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Antagonistic functions of Par-1 kinase and protein phosphatase 2A are required for localization of Bazooka and photoreceptor morphogenesis in *Drosophila*

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Abstract

Establishment and maintenance of apical basal cell polarity are essential for epithelial morphogenesis and have been studied extensively using the *Drosophila* eye as a model system. Bazooka (Baz), a component of the Par-6 complex, plays important roles in cell polarity in diverse cell types including the photoreceptor cells. In ovarian follicle cells, localization of Baz at the apical region is regulated by Par-1 protein kinase. In contrast, Baz in photoreceptor cells is targeted to adherens junctions (AJs). To examine the regulatory pathways responsible for Baz localization in photoreceptor cells, we studied the effects of Par-1 on Baz localization in the pupal retina. Loss of Par-1 impairs the maintenance of AJ markers including Baz and apical polarity proteins of photoreceptor cells but not the establishment of cell polarity. In contrast, overexpression of Par-1 or Baz causes severe mislocalization of junctional and apical markers, resulting in abnormal cell polarity. However, flies with similar overexpression of kinase-inactive mutant Par-1 or unphosphorylatable mutant Baz protein show relatively normal photoreceptor development. These results suggest that dephosphorylation of Baz at the Par-1 phosphorylation sites is essential for proper Baz localization. We also show that the inhibition of protein phosphatase 2A (PP2A) mimics the polarity defects caused by Par-1 overexpression. Furthermore, Par-1 gain-of-function phenotypes are strongly enhanced by reduced PP2A function. Thus, we propose that antagonism between PP2A and Par-1 plays a key role in Baz localization at AJ in photoreceptor morphogenesis.

Keywords: Cell polarity; Photoreceptor; Rhabdomere; Bazooka; Par-1; PP2A

Introduction

Genetic control of apical basal cell polarity is essential for epithelial morphogenesis and asymmetric cell division during cell fate specification. It is also important for development of polarized subcellular structures with specialized functions such as the light sensing organelles of photoreceptor cells. A small number of evolutionarily conserved proteins play important roles in diverse types of apical basal cell polarization. These polarity proteins form two major heterotrimeric cassettes consisting of Crumbs (Crb)-Stardust (Sdt)-Dpatj (Crb complex) and Par-6-aPKC-Baz (Par-6 complex) in the apical cell membrane. Recent studies have shown that these two protein complexes function in a coordinated fashion by direct protein–protein interactions (Hurd et al., 2003; Lemmers et al., 2004; Nam and Choi, 2003; Sotillos et al., 2004). However, the functional significance of these interactions and the mechanisms for precise subcellular targeting of these proteins remain to be elucidated. The *Drosophila* eye provides an excellent system to study *in vivo* functions of these interacting polarity proteins in control of cell polarity and organization of the rhabdomere, the light-sensitive apical structure of photoreceptor cells.

In *Drosophila*, about 800 ommatidial clusters comprising of 8 photoreceptor cells (R1–R8) are generated in the eye disc epithelium during the third instar larval stage, but morphogenesis of

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photoreceptor cells takes place mainly during the following pupal stage. By 40% pupal development (pd), the apical region of each photoreceptor cell is involuted by 90°, which reorients the apical side toward the center of the cluster (Longley and Ready, 1995). In photoreceptor cells, Crb complex proteins are localized immediately apical to AJs (Fig. 1A). At 55% pd, when the rhabdomeres begin to develop from the apical surface of photoreceptor cells, Crb complex proteins are positioned to the region called the rhabdomere stalk, which links the rhabdomere with the AJ. During this time, developing rhabdomeres undergo dramatic vertical extension from the distal region of photoreceptor cells to the proximal base of the retina.

Crb, together with Sdt and Dpatj (Bachmann et al., 2001; Hong et al., 2001; Nam and Choi, 2003, 2006; Richard et al., 2006), is required for extension of rhabdomeres and formation of AJ along the distal–proximal axis of the photoreceptor cell, although it is not essential for establishing apical basal cell polarity (Izaddoost et al., 2002; Pellikka et al., 2002). The mammalian homolog of Crb, CRB1, is also localized to the inner segment of photoreceptors, the structure analogous to the rhabdomere stalk, between the outer segment and the AJ (Pellikka et al., 2002). Furthermore, mutations in *CRB1* cause retinal diseases including retinitis pigmentosa 12 and Leber Congenital Amaurosis (LCA) in humans (den Hollander et al., 1999, 2001).

In addition to Crb, Par-6 complex is also required for proper organization and maintenance of apical photoreceptor membranes in the eye (Hong et al., 2003; Nam and Choi, 2003). Genetic evidence suggests that Par-6 complex is required for localization of the Crb complex to the apical membrane whereas the Crb complex may be necessary for maintenance of Par-6 complex proteins in the apical region (Hong et al., 2003; Nam and Choi, 2003). However, it is unknown whether any one of the Par-6 complex proteins plays a primary role in the Par-6 complex function during the organization of photoreceptor cells. Interestingly, although Par-6 and aPKC, like the Crb complex, are localized to the apical membrane of photoreceptors, Baz is targeted to the AJ domain where Armadillo (Arm, *Drosophila* β-catenin) and DE-cadherin are localized (Nam and Choi, 2003). Recent studies have also shown the localization of Baz to AJ in the embryonic epithelia (Harris and Peifer, 2005). The differential localization of Baz and Par-6/aPKC raises the question of how Baz localization is regulated and whether Baz functions independently from Par-6 and aPKC.

In *C. elegans*, the Baz homolog, Par-3, is asymmetrically localized to the anterior cortex of the embryo whereas the serine/threonine protein kinase Par-1 is positioned to the posterior side (Kemphues et al., 1988). In *Drosophila* follicle cells, Baz localization to the apical membrane is also regulated by basolateral Par-1 (Benton and St Johnston, 2003). Thus, in both *C. elegans* embryo and *Drosophila* follicle cells, Par-3 and Par-1 localize in a complementary cellular pattern. Interestingly, Baz is a phosphorylation substrate for Par-1 kinase, and the apical localization of Baz requires Par-1 phosphorylation (Benton and St Johnston, 2003). In follicle cells, Par-1 is required to exclude Baz from the basolateral membrane. Loss of Par-1 results in the basolateral expansion of Baz–Par-6 complex, indicating that Par-1 is essential for the regulation of apical basal cell polarity (Benton and St Johnston, 2003). As opposed to the essential role for Par-1 in follicle cell polarity, no significant function of Par-1 in regulation of cell polarity in the developing eye disc has been identified (Bayraktar et al., 2006). However, it has not been studied whether Par-1 is required at a later time point in photoreceptor morphogenesis for the control of Baz localization during the pupal stage when the photoreceptor cells undergo massive reorganization of their apical basal cell structure.

Since Par-1 is a protein kinase, an interesting question is whether Baz localization might be regulated by an opposing phosphatase activity. Evidence from mammalian studies has suggested that the formation of tight junctions is regulated by the interaction of protein phosphatase 2A (PP2A) and aPKC (Nunbhakdi-Craig et al., 2002). However, understanding the *in vivo* role of PP2A in cell polarity requires additional study, and the use of an animal model such as *Drosophila* eye can be a powerful tool to address these questions. PP2A is a heterotrimeric serine/threonine phosphatase composed of invariant catalytic ('C') and structural ('A') subunits and a variable regulatory subunit ('B') that directs the AC core complex to different substrates (Janssens and Goris, 2001). In *Drosophila*, the catalytic subunit is encoded by the *microtubule star* (*mts*) gene. *mts* mutants die in embryogenesis with defects in mitosis (Snaith et al., 1996), but the function of Mts in apical basal cell polarity has not been examined.

To understand the role of Baz and the regulation of Baz localization during photoreceptor morphogenesis, we investigated the functional relationship among Baz, Par-1 and PP2A in early/mid pupal eye development. First, we show that Baz plays an essential role in the localization of Par-6-aPKC complex to the apical domain. Second, Par-1 is required for the localization and/or maintenance of Baz and proper morphogenesis of photoreceptors. Thirdly, Baz unphosphorylated at the Par-1 sites is preferentially targeted to AJ whereas phosphorylated Baz is ectopically localized. Lastly, we identify an important function of PP2A in photoreceptor cell organization and its role as an antagonizing factor for Par-1. This study establishes the role for Baz as a central player in the localization of cell polarity proteins during photoreceptor morphogenesis and provides new insight into how Baz is regulated by antagonistic roles of Par-1 and PP2A.

Materials and methods

Genetics

Mitotic recombinations were induced by using FLP/FRT method for clonal analysis (Xu and Rubin, 1993). $apkc^{K06304}$ and baz^{Xi106} mutant clones were produced in the eye by *eyeless* (*ey*)-*FLP* in *y w par-6*$^{\Delta 226}$ *FRT*$^{9\text{-}2}$*/y w Ubi-GFP FRT*$^{9\text{-}2}$; *ey-Flp/+*, *y w ey-Flp/+; FRT42D aPKC*^{K06304}*/FRT42D Ubi-GFP*, and *y w baz*^{Xi106} *FRT*$^{9\text{-}2}$*/y w Ubi-GFP FRT*$^{9\text{-}2}$; *ey-Flp/+*, respectively (Petronczki and Knoblich, 2001; Wodarz et al., 1999, 2000). Overexpression of Mts, dnMts, Par-1 or Baz was induced by crossing *UAS-Mts*, *UAS-dnMts* (Hannus et al., 2002), *UAS-Par-1* (full length of Par-1 *a* form, Sun et al., 2001), *UAS-GFP:Baz*, *UAS-GFP:Baz*^{S151A,S1085A} (Benton and St Johnston, 2003) with *GMR-Gal4* (Freeman, 1996). *mts*^{XE2258}, a null allele of *mts* (Shiomi et al., 1994; Wassarman et al., 1996) and *par-1*$^{\Delta 16}$ or *par-1*^{W3}, null alleles of *par-1* (Cox et al., 2001; Huynh et al., 2001) were