Discussion

Several studies have focus on how epithelial polarity is established and regulated, and how the different regulators cross-talk to each other. Although important insights have resulted from these studies, little is known about the dynamic regulation of epithelial polarity during morphogenesis

It is known that the kinase activity of aPKC is important for the stabilization, activation and localization of several polarity components and AJs. Several studies in MDCK cells and Drosophila showed that absence of aPKC result in loss of epithelial integrity in different epithelial tissues. In this way, aPKC is thought to play a crucial and conserved role in regulating epithelial apico-basal polarity and in maintaining cell integrity (Wodarz, Ramrath et al. 2000; Rolls, Albertson et al. 2003; Harris and Peifer 2007).

Nevertheless it is still unclear to what extent aPKC function is conserved in different epithelia and/or during different morphogenetic events. In order to answer this, we took advantage of a previously isolated temperature-sensitive allele of aPKC. Analyses of this allele suggested the following hypotheses:

1. Analysis of *aPKC^{TS}* mutants.

1.1 Hypothesis - Different epithelial tissues have differential requirements of aPKC activity.

Previously we have isolated a temperature-sensitive allele of aPKC that at 25 º C shows a 100% lethal phenotype in a maternal mutant, whereas zygotic mutant is 100% viable. In contrast at 30ºC the aPKC^{TS} zygotic mutant is 100% lethal. We observed that during GBE, epithelial ectoderm shows loss of integrity at 25ºC (maternal phenotype), whereas follicular epithelium and wing imaginal discs blade are mostly normal at the same temperature (zygotic phenotypes). Also consistent with our hypothesis, very recently was shown that aPKC function in the follicular epithelium is independent of Par6 and kinase activity (Kim, Gailite et al. 2009). Altogether, these observations are consistent

with the hypothesis that different epithelial tissues have differential requirements of aPKC activity.

1.2 Hypothesis - Epithelial tissue requires higher levels of aPKC activity during *de novo* formation of AJs.

In contrast with aPKC^{TS} zygotic mutants that are mostly normal at 25ºC, we observed loss of epithelial integrity during GBE at all tested temperatures. During this stage, AJ belt is still under maturation from spot junctions into ZA. Also the apical domain components, like Crb, are not enriched at the sub apical region, as well, the basolateral components weren't excluded from the apical domain. This suggest that immature epithelium (Muller 2003), are particularly sensitive to lower levels of aPKC activity.

Moreover, in the follicular epithelium, when the temperature is increased to 30ºC or by reducing one copy of aPKC^{TS} (*aPKC^{TS}/DF6482*), we observed defects in germ-line cyst encapsulation. This phenotype suggests defects in mesenquimal to epithelial transition of precursor follicle cells, a process that depends on the formation of new AJ, and that appears to be particulary sensitive to lower levels of aPKC. Interestingly, the mutant cells that manage to do MET give rise to a completely normal epithelia, which suggest that once the epithelium is formed the aPKC^{TS} activity is not rate-limiting anymore.

During metamorphosis a set of abdominal histoblasts (adult epidermal cell precursors) undergo rapid divisions and then grow to replace the adjacent larval epithermal cells. This process is completed upon the fusion of dorsal midline by 36 hours after puparium formation, which is a process dependent of the formation of new AJs (Ninov, Chiarelli et al. 2007). Also supporting our hypothesis, aPKC^{TS} mutants show defects in adult abdomen dorsal closure at semi-permissive temperatures, whereas many other adult structures (wings, eyes, etc.) are mostly normal.

Based on our results, we propose that aPKC requirements are higher, when epithelium is immature and/or is involved in morphogenetic processes that involve the *de novo* formation of AJs (Fig.23).

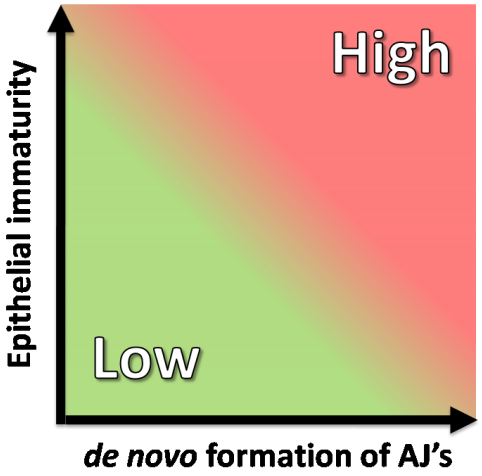

Fig 23. - aPKC requirements within different epithelial tissues. Displayed colors symbolize the requirements of aPKC activity, low (green) and higher (red).

1.3 Hypothesis - *aPKC^{TS}* is most likely a temperature-sensitive kinase.

Although we still need to do an *in vitro* kinase assay to directly test it, there are already several evidences that support this hypothesis.

aPKC^{TS} allele has a point mutation within a highly conserved aminoacid in the kinase domain. Analyses of the conformational changes induced by this mutation (Fig. 24 orange), using cristal structure of the kinase domain of aPKC Human homologue (Messerschmidt, Macieira et al. 2005), suggest that aPKC^{TS} kinase domain is likely to be more unstable. This point mutation is likely to affect the activation loop stability (Fig. 24 green) near to C-lobe since creates some space within a nearby hydrophobic region (Fig. 24 purple). Higher temperatures might cause vibrations in the structure that might destabilize this C-lobe, by exposing the hydrophobic region to water molecules, affecting the protein folding and consequently his function (Fig. 24). Consistent with this hypothesis, other protein kinases that have on amino acid similar to our mutation invariably have a compensatory change of the neighbor amino acid to Trp (a bulky amino acid), presumably to fill the hole and maintain conformational stability.

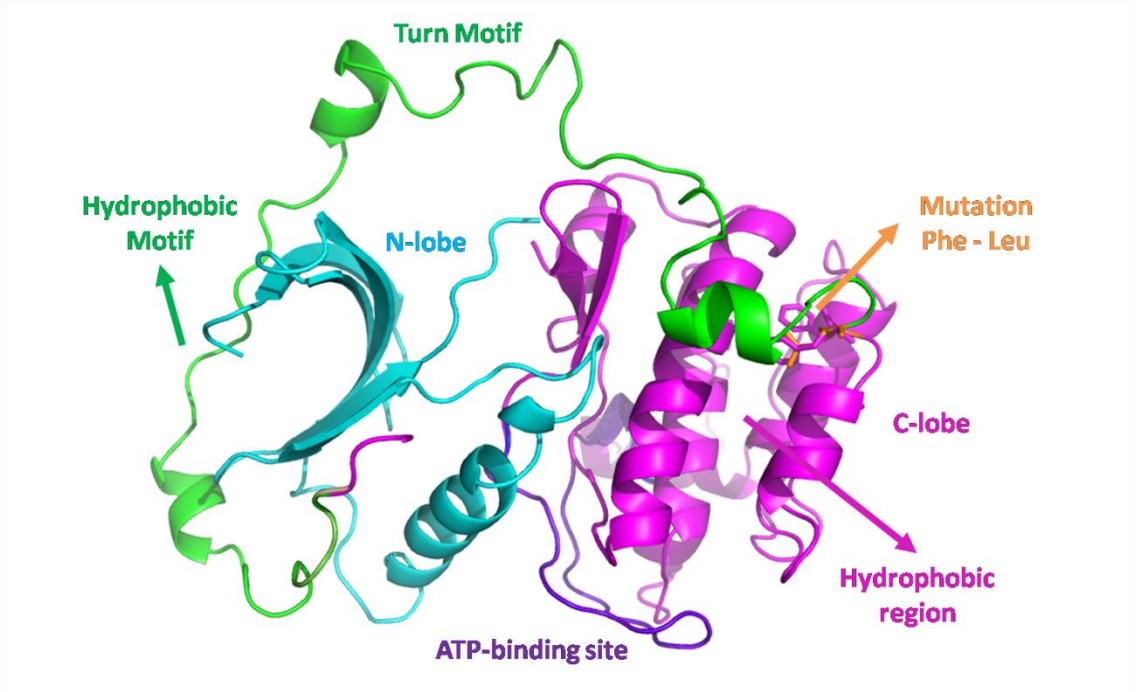

Fig. 24 - Crystal Structure of Catalytic domain from Human Atypical Protein Kinase C-iota. ATP-binding site (blue) is located between N-lobe (cyan) and C-lobe (purple). Protein structure is stabilized by activation loop that contain both hydrophobic and Turn Motifs (green). Mutation of *aPKC*^{TS} allele is marked in orange on a residue near to hydrophobic region of C-lobe. Structure analysis in collaboration with Alekos Athanasiadis.

Currently there are two scenarios for the differential requirement observed in the aPKC^{TS} mutants:

A – Qualitative scenario:

Since the aPKC^{TS} mutation is within the kinase domain, with which it interacts with its substrates, we can think that there can be one or more substrates that aPKC^{TS} is unable to properly phosphorylate. Consider a scenario where two different substrates of aPKC have different affinities for this kinase. The one with lower affinity will be, in these conditions, less likely to be phosphorylated by aPKC^{TS}, whereas the higher-affinity substrate will still be phosphorylated, even in the aPKC^{TS} mutant scenario. If the function of the protein with lower affinity for aPKC is only important in immature epithelial tissues, then this could explain why these tissues are more sensitive to lower levels of aPKC activity.

B – Quantitative scenario:

Alternatively, the differences observed in aPKC^{TS} may result from the differential phosphorylation requirements that the various tissues might have in terms of aPKC substrates. In aPKC^{TS} mutant this substrates might not be properly phosphorylated, but the level of phosphorylation may be sufficient for mature epithelium, whereas for immature tissues they are not (Fig.25). These differences would explain why epithelial tissues are differentially affected in the aPKC^{TS} mutants.

Despite the formalization of the above two hypotheses, it is possible that the aPKC^{TS} phenotype results from the combination of both scenarios.

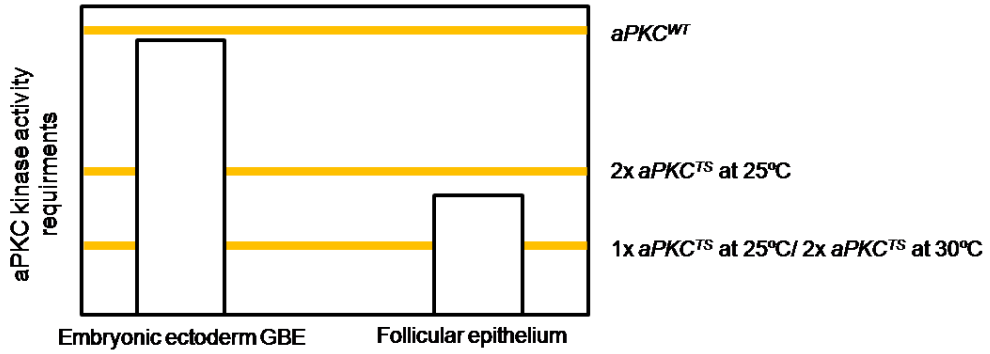

Fig. 25 - Requirements of aPKC activity during *Drosophila* development. White bars represent the hypothetical requirement of aPKC activity during embryonic ectoderm (GBE) and follicular epithelium. The Orange lines represents the aPKC activity in different represented cases.

2. Analysis of *aPKC^{PB1}* mutant.

2.1 Hypotheses - The apical localization of aPKC is essential for maintenance of follicular epithelium, even in the absence of kinase activity

Analyses of aPKC^{PB1} showed a point mutation in a highly conserved amino acid within the PB1 domain (Fig. 26 A and B)(Hirano, Yoshinaga et al. 2005). This domain was shown to be essential for interaction with PAR6, and this interaction is recruited for proper aPKC apical localization and activation. We speculated that in aPKC^{PB1} mutant aPKC does not interacts with PAR6 but we still need to test this biochemically. During GBE the phenotype of aPKC^{PB1} is very reminiscent with aPKC^{TS} mutant. In both

mutants the embryonic epithelium loses the integrity. Surprisingly in follicular epithelium whereas aPKC^{TS} shows a partial failure to induce PFCs MET, in aPKC^{PB1} all PFCs fail MET. In this mutant the developing germ-line cyst is surrounded by mesenquimal-looking follicular tissue, which is very reminiscent with aPKC loss-of-function. Since aPKC^{PB1} protein does not localize apically in the embryo ectoderm cells, but shows normal levels of protein, and since a published aPKC-kinase-dead shows a phenotype reminiscent of the aPKC^{TS} (Kim, Gailite et al. 2009), this suggests that apical localization of aPKC have a potential structural function necessary to induce MET and maintain epithelial integrity.

We hypothesize that apical localization of aPKC recruits proteins to apical domain necessary to maintain epithelial integrity.

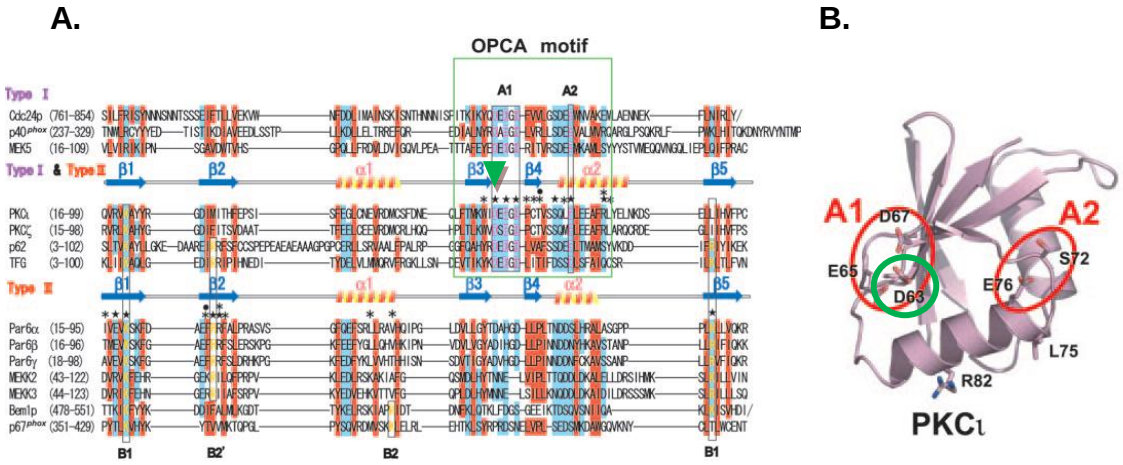

Fig. 26 - Conservation of Aspartic acid (D) residue (green arrowhead in A and green circle in B) in PB1 domain. Conserved type I acidic regions A1 and A2. Residues with side chains involved in an intermolecular salt bridge and/or hydrogen bond *(Stars)*. Residues involved in an intermolecular hydrophobic interaction (*Asterisks).* Adapted from (Hirano, Yoshinaga et al. 2005)

Conclusions

All together, our data point to the fact that different epithelial cells, even sharing similar architecture and dynamic interactions, are faced with different requirements of aPKC dependent of the characteristics and processes related with that specific tissue. Furthermore, analysis of the aPKC^{PB1} allele also suggested kinase-independent functions of aPKC in the apical domain of the follicular epithelium.