, a pattern that might represent the subset of M-phase cells undergoing cell division. In order to test this possibility, double antibody staining using Patj and an M-phase marker, MPM-2, was performed. As shown in Fig. 2F – H3, Patj staining, which is typically weak and punctate, overlapped with that of MPM-2 in prometaphase (Fig. 2F – F3) and metaphase (Fig. 2G – G3) cells, but weakened in late anaphase and telophase cells (Fig. 2H - H3). A small number of cells in RPE and surrounding periocular mesenchyme were also Patj (+) (Fig. 2C green arrows), and Crb polarity complex proteins showed a pattern of limited differential expression in corneal epithelium. Unlike Pals1 and Patj, pan-Crb antibody did not detect

significant levels of the signal in corneal or adjacent surface epithelia (Fig. 2A – C). These findings from the expression survey of the early optic cup, including Patj expression in putative mitotic cells and differential expression of Crb complex proteins in corneal cells, are consistent with complex-independent function during retinal development.

Pals1 is localized in Golgi of retinal cells

In order to determine whether Pals1 also localizes in the non-apical region of the retina, immunofluorescence assay was performed on retinal sections from the mid-embryonic stage to P21, when primary development is completed. Consistent with previous findings (Cho et al., 2012; Park et al., 2011), Pals1 was consistently localized in the apical surface of the developing and neonatal retinal epithelia (Fig. 3A - D). Within the apical surface of the retinal epithelia, Pals1 staining partially overlapped with that of adherens junction proteins such as N-cadherin (Fig. 3E - E2) and of Par apical polarity complex proteins such as aPKC (Fig. 3F - F2). However, retinal Pals1 staining was clearly below the apical surface of RPE (Huang et al., 2009), as shown in Fig. 3G - G2 Additionally, widespread, punctate staining of Pals1 was observed in the inner retina at E13.5 (Fig. 3A, arrows). This pattern of non-apical Pals1 staining continued to be prominent at later embryonic stages such as E16.5 and E19.5 (Fig. 3B & C) and was maintained after the completion of retinal development (Fig. 3D). Co-localization with nuclear Hoechst staining suggested that Pals1 staining was limited to regions around the nuclei of most retinal cells in the outer and inner neuroblastic layers (ONBL and INBL) (Fig. 3H - H2).

This pattern of scattered, widespread, punctate staining in the majority of retinal cells, including both ONBL and INBL, suggests that Pals1 might be localized to common, ubiquitous cellular organelles. One such candidate is the Golgi complex, which modifies, sorts and packages proteins for secretion and/or delivery to the plasma membrane (Conibear and Davis, 2010; Rocks et al., 2010; Salaun et al., 2010). One plausible mechanism by which the Golgi might modify Pals1 is protein palmitoylation. MPP1, a prototypical MPP family protein, was recently shown to be regulated by protein palmitoylation, which is essential for lateral membrane organization of erythrocytes (Biernatowska et al., 2013; Lach et al., 2012). Whether Pals1 is regulated by protein palmitoylation has not been investigated, but MPP1 and MPP5 share high similarity among MAGUK family proteins (de Mendoza et al., 2010). If Pals1 is regulated by palmitoylation similarly to MPP1, the process is likely to occur in the Golgi, the principal site for palmitoylation (Rocks et al., 2010). In order to determine whether the punctate Pals1 staining pattern in developing retinas represents localization in the Golgi, we tested for co-localization with the Golgi marker, GM130, at E14.5 and P1, when non-apical localization of Pals1 is evident. We used two antibodies raised against different regions of Pals1. Consistent with the prediction, most of the GM130 (+) signal co-localized with Pals1 (+) signal, indicating specific Golgi localization, when Pals1 antibody (07-708; 1:200; Millipore) was used (Fig. 4A - C1). In addition to the Pals1 antibody used throughout the study, which was raised with synthetic peptide (amino acids 450 - 461 of human Pals1), another Pals1 (N) antibody produced using N-terminal peptide was tested (See Materials and Methods). Pals1 (N) antibody showed strong staining at the apical surface and in post-mitotic retinal cells in the inner neuroblastic layer, and most cellular Pals1 signal overlapped with that of GM130 (Fig. 4D-F1), further supporting the

notion of Pals1 localization in the Golgi. During the 1st and 2nd postnatal weeks, Golgi of photoreceptor cells was concentrated subjacent to the connecting cilia, where Pals1 was localized (Fig. 4G - H2). Thus, it is likely that Pals1 is localized in two locations in adult retina, at the OLM and around connecting cilia.

In order to investigate whether Golgi-specific localization of Pals1 is a general feature in epithelial cells, we examined MCF7, a breast cancer cell line with characteristic epithelial morphology in culture. As shown in Fig. 4I - K, Pals1 was localized in GM130 (+) Golgi, in addition to apical junctions. Similar to the co-localization that we observed in retinal sections, two different Pals1 antibodies showed Golgi-enriched localization in MCF7 cells. Pals1 staining was evident in the Golgi where cells were not densely populated (Fig. 4L-N), whereas Pals1 preferentially localized at the apical junction when MCF7 cells were highly confluent (Fig. 4I-K and result not shown). Pals1 was similarly localized in the Golgi of subsets of cultured embryonic cortical neurons (Fig. 4O - Q). Although the functional significance of Pals1 localization in Golgi is unclear, a similar result was previously observed in other cells: PALS1 was recruited to the Golgi of Vero E6 epithelial cells upon viral infection (Teoh et al., 2010) and normally distributed in the Golgi of human T lymphocytes (Carvalho et al., 2011). Our findings are further validated by the fact that we found similar results with multiple Pals1 antibodies that recognize different regions of the protein and in multiple cell types (retinal cells, MCF7, dissociated cortical cells).

Crb and Patj are limited to the apical junction of the retinal epithelia

In order to investigate whether other core Crumbs polarity complex proteins, Crumbs homologs and Patj, are localized in the Golgi and/or nuclei of ocular cells, we performed immunofluorescence staining on retinal sections from embryonic to post-developmental stages. As shown in Fig. 5A – F, both Crumbs homologs and Patj were mainly detected at the apical junction of retinal epithelia; this pattern remained after the completion of retinal development. Golgi-specific localization was not detected in the stages examined. Nucleus-specific Patj staining was detectable at the apical surface of the retinal epithelium, but staining was weaker at P0 (Fig. 2E – E2), and undetectable at later stages. Of note, the nuclear staining seen in Fig. 5E and F is likely to be artifactual and caused by extensive antigen retrieval. This conclusion is supported by the frequent presence of nuclear signal in retinal and other tissues after extensive antigen retrieval over 30 min periods regardless of the antibody (result not shown).

Crb polarity complex proteins are expressed in LE of the developing lens

Next, we determined the expression and localization of the three core Crumbs polarity complex proteins in developing lens. We expected that Pals1 would show apical-junctional and Golgi-specific localization, whereas Crb and Patj would show only apical-junctional localization. Consistent with our prediction, all the Crumbs polarity complex proteins, Pals1, Crbs and Patj, were predominantly localized at the apical border of LE and LF, whereas Pals1 showed additional localization in the subapical area (Fig. 6A – C). This non-apical staining pattern is reminiscent of Golgi staining in retinal cells (Fig. 4). In order to test whether subapical staining of Pals1 corresponds to Golgi, we carried out double antibody staining with Pals1 and GM130. Nearly all regions of Pals1 (+) staining were also GM130

(+) although Pals1 staining was often weak and sometimes not as discrete as that of GM130 (Fig. 6D – D2). This result suggests that, as in retinal cells, Pals1 is localized in the Golgi of developing lens. Other apically localizing proteins such as Par3 and ZO-1, which localize at the border between LE and LF during lens development, are shown in parallel for comparison (Fig. 6E – F).

Our extensive study of Crb polarity complex proteins not only confirms their apical junctional localization in multiple cells of epithelial origin, including retina, lens and cells originating in mammary gland, but also reveals novel expression and localization. For example, Patj was unexpectedly observed in subsets of early embryonic retinal progenitor cells undergoing cell division. Within mitotic phases, Patj was preferentially enriched in the nucleus during prometaphase, metaphase and early anaphase. In *Drosophila* Crumb has recently been reported to be engaged in nuclear division through an interaction with spindle-associated complex (Yeom et al., 2014). Although we have not detected its localization in mitotic spindles, Patj localization in the nucleus during selective mitotic phases would be consistent with a role for Crumbs complex proteins in mitotic progression. The zebrafish ortholog of Pals1 *nagie oko* was shown to shuttle between the cytoplasm and nucleus via its putative nuclear export and localization signals (Bit-Avragim et al., 2008b); similar studies have not been performed in mammalian systems. Nuclear localization of Patj in subsets of mitotic cells, if proven, may provide further support for a nuclear function of Crb polarity complex.

Our study also identifies novel localization of Pals1 in the Golgi. It is difficult to determine whether Golgi accumulation of Pals1 represents a cryptic role in Golgi, either functionally or structurally. Alternatively, it may simply represent the intermediate retention of Pals1 in Golgi for protein maturation by fatty acid modification. Pals1 was initially named MPP5 due to its structural similarity to MPP1, a prototypical palmitoylated membrane protein. Although the question has not been studied, Pals1 may be similarly regulated by palmitoylation enzymes known as protein-acyl transferases and protein palmitoyl thioesterases (Blaskovic et al., 2013; Charollais and Van Der Goot, 2009), which belong to the DHHC family (Korycka et al., 2012; Mitchell et al., 2006). Protein palmitoylation on cysteine residues, a reversible posttranslational modification essential for membrane organization, mainly occurs in the ER/Golgi (Aicart-Ramos et al., 2011; Salaun et al., 2010). These findings would be consistent with a diversified function or regulation of Crumbs polarity complex proteins, independent of complex formation around the tight junction of epithelial cells. Intriguingly, our study identified Golgi accumulation of Pals1 in developing retina and lens, dissociated cortical cells and a mammary gland cell line, but not in other CNS tissues, including cerebral cortex and cerebellum (data not shown). This result may reflect differences in rates of Pals1 palmitoylation in different cells but could also be interpreted as tissue-specific modification of Pals1 by palmitoylation. This question warrants further analysis in various tissues.

In summary, our results reveal unconventional sites in which Patj and Pals1 have been localized by reporting their appearance in a distinct subcellular compartment in a subset of retinal cells. Crumbs core complex proteins at the apical junction are essential in establishing apicobasal polarity and adhesion in many epithelial tissues, including retina.

Differential subcellular localization suggests a distinct requirement for additional modification or interaction with other components or even a completely independent function, and provides new insights into the regulatory mechanism utilized by polarity complex proteins.

MATERIALS AND METHODS

Animals and cell culture

Animal handling and housing were approved by the Temple University Institutional Animal Care and Use Committee and conducted in accordance with their guidelines. Embryonic and postnatal retinas from C57BL/6J and Swiss Webster (SW) mice (Taconic Farms and Jackson laboratory) were used after timed mating. In order to avoid complication due to the presence of the rd1 allele, which is frequently present in wild type mice, including SW, we excluded retinal sections from mice older than P10 showing retinal degeneration (thinning of outer nuclear layer). In order to avoid false-positive results, at least 3 independent retinas were examined for each antibody using sections from multiple developmental stages. MCF7 cells (**86012803,** Sigma-Aldrich) were cultured in DMEM culture media containing 10% fetal bovine serum. For immunofluorescence MCF7 cells were plated on chamber slides (177445, Nunc) and cultured 3 - 7 days to achieve intermediate to high confluency. Mid-embryonic cortical cells at E16.5 were dissociated using papain and cultured for 18 hours in neurobasal media containing B27 supplement.

Immunohistochemistry and imaging

Enucleated eyes of postnatal mice or the entire heads of embryonic mice were fixed with 4% paraformaldehyde (PFA), dehydrated, and embedded in paraffin, and 7-um sections were prepared with a microtome (Leica RM2245, Germany). Slides were subjected to extensive antigen retrieval in 10mM sodium citrate using a microwave for 30 minutes. After several washes with PBS, primary antibody was incubated overnight at 4°C. After 3 washes with PBS, slides were incubated with the secondary antibodies for at least 3 hours at room temperature (RT). For cultured cell staining, cells were fixed with 4% PFA for 15 minutes and then incubated in 1% sodium dodecyl sulfate (SDS) for 10 minutes at RT. After washing off SDS with PBS containing 0.02% Tween20 (PBST), primary antibody was added and incubated overnight at 4°C. After washing with PBST, cells were incubated with secondary antibody (1:500) for 3 hours at RT. Nuclear counter-staining was done by incubating in Hoechst33342 solution (Trihydrochloride; H21492; 1:1000; 10mg/ml; Life Technologies). Images were taken using an Axioplan 2 imaging microscope (Carl Zeiss Microimaging GmbH, Germany) or a confocal microscope (TCS SP8, Leica Microsystems GmbH, Germany and Nikon Eclipse Ti). For co-localization assays and most confocal imaging, sequential scanning with 3 different channels was applied to avoid crosstalk of signals from fluorochrome conjugates of the secondary antibodies. Some of the images were pseudocolored to red or green after image acquisition to maintain consistency.

Antibodies

Primary antibodies used in this study were: aPKC (610207; 1:200; BD), ezrin (3C12) (ab4069; 1:200; Abcam), GM130 (610822; 1:200; BD), N-cadherin (610920; 1:200; BD),

MPM-2 (05-368; 1: 200; Millipore), pan-Crb (1:200; produced in the lab), Pals1 (amino acids 450 – 461 of human Pals1 (YNANKNDDYDNE) ; 07-708; 1:200; Millipore), Pals1 (N) (rabbit; previously produced using synthetic N-terminal peptides (amino acids 11-23 of mouse Pals1 (TEESDSGIKNLDL); 1: 200; (Chae et al., 2004)), Par3 (07330; 1:200; Millipore), Patj (gift of Dr. le Bivic), ZO-1 (610966; 1:200; BD). The Pals1 antibody from Millipore was used for most of the studies, except for some of the co-localization experiments shown in Fig. 4. Pan-Crb antibodies were produced using an oligopeptide (20 amino acids) common to all three forms of Crb, Crb1, 2 and 3 (Makarova et al., 2003). Therefore, pan-Crb staining represents the sum of all three Crb isoforms. Secondary antibodies were: Alexa Fluor 488 conjugated to goat anti-mouse or anti-rabbit (A11001, 1:200; Life technologies or A11008, 1:200; Life technologies) and Cy3 conjugated to goat anti-mouse or anti-rabbit (115-165-146; 1:300; Jackson ImmunoResearch, 111-165-045; 1:300; Jackson ImmunoResearch).

Acknowledgments

We thank Dr. le Bivic for providing Patj antibody. This work was supported by National Institute of Health Grant EY020578 (S.-H.C) and NS073112 (S.K.), Curing Retinal Blindness Foundation Grant (S.-H.C.) and by a Research Grant from Shriners Hospitals for Children (S.-H.C. and S.K.).

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HIGHLIGHTS

- Core Crumbs polarity complex proteins display common and distinct localization patterns during eye development
- Patj localizes in the nucleus of a subset of retinal progenitor cells.
- Pals1 localizes in the Golgi of retinal cells.

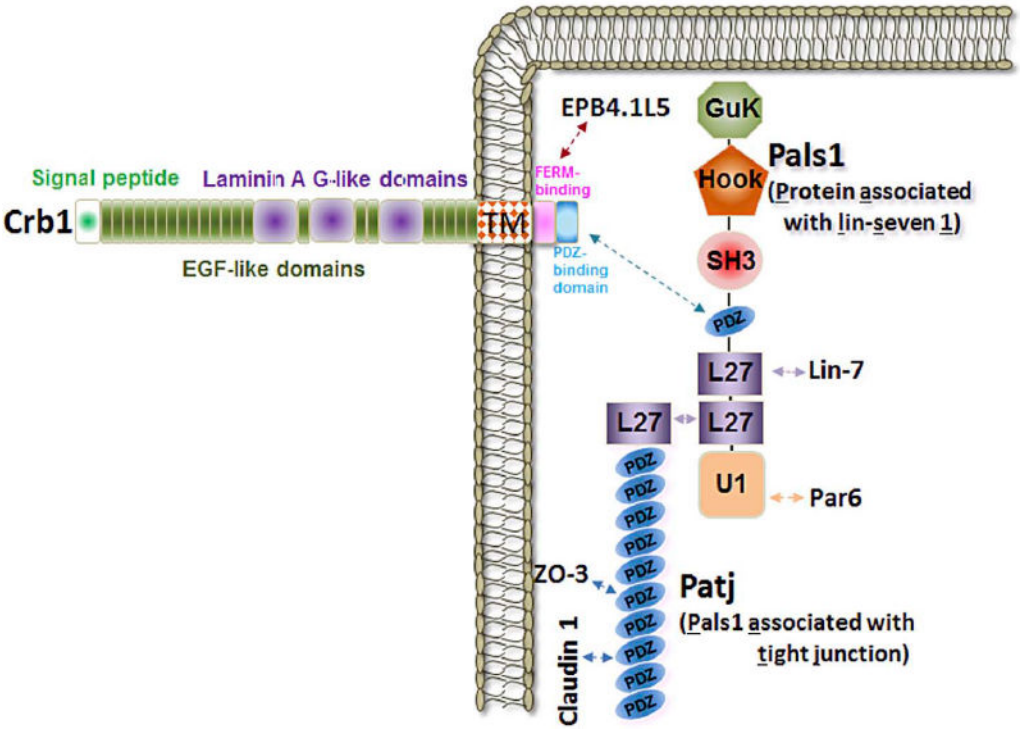

Fig. 1.

Schematic showing domain structure of Crumbs polarity complex proteins and binding partners. Apical corner region of epithelial cells is shown (see text for detail).

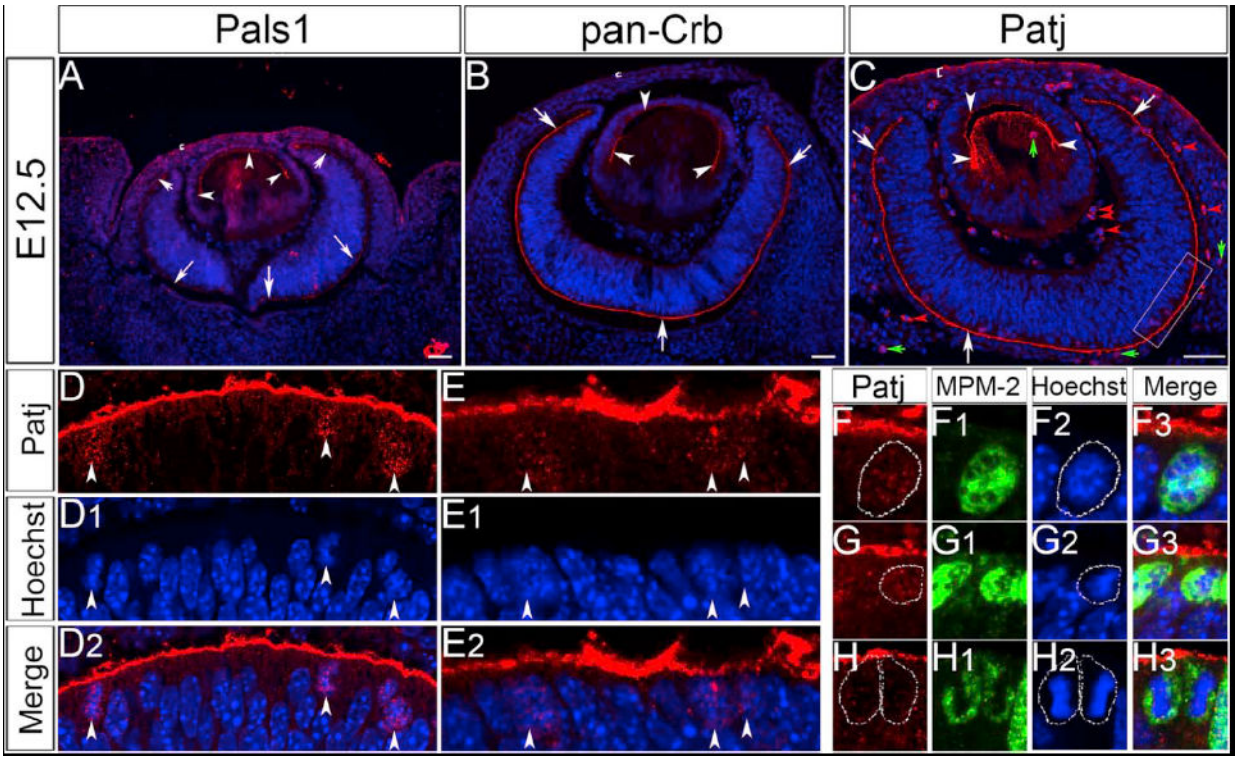

Fig. 2.

Localization of Crb polarity complex proteins in the developing optic cup. (A – E2) Pals1 (A), pan-Crb (B) and Patj (C) are localized at the apical surface (arrows) of optic cup and lens cells (arrowheads) at E12.5. Additionally, Patj is present in the nucleus of subsets of apically localizing cells (C - E (arrowheads)). Patj (+) non-retinal cells in RPE, lens and periocular mesenchyme are marked with green arrows (C). Corneal epithelia showing differential expression of Crb polarity complex proteins and immune cells (C) showing cytoplasmic autofluorescence are marked (brackets and red arrowheads, respectively). Insets in C; D – D2. Nuclear, weak Patj staining is also observed in P0 retina (E – E2). (F – H3) Double antibody staining with Patj and MPM-2 shows co-localization in subsets of mitotic cells (F & G (P0) and H (E12.5)). Hoechst 33342 was used for nuclear counter staining (Blue). Scale bars; 100um.

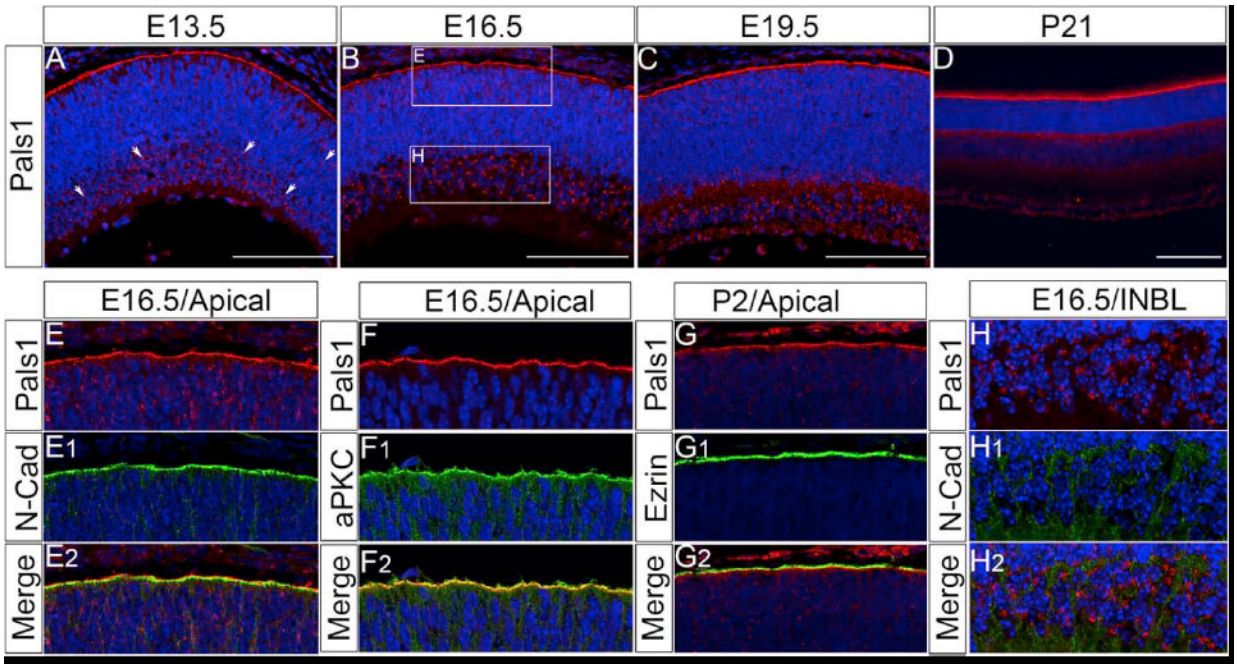

Fig. 3.

Pals1 is localized in the cytoplasm of retinal cells as well as in apical junctions throughout the developmental and postnatal stages examined. (A - D) Expression and localization of Pals1 in developing (E13.5 (A), E16.5 (B) and E19.5 (C)) and postnatal retinas (P21 (D)). While Pals1 is mainly localized at the apical surface of the retina at all the stages examined (A - G2), it partially co-localizes with adherens junction proteins such as N-cadherin (E - E2). Pals1 also partially co-localizes with aPKC, a component of Par complex (F - F2), but minimally overlaps with a marker for apical villi of RPE, ezrin (G - G2). Pals1 is also detected in inner parts of the retina, adjacent to the nuclei (A, arrows, B - D, H - H2). Hoechst 33342 was used for nuclear counter staining (Blue). Scale bars; 100um.

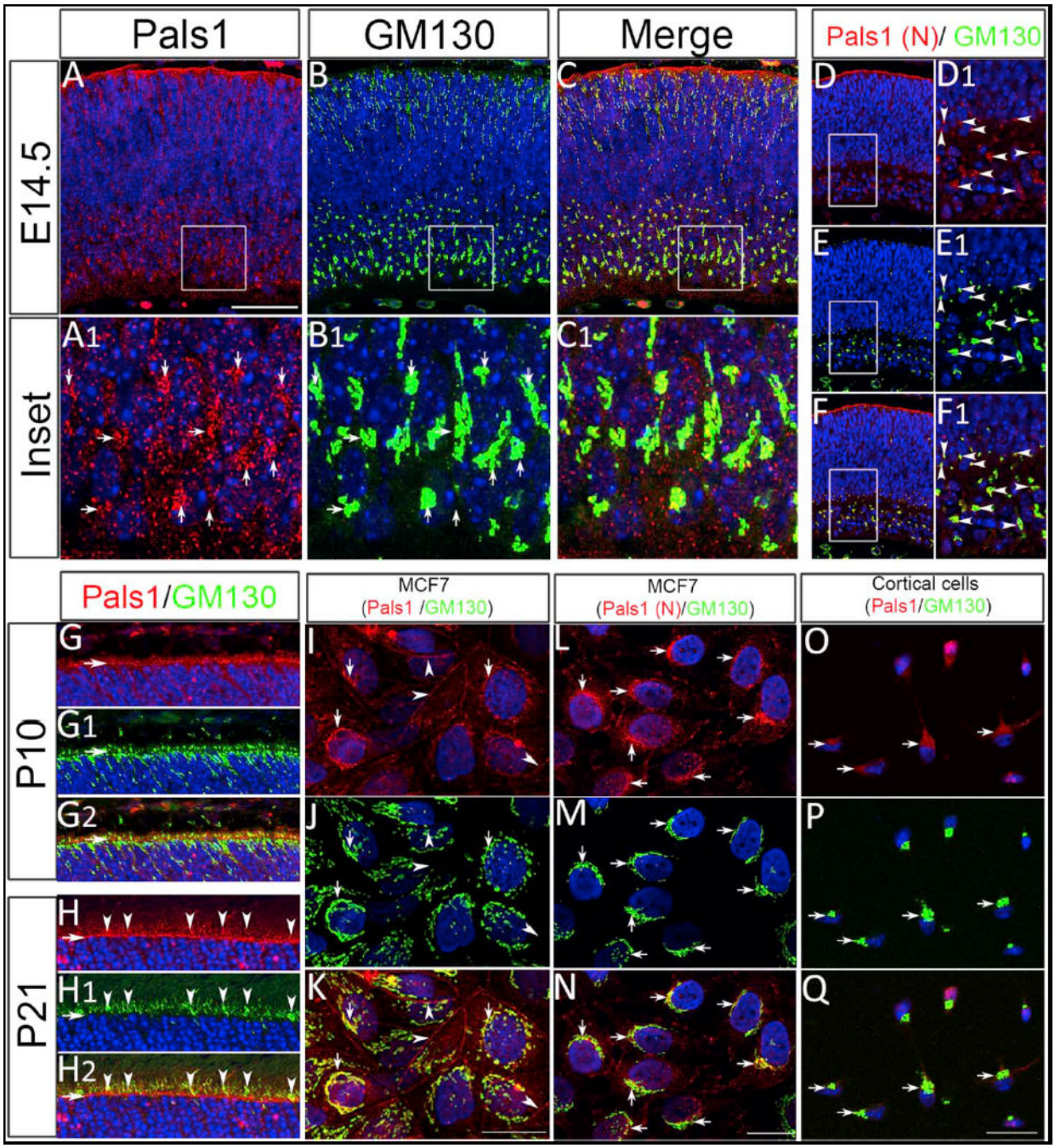

Fig. 4.

Pals1 is localized in the Golgi of developing retina, MCF7 and cultured cortical cells. Non-apical Pals1 staining coincides with that of Golgi marker, GM130 at E14.5 (A - C1) and P1 (D – F1) when two independent Pals1 antibodies were used. Pals1 staining also partially overlaps with that of GM130 when the Golgi of photoreceptors is concentrated at the connecting cilia at P10 (G – G2) and P21 (H – H2). In MCF7 cells, Pals1 cytoplasmic staining (arrows) overlaps with that of GM130; distinct apical Pals1 staining (arrowheads) is apparent in high-density area (I – N and results not shown). In dissociated embryonic

cortical cells, Pals1 staining overlaps with that of GM130 (O – Q). Only a subset of cortical cells shows nearly complete co-localization of Pals1 with the Golgi marker (arrows). Hoechst 33342 was used for nuclear counter staining (Blue). Scale bars; 50um except I to Q (25 um).

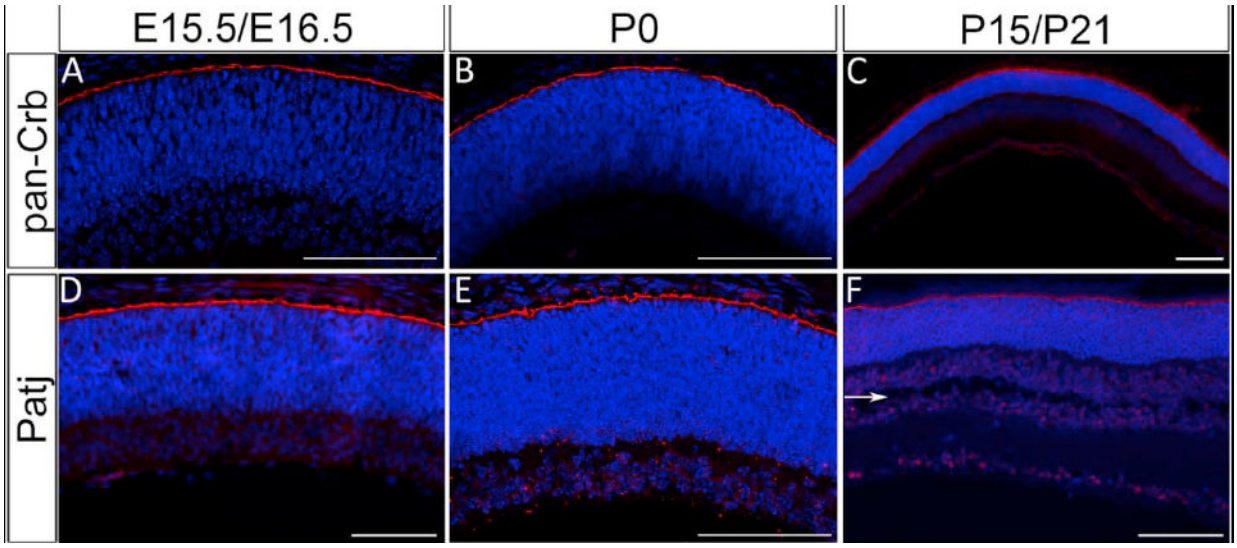

Fig. 5.

Crumbs homologs and Patj are localized in the apical junction of retinal epithelia throughout development and after the completion of development. Pan-Crb (A - C) and Patj (D - F) mainly appear in the apical surface of the retina at mid-embryonic stage (A & D, E15.5 and E16.5, respectively), birth (B & E, P0) and P21/P15 (C & F, respectively). Arrow denotes a crack introduced during tissue processing (F). Hoechst 33342 was used for nuclear counter staining (Blue). Scale bars; 100um.

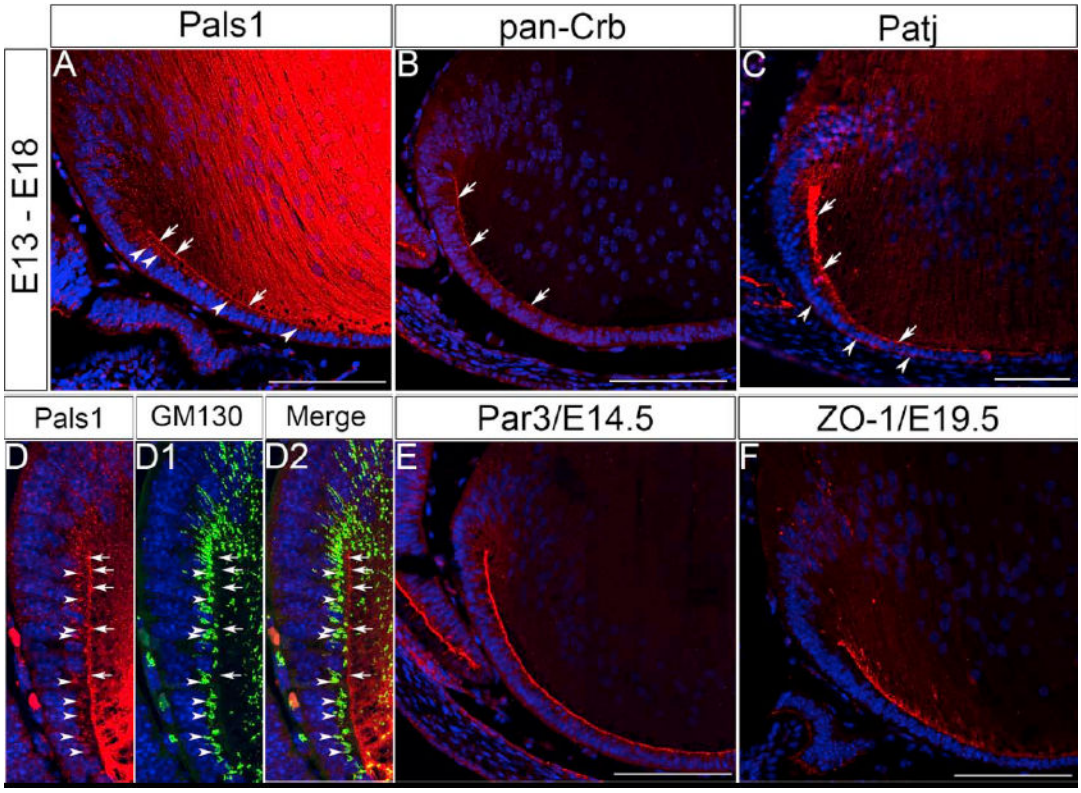

Fig. 6.

Crb polarity complex proteins are expressed in LE and lens progenitors. Pals1 (A, E18.5) appears both at the apical surface (arrows) and at subapical areas in the vicinity of nuclei (arrowheads). Crumbs homologs and Patj proteins are predominantly localized at the apical surface of the LE (arrows in B (E13.5) & C (E16.5), respectively) while weak Patj staining also appears in the basal side of anterior LE (C, arrowheads). Pals1 staining in LE cells near the equator shows co-localization with GM130 (D – D2) at E14.5. This pattern is similar to that of known apically localizing proteins like Par3 (E, E14.5) and ZO-1 (F, E19.5) in the lens. Hoechst 33342 was used for nuclear counter staining (Blue). Scale bars; 100um.