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Contribution of Ref(2)p to regulation of *Drosophila* notum epithelial cell apico-basal polarity and phenotype

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1. Introduction

Cell polarity is an essential characteristic in all cell types from single-cell organisms such as bacteria to multi-cellular organisms (1). The significance of cell polarity is apparent in controlling cell behaviour, outlining the asymmetry of cell shape, and controlling the distribution of different proteins with importance in cell migration, division, and differentiation (2, 3). A number of polarity complexes are implicated in polarity establishment, with the Par complex (aPKC-Par6-Baz) being central in maintaining cell polarity (4). In addition to this role, aPKC as part of Cdc42-Par6-aPKC complex is essential in maintaining cell junction stability and regulating actin based protrusions formation and actin cytoskeleton (5, 6).

Ref(2)p in Drosophila as well as its mammalian homologue p62 interacts physically with aPKC to regulate NF-kappa β signalling pathway activation (7). Using Drosophila melanogaster as a model system this study is investigating whether this interaction is relevant for apico-basal polarity and cell morphology. Interestingly, Ref(2)p has been revealed to have a role in protrusions formation in Drosophila haemocytes through regulating Rho pathway components (8).

2. Methods

Drosophila genetics, immunostaining, confocal microscopy were used.

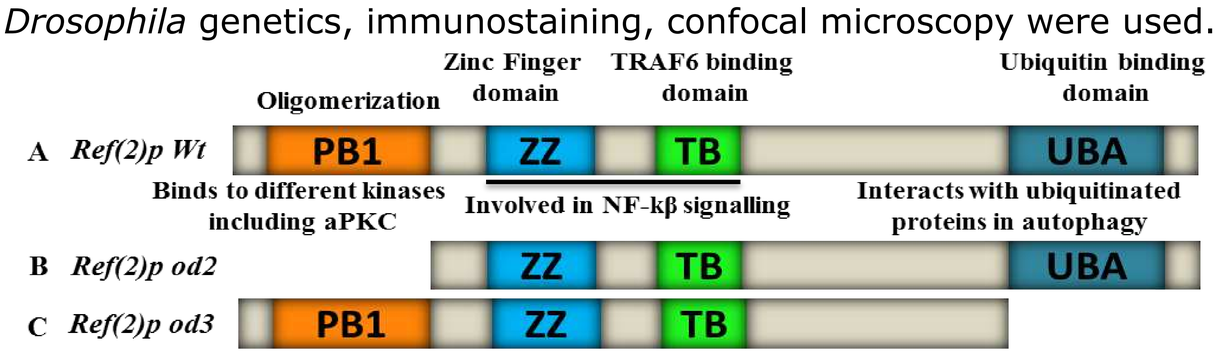

Figure 1: Schematic diagram of Ref(2)p mutants used.
Diagram shows wild type domains of Ref(2)p including PB1, ZZ, and TB domains in (A). *Ref(2)p od2* mutation, which lacks PB1 domain in (B) and *Ref(2)p od3* mutation, which lacks UBA domain in (C).

3. Results

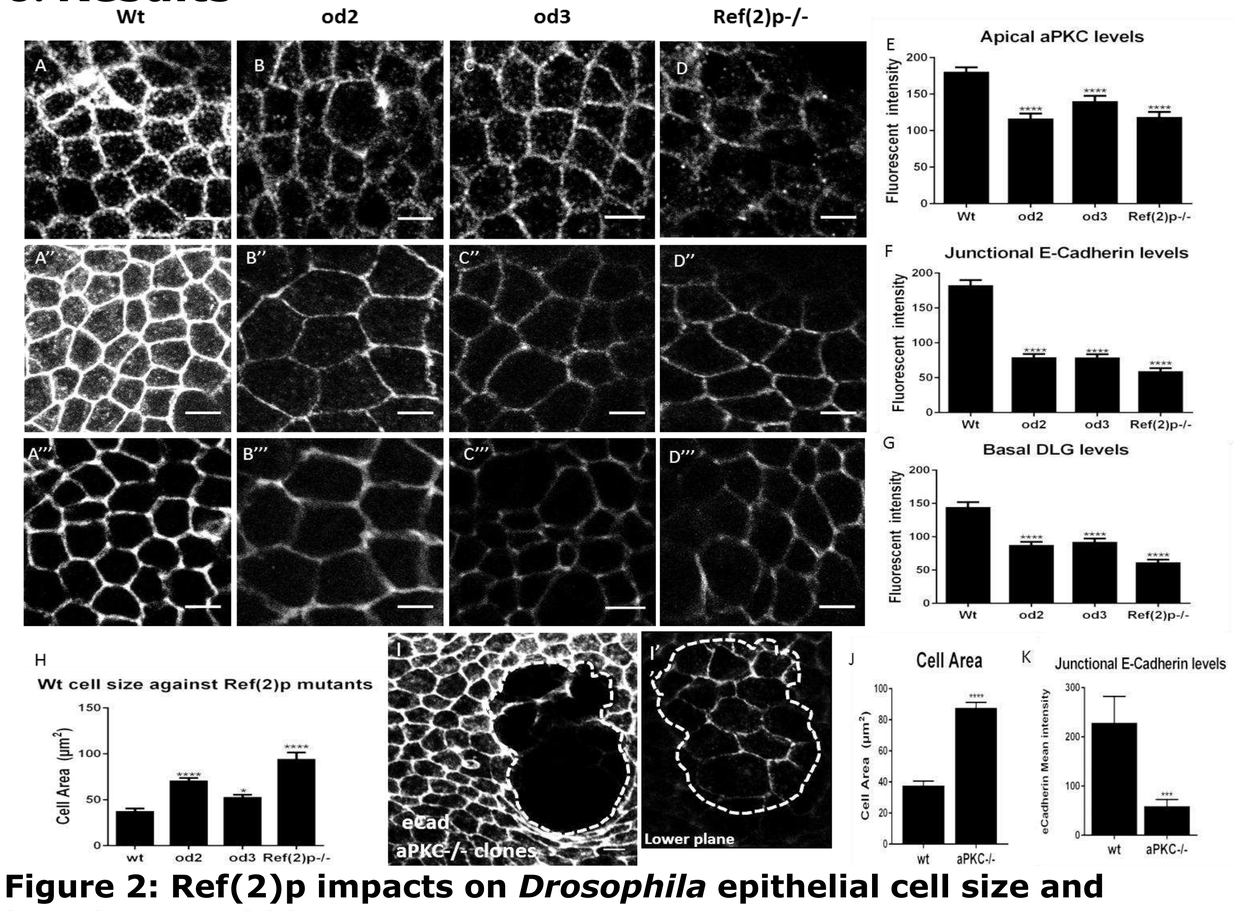

Figure 2: Ref(2)p impacts on *Drosophila* epithelial cell size and junctional stability.
Flies with germline mutations in the *Ref(2)P* gene express mutant proteins which lack different functional domains including homozygous *od2* (B) and *od3* (C). UAS-*Ref(2)p* RNAi is driven to the back of the fly by PnrGal4 (D). Ref(2)p mutants showed junctional breaks and increased cell size (H) in comparison to wild type (A). Polarity proteins levels in *Ref(2)p* mutants and RNAi showed a broad decrease in aPKC (E), eCadherin (F) and DLG (G). Flp/FRT system was used to generate negatively marked clones of homozygous *aPKC* null cells in Drosophila notum using Ubi-nls-GFP construct, which expresses nuclear GFP in wild type cells so aPKC phenotypes could be compared to Ref(2)p phenotypes (I). As published before tissue folding and cell size increase observed in aPKC null clones in the lower plane (I'-J) respectively. eCad levels were decreased in *aPKC* null cells (K). Scale bars represent 5 microns.

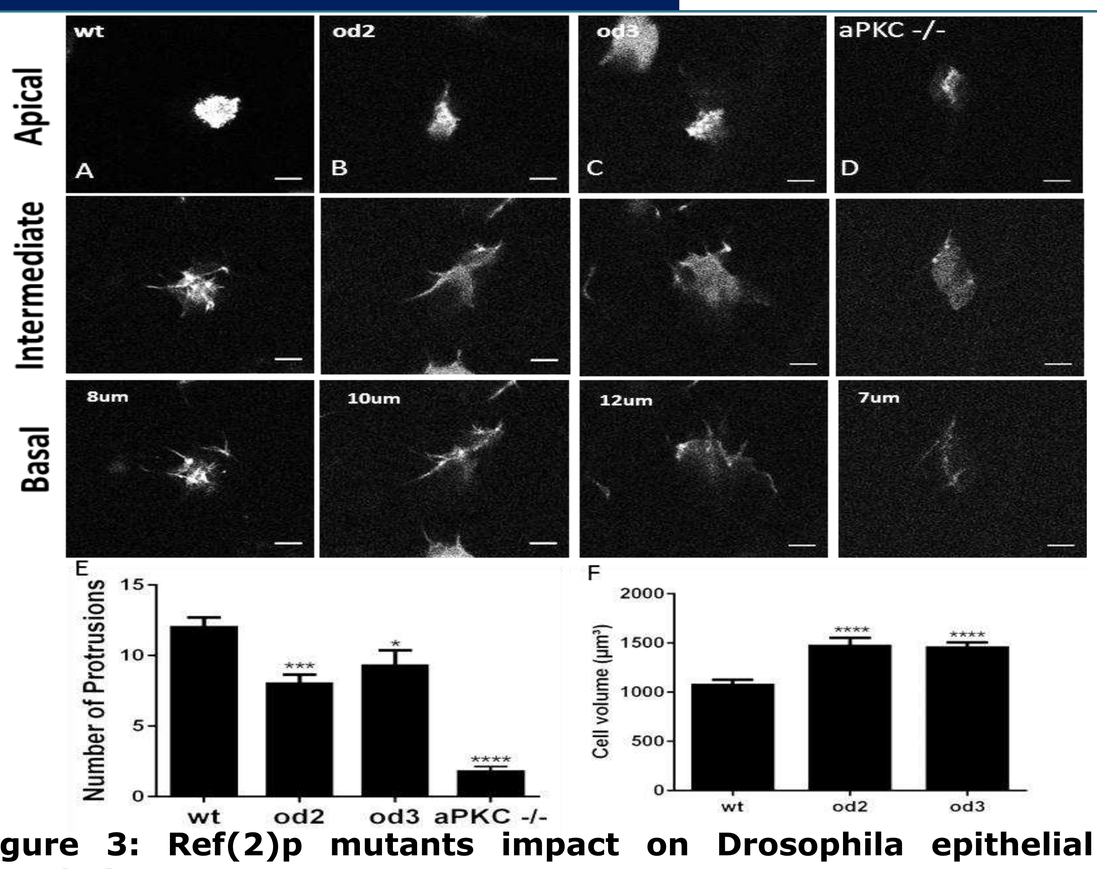

Figure 3: Ref(2)p mutants impact on Drosophila epithelial cell morphology.
NeurGal4-UAS-moeGFP driver was used to study individual cell morphology in *od2* and *od3* homozygous mutants compared to wild-type epithelial cells. Wild type cells appear in (A) with average 8 um cell length; however *od2* (B) and *od3* (C) mutants were more longitudinal. The number of protrusions decreased in mutants (E), and similarly protrusion almost diminished in *aPKC* null mutant cells, sometimes few filopodia appear at the basal domain (D). Further, there is an increase in *Ref(2)p* mutants cell volume (F) in comparison to wild type cells. Scale bars represent 5 microns.

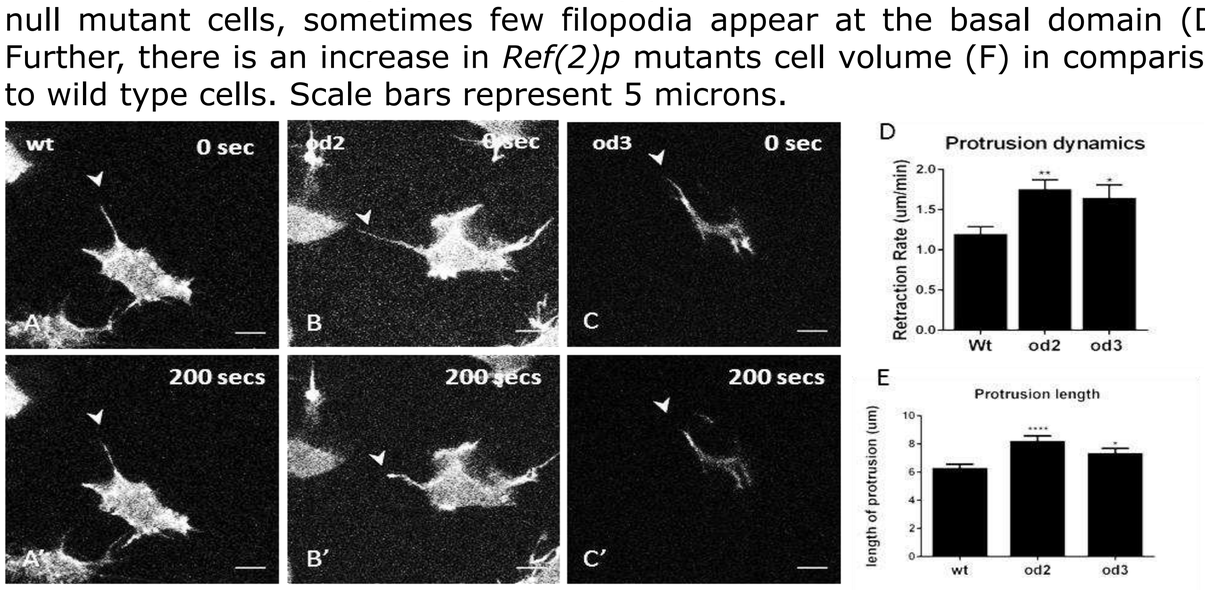

Figure 4: Ref(2)p influences Drosophila epithelial cell protrusion dynamics and morphology.
Using NeurGal4-UAS-moeGFP in *od2* and *od3* homozygous mutants, a change was noted in protrusions dynamics as shown in *od2* (B), *od3* (C) in comparison to wild type (A) and this is represented in the histogram in (D) There is an increase in protrusions retraction rate detected in *Ref(2)p* mutants, however no significant increase in extension rate observed. A lower number of protrusion were found in *Ref(2)p* mutants, whereas they were longer in length (E). Scale bars represent 5 microns.

4. Conclusion

- Ref(2)p has an essential role in regulating polarity proteins.
- Ref(2)p has a role in maintaining cell size, junctional stability and protrusion morphology and dynamics in *Drosophila* epithelial cells.
- Ref(2)p phenotypes are similar to aPKC phenotypes, which suggests a Ref(2)p role in maintaining actin cytoskeleton organization.

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