

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

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## 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 (*dPatj<sup>synhyppo</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>synnull</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>synnull</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

*y<sup>1</sup> w<sup>67c23</sup>; P{GSV2}GS50262/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)BSC123/TM6B, Tb<sup>1</sup>* (BL#9143); *w\**; *FRT-2A* (BL#1997); *w\**; *Dr<sup>1</sup>/TMS, P{ry[+t7.2]=Δ2-3}99B* (BL#1610); *hsFLP<sup>1</sup> y<sup>1</sup> w<sup>1118</sup>; Dr<sup>1</sup>/TM3, Sb<sup>1</sup>* (BL#26902); *w<sup>1118</sup>; Ubi-GFP.nls<sup>3L1</sup> Ubi-GFP.nls<sup>3L2</sup> FRT-2A* (BL#5825); *y<sup>1</sup> vas-φC31<sup>ZH-2A</sup> w\**; *attP-9A<sup>VK00020</sup>* (BL#24867); *w\**; *ovoD<sup>1-18</sup> FRT-2A/st<sup>1</sup> βTub85D<sup>D</sup> ss<sup>1</sup> e<sup>1</sup>/TM3, Sb<sup>1</sup>* (BL#2139); *ovo-FLP, w\** (BL#8727); *y<sup>d2</sup> w<sup>1118</sup> ey-FLP* (BL#5580); and *w<sup>1118</sup>; Dr<sup>Mio</sup>/TM3, P{GAL4-twi.G}2.3, P{UAS-2xEGFP}AH2.3, Sb1 Ser<sup>1</sup>* (BL#6663; hereafter referred to as *w*; *Dr/TM3 twi>GFP*). The knockout null allele *crb<sup>GX24w[-]</sup> (crb<sup>ko</sup>)* was described previously (Huang et al., 2009).

### Generation and characterization of *dPatj* deletion and rescue alleles

*P{GSV2}GS50262* excision mutagenesis was performed according to the standard method (Hummel and Klämbt, 2008). *dPatj<sup>Δ7</sup>*, *dPatj<sup>Δ13</sup>* and *dPatj<sup>Δ14</sup>* were isolated from a total of 253 excision candidates screened by *Df(3L)BSC123* complementation. PCR verifications and sequencing were performed using primers (5'-3') CGAGACGCGCCGCGGACACACCTTATTGTTTTCC and CGAGACGCTAGCCTAGTTCCGCCAGTCGGGAA. *dPatj-L* and *dPatj-S* DNA fragments were PCR amplified from genomic DNA using: *dPatj-S* Forward, CGAGACGCGCCGCGGACACACCTTATTGTTTTCC; *dPatj-L* Forward, CGAGACGCGCCGATTCGGGTGATATTATTTTCATTG; and *dPatj-S* (or -L) Reverse, CGAGACGCGTACTCATTGACCGTACGGAAGGTA.

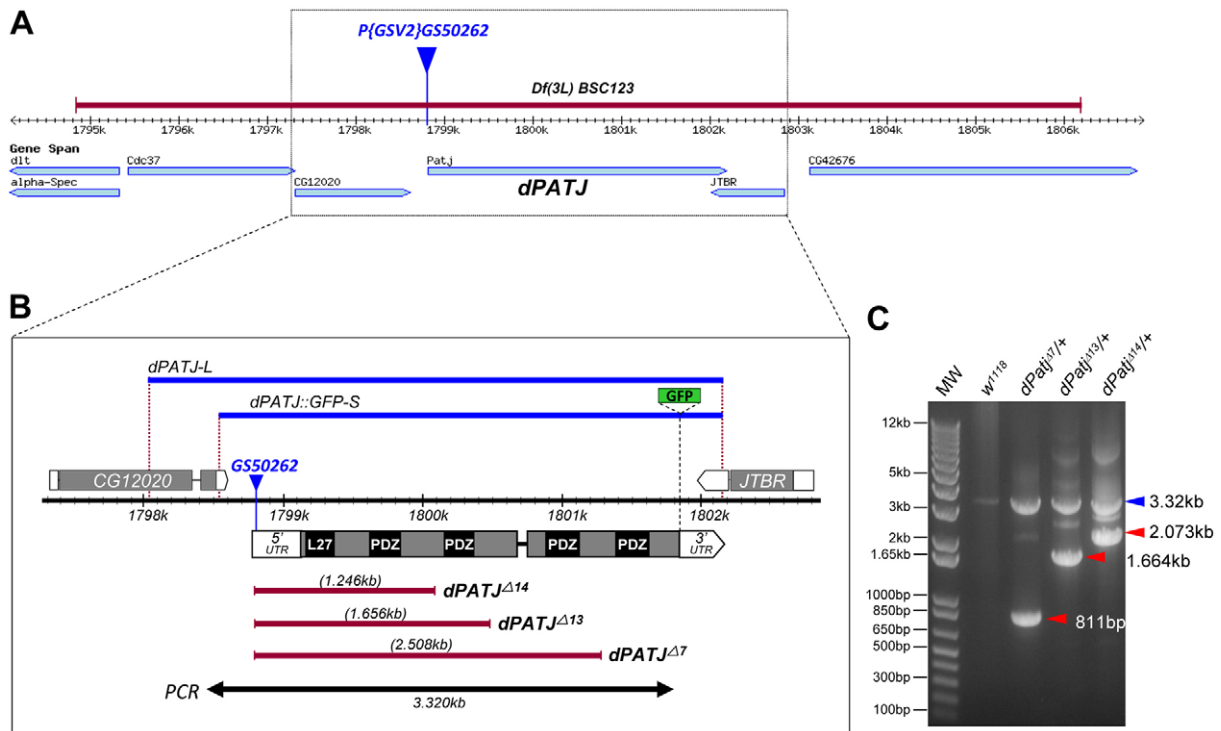
*GFP* was inserted before the *dPatj* stop codon in *dPatj-S* to produce *dPatj::GFP-S*. *dPatj-L* and *dPatj::GFP-S* were integrated into *attP-9A<sup>VK00020</sup>* [VK20 at the cytologic location 99F8 (Venken et al., 2006)] via  $\phi$ C31-mediated DNA integration (Groth et al., 2004; Bischof et al., 2007) to generate *dPatj-L* and *dPatj::GFP-S*, which were then recombined with *dPatj<sup>Δ7</sup>*.

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

*dPatj<sup>Δ7</sup>* germline clone embryos were collected from crosses of *ovo-FLP; dPatj<sup>Δ7</sup> FRT-2A/ovoD FRT-2A* × *dPatj<sup>Δ7</sup>* [or *dPatj<sup>Δ13</sup>* or *Df(3L)BS123* or *dPatj<sup>Δ7</sup> VK20-dPatj-L/TM3, twi>GFP* (Xu and Rubin, 1993; Chou and Perrimon, 1996)]. To generate follicular clones, young females of *hs-FLP/+; dPatj<sup>Δ7</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>Δ7</sup> FRT-2A/TM3, twi>GFP* crosses were collected for 24 hours,

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**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 *dPatj*<sup>Δ7</sup>, *dPatj*<sup>Δ13</sup> and *dPatj*<sup>Δ14</sup> (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 in the middle of the *CG12020* coding sequence, whereas *dPatj::GFP-S* starts immediately after the *CG12020* stop codon; both end in the 3'UTR of *JTBR*. In *dPatj::GFP-S*, GFP is inserted in frame before the *dPatj* stop codon. (C) PCR verification of *dPatj*<sup>Δ7</sup>, *dPatj*<sup>Δ13</sup> and *dPatj*<sup>Δ14</sup> mutants. PCR products are indicated for the wild-type (*w*<sup>1118</sup>) chromosome (3.32 kb, blue arrowhead) versus the *dPatj*<sup>Δ7</sup>, *dPatj*<sup>Δ13</sup> and *dPatj*<sup>Δ14</sup> mutant chromosomes (red arrowheads). MW, DNA ladder.

heat shocked for 1 hour at 38°C 1 day later, and maintained at 25°C until the third instar larvae emerged. To generate *dPatj* photoreceptor clones, white pupae (less than 2-3 hours into pupation) were collected from the *ey-FLP; dPatj*<sup>Δ7</sup> *FRT-2A/TM3* *twi*> *GFP* × *ubi-GFP FRT-2A* cross and aged at 18°C until the desired pupal stages (Hong et al., 2003). Pupae were genotyped by the absence of *twi*> *GFP*.

#### Immunostaining and confocal imaging

Immunostaining of embryos, larval imaginal discs and adult ovaries (Tanentzapf et al., 2000; Hong et al., 2001; Huang et al., 2009) and pupal retina dissection and immunostaining (Hong et al., 2003) were performed as previously described. Primary antibodies were: mouse anti-*dPatj* (full-length) and rabbit anti-*dPatj*-N (the first 517 amino acids) (Huang et al., 2009) 1:500; rabbit anti-GFP (Huang et al., 2009) 1:1500; guinea pig anti-Baz (Huang et al., 2009) 1:500; rabbit and rat anti-Par-6 (Huang et al., 2009) 1:500; rabbit anti-Sdt (Hong et al., 2001) 1:100-200; mouse anti-Crb (Cq4; DSHB) 1:10-100; mouse anti-Arm (N2 7A1; DSHB) 1:50-100; rat anti-DE-Cad (DCAD2; DSHB) 1:100; mouse anti-Dlg (4F3; DSHB) 1:50; and rabbit anti-aPKC (Santa Cruz) 1:1000. Secondary antibodies: Cy2-, Cy3- or Cy5-conjugated goat anti-rabbit IgG, anti-rat IgG, anti-mouse IgG and anti-guinea pig IgG (Jackson ImmunoResearch), all at 1:400. Images were taken on an Olympus FV1000 confocal microscope (Center for Biologic Imaging, University of Pittsburgh Medical School) and processed in Adobe Photoshop for compositions.

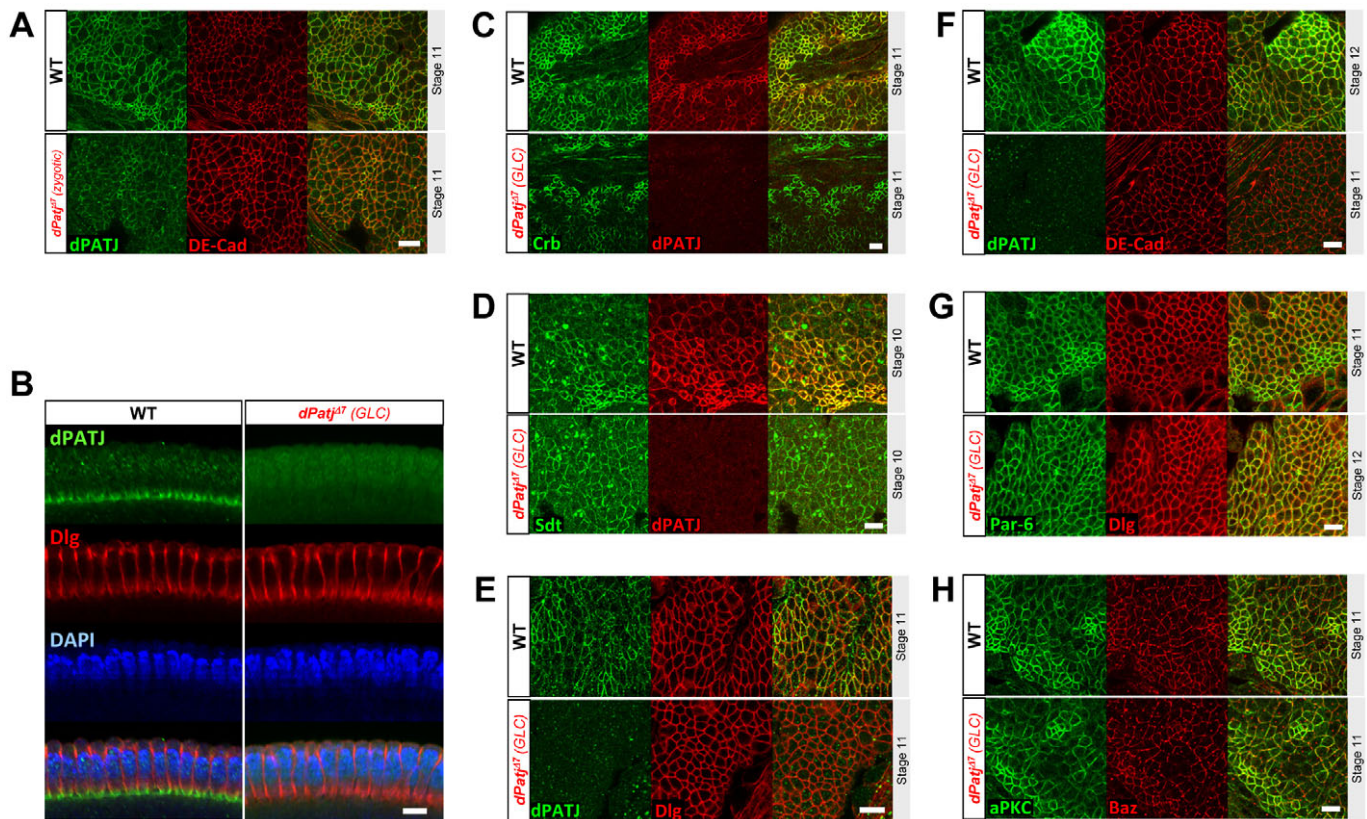
## RESULTS AND DISCUSSION

### *Drosophila Patj* is essential for viability

*P{GSV2}GS50262* is a viable P-element insertion located 303 bp upstream of the first ATG of the *dPatj* coding sequence (Fig. 1A). By mobilizing *GS50262* with transposase (Hummel and Klämbt,

2008), 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). *dPatj*<sup>Δ7</sup>, *dPatj*<sup>Δ13</sup> and *dPatj*<sup>Δ14</sup> contained deletions that all began at the *GS50262* insertion site and removed 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 *dPatj*<sup>Δ7</sup> 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 *dPatj*<sup>Δ13</sup> and *dPatj*<sup>Δ14</sup> 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 *dPatj*<sup>Δ7</sup> zygotic mutant embryos (Fig. 2A). However, in *dPatj*<sup>Δ7</sup> germline clone (*dPatj*<sup>Δ7GLC</sup>) mutant embryos, which removed *dPatj* maternal contributions, *dPatj* staining was absent, confirming that *dPatj*<sup>Δ7</sup> is a genetic null mutant (Fig. 2B-F).

Transheterozygotes among *dPatj*<sup>Δ7</sup>, *dPatj*<sup>Δ13</sup>, *dPatj*<sup>Δ14</sup> and *Df(3L)BSC123* suggested that the zygotic mutants of *dPatj* were pupal lethal, but *dPatj*<sup>Δ7</sup>, *dPatj*<sup>Δ13</sup> and *dPatj*<sup>Δ14</sup> appeared to carry additional background mutations that caused prepupal lethality (supplementary material Table S1). To confirm that 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). *dPatj*<sup>Δ7</sup> *VK20-dPatj-L* or *dPatj*<sup>Δ7</sup> *VK20-dPatj::GFP-S* recombinants fully rescued *dPatj*<sup>Δ7</sup>, *dPatj*<sup>Δ13</sup> and



**Fig. 2. dPatj is not required for initiating or establishing apical-basal polarity during embryogenesis.** (A) Reduced but detectable dPatj expression (green) in the *dPatj*<sup>Δ7</sup> zygotic mutant embryo. DE-Cad (red) marks adherens junctions. (B) Maternal contribution of dPatj (green) was completely removed in *dPatj*<sup>Δ7GLC</sup> stage 5 embryos, but cellularization remained normal, as shown by Dlg (red), which marks the invaginating basolateral membrane. Nuclei are stained with DAPI (blue). The signal in the green channel was boosted in the *dPatj*<sup>Δ7GLC</sup> sample to emphasize the absence of dPatj staining. (C-H) In older *dPatj*<sup>Δ7GLC</sup> embryos at stage 10-11, Crb expression appears to be mildly reduced (C), but both Sdt and Crb show normal subcellular localization patterns (C,D). The expression and subcellular localization of the basolateral polarity protein Dlg (E) and the apical polarity proteins DE-Cad (F), Par-6 (G), aPKC and Baz (H) were also normal in these embryos. With the exception of B, embryos are presented in tangential view and images are maximum z-projections of ~5-10 μm depth. Scale bars: 10 μm.

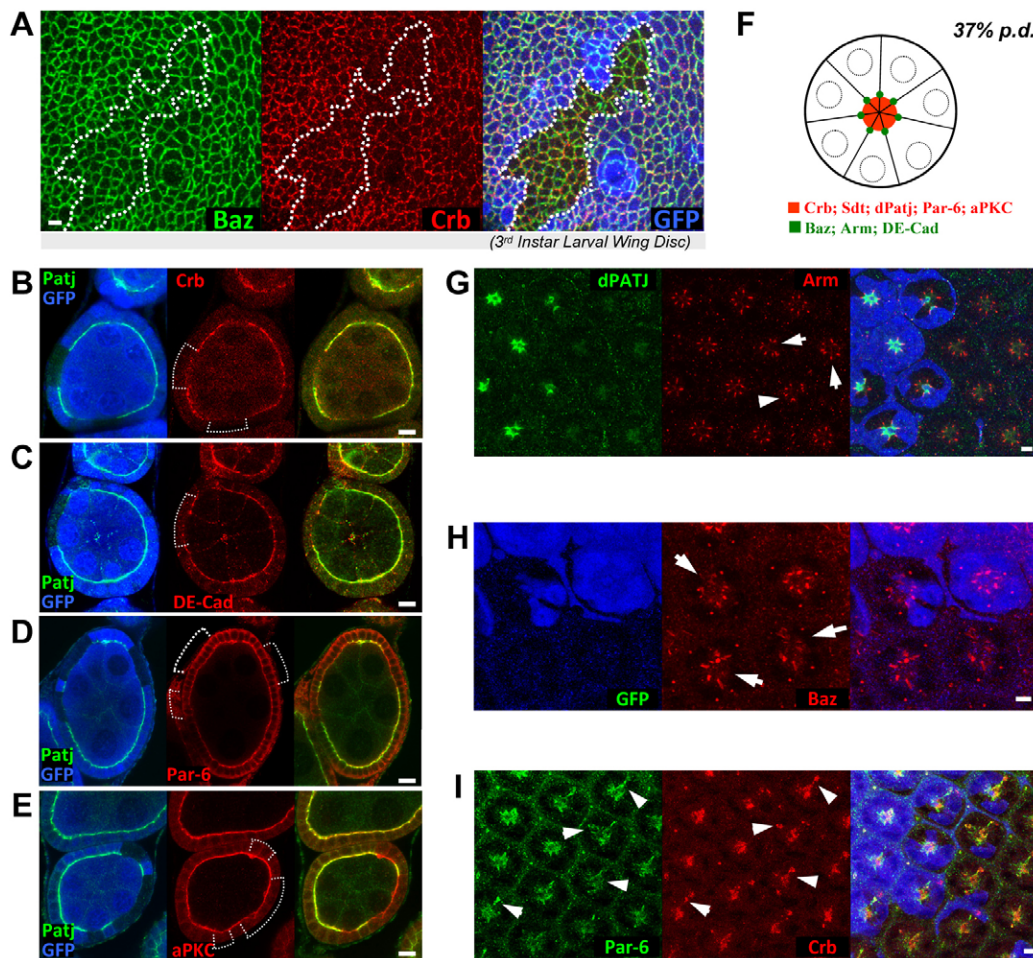
*Df(3L)BSC123* (supplementary material Table S1). *dPatj*<sup>Δ7</sup> *VK20-dPatj-L* also fully rescued the lethality of *dPatj*<sup>Δ7GLC</sup> embryos (supplementary material Table S1), indicating that the zygotic expression of *dPatj* is sufficient for development. It is likely that the early larval lethality observed in the *dPatj*<sup>symnull</sup> 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 *dPatj*<sup>Δ7GLC</sup> embryos ( $n > 10$ ), we found no discernible defects in cellularizing cells in the absence of dPatj (Fig. 2B). Because *dPatj*<sup>Δ7GLC</sup> 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 Pals1 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 *dPatj*<sup>Δ7GLC</sup> embryos, both Crb and Sdt showed normal subcellular localization (Fig. 2C,D). Crb expression was slightly reduced in *dPatj*<sup>Δ7GLC</sup> embryos, but the overall pattern was clearly undisturbed (Fig. 2C). We further examined the subcellular localization of several representative polarity proteins in *dPatj*<sup>Δ7GLC</sup> embryos, including: DE-Cadherin (DE-Cad; Shotgun – FlyBase), which marks the adherens junction; Bazooka (Baz, or Par-3), which is required for



**Fig. 3. dPatj supports Crb expression in polarized epithelial cells and apical-basal polarity in early pupal photoreceptors.** (A) *dPatj<sup>Δ7</sup>* clone (outlined) in a larval imaginal disc shows subtle reduction in Crb expression but normal expression of Baz. (B-E) *dPatj<sup>Δ7</sup>* 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 *dPatj<sup>Δ7</sup>* 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, *dPatj<sup>Δ7</sup>* clones are marked by the loss of GFP (blue). Scale bars: 10  $\mu$ m.

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). As shown in Fig. 2E-H, in *dPatj<sup>Δ7GLC</sup>* embryos none of these polarity proteins showed any discernible changes in their expression level or subcellular localization. We conclude that *Drosophila* Patj is not essential for initiating and establishing apical-basal polarity.

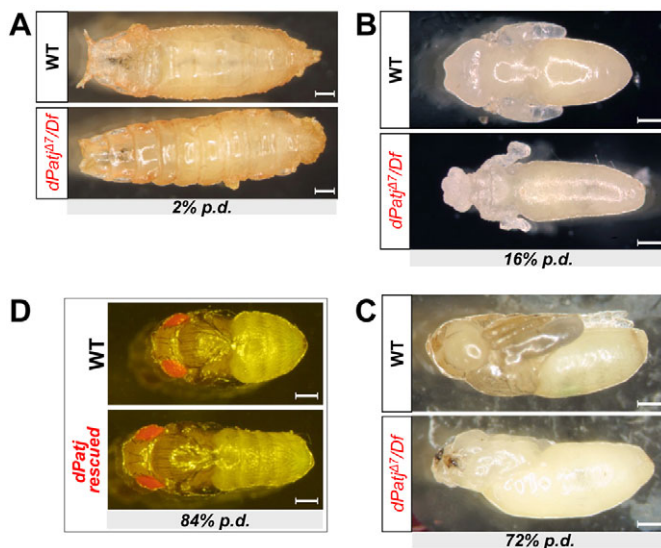
#### dPatj supports Crb expression and subcellular localization in polarized epithelial cells

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 *dPatj<sup>MY10</sup>* allele with multiple gene deletions

(Bhat et al., 1999), we generated *dPatj<sup>Δ7</sup>* clones in larval imaginal discs and adult follicular cells to investigate their phenotypes in polarized epithelial cells (Fig. 3A-E). The *dPatj<sup>Δ7</sup>* 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



**Fig. 4. dPatj is essential for pupal development.** (A) Deformed *dPatj<sup>Δ7</sup>/Df(3L)BSC123* (*dPatj<sup>Δ7</sup>/Df*) pupa at the beginning of pupation. (B,C) *dPatj<sup>Δ7</sup>/Df* and wild-type pupae at 16% pd and 72% pd, respectively. (D) Pupal development in *dPatj<sup>Δ7</sup> VK20-dPatj-L/Df(3L)BSC123* is completely rescued. WT are (A-C) *dPatj<sup>Δ7</sup> [or Df(3L)BSC123]/TM3 Sb twi>GFP* and (D) *dPatj<sup>Δ7</sup> VK20-dPatj-L/TM6 Tb*. In B-D, pupal cases were removed prior to imaging. Scale bars: 300 μm.

in the photoreceptor soma are generated through a complex morphogenetic process requiring many polarity proteins, including Baz, Crb, Sdt, Par-6 and aPKC (Izaddoost et al., 2002; Pellikka et al., 2002; Hong et al., 2003). It has been reported previously that in *dPatj<sup>synnull</sup>* photoreceptors, the apparent loss of dPatj causes the disruption of apical-basal polarity in the soma (Nam and Choi, 2003; Nam and Choi, 2006). To conclusively show that such phenotypes are indeed specific to dPatj, we generated *dPatj<sup>Δ7</sup>* mutant photoreceptor clones and examined their apical-basal polarity in early pupal retina at ~37% pupal development (pd) (Fig. 3F-I). The *dPatj<sup>Δ7</sup>* photoreceptors showed no dPatj expression (Fig. 3G) but did exhibit frequent mislocalization of major apical polarity proteins. Armadillo (Arm; *Drosophila* β-Catenin homolog) and Baz, which marks adherens junctions, were often disrupted in the *dPatj<sup>Δ7</sup>* photoreceptors (Fig. 3G,H), in addition to the basolateral mislocalization of Par-6 and Crb (Fig. 3I). Therefore, although it is dispensable in normal epithelia, dPatj might be crucial to maintain apical-basal polarity under more complex and stringent developmental contexts.

### **dPatj is required for pupal development**

We did not observe any obvious morphological abnormalities, such as the loss of imaginal discs, in *dPatj<sup>Δ7</sup>/Df(3L)BSC123* (hereafter *dPatj<sup>Δ7</sup>/Df*) larvae (data not shown). However, within several hours of pupal formation, the *dPatj<sup>Δ7</sup>/Df* pupae exhibited deformities in shape, with a flattened anterior end and rounded posterior (Fig. 4A). At 16% pd, the head region was underdeveloped, although the imaginal discs appeared to have successfully everted (Fig. 4B), suggesting that even without dPatj the imaginal disc cells could still modulate their cell junctions to accomplish disc eversion (Pastor-Pareja et al., 2004). Older *dPatj<sup>Δ7</sup>/Df* pupae failed to develop head and thoracic regions and growth of the everted imaginal discs stalled (Fig. 4C). The pupal defects and lethality were completely

rescued in *VK20-dPatj-L* or *dPatj<sup>Δ7</sup> 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<sup>Δ7</sup>/Df(3L)BSC123 crb<sup>ko</sup>* or *dPatj<sup>Δ7</sup> crb<sup>ko</sup>/dPatj<sup>Δ13</sup>* did not enhance pupal lethality (supplementary material Table S1). The viability of *dPatj<sup>synhypo</sup>* (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).

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### **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>

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**Table S1. Phenotypes of *dPatj* deletion alleles**

Allele/genotype	Phenotype
<i>dPatj<sup>Δ7</sup>/Df(3L)BSC123</i>	Pupal lethal
<i>dPatj<sup>Δ13</sup>/Df(3L)BSC123</i>	Pupal lethal
<i>dPatj<sup>Δ14</sup>/Df(3L)BSC123</i>	Pupal lethal
<i>dPatj<sup>Δ7</sup>/dPatj<sup>Δ7</sup></i>	Embryonic lethal
<i>dPatj<sup>Δ7</sup>/dPatj<sup>Δ13</sup></i>	Pupal lethal
<i>dPatj<sup>Δ7</sup>/dPatj<sup>Δ14</sup></i>	First instar lethal
<i>dPatj<sup>Δ13</sup>/dPatj<sup>Δ13</sup></i>	Second instar lethal
<i>dPatj<sup>Δ13</sup>/dPatj<sup>Δ14</sup></i>	Pupal lethal
<i>dPatj<sup>Δ14</sup>/dPatj<sup>Δ14</sup></i>	Embryonic lethal
<i>dPatj<sup>Δ7</sup> VK20-dPatj-L/dPatj<sup>Δ7</sup></i>	Viable
<i>dPatj<sup>Δ7</sup> VK20-dPatj-L/dPatj<sup>Δ13</sup></i>	Viable
<i>dPatj<sup>Δ7</sup> VK20-dPatj-L/Df(3L)BS123</i>	Viable
<i>dPatj<sup>Δ7</sup> VK20-dPatj::GFP-S/dPatj<sup>Δ7</sup></i>	Viable
<i>dPatj<sup>Δ7</sup> VK20-dPatj::GFP-S/dPatj<sup>Δ13</sup></i>	Viable
<i>dPatj<sup>Δ7</sup> VK20-dPatj::GFP-S/Df(3L)BS123</i>	Viable
<i>dPatj<sup>Δ7GLC</sup>/dPatj<sup>Δ7</sup></i>	Embryonic lethal
<i>dPatj<sup>Δ7GLC</sup>/dPatj<sup>Δ13</sup></i>	Pupal lethal
<i>dPatj<sup>Δ7GLC</sup>/Df(3L)BSC123</i>	Pupal lethal
<i>dPatj<sup>Δ7GLC</sup>/dPatj<sup>Δ7</sup> VK20-dPatj-L</i>	Viable
<i>Df(3L)BSC123 crb<sup>ko</sup>/dPatj<sup>Δ7</sup>*</i>	Pupal lethal
<i>dPatj<sup>Δ7</sup> crb<sup>ko</sup>/dPatj<sup>Δ13</sup>*</i>	Pupal lethal

\**crb<sup>ko</sup>* is a null allele of *crb* generated by gene targeting (Huang et al., 2009).