**Drosophila** Patj plays a supporting role in apical-basal polarity but is essential for viability

Wenke Zhou and Yang Hong*

**SUMMARY**

Patj has been characterized as one of the so-called polarity proteins that play essential and conserved roles in regulating cell polarity in many different cell types. Studies of *Drosophila* and mammalian cells suggest that Patj is required for the apical polarity protein complex Crumbs-Stardust (Pals1 or Mpp5 in mammalian cells) to establish apical-basal polarity. However, owing to the lack of suitable genetic mutants, the exact in vivo function of Patj in regulating apical-basal polarity and development remains to be elucidated. Here, we generated molecularly defined null mutants of *Drosophila* Patj (*dPatj*). Our data show conclusively that *dPatj* only plays supporting and non-essential roles in regulating apical-basal polarity, although such a supporting role may become crucial in cells such as photoreceptors that undergo complex cellular morphogenesis. In addition, our results confirm that *dPatj* possesses an as yet unidentified function that is essential for pupal development.

**KEY WORDS:** *Drosophila* Patj, Polarity proteins, Apical-basal polarity, Embryonic epithelium, Follicular epithelium, Photoreceptor

**INTRODUCTION**

Polarity proteins play evolutionarily conserved roles in regulating apical-basal polarity in epithelial cells (Wang and Margolis, 2007). Pals1-associated tight junction protein (Patj) is a multi-PDZ domain protein that binds the apical polarity protein complexes Crumbs (Crb)-Stardust (Sdt; Pals1) (Roh et al., 2002; Roh et al., 2003) and Par-6–aPKC (Nam and Choi, 2003; Wang et al., 2004). In *Drosophila*, the homolog of Patj was first identified as the product of *discs lost* (*dlt*) (Bhat et al., 1999), but Pielage et al. later showed that *dlt* actually encodes a protein involved in cell cycle control and the original *dlt* was renamed *Drosophila* Patj (*dPatj*) (Pielage et al., 2003). RNAi knockdown results suggested that *dPatj* is an essential gene that is required for initiating and establishing apical-basal polarity, primarily through its regulation of Crb (Bhat et al., 1999). However, Pielage et al. generated a synthetic *dPatj* mutant by rescuing all of the deleted genes with the exception of *dPatj* in a small deficiency and found it to be viable, disputing essential roles for *dPatj* in polarity and development (Pielage et al., 2003). Such conflicting results were partially reconciled when it was shown that this synthetic *dPatj* mutant is in fact a hypomorphic allele of *dPatj* (*dPatj*<sup> hyp/ hyp </sup>) that expresses a truncated *dPatj* protein from one of the rescuing DNA fragments (Nam and Choi, 2006). They constructed a new synthetic null mutant, *dPatj*<sup> hyp/ hyp </sup>, that was free of *dPatj* coding sequences and found that it was early larval lethal. Unfortunately, polarity defects could not be assessed in *dPatj*<sup> hyp/ hyp </sup> embryos due to difficulties in removing *dPatj* maternal contributions (Nam and Choi, 2006).

Thus, owing to the lack of suitable genetic mutants, it remains to be determined whether Patj is indeed essential for apical-basal polarity and general development. Here, we generated multiple molecularly and genetically defined null mutants of *dPatj* to address this key issue.

**MATERIALS AND METHODS**

*Drosophila* stocks

yw<sup>65C2</sup>; P[GSV2]GS0262/TM3, Sb<sup>1</sup>Ser<sup>1</sup> (stock #204965) was obtained from the Kyoto *Drosophila* Genetic Resource Center. Stocks obtained from the Bloomington *Drosophila* Stock Center: w<sup>1118</sup>; Df(3L)BS123/TM6B, Tb<sup>1</sup> (BL#9145); w<sup>+</sup>; FRT-2A (BL#1997); w<sup>+</sup>; Dr<sup>-</sup>/TM6B, P[ryl+17.2]/w<sup>1118</sup>; Df(2R)2-3/99B (BL#1610); hsFLP<sup>+</sup> y<sup>+</sup>; w<sup>+</sup>; Dr<sup>-</sup>/TM3, Sb<sup>1</sup> (BL#26902); w<sup>+</sup>; Ubi-GFP<sup>fls2</sup>; Ubi-GFP<sup>fls2</sup> FRT-2A (BL#5825); y<sup>+</sup>; vasa-4C31B10-14 w<sup>+</sup>; attP-9A<sup>Sb1</sup> (BL#24867); w<sup>+</sup>; ovoD1-18 FRT-2A/s<sup>0</sup> (Makarova et al., 1995); Sb<sup>1</sup> (BL#2319); ovo-FLP, w<sup>+</sup>; (BL#8727); y<sup>+</sup>; w<sup>+</sup>; ey-FLP (BL#5580); and w<sup>+</sup>; Ubi-GFP<sup>fls2</sup>; Ubi-GFP<sup>fls2</sup>/TM3, Sb<sup>1</sup> Ser<sup>1</sup> (BL#6663; hereafter referred to as w<sup>+</sup>; Dr<sup>-</sup>/TM3 twi<sup>–</sup>–GFP). The knockout null allele *crb*<sup>YX42663</sup> (<sup>crb</sup><sup>kn</sup>) was described previously (Huang et al., 2009).

Generation and characterization of *dPatj* deletion and rescue alleles

P[GSV2]GS0262 excision mutagenesis was performed according to the standard method (Hummel and Klämbt, 2008). *dPatj<sup> Flp</sup>*<sup>–</sup> and *dPatj<sup> Flp</sup>–<sup>–</sup> were isolated from a total of 253 excision candidates screened by Df(3L)BS123 complementation. PCR verifications and sequencing were performed using primers (5′-3′): CGAGACGCGGCCGCAGACACCATTATGTTCCTCCAGACAGCTGATCCATTGGTGTCCC and CGAGACGGCGAGTCGGAGA. *dPatj-L* and *dPatj-S* DNA fragments were PCR amplified from genomic DNA using: dPatj-S Forward, CGAGACGGCGGCCGCGCGCGACACCATTTAATTTGCC; and dPatj-S Reverse, CGAGACGCGGCCGCGCGCGACACCATTTAATTTGCC; dPatj-L Forward, CGAGACGGCGAGTCGGAGA. *dPatj<sup> Flp</sup>–<sup>–</sup>* was inserted before the stop codon in *dPatj-S* to produce *dPatj<sup> Flp</sup>–<sup>–</sup>*<sup>GFP</sup> was inserted before the stop codon in *dPatj<sup> Flp</sup>–<sup>–</sup>*

**Generation of somatic and germline clones of *dPatj***

*dPatj<sup> Flp</sup>* germline clone embryos were collected from crosses of *ovo-FLP*; *dPatj<sup> Flp</sup>* FRT-2A/ovo<sup>–</sup>FRT<sup>2A</sup> × *dPatj<sup> Flp</sup>*<sup>–</sup> or *dPatj<sup> Flp</sup>*<sup>–</sup> or Df(3L)BS123 or *dPatj<sup> Flp</sup>*<sup>–</sup> VK20-dPatj-L/TM3, twi<sup>–</sup>–GFP (Xu and Rubin, 1993; Chou and Perrimon, 1996). To generate follicular clones, young females of *hs-FLP*<sup> Flp</sup>–<sup>–</sup>; *dPatj<sup> Flp</sup>* FRT-2A/ubi-GFP FRT-2A were heat shocked for 1 hour at 38°C and then aged for 2-4 days prior to ovary dissection. To generate larval imaginal disc clones, embryos from the *hs-FLP*; *ubi-GFP* FRT-2A × *dPatj<sup> Flp</sup>* FRT-2A/TM3, twi<sup>–</sup>–GFP were collected for 24 hours,

Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA.

*Author for correspondence (yong@pitt.edu)

Accepted 12 June 2012


© 2012. Published by The Company of Biologists Ltd

Development Advance Online Articles. First posted online on 12 July 2012 as 10.1242/dev.083162

Access the most recent version at http://dev.biologists.org/lookup/doi/10.1242/dev.083162

Development ePress online publication date 12 July 2012

http://dev.biologists.org/lookup/doi/10.1242/dev.083162

Development ePress online publication date 12 July 2012

http://dev.biologists.org/lookup/doi/10.1242/dev.083162
Fig. 1. Characterization of dPatj null mutants generated by imprecise P-element excision. (A) The Drosophila Patj (dPatj) locus. GS50262 (blue triangle) is inserted at 1,798,845 bp of the third chromosome (http://flybase.org/reports/FBti0109626.html). The Df(3L)BSC123 deletion is indicated (red bar). (B) Deletions in dPatjΔ7, dPatjΔ13 and dPatjΔ14 (red bars), the region covered by the PCR in C (black bar), and the genomic fragments used in VK20-dPatj-L and VK20-dPatj-GFP-S (blue bars) are shown. dPatj-L starts immediately after the CG12020 stop codon; both end in the 3′UTR of Jembrin. In dPatj::GFP-S, GFP is inserted in frame before the dPatj stop codon. (C) PCR verification of dPatjΔ7, dPatjΔ13 and dPatjΔ14 mutants. PCR products are indicated for the wild-type (w1118) chromosome (3.32 kb, blue arrowhead) versus the dPatjΔ7, dPatjΔ13 and dPatjΔ14 mutant chromosomes (red arrowheads). MW, DNA ladder.

Immunostaining and confocal imaging

Drosophila photoreceptor clones, white pupae (less than 2-3 hours into pupation) were collected from the third instar larvae emerged. To generate dPatj photoreceptor clones, white pupae were heat shocked for 1 hour at 38°C 1 day later, and maintained at 25°C until the desired pupal stages (Hong et al., 2003). Pupae were genotyped by the absence of transposase (Hummel and Klämbt, 1995). By mobilizing dPatj upstream of the first ATG of the dPatj coding sequence (Fig. 1A), we recovered several imprecise excision mutants that failed to complement Df(3L)BSC123, which is a 11,312 bp deficiency encompassing the dPatj locus (Cook et al., 2012) (Fig. 1A-C and supplementary material Table S1). dPatjΔ7, dPatjΔ13 and dPatjΔ14 contained deletions that all began at the 5′UTR plus the conserved L27 motif required for the binding of Sdt or Pals1 (Roh et al., 2002; Li et al., 2004; Feng et al., 2005) (Fig. 1A-C). The 2,509 bp deletion in dPatjΔ7 further removed the first three PDZ domains that are required for interaction with Crb (Bhat et al., 1999) and Par-6 (Nam and Choi, 2003), whereas deletions in dPatjΔ13 and dPatjΔ14 also removed the first two and the first PDZ domain, respectively (Fig. 1B). Antibodies against the N-terminal and full-length dPatj proteins (Huang et al., 2009) revealed maternal contributions of dPatj in zygotic mutant embryos (Fig. 2A). However, in dPatjΔ7 germline clone (dPatjΔ7/LC) mutant embryos, which removed dPatj maternal contributions, dPatj staining was absent, confirming that dPatjΔ7 is a genetic null mutant (Fig. 2B-F).

Transheterozygotes among dPatjΔ7, dPatjΔ13, dPatjΔ14 and Df(3L)BSC123 suggested that the zygotic mutants of dPatj were pupal lethal, but dPatjΔ7, dPatjΔ13 and dPatjΔ14 appeared to carry additional background mutations that caused prepupal lethality (supplementary material Table S1). To confirm the loss of dPatj specifically caused pupal lethality, we developed two independent genomic rescue lines, VK20-dPatj-L and VK20-dPatj::GFP-S, that each only contained dPatj as the single intact gene (Fig. 1B). dPatjΔ7 VK20-dPatj-L or dPatjΔ7 VK20-dPatj::GFP-S recombinants fully rescued dPatjΔ7, dPatjΔ13 and...
Df(3L)BSC123 (supplementary material Table S1), dPatjΔ7 VK20-dPatj-L also fully rescued the lethality of dPatjΔ7GLC embryos (supplementary material Table S1), indicating that the zygotic expression of dPatj is sufficient for development. It is likely that the early larval lethality observed in the dPatjsynnull mutants (Nam and Choi, 2006) was due to additional background mutations or incomplete rescue. The expression pattern of GFP-tagged dPatj from VK20-dPatj::GFP-S was indistinguishable from the dPatj immunostaining pattern (data not shown).

dPatj is not required for establishing apical-basal polarity during embryogenesis

During Drosophila embryogenesis, cellularization is the earliest event in the formation of the polarized embryonic epithelia, and one striking feature of dPatj is its dynamic association with the leading edge of the invaginating membrane in cellularizing cells (Fig. 2B) (Bhat et al., 1999). It was reported that dPatj RNAi knockdown impairs cellularization and results in shortened epithelial cells (Bhat et al., 1999), suggesting that dPatj is required in membrane invagination and the initiation of apical-basal polarity. Surprisingly, in stage 5 dPatjΔ7GLC embryos (n>10), we found no discernible defects in cellularizing cells in the absence of dPatj (Fig. 2B). Because dPatjΔ7GLC embryos completed their development through the embryonic and larval stages (supplementary material Table S1), even if the loss of dPatj caused cellularization defects that were too subtle to be observed by immunostaining, such defects were not detrimental to normal development. Our data confirm that dPatj is dispensable for cellularization and initiating apical-basal polarity.

After cellularization, establishing apical-basal polarity in polarizing embryonic epithelia requires the Crb-Sdt complex (Tepass and Knust, 1993; Bachmann et al., 2001; Hong et al., 2001). Again, RNAi knockdown of dPatj severely disrupted the apical localization of Crb and apical-basal polarization (Bhat et al., 1999). Similarly, in polarizing MDCK cells, RNAi knockdown of Patj caused the loss of Palš1 from tight junctions and delayed tight junction formation (Straight et al., 2004; Shin et al., 2005). These data support an essential role of dPatj in establishing apical-basal polarity. Nonetheless, we found that in stage 10 and 11 dPatjΔ7GLC embryos, both Crb and Sdt showed normal subcellular localization (Fig. 2C,D). Crb expression was slightly reduced in dPatjΔ7GLC embryos, but the overall pattern was clearly undisturbed (Fig. 2C). We further examined the subcellular localization of several representative polarity proteins in dPatjΔ7GLC embryos, including: DE-Cadherin (DE-Cad; Shotgun – FlyBase), which marks the adherens junction; Bazooka (Baz, or Par-3), which is required for...
the early stage of apical-basal polarization; Par-6 and atypical PKC (aPKC), which form an evolutionarily conserved apical complex that regulates multiple polarity proteins through aPKC-mediated phosphorylation (Suzuki et al., 2001; Betschinger et al., 2003; Plant et al., 2003; Sotillos et al., 2004; Krahn et al., 2010; Morais-de-Sá et al., 2010); and Discs large (Dlg, or Dlg1), which is required for specifying the basolateral membrane domain and junctions (e.g. septate junctions). Before 40% pupal development (pd), the apical membranes of all photoreceptors are converged at the center of the ommatidium and show enriched localization of Crb, Sdt, dPatj, Par-6 and aPKC (red). Adherens junctions that form between photoreceptors show ring-like patterns that are marked by DE-Cad, Arm and Baz (green). (G-I) In dPatjΔ7 mutant photoreceptors, Arm (G) and Baz (H) show mild disruptions (examples indicated by arrows), whereas Crb and Par-6 exhibit more severe mislocalization (I, arrowheads). All samples are from 37% pd pupae. In all panels, dPatjΔ7 clones are marked by the loss of GFP (blue). Scale bars: 10 μm.

**Fig. 3.** dPatj supports Crb expression in polarized epithelial cells and apical-basal polarity in early pupal photoreceptors. (A) dPatjΔ7 clone (outlined) in a larval imaginal disc shows subtle reduction in Crb expression but normal expression of Baz. (B-E) dPatjΔ7 clones in follicular epithelia show more marked loss of Crb (B) but no obvious disruption of DE-Cad (C), Par-6 (D) or aPKC (E). (F) Illustration of a transverse view of an early pupal ommatidium. Before 40% pupal development (pd), the apical membranes of all photoreceptors are converged at the center of the ommatidium and show enriched localization of Crb, Sdt, dPatj, Par-6 and aPKC (red). Adherens junctions that form between photoreceptors show ring-like patterns that are marked by DE-Cad, Arm and Baz (green). (G-I) In dPatjΔ7 mutant photoreceptors, Arm (G) and Baz (H) show mild disruptions (examples indicated by arrows), whereas Crb and Par-6 exhibit more severe mislocalization (I, arrowheads). All samples are from 37% pd pupae. In all panels, dPatjΔ7 clones are marked by the loss of GFP (blue). Scale bars: 10 μm.

**dPatj supports Crb expression and subcellular localization in polarized epithelial cells**

The reduction of Crb expression in the absence of dPatj has also been reported previously in polarized epithelial cells, such as the follicular cells of ovaries (Tanentzapf et al., 2000). Because such studies also used a deficiency-based dPatjΔ10 allele with multiple gene deletions (Bhat et al., 1999), we generated dPatjΔ7 clones in larval imaginal discs and adult follicular cells to investigate their phenotypes in polarized epithelial cells (Fig. 3A-E). The dPatjΔ7 mutant clones in the imaginal epithelia showed mild reductions in the expression of Crb but not Baz (Fig. 3A), whereas clones in the follicular cells showed much stronger reductions in Crb expression (Fig. 3B). Therefore, dPatj appears to be specifically required to maintain high levels of Crb expression in polarized epithelial cells. Consistent with the observations of Tanentzapf et al. (Tanentzapf et al., 2000), reduced Crb expression in dPatj mutant cells did not disrupt their apical-basal polarity: other polarity markers, such as Baz, DE-Cad, Par-6 and aPKC, all appeared to be unaffected (Fig. 3A-E).

**dPatj is required for apical-basal polarity in early pupal photoreceptors**

For cells that must undergo dramatic remodeling of their apical-basal polarity, the supporting role of dPatj in maintaining the expression and subcellular localization of Crb might become crucial. In Drosophila, the highly polarized subcellular structures
rescued in VK20-dPatj-L or dPatj\textsuperscript{7} VK20-dPatj::GFP-S (Fig. 4D and supplementary material Table S1). The mild reduction in Crb expression observed in the dPatj mutants was unlikely to be responsible for the pupal lethality because the additional removal of one copy of crb in dPatj\textsuperscript{7}/Df(3L)BSC123 crb\textsuperscript{66} or dPatj\textsuperscript{7} crb\textsuperscript{66}/dPatj\textsuperscript{713} did not enhance pupal lethality (supplementary material Table S1). The viability of dPatj\textsuperscript{7} (Pielage et al., 2003; Nam and Choi, 2006) suggests that the N-terminal 260 amino acids of dPatj might be sufficient for pupal development, but at present the specific function of dPatj in pupal development remains to be identified.

In summary, our molecularly defined dPatj null alleles conclusively show that dPatj is not essential for establishing or maintaining apical-basal polarity. The mild reduction in Crb expression in the absence of dPatj suggests that dPatj plays a supporting role in maintaining the Crb-Sdt complex in polarized epithelial cells. Such a scenario exhibits similarities with the situation for p120catenin (Adherens junction protein p120 – FlyBase) mutants: despite RNAi results suggesting that p120catenin is essential for cell adhesion, genetic mutants revealed that it only plays a supporting and non-essential role in cell adhesion in Drosophila (Myster et al., 2003). It remains possible, however, that mammalian Patj might have evolved increasingly important roles in apical-basal polarity, which might explain the differences between our dPatj null mutant phenotypes and those of mammalian cells with RNAi knocked-down Patj levels (Straight et al., 2004; Wang et al., 2004; Shin et al., 2005).

Acknowledgements

We thank the Bloomington Drosophila Stock Center and Kyoto Drosophila Genetic Resources Center for fly stocks; the Developmental Studies Hybridoma Bank (DSHB) for antibodies; and the University of Pittsburgh Medical School Center for Biologic Imaging for providing access to confocal microscopes.

Funding

This work was supported by National Institutes of Health (NIH) grants R21HG024869 from the National Center for Research Resources (NCRR), R01GM086423 from the National Institute of General Medical Sciences (NIGMS) to Y.H. Deposited in PMC for release after 12 months.

Competing interests statement

The authors declare no competing financial interests.

Supplementary material

Supplementary material available online at http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.083162/-/DC1

References


